Pharmacological Reports 66 (2014) 335–342

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Review article

Modulation of adenosinergic system and its application for the treatment of epilepsy

Mariusz J. Świąder [a,*], Jacek Kotowski [a], Jarogniew J. Łuszczki [b,c]

[a] Department of Experimental and Clinical Pharmacology, Medical University, Lublin, Poland
[b] Department of Pathophysiology, Medical University, Lublin, Poland
[c] Isobolographic Analysis Laboratory, Institute of Rural Health, Lublin, Poland

ARTICLE INFO

Article history:
Received 27 March 2013
Received in revised form 15 October 2013
Accepted 31 October 2013
Available online 13 April 2014

Keywords:
Adenosine
Antiepileptic drugs
Seizures

ABSTRACT

Adenosine is present in all cells and is implicated in the control of the function of every tissue and organ. The elevated adenosine levels seem to play a significant role in a protection against cellular damage in the regions with increased metabolic demand and prevent the subsequent dysfunction of the affected organs. Furthermore, adenosine has been shown to play an important role not only in the regulation of pathophysiological processes, but also in the modulation of normal physiological processes, for example, the regulation of sleep and arousal as well as by impact on pre- or postsynaptic receptors involved in releasing neurotransmitters (*e.g.* glutamate, acetylcholine, norepinephrine, 5-hydroxytryptamine, dopamine, GABA and others).

Experimental studies provide evidence supporting the role of adenosine as an endogenous anticonvulsant agent. Numerous adenosine agonists acting through A_1, A_2 and A_3 receptors were proven as potent anticonvulsant compounds in a wide variety of animal models of epilepsy. However, despite their efficacy in such models, adenosine receptor agonists do not appear to be good candidates for successful clinical applications. The therapeutic range of systemically administered adenosine receptor agonists is very narrow and they often produce profound adverse events. It seems, therefore, that adenosine receptor agonists could only be used clinically when co-administered with other antiepileptic drugs or when used in local therapies, where their side effect profile is much more tolerable. An alternative strategy would be to enhance the natural adenosinergic feedback mechanism triggered by seizures by using adenosine uptake inhibitors. This approach seems very attractive as it would allow limiting the action only in the active areas such as seizure foci and thus, preventing the systemic side effects.

Contents

* Corresponding author.
E-mail address: mariusz.swiader@am.lublin.pl (M.J. Świąder).

http://dx.doi.org/10.1016/j.pharep.2013.10.005

Introduction

Many epileptic patients are not adequately treated with currently available antiepileptic drugs. It is estimated that seizures are intractable in as much as 35% of patients with partial complex seizures, which are the most frequent type of epilepsy in humans [1,2]. Non-pharmacological therapies, such as surgical resection, are applicable for only some patients as it is often extremely difficult or even impossible to localize the epileptic focus. In many cases, the epileptic focus is located in the close proximity of eloquent brain areas, which creates serious surgical risks [3]. Moreover, the potential for significant side effects caused by the drug actions in or outside of the brain limits the optimal systemic drug delivery in a high percentage of cases. There is, therefore, an unfulfilled need for alternative treatment strategies that would allow achieving improved efficacy in these drug resistant forms of epilepsy. Recently, several novel approaches are being investigated and a number of them concentrate on therapies that involve mechanisms of action different from those exhibited by the currently available antiepileptic drugs. The approach that seems to be particularly attractive and promising involves modulation of excitatory neurotransmission in the brain with agents suppressing neuronal activity, including the adenosinergic system.

Adenosine exists in all cells and it is implicated in the control of the function of every tissue and organ. Extracellular levels of adenosine rise during conditions involving increased metabolic demand and/or lack of oxygen. Such conditions occur during seizures, ischemia, stress, hypoglycemia, inflammation and trauma. The increased adenosine-5′-triphosphate (ATP) metabolism taking place in such conditions leads to the production of adenosine through the breakdown of ATP *via* adenosine-5′-diphosphate (ADP) and adenosine-5′-monophosphate (5′-AMP) [4]. The elevated adenosine levels seem to play a role in a protection against cellular damage in the regions with increased metabolic demand and prevent the subsequent dysfunction of the affected organs. Numbers of study reports available to date provide evidence that adenosine exerts a protective action throughout all organs of the body and its effects range from the amelioration of brain and heart injury caused by ischemia, suppression of inflammation and suppression of seizures. Furthermore, adenosine has been shown to play an important role not only in the regulation of pathophysiological processes, but also in the modulation of normal processes, especially, the regulation of sleep and arousal [5]. Adenosine is a natural sleep-promoting agent and its level increasing during periods of wakefulness and decreasing during sleep [6]. Adenosine is involved in the autoregulation of cerebral blood flow modulating vascular resistance and causing vasodilation, inhibits locomotor activity and motor coordination [7,8], causes sedation [9] and leads to depression of cardiovascular and respiratory functions (for review see Dunwiddie and Masino [10]).

Adenosine enters the extracellular space either following the dephosphorylation of adenine nucleotides or through a direct release from cells. This release does not appear to be calcium-dependent and takes place *via* facilitated diffusion nucleoside transporters, which are driven by adenosine concentration gradients. Active sodium-dependent adenosine transporters have also been identified, however, their importance is yet to be established [11].

Adenosine is removed from the extracellular space by reuptake transported to the cells (facilitated diffusion or active transport) or by inactivation with adenosine deaminase resulting in transformation to inosine [12,13,14].

Adenosine behaves as an extracellular signal molecule and affects synaptic transmission without being itself a neurotransmitter, and thus modulating the activity of the nervous system at cellular level. This modulation takes place either presynaptically by inhibiting or facilitating transmitter release or postsynaptically by depolarizing or hyperpolarizing neurons [15]. Stimulation of the adenosine receptors causes the inhibition of the release of the various neurotransmitters like glutamate, acetylcholine, norepinephrine, 5-hydroxytryptamine, dopamine, GABA and others [16,17]. The strongest inhibitory action of adenosine occurs in the excitatory glutamatergic system, where it is capable of completely blocking synaptic transmission. The action on the excitatory system is much stronger than that on the inhibitory modulation of inhibitory systems, therefore, in general the activation of adenosine receptors leads to reduced excitability of neurons [18].

