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Modulators of Nucleoside Metabolism in the Therapy of Brain Diseases

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Abstract

Nucleoside receptors are known to be important targets for a variety of brain diseases. However, the therapeutic modulation of their endogenous agonists by inhibitors of nucleoside metabolism represents an alternative therapeutic strategy that has gained increasing attention in recent years. Deficiency in endogenous nucleosides, in particular of adenosine, may causally be linked to a variety of neurological diseases and neuropsychiatric conditions ranging from epilepsy and chronic pain to schizophrenia. Consequently, augmentation of nucleoside function by inhibiting their metabolism appears to be a rational therapeutic strategy with distinct advantages: *(i)* in contrast to specific receptor modulation, the increase (or decrease) of the amount of a nucleoside will affect several signal transduction pathways simultaneously and therefore have the unique potential to modify complex neurochemical networks; *(ii)* by acting on the network level, inhibitors of nucleoside metabolism are highly suited to fine-tune, restore, or amplify physiological functions of nucleosides; *(iii)* therefore inhibitors of nucleoside metabolism have promise for the “soft and smart” therapy of neurological diseases with the added advantage of reduced systemic side effects. This review will first highlight the role of nucleoside function and dysfunction in physiological and pathophysiological situations with a particular emphasis on the anticonvulsant, neuroprotective, and antinociceptive roles of adenosine. The second part of this review will cover pharmacological approaches to use inhibitors of nucleoside metabolism, with a special emphasis on adenosine kinase, the key regulator of endogenous adenosine. Finally, novel gene-based therapeutic strategies to inhibit nucleoside metabolism and focal treatment approaches will be discussed.

Keywords

adenosine; ADK; nucleosides; focal drug delivery; neurology; psychiatry

INTRODUCTION

Half a century ago inhibitors of nucleoside metabolism received much attention as inhibitors of nucleic acid synthesis, and therefore as potent anti-neoplastic and anti-viral agents [1–3]. The past decade has brought a wealth of new understanding how nucleoside metabolism is involved in normal brain function and how dysfunctional nucleoside metabolism is implicated in the etiopathology of a wide spectrum of neurological, neurodegenerative, and neuropsychiatric disorders. Consequently, the pharmaceutical development of inhibitors of nucleoside metabolism has gained renewed interest as potential agents to treat brain disease.

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Augmentation of nucleoside function by inhibiting their metabolism is expected to be advantageous compared to traditional approaches that involve the direct modulation of specific receptors: A subtle change in the amount of a nucleoside will affect several signal transduction pathways simultaneously with the potential to modify complex neurochemical networks at the same time. Inhibitors of nucleoside metabolism are highly suited to fine-tune, restore, or amplify physiological functions of nucleosides with the added advantage of reduced systemic side effects. This review will focus on the nucleosides adenosine, guanosine, cytidine, uridine, and inosine, inhibitors of their metabolism, and nucleoside-based therapeutic approaches to treat brain disease. The transport of nucleosides has recently been covered elsewhere [4] and will not be discussed here.

NUCLEOSIDES IN BRAIN FUNCTION

Adenosine

The purine ribonucleoside adenosine is a ubiquitous neuromodulator and upstream regulator of diverse brain functions [5–12]. In brain, adenosine is an endogenous distress signal [9] accumulating rapidly as an acute response to stress, e.g. under conditions of oxygen deprivation during stroke [13–15] or under conditions of excessive energy consumption during seizures [16]. Adenosine modulates many neuronal [17] and glial [10] functions in physiological and pathophysiological conditions [11,12] such as seizure susceptibility [18], tissue damage and repair [9], and immune functions of the brain [19]. In addition, adenosine has direct impacts on other neurotransmitter systems and is an important upstream regulator to integrate and fine-tune glutamatergic and dopaminergic neurotransmission [17]. The downstream effects of adenosine are mediated by G protein-coupled adenosine receptors (ARs) A_1, A_{2A}, A_{2B}, and A_3 [20,21], which are important targets for drug development [22,23]. Inhibitory A_1Rs and facilitatory A_{2A}Rs have in common a high affinity for adenosine, but have opposing activities based on coupling to different G proteins and a spatially distinct expression within the brain [21]. Ambient levels of adenosine (i.e. the “tone” of adenosine) within the brain is largely regulated by the astrocyte-based enzyme adenosine kinase (ADK), which is regarded as the key “upstream-regulator” of adenosinergic neuromodulation [24,25] (Figure 1).

The opposing activities of A_1 and A_{2A}Rs indicated above imply that adenosine can exert both inhibitory and excitatory functions within the brain. A_1Rs mediate inhibitory neuromodulation by coupling to inhibitory G_i or G_o containing G proteins [12,23]. As a result of this interaction adenylyl cyclase is stimulated, inwardly rectifying K^+ channels are activated, presynaptic Ca^{2+} channels are inhibited [26], and phospholipase C is activated. Consequently, the release of predominantly excitatory neurotransmitters, such as dopamine, serotonin and acetylcholine, is inhibited, while the postsynaptic neurons are hyperpolarized. Thus, therapeutic activation of A_1Rs has profound antiepileptic [27] and neuroprotective [28] functions.

In contrast to the inhibitory activity of A_1Rs, excitatory functions of adenosine are largely mediated by activation of A_{2A}Rs. Thus, inhibitors of the A_{2A}R have profound neuroprotective functions [28] and prevent apoptosis [29]. The A_{2A}R has important functions related to the control of dopaminergic neurotransmission [17,30–33]. Thus, the highest expression of A_{2A}Rs is found in striatal neurons, which are involved in the control of motor function [34]. A_{2A}Rs can form heteromers with other receptors, e.g. A_1/A_{2A} heteromers, or A_{2A}/D_2 heteromers and thus can modulate adenosinergic and dopaminergic neuromodulation by direct receptor interactions [31,35]. Due to these interactions with other transmitter systems and due to its action on receptors with opposing activity (A_1 *versus* A_2), adenosine is uniquely positioned as an upstream regulator to integrate and fine tune excitatory and inhibitory functions within the central nervous system (CNS).

Ambient levels of brain adenosine appear to be largely under the control of astrocytes [36–40]. Under physiological conditions a significant part of synaptic adenosine is derived from astrocytic vesicular release of adenosine-5′-triphosphate (ATP) and its extracellular degradation to adenosine by several ectonucleotidases [41]. Extra- and intracellular levels of adenosine are rapidly equilibrated by nucleoside transporters [42–44]. Within astrocytes, adenosine is rapidly phosphorylated and removed from the adenosine pool by ADK [24,39]. Thus, ADK controls synaptic adenosine by driving its influx into astrocytes via the nucleoside transporters [24].

Guanosine

Guanine-based purines are well recognized as intracellular modulators of G protein activity. In addition, several extracellular effects of guanosine that are not related to G proteins have recently been identified [45]. Extracellular guanosine is largely derived from astrocytic sources [46], fulfills important neurotrophic functions in the CNS [46] and stimulates astrocyte proliferation likely by increasing the endogenous extracellular adenosine concentration [47]. Current evidence suggests that guanosine stimulates glutamate uptake by astrocytes [48–50]. Consequently, guanosine reduces seizures induced by glutamatergic agents [51,52], and is neuroprotective in response to ischemia-hypoxia [53]. On the behavioral side, guanosine impaired retention on the inhibitory avoidance task and decreased locomotor activity on the open field test [54]. Although the receptors and molecular pathways affected by guanosine are still incompletely understood, several studies suggest guanosine as potential new target for neuroprotection and neuromodulation.