Types of adenosine receptors

Adenosine exerts its effects by interacting with specific cell-surface G-protein coupled receptors. Activation of these receptors leads to the inhibition of calcium influx and opening of presynaptic potassium channels (GIRKs), which causes hyperpolarization and subsequent decrease in the release of excitatory neurotransmitters. So far, four adenosine receptors have been identified, cloned, pharmacologically characterized and classified into the following subtypes: A_1, A_{2A}, A_{2B} and A_3 [19]. The A_1 and A_3 receptors interact with G and G_0 proteins and inhibit adenyl cyclase, whereas the A_{2A} and A_{2B} receptors stimulate the adenyl cyclase through interaction with members of the G_s family of G proteins. A_1, A_{2B} and A_3 receptors can also activate phospholipase C, and this action is mediated by activation of G_q proteins [20].

Adenosine receptors show an uneven distribution with A_1 receptors widely presents throughout the brain, but particularly concentrated in the cerebral cortex, cerebellum, hippocampus, olfactory tubercles and ventral globus pallidus [21,22]. The A_1 receptors are also found in the heart, aorta, liver, kidney, bladder and eye. The high affinity A_{2A} receptors are present at high levels in only a few parts of the brain, such as the striatum [23], nucleus accumbens and olfactory tubercles [24]. However, a highly sensitive RT-PCR technique (reverse transcription-polymerase chain reaction) can detect low concentrations of A_{2A} receptor

mRNA in all brain regions [21]. The A_{2B} and A_3 receptors are not well characterized and neither are their exact locations in the nervous system. In rats, the expression of A_3 receptor encoding gene was shown to be widespread, while mRNA was detected across the central nervous system and in several peripheral tissues. These findings seem consistent with the pattern in man and sheep [21].

As mentioned above adenosine is considered to provide an inhibitory tone in the central nervous system and is involved in the modulation of various physiological and pathophysiological processes. In several pathological conditions, the elevated extracellular concentrations of adenosine offer protection against damage induced by hypoxic or ischemic events. Other than acting as a neuroprotective agent, adenosine has also been proposed to play a role of an endogenous anticonvulsant [25]. This hypothesis is supported by various experimental studies showing that adenosine levels rise dramatically during seizures, studies directly demonstrating that adenosine can suppress seizures, studies showing that adenosine receptor antagonists increase the duration and severity of seizures and therefore are proconvulsant, studies showing that adenosine receptor agonists ameliorate seizures in a dose-dependent manner, and finally, studies demonstrating that seizure suppression and prevention can be achieved with agents acting as adenosine uptake inhibitors. These studies are reviewed below.

Adenosine levels rise during seizure activity

The implantation of depth electrodes with a microdialysis probe in the hippocampi of patients with intractable complex seizures showed a rapid rise in the levels of adenosine directly after the onset of seizures and elevated levels continuing postictally. The extracellular concentrations of adenosine were shown to increase 6- to 31-fold, with a significantly higher increase observed in the hippocampus, in which seizures originated [26]. Similarly, *in vivo* microdialysis performed in rats showed an increase in hippocampal adenosine release and metabolism associated with seizures induced by pentylenetetrazole, kainic acid and bicuculline [27].

Adenosine suppresses seizures

In electrically kindled rats (a model of partial epilepsy) the implantation of fibroblasts that were genetically engineered to release adenosine (adenosine kinase and adenosine deaminase were inactivated so that adenosine could not be metabolized) into the brain ventricles provided almost complete protection from behavioral seizures and nearly complete suppression of afterdischarges in the electroencephalogram (EEG) recordings [28].

Adenosine levels less than 25 nM at critical sites within the brain were demonstrated as sufficient to suppress seizures [29]. In a similar study, mouse C2C12 myoblasts genetically engineered to release adenosine by inactivation of adenosine kinase were encapsulated and then grafted into the lateral brain ventricles of rats kindled in the hippocampus. These adenosine-releasing implants caused complete protection from seizures and a reduction of afterdischarges in EEG recordings. No sedative effects were observed in this experiment and adenosine A_1 receptors remained responsive to selective agonists and antagonists suggesting a lack of desensitization of A_1 receptors after local long-term exposure to adenosine [30]. In another study, kindled seizures were suppressed following the intraventricular implantation of adenosine-releasing embryonic stem cells (such cells were created by disruption of both alleles of adenosine kinase and then encapsulated into semipermeable polymer membranes).

Implanted rats displayed transient protection from convulsive seizures and a significant reduction of afterdischarge activity in EEG recordings. Due to the limited viability of the encapsulated cells only short-term seizure suppression was observed [31]. In another study with electrically kindled rats, similar results were achieved by intraventricular implantation of synthetic polymers that release adenosine. In this experiment the anticonvulsant activity was observed when adenosine was released in an amount of 20–50 ng per day [32].

Further evidence supporting the role of adenosine as an endogenous regulator of seizure susceptibility is provided by *in vitro* experiments. For example, the release of adenosine from the hippocampal slice preparation was shown to exert a tonic inhibitory effect on spontaneous interictal discharges and these discharges appeared to be slowed or suppressed by exogenously applied adenosine [33]. In a similar study, but involving slices of human epileptogenic neocortex, adenosine (40–50 nM) affected magnesium-free epileptogenesis causing a significant decrease in the frequency of spontaneous epileptiform discharges. Identical effects were observed during bath application of the adenosine uptake inhibitor nitrobenzylthioinosine (10–50 μM) [34].

Another fact is worth mentioning while explaining the role of adenosine in seizure activity. Generally, intense activation of the GABAergic system during seizures leads to a transient switch in the action of $GABA_A$ receptors from inhibitory to excitatory [35]. In such a situation, adenosine does not change chloride homeostasis mechanisms or conductance of $GABA_A$ receptors, but it reduces $GABA_A$ receptor responses *via* an adenosine A_1 receptor-dependent activation of K^+ channels that hyperpolarize the membrane potential and increase membrane conductance of neurons [36,37,38]. The adenosine-induced attenuation of depolarizing $GABA_A$ receptor signaling represents an important mechanism by which adenosine limits seizure activity. Pharmacological experiments showed that blocking adenosine receptors during seizures increased the excitatory actions of GABAergic signaling [39]. Additionally, adenosine affects the activity of interneurons either directly or indirectly *via* reducing their glutamatergic inputs, which decrease the amount of GABA released. It is widely known that GABAergic interneurons can remain active during seizures and can in fact maintain seizure activity and after-discharge events when glutamatergic transmission is blocked [40–42]. Recordings from hippocampal neurons have revealed that adenosine does not have a direct effect on the presynaptic release of GABA [43–47], but adenosine modulates the postsynaptic effects of GABAergic inputs during seizures [39].

Adenosine antagonists are proconvulsant

Treatment with aminophylline – an adenosine receptor antagonist was found to be proconvulsant in rats that were kindled either from amygdaloid or neocortical sites. In these rats, aminophylline acted in a dose-dependent manner and caused dramatically longer afterdischarge durations [48]. Another adenosine antagonist, theophylline, prolonged the post-kindling afterdischarge threshold, and motor seizure durations as well as facilitated partially kindled seizures, however, it did not alter the pre-kindling or post-kindling afterdischarge thresholds of amygdala-elicited seizures in rats [49]. In another study on amygdala-kindled rats, similar proconvulsant effects, which primarily involved the increase in the afterdischarge duration, were produced not only by aminophylline, but also by such adenosine antagonists as isobutylmethylxanthine and caffeine. In the same study, aminophylline produced seizures when it was injected repeatedly in the absence of electrical stimulation [50]. As these seizure-prolonging and proconvulsant effects of aminohylline were prevented by adenosine A_1 agonists, they are, therefore, considered to be due to the blockage of A_1 adenosine receptors [51].

In a model of limbic status epilepticus, where status epilepticus was induced with pulsed-train current delivered to amygdala in successive 5-min current-on sessions, aminophylline increased major convulsive activity during stimulation and significantly accelerated the entry into convulsive status epilepticus. In animals already in the status epilepticus, administration of aminophylline led to lethal convulsions [52]. In the same study, the A_1 receptor agonist 2-chloroadenosine (2-CLA) had the opposite effect and suppressed major convulsive activity during stimulation, blocked or delayed status epilepticus entry and in animals already in the exploratory status epilepticus, it suppressed status epilepticus behaviorally and electroencephalographically as well as protected the animals from cerebral damage associated with seizures [52]. Similarly, in animals pretreated with aminophylline at doses of 25–100 mg/kg, pilocarpine at a non-convulsant dose of 100 mg/kg caused severe motor limbic seizures, which developed into status epilepticus and resulted in extensive damage in several areas of the brain (hippocampus, thalamus, amygdala, olfactory cortex, substantia nigra and neocortex) [53].

Adenosine receptor antagonists were also shown to be proconvulsant in audiogenic seizure-sensible DBA/2 mice [54]. They increased the incidence of both clonic and tonic seizures in the absence of auditory stimulation. The adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) had the highest convulsive potency, followed by different A_{2A} receptor antagonists as 3,7-dimethylpropargilxanthine (DMPX), 8-(3-chlorostyryl) caffeine (CSC), (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF-17837) and caffeine. Following a subconvulsant audiogenic stimulus, all adenosine receptor antagonists induced both tonic and clonic seizures DBA/2 mice [54].

Adenosine receptor antagonists have a proconvulsant effect in pentylenetetrazole-induced seizures, especially, theophylline (at doses ranging from 15 to 60 mg/kg, i.p.) was shown to exert a strong effect in the rat model. Moreover, pretreatment with theophylline (5 mg/kg, i.v.) also antagonized the elevation of the threshold for pentylenetetrazole-induced seizures by 2-CLA, again supporting the idea that endogenous adenosine acts as a regulator of seizure susceptibility [55].

Adenosine receptor agonists prevent or ameliorate seizures

Electrically-evoked seizures

The adenosine A_1 receptor agonists proved effective in the following models of epilepsy: Maximal electroshock seizures (MES) – a widely accepted animal model of generalized tonic–clonic convulsions in humans [56]. In this model the adenosine A_1 receptor agonist 2-CLA (0.25–1 mg/kg) significantly raised the threshold for electroconvulsions in mice [57], while focal injection of 2-CLA into the substantia nigra provided protection against electroshock seizures in rats. This protection was antagonized by aminophylline [58].

Pentylenetetrazole seizures

2-CLA (1 mg/kg) was found to be effective against pentylenetetrazole-induced seizures in mice [56] and it significantly raised the median convulsive dose (CD_{50} value) for pentylenetetrazole [57]. When injected through a tail vain, the adenosine receptor agonists, 2-CLA, cyclohexyladenosine (CHA) and L- and D-phenylisopropyladenosine (L- and D-PIA), all produced a dose-dependent elevation of the threshold for pentylenetetrazole-induced seizures in rats. L-PIA proved most potent followed by 2-CLA, CHA and D-PIA [55]. Adami et al. [59] showed that in the pentylenetetrazole-induced seizures the anticonvulsant effects caused by repeated stimulation of adenosine A_1 receptors are subject to tolerance. In acute studies, the selective adenosine A_1 receptor agonist, 2-chloro-N(6)-cyclopentyladenosine (CCPA), provided a dose-dependent reduction in seizures induced by intraperitoneal injection of pentylenetetrazole [60], whereas repeated treatment with CCPA resulted in a marked decrease of its effects [59]. In another study, intrathalamic micro-injections of 2-CLA caused significant decreases in both seizure duration and seizure severity in pentylenetetrazole-induced seizures in rats. Pretreatment with theophylline prevented the protective effect of 2-CLA on seizure activity and increased both seizure duration and seizure severity [61].

Pilocarpine-induced seizures

This model is useful to investigate the pathophysiological mechanisms of temporal lobe epilepsy. 2-CLA in doses of 5–10 mg/kg blocked the appearance of behavioral and EEG seizures produced by a convulsant dose of pilocarpine (380 mg/kg) and prevented brain damage in rats [62]. In another rat study, 2-CLA co-perfused (intrahippocampal perfusion) or injected systemically with pilocarpine (7.5 mg/kg), prevented the development of seizures as well as pilocarpine-evoked augmentation of the glutamate and dopamine levels [63]. It did not, however, prevent the delayed increase in glutamate overflow. Interestingly, intraperitoneal injection of the selective adenosine A_{2A} receptor antagonist, 5-amino-7-(β-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]-pyrimidine (SCH 58261), reversed the 2-CLA-elicited attenuation of pilocarpine-induced change in dopamine efflux and completely eliminated the delayed augmentation of glutamate levels, irrespective of perfusion with pilocarpine. This may indicate that the mechanism of action of 2-CLA is more complex and its protective action may be partially dependent on its agonistic action upon hippocampal adenosine A_{2A} receptors.