Cytidine and uridine

Cytidine and uridine are not known to have direct effects on CNS function. Via pyrimidine salvage they will be transformed into cytidine-5′-triphosphate (CTP) and uridine-5′-triphosphate (UTP) and can contribute to brain phosphatidylcholine and phosphatidylethanolamine synthesis via the Kennedy pathway [55]. Based on these metabolic links cytidine and uridine metabolism might however play roles in neurodegenerative conditions.

Inosine

Inosine is a metabolite of adenosine and like adenosine, inosine fulfills important neuroprotective functions. Apart from being a degradation product of adenosine, inosine can directly be released from neurons in a *N*-methyl-d-aspartate receptor (NMDAR) dependent manner requiring extracellular calcium [56]. In addition, inosine can stimulate neurons to extend axons in culture and, *in vivo*, augments the ability of undamaged neurons to form axon collaterals after brain damage. Using a unilateral injury model limited to the sensorimotor cortex, it was recently shown that inosine increased the number of corticospinal tract axons that project from the unaffected hemisphere and enhanced the ability of these neurons to form connections on the denervated side of the spinal cord, which led to improved performance of the impaired limb [57]. This effect was attributed to inosine-induced alteration of gene expression in neurons contralateral to a stroke. Apart from direct effects of inosine within the CNS it was recently shown that inosine can contribute to beneficial effects in multiple sclerosis that have been attributed to a rise in serum urate levels [58].

NUCLEOSIDES IN BRAIN PATHOLOGY

The complex nature and ubiquitous distribution of nucleosides within the CNS implies that any dysfunction of nucleoside levels or distribution may translate into a wide spectrum of neurological diseases and psychiatric conditions. Research from recent years has provided

novel evidence that dysregulation of endogenous nucleosides, in particular of adenosine, might play key roles in the pathogenesis of a wide range of neurological and neuropsychiatric conditions. The following sections will elucidate in selected examples – using adenosine as a prototype nucleoside – that any pathological dysregulation of nucleoside metabolism is expected to contribute to a vast array of neurological and neuropsychiatric disorders. Therefore, inhibitors of nucleoside metabolism hold great potential for the treatment of a vast spectrum of conditions (Table 1).

Epilepsy

Adenosine is widely considered to be an endogenous anticonvulsant of the brain [16,18,59–61]. Recent findings indicate that dysfunction of astrocytes [37,38,40,62–66] and in particular dysfunction of the adenosine system [24,67] can cause seizures [18], or augment the spread of kainic acid- or traumatic brain injury- induced seizures and associated neuronal cell loss [67,68]. Apart from other histopathological changes [69–73] including mossy fiber sprouting, dispersion of granule cells, and pyramidal cell loss, astrogliosis [74,75] is one hallmark of the epileptic brain. In contrast, the loss of astrocytes – which provide a major source for adenosine – has been described in epileptic brain specimens obtained from patients with Rasmussen's encephalitis [76].

Astrogliosis depends on the proliferation and hypertrophy of astrocytes, which is regulated in part by the ratio of A_1R and A_{2A}R expression on astrocytes [10]. Thus, activation of the A_{2A}R potentiates synaptic actions of brain-derived neurotrophic factor (BDNF) in the hippocampus [77], stimulates glutamate outflow and leads to excessive glial activation [78]. Experimentally, astrogliosis could be increased in rat cortex by micro-infusion of the A_{2A}R agonist 5′-(N-cyclopropyl)-carboxamidoadenosine (CPCA); this effect could be abolished by co-infusion of the adenosine A_{2A}R antagonist 1,3-dipropyl-7-methylxanthine (DPMX) [79]. In line with these findings, A_{2A}R blockade abolished reactive gliosis in rat striatal primary astrocytes that was induced by basic fibroblast growth factor [80]. These findings suggest that A_{2A}R stimulation promotes astrogliosis. Since upregulation of A_{2A}Rs [28] as well as acute increases in adenosine [81] have all been described as a consequence of brain injury, hypoxia, or inflammation, it is tempting to speculate that astrogliosis might be triggered via an adenosine-based mechanism. Likewise, stimulation of A_1Rs, which are downregulated during epileptogenesis [82–84], was demonstrated to lead to a reduction of astrocyte proliferation [47].

Due to astrogliosis, changes in the homeostasis of the astrocyte-based adenosine cycle are to be expected in epileptic brain. Thus, as a consequence of astrogliosis, the astrocyte-based adenosine-removing enzyme ADK is upregulated, leading to a reduction in ambient adenosine [24]. Interestingly, upregulation of ADK coincides – both spatially as well as temporally – with the emergence of spontaneous chronic seizures [24]. The hypothesis that upregulated ADK can be a direct cause for seizures was validated by creating transgenic mice with brain-wide overexpression of ADK (Adk-tg mice); as a consequence of this genetic manipulation Adk-tg mice display reduced seizure thresholds and spontaneous seizures [85]. These findings provide several rationales for therapeutic interventions in epilepsy: *(i)* Reconstitution of the physiological adenosine tone by increasing adenosine – ideally restricted to an astrogliotic scar; *(ii)* inhibition of ADK; *(iii)* prevention of astrogliosis by adenosine receptor modulation.

Cerebral Stroke

Adenosine, via activation of A_1Rs is considered to be an important neuroprotectant against ischemic damage in the adult brain, although there is still controversy regarding the underlying mechanisms [15,86–89]. Surprisingly, A_1R activation does not protect the

developing brain and impairs cerebral maturation [90] and adenosine receptor antagonists reduce ischemic brain injury in neonatal mice [91]. Thus, transient high neuronal expression of ADK in the early postnatal development of the mouse brain [39] might be a physiological mechanism to keep adenosine levels low.

In adult brain a rapid surge of adenosine following cerebral ischemia [15,92] as an endogenous neuroprotective mechanism might initially lead to a cerebroprotective state known as ischaemic tolerance [93], a phenomenon, in which astrocytes appear to play a prominent role [94]. In line with this notion, the astrocytic enzyme ADK was downregulated within 3 hours following mild or severe stroke in the mouse [13]. Downregulation of ADK was associated with an increase in ambient adenosine [13]. Thus, activity levels of ADK in astrocytes might control the brain's susceptibility to ischemic cell death. In support of this hypothesis Adk-tg mice overexpressing ADK in brain are hypersensitive to middle cerebral artery occlusion (MCAO) induced cell death [95]. If increased ADK in brain renders the brain more vulnerable, then focal augmentation of adenosine in the brain should be protective. This was demonstrated recently by transplantation of ADK-deficient neural or glial progenitor cells into the striatum of mice one week prior to MCAO; adenosine-releasing brain implants led to a striking reduction of the injured brain volume [96]. Thus, susceptibility to ischemia-induced brain injury seems to be tightly balanced by the tone of ambient adenosine. Consequently, as a rationale for therapeutic cerebroprotection, augmentation of the adenosine system e.g. by focal cell implants or inhibition of ADK appears to have promising potential.