Kindled seizures

The adenosine A_1 receptor agonists were also effective in amygdala kindled seizures – a model of partial complex seizures. 2-CLA was demonstrated to shorten the duration of the afterdischarge in amygdala kindled rats, whereas aminophylline prolonged these afterdischarges [48]. In another study, 2-CLA was shown not only to completely block the evolution of amygdala- but also hippocampal-kindled seizures [62]. Another A_1 receptor agonist L-PIA injected into the lateral cerebral ventricle of amygdala kindled rats produced a dose-related reduction in the severity of seizures [64]. L-PIA was also shown to antagonize seizures, where kindling originated from hippocampus or caudate nucleus [65]. Amygdala kindled seizures were also antagonized by cyclohexyladenosine which, additionally, was demonstrated to prevent the seizure-prolonging action of aminophylline [51].

Status epilepticus

Selective A_1 receptor agonists, N6-cyclohexyladenosine (CHA) and N6-cyclopentyladenosine (CPA), were able to prevent the development of electrically elicited status epilepticus in rats (a constant electrical stimulation model). CHA was also effective in terminating status epilepticus after it had progressed for 20 min. Adenosine A_1 receptor antagonists induced status epilepticus (recurrent electrical stimulation model of status epilepticus) suggesting that status epilepticus develops as a result of central adenosine receptor antagonism [66]. In another study utilizing a rat model where status epilepticus was induced with pulsed-train current delivered to amygdala in successive 5-min current-on sessions, 2-CLA suppressed major convulsive activity during stimulation and blocked or delayed status epilepticus entry, whereas in animals already in exploratory status epilepticus, 2-CLA suppressed status epilepticus behaviorally and electroencephalographically, offering protection against cerebral damage induced by seizures. These results prove that endogenous adenosine mechanisms prevent the development of status

epilepticus, modulate the severity of ongoing status epilepticus, and limit the anatomic spread of seizure activity, again supporting the role of adenosine as an endogenous anticonvulsant [52].

NMDA seizures

In N-methyl-D-aspartate (NMDA)-evoked seizures in mice, prior administration of CPA resulted either in a delay of seizure onset and unchanged mortality (0.5 mg/kg CPA, 60 mg/kg NMDA) or in elimination of tonic episodes and a significant reduction in postictal mortality (1 and 2 mg/kg CPA; 60 and 125 mg/kg NMDA) [67]. Interestingly, when CPA was administered chronically, tonic–clonic episodes were more frequent and a rise in postictal mortality was observed. In the same study, chronic administration of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) led to a total elimination of clonic–tonic episodes, increased average survival time and reduced postictal mortality. Since the density of adenosine receptor binding sites was unchanged after chronic treatment with either CPX or CPA, it is likely that the mechanism behind the observed protection involved the second messenger systems coupled to adenosine A_1 receptors [68].

Bicuculline seizures

Administration of the adenosine receptor agonists such CHA, CPA, 2-CLA and D- and L-PIA as well as the nonselective A_1/A_2 agonist, 5′-N-ethylcarboxamidoadenosine (NECA), into the rat prepiriform cortex (a forebrain area which may play a significant role in the pathology of epilepsy) resulted in suppression of seizures elicited with bicuculline methiodide. NECA proved most potent followed by CPA, D-PIA, 2-CLA and L-PIA. On the other hand, high doses of the A_2 selective agonist, 2-phenylaminoadenosine, had no influence on seizures [69].

Audiogenic seizures

Focal injection of 2-CLA into the substantia nigra protected genetically epilepsy prone rats against audiogenic seizures [58]. Similarly, dose-dependent protection against audiogenic seizures was observed when audiogenic-seizure-sensitive DBA/2 mice were treated with the adenosine A_1 receptor agonist, 2-chloro-N6-cyclopentyladenosine (CCPA) [54].

Organophosphate seizures

In an *in vitro* model of organophosphate-induced seizures, (where epileptiform activity was induced by the organophosphate sarin, in the CA1 stratum pyramidale of the guinea pig hippocampal slice) application of the adenosine A_1 receptor agonist N(6)-cyclopentyladenosine (CPA), or the partial adenosine A_1 receptor agonists, 2-deoxy-N(6)-cyclopentyladenosine (2-deoxy-CPA) and 8-butylamino-N(6)-cyclopentyladenosine (8-butylamino-CPA), suppressed epileptiform activity in a concentration-dependent manner [70]. The adenosine A_1 receptor agonists, 2-CLA and D-N6-phenylisopropyladenosine, also proved effective in seizures evoked by mitochondrial toxin, 3-nitropropionic acid (3-NPA) in mice. Both agonists decreased the occurrence of seizures elicited by peripheral application of 3-NPA and they both prevented 3-NPA-induced mortality. When seizures were induced by intracerebral injection of 3-NPA, administration of the adenosine A_1/A_2 receptor agonist, NECA had a protective effect, which could be reversed by the adenosine receptor antagonist, 8-(p-sulfophenyl)theophylline [71].

Adenosine A_2 receptor agonists

The studies described above provide clear evidence that activation of adenosine A_1 receptors can exert anticonvulsant effects in a variety of experimental models of epilepsy. The role of adenosine A_2 receptors in seizure suppression is not clearly characterized. In comparison with the wide expression of A_1 receptors, the adenosine A_{2A} receptors show a much more limited distribution [72], and are present in high concentrations only in a few brain structures, mainly in the striatum, nucleus accumbens and olfactory tubercles [23]. In contrast to the general inhibitory role of adenosine A_1 receptors, the A_{2A} receptors have been found to mediate both inhibitory and excitatory responses [72]. It has been shown that adenosine A_{2A} receptor activation induces excitatory responses in the hippocampus [73]. It is still not completely clear how A_{2A} receptors lead to excitatory action, but it has been implicated that it happens due to increase in glutamate and acetylcholine release [74]. The potential of adenosine A_{2A} receptor activation for seizure suppression has been investigated only in a few studies and the results are controversial. For example, the adenosine A_{2A} receptor agonist 5′-(N-cyclopropyl)-carboxamido-adenosine (CPCA) did not antagonize pentylenetetrazole-induced seizures [75] and very high doses of the selective adenosine A_2 agonist, 2-phenylaminoadenosine, had no influence on seizures evoked by bicuculline methiodide [69], even though adenosine A_1 receptor agonists proved very potent in both of these experimental models.

On the other hand, there are several studies documenting that adenosine A_{2A} receptor activation can lead to anticonvulsant activity. The non-selective adenosine A_2 receptor agonist, NECA, produced a dose-related reduction of amygdala-kindled seizures and its effects were slightly more potent than these evoked by the adenosine A_1 receptor agonist, L-PIA. The anticonvulsant effects of NECA were antagonized by parenteral injections of caffeine, at a dose which had no effect on seizure parameters [64]. However, in another study where adenosine receptor agonists were injected directly into the seizure focus of rats, in which seizures were kindled from various brain structures (amygdala, hippocampus or caudate nucleus), NECA proved effective only in suppressing the seizures started in the caudate nucleus (which has a high concentration of adenosine A_2 receptors), whereas L-PIA had potent anticonvulsant effects when injected directly into the kindled seizure focus in all three sites [65]. NECA was able to completely prevent seizures elicited by bicuculline and proved to be more potent than CHA, CPA, D-PIA, 2-CLA and L-PIA. However in the same study, the selective adenosine A_2 agonist, 2-phenylaminoadenosine, offered no protection against seizures, which suggests that the anticonvulsant effects of NECA against bicuculline evoked seizures are due to its action on adenosine A_1 rather than A_2 receptors [69]. NECA was also highly potent in preventing the development of audiogenic seizures in DBA/2 mice. Selective adenosine A_{2A} receptor ligands such as 2-(4-(2-carboxyethyl)-phenylamino)-5′-N-ethylcarboxamidoadenosine (CGS 21680) and 2-hexynyl-5′-N-ethylcarboxamidoadenosine (2-HE-NECA) also had the anticonvulsant effects in this model, however, they were not as potent as NECA [54]. NECA and 2-HE-NECA showed a dose-dependent protective activity against pentylenetetrazole-induced seizures in rats. Contrary to the selective adenosine A_1 receptor agonist, CCPA, repeated administration of 2HE-NECA and NECA did not cause tolerance and their protective activity against pentylenetetrazole-induced seizures was maintained [59].

Adenosine A_3 receptor agonists

The low affinity adenosine A_3 receptors can be activated only by high concentrations of adenosine [76]. There are data suggesting that activation of the adenosine A_3 receptors may counteract the inhibitory effects of adenosine A_1 receptors. For example, activation of adenosine A_3 receptors with a selective adenosine A_3 receptor agonist, 2-chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (Cl-IB-MECA) antagonized the inhibition of

excitatory neurotransmission mediated by adenosine A_1 receptors [77]. In an *in vitro* experiment on the rat hippocampus, administration of 3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS-1191), an adenosine A_3 receptor antagonist, decreased the duration and intensity of seizures [78]. In amygdala-kindled rats, (N(6)-2-(4-aminophenyl)ethyl-adenosine (APNEA – a non-selective adenosine A_3 receptor agonist) remained without any significant effect on seizure parameters (seizure severity, seizure duration and after-discharge duration) [79].

On the other hand, there are studies suggesting that activation of adenosine A_3 receptors does have the anticonvulsive effects, for example in the mouse MES-induced seizure model, the threshold for electroconvulsions was significantly raised following the administration of APNEA [80]. APNEA was also shown to be effective in preventing the development of seizures in audiogenic-seizure-sensitive DBA/2 mice, although it was not as potent as either adenosine A_1 or A_2 receptor agonists used in this experiment. In the same study, however, the adenosine A_3 selective receptor agonist, N6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine (IB-MECA) afforded no protection against seizures [54]. Acutely administered IB-MECA was also ineffective against electrically induced seizures, but when administered chronically, it significantly reduced post-epileptic mortality even though it did not affect the threshold voltage. In seizures induced by NMDA and pentylenetetrazole, IB-MECA offered significant protection both during acute and chronic administration. It is possible however, that in case of the chemically induced seizures, the protection offered by the acutely administered drug was caused by inadequate delivery of chemoconvulsants to the brain due to both arteriolar constriction and severe hypotension related to the acute stimulation of adenosine A_3 receptors. Thus, it is unknown whether the anticonvulsant effects observed during the chronic administration of IB-MECA were caused by the drug's influence on neurotransmission, blood flow or both mechanisms simultaneously [81].

Adenosine-regulating agents

As indicated above, administration of adenosine receptor agonists can ameliorate or prevent seizures induced by electrical and chemical stimuli in a variety of experimental paradigms. However, in spite of their efficacy in such a wide range of models, adenosine receptor agonists do not appear to be good candidates for successful clinical applications. When administered systemically, adenosine receptor agonists have a very narrow therapeutic range and produce a number of profound adverse effects, including a significant reduction in blood pressure and heart rate, pronounced hypothermia and motor depression [9,75]. An alternative strategy would be to enhance the natural adenosinergic feedback mechanism triggered by seizures. It may be achieved by blocking the uptake and metabolism of adenosine with so called adenosine-regulating agents. In cells subjected to cellular stress or hypoxia the repletion of ATP is not fast enough to meet the demand for energy and since the extracellular adenosine comes mainly from the intracellular breakdown of ATP, the use of agents that direct ATP breakdown toward increased production of adenosine, or agents that slow the metabolism of adenosine should increase its extracellular levels only within the hypoxic region.

Inactivation of adenosine kinase provides one such approach leading to increased intracellular adenosine, which then diffuses or is transported *via* nucleoside transporters to the extracellular space, where it can activate the cellular adenosine receptors. Adenosine kinase inhibition could represent an alternative mechanism for the activation of adenosine receptors offering a better therapeutic window and fewer side effects than adenosine receptor agonists.