Sleep Disorders

Adenosine is considered to be an endogenous sleep inducer based on a variety of pharmacological and behavioral data. Its biological function appears to be the restoration of brain energy metabolism during sleep [97,98]. Administration of adenosine or its analogues can induce sleep when administered to experimental animals [99]. In behavioral studies sleep deprivation leads to increases in extracellular adenosine levels in the basal forebrain, but to decreases during sleep recovery [99,100]. Sleep induction seems to be dependent on the activation of both A_1 and A_{2A}Rs, however their relative contribution to sleep induction remains unclear [99,101]. Thus, the electroencephalographic effects of sleep deprivation could be mimicked by A_1R stimulation [102]. Adenosine stimulated calcium release in cholinergic but not non-cholinergic neurons via the A_1R [103]. Microdialysis perfusion of A_1R antisense oligonucleotides into the basal forebrain resulted in a significant reduction in non-rapid eye movement (NREM) sleep and electroencephalogram delta power with an increase in wakefulness within the spontaneous levels of sleep-wakefulness [104]. These and the above data support the hypothesis that adenosine, acting via the A_1R in the basal forebrain is a key component in the homeostatic regulation of sleep. In contrast to adenosine, the non-selective A_1R and A_{2A}R antagonist caffeine promotes wakefulness [105,106].

More recent data demonstrate that the A_{2A}R might play a dominant role in sleep regulation. Thus, it was recently demonstrated that caffeine promoted wakefulness in wildtype (WT) and A_1R knockout (KO) mice, but not in A_{2A}R KO mice, indicating that the arousal effect of caffeine was caused by blockade of the A_{2A}R [107]. Likewise, caffeine was shown to reduce the hypnotic effects of alcohol via the A_{2A}R [108]. Together, these data imply a predominance of the A_{2A}R for sleep regulation, although the subtype of adenosine receptor responsible for sleep regulation is still a matter of debate [99,109].

In regard to the ongoing debate on the contribution of different adenosine receptor subtypes to sleep regulation, both A_1R and A_{2A}R KO mice demonstrated clear circadian profiles of sleep-stage distribution and identical amounts of NREM and rapid eye movement (REM)

sleep compared to WT mice under basal conditions [107,110]. A major difference however is that A_{2A}R KO mice did not show NREM sleep rebound after sleep deprivation; in contrast they exhibited a robust REM sleep rebound [109]. In contrast, A_1R KO displayed a phenotype similar to WT in having a robust rebound of both NREM and REM sleep after sleep deprivation [110]. These results indicate that the A_{2A}R, but not the A_1R, is essential for the homeostatic regulation of NREM sleep and imply that any dysregulation within the adenosine system affects sleep properties.

Chronic Pain

Pro- and antinociceptive activity can both be mediated by adenosine depending on the site of application or the receptor(s) involved [8]. Pain pathways involving the A_1R, which dominates in mediating the antinociceptive effects of adenosine, have received most attention [8]. Thus, A_1R agonists – acting at spinal sites – are effective antinociceptive agents in conditions of neuropathic and inflammatory pain, therapeutic effects mediated probably by prejunctional inhibition of transmitter release in pain pathways [111]. In line with these findings, A_1 receptor knockout mice are characterized by hyperalgesia and increased susceptibility to chronic pain [112,113]. These findings have been translated into clinical trials, in which adenosine, infused into the intrathecal space, ameliorated hypersensitivity due to central sensitization following peripheral capsaicin injection [114]. Likewise, intravenous adenosine led to a remarkable reduction of neuropathic pain in a double blind placebo controlled crossover phase I trial [115]. More recently, antagonism of A_{2A} receptors has been considered for the management of chronic pain [33]. In particular, spinal effects of A_{2A} receptors are likely based on combined immunohistochemical and electrophysiological studies demonstrating responses to the A_{2A} selective agonist 3-[4-[2-[[6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl] propanoic acid (CGS 21680) in spinal cord, leading to the proposal that A_{2A} receptors are located on presynaptic inhibitory terminals of descending fibers from higher centers [116]. Consistent with these findings, the A_{2A} selective antagonist 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261) produced antinociception in two acute thermal pain tests [117]. In addition, A_{2A} receptor knockout mice are characterized by significantly reduced NMDAR binding in all regions of the spinal cord, which may be due to altered glutamate signaling in C-fibers [118]. Following intraplanar formalin injection A_{2A} receptor knockout mice have reduced biting and flinching responses in the first acute response phase (0–15 min) and reduced flinching only in the second phase (15–60 min) of the test [118]. The potential for A_{2A} receptor antagonists in inflammatory pain is supported by findings that SCH 58261 blocks both phases of the formalin response in wild-type mice [118]. Interactions between A_{2A} receptors and the opioid system is suggested by findings that A_{2A} receptor knockout mice show altered expression of the opioid peptide precursor pro-opiomelanocortin [119]. In summary, the available evidence suggests that A_1 receptors exert antinociceptive effects at peripheral and spinal sites, while A_{2A} receptors play an opposing role. Thus, a combination of A_1 receptor agonism with A_{2A} receptor antagonism might be an effective strategy for pain control.

Alzheimer's Disease

Alzheimer's disease (AD) affects 20–30 million people worldwide and is characterized by progressive memory deficits, cognitive impairment and personality changes. The main cause of AD is generally attributed to the increased production and extracellular accumulation of amyloid-beta (Aβ), in association with intracellular neurofibrillary tangle (NFT) formation [120–122]. In addition, inflammatory processes appear to be crucial in the pathophysiology of AD [123] and activated astrocytes were found to be closely associated with amyloid plaques in tissue from individuals with AD [124]. Astrocytes are thought to be activated by

human Aβ indicating a correlation between this protein and astrocyte dysfunction [125]. Conversely, activated glial cells appear to contribute to the development of amyloid plaques [126]. Clinically, hippocampal sclerosis is a common finding in AD autopsies [127] and the glial activation marker S100β was elevated in AD [128,129]. Although it is generally accepted that Aβ-deposition is a potent glial activator, astrocyte and microglial activation could be an early event in the disease, occurring even in the absence of Aβ-deposition [130,131]. Thus, in a clinical study glial activation was detected at very early stages of AD [132] and in a recent population-based study increasing gliosis has been found before the development of AD-lesions [133]. Likewise, in amyloid precursor protein mutant mice focal glial activation preceded Aβ deposition [134]. Remarkably, several transgenic AD-models show cognitive deficits well before Aβ deposition [135]. Together, these findings suggest that glial dysfunction is involved in the pathophysiology of AD.

Studies from our lab concerning the role of ADK in epilepsy have demonstrated, that – as a homotypic chronic response of the brain to injury (e.g. status epilepticus) – astrogliosis is always accompanied by upregulation of ADK, which in turn leads to a focal adenosine deficiency that triggers seizures [85,136–141].

The neuromodulator adenosine fulfils a unique role in the brain affecting glutamatergic neurotransmission and dopaminergic signalling via activation of adenosine A_1 and A_{2A} receptors, respectively. The adenosine system is thus ideally positioned to integrate glutamatergic and dopaminergic neurotransmission, which in turn could affect behavior and cognition. To test this hypothesis, transgenic mice with an overexpression of ADK in brain (Adk-tg), and therefore reduced brain adenosine levels, were evaluated in a panel of behavioral and psychopharmacological assays to assess possible glutamatergic and dopaminergic dysfunction [142]. In comparison to non-transgenic control mice, Adk-tg mice were characterized by severe learning deficits in the Morris water maze task and in Pavlovian conditioning. These results confirmed that ADK overexpression could lead to functional concomitant alternations in dopaminergic and glutamatergic functions, which in turn affect cognition.