By elevating extracellular adenosine levels, uptake inhibitors can be expected to offer amelioration or protective effects in various diseases. Up to date, such effects have been reported in ischemic cerebral and cardiac injury, thrombosis, inflammatory diseases, insomnia, pain and seizures [82]. Adenosine kinase inhibitors proved to be very effective in the rat MES-induced seizure model and exerted antiepileptic effects in a rat neocortical preparation *in vitro* [83]. Some of the adenosine kinase inhibitors such as 4-(N-phenylamino)-5-phenyl-7-(5′-deoxyribofuranosyl)-pyrrolo[2,3-d]pyrimidine (GP683), possess a substantially better side-effect profile than adenosine receptor agonists. GP683 did not reduce mean arterial blood pressure and had little effect on heart rate, even at a dose 36-fold above its ED_{50} for inhibition of MES-induced seizures. In contrast, CPA reduced blood pressure and heart rate to one-third of baseline level when administered at its ED_{50} for MES-induced seizures. Furthermore, the hypothermic effect associated with adenosine receptor agonists in rats was largely gone with GP683, and the inhibition of locomotor activity by GP683 was reduced compared with CPA. It is possible that this improved profile results from the amplification of the rise in adenosine levels associated with epileptiform activity within seizure foci [84]. The studies demonstrating anticonvulsant activity with various structurally diverse classes of adenosine kinase inhibitors and studies demonstrating that this activity can be antagonized with adenosine receptor antagonists strongly suggest that adenosine kinase inhibitors could be used as potent antiepileptic drugs. For review of adenosine kinase inhibitors, see McGaraughty et al. [85] and Kowaluk and Jarvis [86].

Effects of adenosine agonists on other anticonvulsant agents

Various adenosine A_1, A_2 and A_3 receptor agonists seem to possess different effects upon the protective action of traditional antiepileptic drugs. The adenosine A_1 receptor agonist L-PIA offered no protection against electroconvulsions in mice, but potentiated the anticonvulsant action of diazepam and valproate against MES-induced seizures, significantly decreasing their ED_{50} values. However, it remained without effect on the protective activity of phenobarbital, carbamazepine and phenytoin [87]. NECA, a preferential adenosine A_2 receptor agonist also potentiated the anticonvulsant potency of valproate, but diminished the anticonvulsant action of phenobarbital while having no effect upon the protective action of carbamazepine against MES-induced seizures in mice [87]. APNEA, a nonselective adenosine A_1/A_3 receptor agonist used at subprotective doses against electroconvulsions, significantly potentiated the anticonvulsive action of phenobarbital, phenytoin, valproate and carbamazepine. The enhancement of the anticonvulsant activity of phenobarbital, phenytoin and valproate seems to be due to the activation of adenosine A_1 receptors, whereas influence on the action of carbamazepine seems to be mediated by the activation of adenosine A_3 receptors [88].

In amygdala-kindled rats, APNEA had no significant effect on various seizure parameters (seizure severity, seizure duration and afterdischarges duration), however, it significantly enhanced the protective activity of carbamazepine, valproate, phenobarbital and clonazepam without affecting the protective action of phenytoin. The strongest positive interaction has been observed between APNEA and carbamazepine. This seems to be mediated by adenosine A_1 and A_3 receptors, in contrast to other combinations, which depend on the stimulation of adenosine A_1 receptors [79].

The anticonvulsant activity of carbamazepine against MES-induced seizures in mice was significantly potentiated by 2-CLA administered at a subprotective dose of 0.125 mg/kg. 2-CLA also

significantly enhanced the protective action of clonazepam against pentylenetetrazole-induced clonic seizures, while the adenosine antagonist such as aminophylline (5 mg/kg) and the adenosine A_1 selective antagonist 8-cyclopentyl-l,3-dimethylxanthine (5 mg/kg) reversed the effects of 2-CLA on these antiepileptic drugs [57,89].

When NECA or APNEA were co-administered with the selective noncompetitive antagonist of AMPA/kainate receptors, LY 300164, the combined effect of these adenosine agonists was superior to a single drug's protective action in the MES-induced seizure model in mice. The combined treatment of LY 300164 with either NECA or APNEA did not cause any motor impairment, although it caused a significant long-term memory deficit [80].

The studies reviewed above provide evidence supporting the role of adenosine as an endogenous anticonvulsant agent. Numerous adenosine agonists acting through A_1, A_{2A}, A_{2B} and A_3 receptors were proven as potent anticonvulsant compounds in a wide variety of animal models of epilepsy. However, despite their efficacy in such models, adenosine receptor agonists do not appear to be good candidates for successful clinical application. The therapeutic range of systemically administered adenosine receptor agonists is very narrow and they often produce profound adverse events. It seems, therefore, that adenosine receptor agonists could only be used clinically when co-administered with other antiepileptic drugs or when used in local therapies, where their side effect profile is much more tolerable. An alternative strategy would be to enhance the natural adenosinergic feedback mechanism triggered by seizures by using adenosine uptake inhibitors. This approach seems very attractive as it would allow limiting the action only in the active areas such as seizure foci and thus preventing the systemic side effects.

Conflicts of interest

There are no known conflicts of interest associated with this publication.

Funding

There has been no financial support for this work.

References

[1] Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med 2000;342:314–9.
[2] Kwan P, Brodie MJ. Epilepsy after the first drug fails: substitution or add-on? Seizure 2000;9:464–8.
[3] Jette N, Wiebe S. Update on the surgical treatment of epilepsy. Curr Opin Neurol 2013;26:201–7.
[4] Brundege JM, Diao L, Proctor WR, Dunwiddie TV. The role of cyclic AMP as a precursor of extracellular adenosine in the rat hippocampus. Neuropharmacology 1997;36:1201–10.
[5] Rainnie DG, Grunze HC, McCarley RW, Greene RW. Adenosine inhibition of mesopontine cholinergic neurons: implications for EEG arousal. Science 1994;263:689–92.
[6] Benington JH, Kodali SK, Heller HC. Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. Brain Res 1995;692:79–85.
[7] Barraco RA, Coffin VL, Altman HJ, Phillis JW. Central effects of adenosine analogs on locomotor activity in mice and antagonism of caffeine. Brain Res 1983;272:392–5.
[8] Marston HM, Finlayson K, Maemoto T, Olverman HJ, Akahane A, Sharkey J, et al. Pharmacological characterization of a simple behavioral response mediated selectively by central adenosine A1 receptors, using in vivo and in vitro techniques. J Pharmacol Exp Ther 1998;285:1023–30.
[9] Dunwiddie TV, Worth T. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J Pharmacol Exp Ther 1982;220:70–6.
[10] Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001;31–55.
[11] Johnston ME, Geiger JD. Adenosine transport systems on dissociated brain cells from mouse, guinea-pig, and rat. Neurochem Res 1990;15:911–5.
[12] Boissard CG, Gribkoff VK. The effects of the adenosine reuptake inhibitor soluflazine on synaptic potentials and population hypoxic depolarizations in area CA1 of rat hippocampus in vitro. Neuropharmacology 1993;32:149–55.
[13] Eldridge FL, Paydarfar D, Scott SC, Dowell RT. Role of endogenous adenosine in recurrent generalized seizures. Exp Neurol 1989;103:179–85.
[14] Gonzales RA, Leslie SW. [3H]adenosine uptake and release from synaptosomes, Alterations by barbiturates. Biochem Pharmacol 1985;34:1619–25.
[15] Haas HL, Selbach O. Functions of neuronal adenosine receptors. Naunyn Schmiedebergs Arch Pharmacol 2000;362:375–81.
[16] Langer SZ, Arbilla S. Presynaptic receptors and modulation of the release of noradrenaline, dopamine and GABA. Postgrad Med J 1981;57(Suppl 1):18–29.
[17] von Kügelgen I, Kurz K, Bültmann R, Driessen B, Starke K. Presynaptic modulation of the release of the co-transmitters noradrenaline and ATP. Fundam Clin Pharmacol 1994;8:207–13.
[18] Gomes CV, Kaster MP, Tome AR, Agostinho PM, Cunha RA. Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta 2011;1808:1380–99.
[19] Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Gimenez-Llort L, et al. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. Proc Natl Acad Sci U S A 2001;98:9407–12.
[20] Klinger M, Freissmuth M, Nanoff C. Adenosine receptors: G protein-mediated signalling and the role of accessory proteins. Cell Signal 2002;14:99–108.
[21] Dixon AK, Gubitz AK, Sirinathsinghji DJ, Richardson PJ, Freeman TC. Tissue distribution of adenosine receptor mRNAs in the rat. Br J Pharmacol 1996;118:1461–8.
[22] Fastbom J, Pazos A, Palacios JM. The distribution of adenosine A1 receptors and 5'-nucleotidase in the brain of some commonly used experimental animals. Neuroscience 1987;22:813–26.
[23] Ongini E, Fredholm BB. Pharmacology of adenosine A2A receptors. Trends Pharmacol Sci 1996;17:364–72.
[24] Moreau JL, Huber G. Central adenosine A(2A) receptors: an overview. Brain Res Brain Res Rev 1999;31:65–82.
[25] Dunwiddie TV. The physiological role of adenosine in the central nervous system. Int Rev Neurobiol 1985;27:63–139.
[26] During MJ, Spencer DD. Adenosine: a potential mediator of seizure arrest and postictal refractoriness. Ann Neurol 1992;32:618–24.
[27] Berman RF, Fredholm BB, Aden U, O'Connor WT. Evidence for increased dorsal hippocampal adenosine release and metabolism during pharmacologically induced seizures in rats. Brain Res 2000;872:44–53.
[28] Kline AE, Montanez S, Bradley HA, Millar CJ, Hernandez TD. Distinctive amygdala kindled seizures differentially affect neurobehavioral recovery and lesion-induced basic fibroblast growth factor (bFGF) expression. Brain Res 2000;880:38–50.
[29] Huber A, Padrun V, Deglon N, Aebischer P, Mohler H, Boison D. Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. Proc Natl Acad Sci U S A 2001;98:7611–6.
[30] Guttinger M, Padrun V, Pralong WF, Boison D. Seizure suppression and lack of adenosine A1 receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. Exp Neurol 2005;193:53–64.
[31] Guttinger M, Fedele D, Koch P, Padrun V, Pralong WF, Brustle O, et al. Suppression of kindled seizures by paracrine adenosine release from stem cell-derived brain implants. Epilepsia 2005;46:1162–9.
[32] Boison D, Scheurer L, Tseng JL, Aebischer P, Mohler H. Seizure suppression in kindled rats by intraventricular grafting of an adenosine releasing synthetic polymer. Exp Neurol 1999;160:164–74.
[33] Dunwiddie TV. Endogenously released adenosine regulates excitability in the in vitro hippocampus. Epilepsia 1980;21:541–8.
[34] Kostopoulos G, Drapeau C, Avoli M, Olivier A, Villemeure JG. Endogenous adenosine can reduce epileptiform activity in the human epileptogenic cortex maintained in vitro. Neurosci Lett 1989;106:119–24.
[35] Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M. Excitatory GABA input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells. Neuroscience 2003;119:265–75.
[36] Clark BD, Kurth-Nelson ZL, Newman EA. Adenosine-evoked hyperpolarization of retinal ganglion cells is mediated by G-protein-coupled inwardly rectifying K^+ and small conductance Ca^{2+}-activated K^+ channel activation. J Neurosci 2009;29:11237–45.
[37] Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. Int Rev Neurobiol 2005;63:191–270.
[38] Hosseinzadeh H, Stone TW. Tolbutamide blocks postsynaptic but not presynaptic effects of adenosine on hippocampal CA1 neurones. J Neural Transm 1998;105:161–72.
[39] Ilie A, Raimondo JV, Akerman CJ. Adenosine release during seizures attenuates GABAA receptor-mediated depolarization. J Neurosci 2012;32:5321–32.
[40] Fujiwara-Tsukamoto Y, Isomura Y, Imanishi M, Fukai T, Takada M. Distinct types of ionic modulation of GABA actions in pyramidal cells and interneurons during electrical induction of hippocampal seizure-like network activity. Eur J Neurosci 2007;25:2713–25.
[41] Fujiwara-Tsukamoto Y, Isomura Y, Imanishi M, Ninomiya T, Tsukada M, Yanagawa Y, et al. Prototypic seizure activity driven by mature hippocampal fast-spiking interneurons. J Neurosci 2010;30:13679–89.
[42] Fujiwara-Tsukamoto Y, Isomura Y, Takada M. Comparable GABAergic mechanisms of hippocampal seizurelike activity in posttetanic and low-Mg^{2+} conditions. J Neurophysiol 2006;95:2013–9.
[43] Han TH, Jang SH, Lee SY, Ryu PD. Adenosine reduces GABAergic IPSC frequency via presynaptic A(1) receptors in hypothalamic paraventricular neurons projecting to rostral ventrolateral medulla. Neurosci Lett 2011;490:63–7.
[44] Kruglikov I, Rudy B. Perisomatic GABA release and thalamocortical integration onto neocortical excitatory cells are regulated by neuromodulators. Neuron 2008;58:911–24.