It is tempting to hypothesize that astrogliosis in AD leads to upregulation of ADK and adenosine deficiency: this has twofold consequences for the pathophysiology of AD: *(i)* A reduction in the tone of ambient adenosine (by overexpressed ADK) implies the loss of a major endogenous neuroprotectant [28]; thus upregulation or overexpression of ADK is associated with increased stroke- or seizure-induced neuronal cell death [95,136]; consequently, upregulation of ADK in AD is likely to contribute to increased neuronal cell death in AD. *(ii)* Transgenic overexpression of ADK in mice causes severe cognitive impairment [142]; thus upregulated ADK in AD is expected to contribute to cognitive impairment. Adenosine-augmentation therapies are thus expected to directly improve cognitive functionality in AD and at the same time promote neuronal survival. This dual therapeutic approach will combine two endogenous properties of adenosine: neuroprotection and cognitive enhancement. Future directions thus may include the development of adenosine-augmenting therapies for AD.

Parkinson's Disease

The adenosine A_{2A} receptor has emerged as a leading non-dopaminergic therapeutic target in Parkinson's disease (PD). Two lines of evidence suggest that pharmacological inhibition of A_{2A}Rs will be of benefit for patients with PD: (1) A_{2A}Rs and dopamine D_2 receptors (D_2Rs) are colocalized in striatopallidal neurons and display antagonistic interactions at the molecular, neurochemical and behavioral level. These interactions have been used to explain the motor stimulant effects of A_{2A}R blockade [143–145]. Based on these considerations A_{2A}R antagonists such as KW-6002 (istradefylline) and SCH 420814 are currently

investigated in clinical trials. Phase II and III studies have confirmed that selective $A_{2A}R$ antagonists can stimulate motor activity by potentiating the L-dopa effect in advanced PD patients [146,147]. (2) $A_{2A}R$ antagonists may also have a more direct effect to attenuate dopaminergic neurodegeneration. Several large-cohort prospective studies have confirmed a similar inverse relationship between the consumption of caffeine and the risk of developing PD [148], [149], [150]. These findings are corroborated by animal models of PD that demonstrated potentially protective effects of caffeine. Thus, it was demonstrated that pharmacological blockade or genetic disruption of the $A_{2A}R$ attenuates dopaminergic neurodegeneration[151–153]. Together, these findings suggest, that any (pathological) imbalance in adenosinergic neuromodulation might impact the pathophysiology of PD.

Amyotrophic Lateral Sclerosis (ALS)

Most current knowledge on ALS pathogenesis is based on the landmark discovery that 20% of all familiar and 1% of all sporadic ALS cases are linked to dominant mutations of the ubiquitously expressed enzyme Cu/Zn superoxide dismutase (SOD1) [154]. Importantly, ALS could be replicated in transgenic rodents that carry single-amino acid mutations of human SOD1; for a review see [155]. These findings suggested that mutant SOD1 might become toxic to motor neurons because of a gain of function. Whereas the primary toxic nature of mutant SOD1 is still unresolved, a seminal study demonstrated that non-cell-autonomous processes that involved the interaction with neighboring non-neuronal cells, in particular microglia and astrocytes, contributed to the death of motor neurons [156]. In line with these findings massive activation of microglia and astrocytes was found in human cases as well as in transgenic animal models [157–159]. A wealth of information now demonstrates that astrogliosis is a pathological hallmark of ALS that might contribute significantly to the etiology of ALS-pathogenesis and has extensively been covered in recent literature [159–162]. Thus, reactive astrocytosis has been identified as a uniform pathological feature in rodent models of ALS [163–165] as well as in human cases of ALS [166–169]. A recent study using genetic ablation of dividing $GFAP^+$ astrocytes suggests that $Olig2^+$ and $NG2^+$ glial progenitor cells likely play a prominent role in the astroglial pathology of ALS [170].

As outlined above in “Epilepsy” and “Alzheimer’s Disease” an astroglial contribution to ALS pathology might lead to adenosine deficiency caused by a hypothesized astrogliotic overexpression of ADK. Thus, a deficiency in the neuroprotective adenosine might contribute to the loss of motor neurons in ALS and might explain the comorbidity of frontal dementia.

Schizophrenia (SZ)

The dopamine hypothesis of SZ is largely based on complementary effects of dopamine receptor (DR) antagonists and agonists to suppress and to promote psychotic symptoms, respectively. Thus, the efficacy of many antipsychotics correlates with their ability to block dopamine D_2Rs [171,172], dopamine-releasing drugs such as amphetamine can exacerbate psychotic symptoms in schizophrenia, and its prolonged abuse can produce psychotic symptoms in healthy subjects [173]. In addition, elevated basal occupation of D_2Rs by dopamine [174], increased dopamine turnover [175], and enhanced amphetamine-induced dopamine-release [173] have been shown in SZ patients using positron emission tomography (PET) imaging. The latter two findings in particular are compatible with a deficiency in adenosine. An adenosine deficit can *(i)* enhance dopamine activity by reducing the inhibitory effect of adenosine A_1Rs on dopamine release [176], and within the striatum it can *(ii)* potentiate amphetamine-induced locomotion [177] and dopamine release in the nucleus accumbens as suggested by the effects of A_1R antagonists [178]. Interactions between $A_{2A}Rs$ and D_2Rs [179,180] allow further opportunity for mutual modulation

between the adenosine and dopamine systems [181]. Thus, increased basal D_2R occupancy in SZ patients [174] could reduce the $A_{2A}R$'s effect on D_2Rs, and thereby increase the affinity of the D_2R for dopamine [179,180,182]. These mechanisms could provide the rationale for a typical neuroleptic-like profile of adenosine receptor agonists.

According to the glutamate hypothesis of SZ, reduced NMDAR function may contribute to cognitive and negative symptoms of schizophrenia [183,184]. Blockade of NMDARs is known to impair the induction of long term potentiation (LTP) and learning in animals [185–187]. Moreover, NMDAR blockade can give rise to behavioral dysfunction such as impulsivity [188] and psychotic-like behavior; e.g., phencyclidine and ketamine (two non-competitive NMDAR antagonists classified as dissociative anesthetics) are well known psychomimetics [183]. In contrast, co-agonists of the NMDAR, such as D-serine and glycine, improve cognition and negative symptoms in SZ [184]. Similarly, disruption of glycine transporter 1 has also been found to possess promnesic and antipsychotic potentials [189–192]. Adenosine is an endogenous modulator of glutamatergic activity. It inhibits glutamate release and the post-synaptic action of excitatory neurotransmitters upon neuronal hyperpolarization via A_1Rs [81]. In animal models of SZ, A_1 and $A_{2A}R$ agonists have repeatedly been shown to be effective against the behavioral as well as neurophysiological (EEG and prepulse inhibition) effects induced by NMDAR antagonists [193,194], thus lending support for their potential antipsychotic efficacy in humans.

Several lines of evidence support dysfunction of the adenosine system in patients with schizophrenia. Thus, increased density of $A_{2A}Rs$ was found in the striatum of patients in a post mortem study [195]. Upregulation of striatal $A_{2A}Rs$ could be a compensatory response triggered by reduced adenosinergic activity, which in turn could produce a hyperdopaminergic state [196,197]. Interestingly, the G_{olf} protein that is coupled to $A_{2A}Rs$ [198] is a candidate gene for schizophrenia [199,200]. Although adenosine-based drugs have not yet been tested in schizophrenia, the purine metabolic drug allopurinol was studied as add-on therapy for schizophrenia [201–203]. Adjunctive allopurinol was effective on positive and general symptoms. Similarly, the adenosine transport inhibitor dipyramidole (by raising adenosine) was beneficial in patients with schizophrenia [204]. Thus, preliminary clinical data support the adenosine hypothesis of schizophrenia.

INHIBITORS OF NUCLEOSIDE METABOLISM

The preceding sections illustrate that any imbalance in the level or distribution of nucleosides is expected to be associated with a wide spectrum of neurological and/or neuropsychiatric disturbances. Therefore inhibitors of nucleoside metabolism hold great promise for the treatment of a wide spectrum of conditions. In the following the major enzymes and inhibitors of nucleoside metabolism and their specific therapeutic relevance will be reviewed. Inhibitors of nucleotide metabolism (e.g. ecto-ATPases) will not be covered here.

5′nucleotidase (5′NT)

5′-nucleotidase (EC 3.1.3.5) degrades guanosine-5′-monophosphate (GMP), inosine-5′-monophosphate (IMP) and adenosine-5′-monophosphate (AMP) to guanosine, inosine, and adenosine, respectively. In addition to the regulation of endogenous nucleosides the enzymes regulate the activation of nucleoside analogues, a class of anticancer and antiviral agents that rely on nucleoside kinases for phosphorylation to their active forms. Both clinical and in vitro studies suggest that an increase in nucleotidase activity can inhibit nucleoside analogue activation and result in drug resistance [205]. The enzyme can either be located in the plasma membrane (ecto-5′-nucleotidase) or in the cytoplasm (cytosolic 5′-nucleotidase). Cytosolic 5′-nucleotidase forms a substrate cycle with ADK to the effect that

small activity changes in either of those enzymes can result in large changes in ambient adenosine [206,207]. In particular, cytosolic 5′-nucleotidase is an important contributor to the generation of adenosine and has been implicated in coupling adenosine to the energy state of a cell [208].

Within a pathological context increased activity of ecto-5′-nucleotidase has been associated with pilocarpine-induced epilepsy in rats, possible an endogenous protective mechanism in an attempt to raise levels of the endogenous anticonvulsant adenosine [209]. In addition, the enzyme is implicated in regulating vascular inflammatory responses that play a major role in many CNS pathologies [210].

Nucleotide and nucleoside analogues have been developed as inhibitors of cytosolic 5′-nucleotidase I from heart and brain, mostly as a biochemical tool to discriminate between functions of different 5′-nucleotidases [211,212]. Similar analogues have also been used to study mechanisms how cells acquire nucleoside analogue resistance [205,213]. Treatment with 0.001 mM adenosine 5′-(alpha, beta-methylene)diphosphate, a competitive ecto-5′-NT/CD73 inhibitor, caused a significant reduction of 30% in glioma cell proliferation [214]. Apart from a potential use as anti-neoplastic agents, inhibitors of 5′-nucleotidase activity are likely of limited therapeutic value as they would lead to a reduction of neuromodulatory nucleosides. In contrast, however, statins were found to increase 5′-nucleotidase activity. Thus, rosuvastatin increased extracellular adenosine formation in humans *in vivo* and was shown to provide protection against ischemia-reperfusion injury in humans [215].

Purine nucleoside phosphorylase (PNP)

Purine nucleoside phosphorylase (EC: 2.4.2.1) can catalyze guanosine to guanine, inosine to hypoxanthine, and adenosine to adenine conversions. Genetic deficiency in PNP causes immunodeficiency similar to immunodeficiency caused by adenosine deaminase deficiency [216]. The therapeutic potential for PNP inhibitors seems to be limited to the realm of oncology. Forodesine is a highly potent, orally active, rationally designed PNP inhibitor, that is active in preclinical studies with malignant cells and clinical utility against T-cell acute lymphoblastic leukemia and cutaneous T-cell lymphoma [217]. Therapeutic potential for CNS conditions is currently not known.

Adenosine kinase (ADK)

Adenosine kinase (EC 2.7.1.20) phosphorylates adenosine into AMP and is part of a highly active substrate cycle between adenosine and AMP [206,207]. In adult brain, ADK is mostly expressed in astrocytes [39]. Two alternatively spliced isoforms exist, a short isoform that is specific for the cytoplasm and a long isoform that is specific for the nucleus [218]. In adult rodent brain ADK is the key regulator for ambient levels of adenosine that are part of an astrocyte-based adenosine cycle [137,219,220]. It was demonstrated more than 15 years ago that inhibition of ADK, but not of adenosine deaminase depressed neuronal activity in hippocampal slices [221]. Increased expression of ADK is linked to astrogliosis in several CNS pathologies leading to a reduction in ambient levels of adenosine [136,137,222]. In epilepsy, it was demonstrated that increased levels of ADK, rather than any other epileptogenetic event is sufficient to trigger spontaneous recurrent seizures [136,222]. Likewise, increased levels of ADK render the brain more susceptible to neuronal injury [95,223]. Due to its key role in regulating the brain's endogenous anticonvulsant and neuroprotectant adenosine, inhibitors of ADK hold great promise for the treatment of neurological conditions that are based on adenosine deficiency, or that can be treated by adenosine augmentation. Therefore, ADK inhibitors have been developed with promising activities including seizure suppression, antinociception, anti-inflammatory action, and neuroprotection [224–242]. Importantly, ADK inhibitors have the potential to potentiate an

event-specific endogenous surge in adenosine (by blocking its degradation) and thereby avoiding unspecific side effects that are common with adenosine A_1R agonists [224–226]. The wide potential utility of ADK inhibitors is illustrated by the following examples: Following systemic administration the nucleoside ADK inhibitors 5′-amino-5′-deoxy-adenosine, 5′-deoxy-5-iodotubercidin, 5-iodotubercidin alleviated acute thermal nociception as measured by the hot-plate test in mice [227]. In addition to antinociception, ADK inhibitors are highly effective anticonvulsants [228,229] with proven efficacy in animal models of pharmacoresistant epilepsy [141,230].

Adenosine deaminase (ADA)

Adenosine deaminase (EC 3.5.4.4) deaminates adenosine into inosine and constitutes a major catabolic pathway for adenosine. In contrast to ADK that plays a major role in the adult CNS, ADA is expressed at high levels in placenta and seems to play a crucial role for fetal and perinatal development [231–233]. During early brain development transient ADA expression was found in specific subsets of neurons [234]. In adulthood, the highest expression levels of ADA are found in tongue and in cells lining the intestinal tract [235,236]. In adult brain ADA is associated with neurons and exhibits an uneven expression pattern [237]. Whereas many brain areas such as hippocampus are characterized by very low levels of ADA, higher ADA levels are only found in specialized nuclei such as the posterior hypothalamic magnocellular nuclei [237]. It is important to note that ADA activity decreases during brain maturation in several brain areas such as superior colliculus, cortex, hippocampus, cerebellum, olfactory bulbs, and olfactory nucleus indicating further that ADA plays important roles during brain development rather than in mature brain [238]. In contrast, ADK expression in astrocytes increases during brain maturation [39] indicating that the brain undergoes a developmental switch from ADA to ADK as the key regulators of ambient brain adenosine. Thus, in slices from adult brain, pharmacological inhibition of ADK, but not of ADA leads to adenosine-induced inhibition of neuronal activity [221].

Inhibitors of ADA include erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA, specific for isozyme ADA1, no activity on ADA2), 2-deoxycoformycin (pentostatin; (8R)-3-[(2R,4S, 5R)-4-hydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]-3,6,7,8-tetrahydroimidazo[4,5-d] [1,3]diazepin-8-ol) and the non-nucleoside inhibitor 1-((1R,2S)-2-hydroxy-1-(2-(1-naphthyl)ethyl)propyl)1H-imidazole-4-carboxamine (FR234938). Pentostatin has been (Federal Drug Administration (FDA) approved for the treatment of hematological malignancies [239–241], whereas the non-nucleoside inhibitor FR234938 has good potential as a new type of anti-rheumatic and anti-inflammatory drug, by modulating host-defense concentrations of adenosine [242]. While most attention to ADA inhibitors is directed at anti-neoplastic and anti-inflammatory activity, few studies have explored the potential use of ADA inhibitors for CNS disorders. Thus, it was shown that the novel putative anticonvulsant drug 1-[2,6-difluorophenyl)-methyl]-1H-1,2,3-triazolo[4,5-c]) pyridine-4-amine monohydrochloride (BW534U87) effectively reduced seizures induced in rodents by threshold maximal and supramaximal electroshock, electrical kindling, pentylenetetrazole (PTZ) infusion and by vestibular stimulation in the genetically seizure-prone epilepsy-like (EL) mouse, likely via inhibition of ADA [243,244]. Protection against focal ischemic brain damage in rats was evident when deoxycoformycin (500 micrograms/kg), was administered prior to the onset of ischemia, whereas delayed dosage was without any effect [245].

Guanase

Guanine deaminase (guanase; EC 3.5.4.3) catalyzes the deamination of guanine to xanthine. Like ADA, guanase plays a prominent role for purine catabolism in the small intestine [235] and is expressed in liver, kidney and brain [246]. In the CNS extracellular guanine is known to exert trophic effects that are likely regulated by conversion of extracellular guanosine into

guanine by a membrane located purine nucleoside phosphorylase (ecto-PNP) and by conversion of extracellular guanine to xanthine by guanase [46]. In addition, guanase appears to be essential for ammoniagenesis in brain [247]

Azepinomycin is a natural guanase inhibitor with antitumor and antibiotic activities [248,249], however guanase inhibitors are currently not used for any CNS indications.

Xanthine oxidase (XO)

Xanthine oxidase (EC 1.17.3.2) degrades hypoxanthine to xanthine and xanthine to uric acid and is part of the major catabolic pathway for purines. The enzyme also produces free radicals, which contributes to neurotoxicity; XO-induced free radical production is therefore thought to contribute to the pathophysiology of stroke, traumatic brain injury, as well as to the development of neurodegenerative diseases [250–252]. Inhibition of XO is therefore a rational neuroprotective strategy. The most widely used inhibitors of XO are allopurinol and oxypurinol, which are used primarily in the treatment of hyperuricemia and gout. XO inhibitors lower uric acid but also attenuate expression of inflammatory adhesion molecules in murine models, reduce oxidative stress in the vasculature, and improve endothelial function. Two distinct mechanisms may account for the therapeutic activity of XO-inhibitors: *(i)* suppression of free radical formation and *(ii)* inhibition of purine degradation, which ultimately leads to an increase in adenosine. A recent study demonstrates that allopurinol exerts potent anti-nociceptive activity via augmentation of adenosine [253]. In this study allopurinol presented dose-dependent anti-nociceptive effects in four different chemical or thermal pain models. The observed anti-nociceptive effects were not affected by the opioid antagonist naloxone. However, the non-selective adenosine-receptor antagonist caffeine and the selective A_1R antagonist DPCPX, but not the selective $A_{2A}R$ antagonist SCH58261, completely prevented allopurinol-induced anti-nociception. Allopurinol, at doses up to 200 mg/kg did not produce any obvious motor deficits. In addition, allopurinol raised cerebrospinal fluid levels of purines, including the nucleosides adenosine and guanosine. Together, these findings suggest that allopurinol exerts beneficial therapeutic effect by leading to a rise in the endogenous anti-nociceptive nucleoside adenosine [253]. Furthermore, adenosine-based antinociception might further be enhanced by reduction of pro-nociceptive reactive oxygen species via XO-inhibition [254]. Allopurinol has clinically been used. In a double-blind adjunctive allopurinol trial significant symptom improvement was noted in patients with schizophrenia, however the underlying mechanism responsible for the therapeutic benefit was not further investigated [255]. In stroke patients allopurinol was shown to have beneficial effects on inflammatory [256] and vascular [257] parameters. Due to its relative safety, allopurinol may hold promise for future adenosine augmentation therapies.

In contrast to the beneficial actions of XO-inhibitors, recent findings demonstrate that the obligatory end-product of purine catabolism, uric acid has neuroprotective functions in Parkinson's Disease [258]. Based on these new findings XO-inhibitors might reduce uric acid-based neuroprotection.

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)

Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) is a purine salvage pathway enzyme, which can catalyze the guanine and hypoxanthine conversion to GMP and IMP, respectively. The gene for HGPRT is considered to be a housekeeping gene and commonly used as a reference standard [259,260]. Deficiency of HGPRT in humans is associated with Lesch-Nyhan syndrome and severe neurological deficits that include self-mutilation [261,262]. It has been suggested that Lesch-Nyhan syndrome is primarily due to deficiency of dopamine in basal ganglia. This neurochemical lesion may result from a deficiency of

purine nucleotides, which impairs arborization of nigrostriatal neurons during perinatal development [263]. Enzyme inhibitors include 6-thioguanine and 6-mercaptoguanine, however their therapeutic use is largely restricted to inhibit the growth of parasites. Due to the general toxicity of HGPRT inhibition, HGPRT inhibitors are of little value for any CNS applications.

Uridine phosphorylase (UP)

Uridine phosphorylase (EC 2.4.2.3) catalyzes the uridine – uracil conversion. Recent evidence suggests that uridine protects neurons against ischemic insult-induced neuronal death, possibly through the action of UP [264]. In addition, the enzyme seems to underlie circadian regulation. Thus, the circadian rhythm observed in the plasma concentration of uridine was found to be inverse of that for uridine phosphorylase activity [265]. It was further suggested that hepatic uridine phosphorylase plays an important role in the regulation of uridine levels in blood which, in turn, may be involved in the humoral control of sleep by uridine [265]. Enzyme inhibitors fall largely into the class of alkylated or benzylated uridine derivatives. Those drugs, such as benzylacyclouridine are largely of interest for their antineoplastic activity [266–268], however benefit for CNS applications is unknown.

Other nucleoside metabolizing enzymes

Several other nucleoside metabolizing enzyme exist, however their relevance for CNS pathology and therapy are not yet defined. Among those enzymes not discussed here are: S-adenosyl-homocysteine hydrolase, guanosine kinase, uridine kinase, uridine-cytidine kinase, cytidine kinase, cytidine deaminase, and enzymes of inosine metabolism.

THERAPEUTIC RATIONALE

The examples above demonstrate that relatively few inhibitors of nucleoside metabolism are of interest for applications within the CNS. Among those drugs, ADA inhibitors, ADK inhibitors, and allopurinol are of most interest, all acting by augmenting adenosinergic signalling with its proven benefit in a wide spectrum of neurological and neuropsychiatric conditions. The following discussion therefore centers on the therapeutic benefit of adenosine augmentation therapies (AATs).

Effects on multiple neurochemical networks

Adenosine is an important upstream regulator of neuronal function [269–273]. By activation of presynaptic A_1Rs it mediates presynaptic inhibition [274,275]. In addition, adenosine promotes hyperpolarization of the postsynaptic neuron [276–278]. Furthermore, adenosine interacts on multiple levels with complex neuronal networks and directly modulates the activity of all major neurotransmitters [5,269]. Attenuation of the glutamate response can explain the potent antinociceptive, neuroprotective, and antiepileptic effects of AATs [253,279–281]. In addition, the ambient adenosine concentration determines the stability of $GABA_A$ receptors [272,282]. Adenosine controls dopamine signalling largely via heteromerization of adenosine A_{2A} and dopamine D_2 receptors; therefore A_{2A}R antagonists are of high therapeutic interest for Parkinson's disease [31,32,146,283,284]. Adenosine A_{2A} receptors interact with trkB receptors and thereby influence BDNF signalling [77,285]. These examples illustrate that any pathological imbalance in adenosine levels is likely to affect several different downstream pathways simultaneously, contributing to the complex phenotypes of most neurological and neuropsychiatric disorders. On the other hand, AAT is uniquely suited to affect several signalling pathways simultaneously and thereby to offer the potential for soft and smart therapeutic applicability. This therapeutic concept is in contrast to industrial drug development efforts that seek to find highly selective compounds to affect

only one specific pathway, e.g. by blocking or activating a specific receptor subtype. However, those approaches are not able to affect the complex pathophysiology of a given disease; they rather provide symptomatic relief for a specific endophenotype of a disorder, and are associated with side effects. As an example pharmacological blockade of NMDA receptors is not an option to treat epilepsy, as NMDA receptor blockade is associated with psychosis. However, AAT may provide an indirect and more elegant way to reduce NMDA receptor function, and to prevent seizures, however, without inducing psychosis.

Amplification of physiological functions of nucleosides

The section above illustrates that AAT allows modulating several downstream pathways simultaneously, which is considered to be a major advance compared to more standard pharmacotherapeutic approaches. The potential efficacy of AAT is further enhanced by its ability to potentiate an endogenous protective response of the brain in an event and site-specific manner. This has best been illustrated with ADK inhibitors [224–228,286–294]. After any type of brain injury, the brain reacts with a surge of endogenous adenosine as an acute protective response of the brain [24]. If this surge of adenosine can be amplified by reducing adenosine clearance, the endogenous protective response of the brain should be potentiated. In a seminal study by Britton et al., KA-evoked striatal adenosine release was augmented in animals receiving systemic treatment with the ADK inhibitor 5′deoxy-5-iodotubercidin (5′d-5IT) (cumulative dose of 7.5 μmol/kg, i.p.) compared with i.p. vehicle controls. In contrast, 5′d-5IT administration had no significant effect on basal or contralateral (artificial CSF-perfused) striatal adenosine levels. This experiment demonstrates that inhibition of adenosine clearance can potentiate the beneficial effects of adenosine in a site- and event-specific manner. However, blocking the clearance of an endogenous adenosine surge can have also detrimental effects as has recently been suggested in a model of sudden unexpected death in epilepsy, in which a combined surge in KA-triggered adenosine release with defective adenosine clearance, led to prolonged apnea in mice with lethal outcome [295].

Reduced side effects

The second generation of non-nucleoside ADK inhibitors, e.g. ABT-702 (5-(3-bromophenyl)-7-(6-morpholin-4-ylpyridin-3-yl)pyrido[2,3-d]pyrimidin-4–ylamine) was shown to exhibit potent antinociceptive and anti-inflammatory activity with a favorable therapeutic window [289,292,293] in particular, when compared to adenosine receptor agonists [288]. Based on ABT-702 novel 4-amino-5-aryl-6-arylethynylpyrimidines have recently been developed [227].

THERAPEUTIC FUTURE FOR ADK INHIBITORS

Despite an excellent therapeutic rationale and demonstrated efficacy in chronic pain and epilepsy, the systemic use of ADK inhibitors interferes with methionine metabolism in liver [296,297] and brain hemorrhage after systemic administration of an ADK inhibitor has been reported in dogs [228]. Although effective in pharmacoresistant epilepsy, the ADK inhibitor iodotubercidin still caused significant sedative side effects [141]. The bulk of pharmaceutical studies on ADK-inhibitor development was published between 1998 and 2005 with a peak in 2001. These findings suggest that systemic ADK inhibitors are no longer considered to be promising drugs for pharmaceutical drug development. Although therapeutically very effective in augmenting adenosine signalling, any systemic adenosine augmentation will affect multiple organ systems and will lead to unspecific augmentation of a multitude of signalling pathways that are regulated by adenosine. One therapeutically viable strategy to circumvent this conceptual problem constitutes the development of focal AATs. The underlying rationale for the use of focal AATs is first to define a local area of

adenosine dysfunction as contributor to disease pathology, and second to augment adenosine selectively within this pathological area. Partial epilepsy is an ideal target for those approaches. In particular, mesial temporal lobe epilepsy is characterized by astrogliosis and focal adenosine deficiency caused by pathological overexpression of ADK. Therefore reconstitution of normal (rather than providing excessive levels of adenosine) within an epileptogenic focus is considered to be a rational and effective approach for seizure control.

NOVEL THERAPEUTIC AVENUES

Based on the neurochemical rationale outlined above, we have developed a wide spectrum of focal AAT approaches for the treatment of seizures in epilepsy [298–300].

Polymer-based adenosine delivery

The biomaterial silk constitutes an ideal system for the controlled and sustained focal delivery of small molecule drugs. Silk is biocompatible and biodegradable, can be used for both drug- and cell-encapsulation and has been used for a wide spectrum of therapeutic drug-delivery approaches [301–303]. We recently generated and tested silk-based brain implants for the focal delivery of adenosine to the epileptic brain. Silk polymers were generated to deliver target doses of 40, 200, or 1000 ng adenosine per day [304]. Implantation of those polymers into the infrahippocampal fissure of rats prior to the onset of electrical kindling suppressed the development of kindled seizures in a dose-dependent way [304]. Polymers engineered to release a target dose of 1000 ng over a time span of 10 days completely suppressed stage 5 (highest possible seizure grade) seizures in fully kindled epileptic rats. Duration of suppression correlated with the release profile of adenosine. As soon as adenosine release from the polymers was expired, seizure activity resumed, indicating that seizure suppression was due to implant-based adenosine release [305]. In addition, when implanted before kindling initiation, the same polymers almost completely prevented the expression of any seizures. After expiration of adenosine release from the polymers kindling was resumed and the subsequent curve of kindling development paralleled kindling development in control animals. These data strongly indicate that the focal delivery of adenosine to the brain might not only suppress seizures but also the process leading to epilepsy, i.e. epileptogenesis [305].

Stem cell-based adenosine delivery

To provide a source for the sustained release of adenosine we engineered mouse embryonic stem cells (mESCs) to release adenosine based on a biallelic genetic disruption of the *Adk*-gene [306]. The cells were subjected to an *in vitro* differentiation paradigm [307] to induce the formation of adenosine-releasing neural precursor (NP) cells [306]. These cells were transplanted into the infrahippocampal fissure of rats prior to the onset of kindling. Recipients of adenosine-releasing mESC-derived NPs were characterized by robust suppression of kindling development compared to sham or wild-type cell-treated control animals [308]. In a similar approach, the same cells were transplanted into mice 24 hours after the intraamygdaloid injection of KA (= trigger for epileptogenesis). Three weeks later, recipients of wild-type cells or of a sham procedure were characterized by focal CA3-selective astrogliosis that included overexpression of ADK and the expression of about 4 spontaneous electrographic seizures per hour. In contrast, recipients of adenosine-releasing cells were characterized by a significant reduction in astrogliosis, by almost normal expression levels of ADK and by complete lack of seizures [136]. Together, these experiments demonstrate antiepileptogenic activity of focal stem cell-based adenosine delivery. In both experiments [136,308], but also in recipients of human ESCs [300], 4 weeks after transplantation dense grafts were found within the infrahippocampal fissure, likely forming a reservoir for paracrine adenosine release, whereas some cells migrated into

the ipsilateral CA1 and assumed a neuronal morphology. To develop human stem cells for future clinical use in cell-based AAT approaches, we developed a lentiviral expression system to express a micro-RNA (miRNA) directed against ADK. Human mesenchymal stem cells (hMSCs) can easily be derived from the bone marrow of a patient with epilepsy and used for autologous cell grafting approaches without the need for immunosuppression. ADK knockdown and adenosine release was induced in hMSCs after lentiviral transduction with the anti ADK miRNA construct, whereas a scrambled control sequence had no effect [309]. Infrahippocampal grafts of adenosine-releasing hMSCs in mice attenuated acute KA-induced neuronal injury and attenuated seizure development after KA-injection [309,310]. These experiments suggest that stem cells derived from a patient might be engineered for therapeutic adenosine delivery.

Gene therapy

Since pathogenetic overexpression of ADK is directly related to seizure generation, the enzyme constitutes an ideal target for future gene therapy approaches. Whereas in conventional gene therapy a therapeutic gene is added, in this case the therapeutic target would be to construct a gene therapy vector capable of downregulating ADK, ideally within an astrogliotic area of increased ADK expression. Limitation of such an approach to the focus of seizure generation might provide a rational long-term strategy for sustain seizure suppression or prevention.

CONCLUSIONS AND FUTURE PERSPECTIVES

Nucleosides are ubiquitous regulators of a wide spectrum of physiological functions that are of importance for every organ system. While inhibitors of nucleoside metabolism are primarily of therapeutic interest for antimicrobial or anti-neoplastic therapies, surprisingly few of those inhibitors have been developed for CNS applications. Even though highly effective in a variety of paradigms (e.g. epilepsy, inflammation, or chronic pain) therapeutic use of systemic nucleoside inhibitors appears to be of limited use due to lack of regional specificity and due to wide-spread side effects. Eventually, focal augmentation of nucleoside function might represent a more promising option. Focal augmentation of nucleosides is ideally suited to limit their therapeutic action to only those regions where nucleoside augmentation appears to be rationally justified.

The bulk of research has focused on adenosine, however the emerging roles of guanosine and inosine as neuromodulators need to be further explored and respective therapeutic tools need to be developed. Eventually, the further development of focal nucleoside augmentation approaches, e.g. by polymeric brain implants, or the development of stem cell or gene therapies may provide the basis for a soft and intelligent management of a wide range of neurological and neuropsychiatric disorders.

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ABBREVIATIONS

Aβ	Amyloid-beta
AAT	Adenosine Augmentation Therapy
AD	Alzheimer’s Disease

ADA	Adenosine Deaminase
ADK	Adenosine Kinase
ALS	Amyotrophic Lateral Sclerosis
AMP	Adenosine-5′-monophosphate
AR	Adenosine Receptor
ATP	Adenosine-5′-triphosphate
BDNF	Brain Derived Neurotrophic Factor
CNS	Central Nervous System
CPCA	5-(N-cyclopropyl)-carboxamido-adenosine
CTP	Cytidine-5′-triphosphate
DPMX	1,3-dipropyl-7-methyl-xanthine
DR	Dopamine Receptor
ESC	Embryonic Stem Cell
GABA	Gamma Amino Butyric Acid
GMP	Guanidine-5′-monophosphate
HGPRT	Hypoxantine Guanine Phosphoribosyltransferase
IMP	Inosine-5′-monophosphate
KA	Kainic Acid
KO	Knockout
KW-6002	Istradefylline
LTP	Long Term Potentiation
MCAO	Middle Cerebral Artery Occlusion
miRNA	micro-RNA
MSC	Mesenchymal Stem Cell
NFT	Neurofibrillary Tangle
NMDAR	*N*-methyl-D-aspartate Receptor
NP	Neural Precursor
NREM	Non-Rapid Eye Movement
PET	Positron Emission Tomography
PD	Parkinson’s Disease
PNP	Purine Nucleoside Phosphorylase
PTZ	Pentylene Tetrazole
REM	Rapid Eye Movement
SOD-1	Cu/Zn Superoxide Dismutase 1
SZ	Schizophrenia
UP	Uridine Phosphorylase

UTP	Uridine-5′-Triphosphate
WT	Wild Type
XO	Xanthine Oxidase

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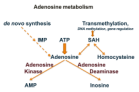

Figure 1. Major pathways of adenosine metabolism

Within the central nervous system degradation of ATP constitutes the major source for synaptic adenosine, whereas the *de novo* synthesis plays only a minor role. In brain the major pathway for the removal of adenosine is its phosphorylation to AMP by adenosine kinase; alternatively, adenosine can be dephosphorylated by adenosine deaminase into inosine. Adenosine is a ubiquitous endproduct of transmethylation reactions. However, when adenosine levels increase the equilibrium of the s-adenosyl homocysteine (SAH) hydrolase reaction is shifted towards synthesis of SAH, resulting in the inhibition of transmethylation reactions.

TABLE 1

THERAPEUTIC POTENTIAL OF ADENOSINERGIC DRUGS

Selected examples are given how therapeutic modulation of the adenosine system might be therapeutically effective in a variety of conditions. See main text for disease specific discussions.

	ADK-inhibitors	**A_1R agonists**	**A_{2A}R antagonists**
Epilepsy	Potent anticonvulsants	Potent anticonvulsants	Potential benefit for seizures DPMX: prevention of astrogliosis
Cerebral Stroke	Potential neuroprotectant?	Beneficial	Beneficial
Sleep Disorders	Potential use as sleep inducers?		Promotion of wakefulness (caffeine)
Chronic Pain	Analgesia	Analgesia	SCH 58261: antinociception
Alzheimer's Disease	Nootropic function?	Neuroprotection	Neuroprotection
Parkinson's Disease	Neuroprotection?	Neuroprotection	KW-6002: motor stimulation
Amyotrophic Lateral Sclerosis	Neuroprotection?	Neuroprotection?	Neuroprotection?
Schizophrenia	ADK-inhibitors and AR agonists/antagonists have not directly been tested, however adenosine regulating agents might be beneficial: Allopurinol: effective on positive symptoms Dipyramidole: beneficial effects in patients		Receptor agonists might be useful to reduce hyperdopaminergic functions.