[45] Thompson SM, Capogna M, Scanziani M. Presynaptic inhibition in the hippocampus. Trends Neurosci 1993;16:222–7.
[46] Thompson SM, Haas HL, Gahwiler BH. Comparison of the actions of adenosine at pre- and postsynaptic receptors in the rat hippocampus in vitro. J Physiol 1992;451:347–63.
[47] Yoon KW, Rothman SM. Adenosine inhibits excitatory but not inhibitory synaptic transmission in the hippocampus. J Neurosci 1991;11:1375–80.
[48] Albertson TE, Stark LG, Joy RM, Bowyer JF. Aminophylline and kindled seizures. Exp Neurol 1983;81:703–13.
[49] Dragunow M, Goddard GV, Laverty R. Is adenosine an endogenous anticonvulsant? Epilepsia 1985;26:480–7.
[50] Dragunow M, Goddard GV. Adenosine modulation of amygdala kindling. Exp Neurol 1984;84:654–65.
[51] Dragunow M. Adenosine receptor antagonism accounts for the seizure-prolonging effects of aminophylline. Pharmacol Biochem Behav 1990;36:751–755.
[52] Handforth A, Treiman DM. Effect of an adenosine antagonist and an adenosine agonist on status entry and severity in a model of limbic status epilepticus. Epilepsy Res 1994;18:29–42.
[53] Turski WA, Cavalheiro EA, Ikonomidou C, Mello LE, Bortolotto ZA, Turski L. Effects of aminophylline and 2-chloroadenosine on seizures produced by pilocarpine in rats: morphological and electroencephalographic correlates. Brain Res 1985;361:309–23.
[54] De Sarro G, De Sarr A, Di Paola ED, Bertorelli R. Effects of adenosine receptor agonists and antagonists on audiogenic seizure-sensible DBA/2 mice. Eur J Pharmacol 1999;371:137–45.
[55] Murray TF, Sylvester D, Schultz CS, Szot P. Purinergic modulation of the seizure threshold for pentylenetetrazol in the rat. Neuropharmacology 1985;24:761–6.
[56] Löscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res 1988;2:145–81.
[57] Borowicz KK, Łuszczki J, Czuczwar SJ. 2-Chloroadenosine, a preferential agonist of adenosine A1 receptors, enhances the anticonvulsant activity of carbamazepine and clonazepam in mice. Eur Neuropsychopharmacol 2002;12:173–179.
[58] De Sarro G, De Sarro A, Meldrum BS. Anticonvulsant action of 2-chloroadenosine injected focally into the inferior colliculus and substantia nigra. Eur J Pharmacol 1991;194:145–52.
[59] Adami M, Bertorelli R, Ferri N, Foddi MC, Ongini E. Effects of repeated administration of selective adenosine A1 and A2A receptor agonists on pentylenetetrazole-induced convulsions in the rat. Eur J Pharmacol 1995;294:383–389.
[60] Tintos-Hernandez JA, Davalos-Rodriguez IP. Deficiency of adenosine desaminase (ADA): clinical, biochemical, molecular and treatment aspects. Rev Invest Clin 2011;63:75–83.
[61] Ates N, Ilbay G, Sahin D. Suppression of generalized seizures activity by intrathalamic 2-chloroadenosine application. Exp Biol Med (Maywood) 2005;230:501–5.
[62] Cavalheiro EA, Calderazzo Filho LS, Bortolotto ZA, Mello L, Turski L. Anticonvulsant role of adenosine. Pol J Pharmacol Pharm 1987;39:537–43.
[63] Khan GM, Smolders I, Ebinger G, Michotte Y. Anticonvulsant effect and neurotransmitter modulation of focal and systemic 2-chloroadenosine against the development of pilocarpine-induced seizures. Neuropharmacology 2000;39:2418–32.
[64] Barraco RA, Swanson TH, Phillis JW, Berman RF. Anticonvulsant effects of adenosine analogues on amygdaloid-kindled seizures in rats. Neurosci Lett 1984;46:317–22.
[65] Rosen JB, Berman RF. Differential effects of adenosine analogs on amygdala, hippocampus, and caudate nucleus kindled seizures. Epilepsia 1987;28:658–66.
[66] Young D, Dragunow M. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. Neuroscience 1994;58:245–61.
[67] von Lubitz DK, Paul IA, Carter M, Jacobson KA. Effects of N6-cyclopentyl adenosine and 8-cyclopentyl-1,3-dipropylxanthine on N-methyl-D-aspartate induced seizures in mice. Eur J Pharmacol 1993;249:265–70.
[68] von Lubitz DK, Paul IA, Ji XD, Carter M, Jacobson KA. Chronic adenosine A1 receptor agonist and antagonist: effect on receptor density and N-methyl-D-aspartate induced seizures in mice. Eur J Pharmacol 1994;253:95–9.
[69] Franklin PH, Zhang G, Tripp ED, Murray TF. Adenosine A1 receptor activation mediates suppression of (−) bicuculline methiodide-induced seizures in rat prepiriform cortex. J Pharmacol Exp Ther 1989;251:1229–36.
[70] Harrison PK, Bueters TJ, Ijzerman AP, van Helden HP, Tattersall JE. Partial adenosine A(1) receptor agonists inhibit sarin-induced epileptiform activity in the hippocampal slice. Eur J Pharmacol 2003;471:97–104.
[71] Zuchora B, Turski WA, Wielosz M, Urbanska EM. Protective effect of adenosine receptor agonists in a new model of epilepsy – seizures evoked by mitochondrial toxin, 3-nitropropionic acid, in mice. Neurosci Lett 2001;305:91–4.
[72] Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology, XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 2001;53:527–52.
[73] Cunha RA, Ribeiro JA. Adenosine A2A receptor facilitation of synaptic transmission in the CA1 area of the rat hippocampus requires protein kinase C but not protein kinase A activation. Neurosci Lett 2000;289:127–30.
[74] Sebastiao AM, Ribeiro JA. Adenosine A2 receptor-mediated excitatory actions on the nervous system. Prog Neurobiol 1996;48:167–89.
[75] Malhotra J, Gupta YK. Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats. Br J Pharmacol 1997;120:282–8.
[76] Dunwiddie TV. Adenosine and suppression of seizures. Adv Neurol 1999;79:1001–10.
[77] Dunwiddie TV, Diao L, Kim HO, Jiang JL, Jacobson KA. Activation of hippocampal adenosine A3 receptors produces a desensitization of A1 receptor-mediated responses in rat hippocampus. J Neurosci 1997;17:607–14.
[78] Etherington LA, Frenguelli BG. Endogenous adenosine modulates epileptiform activity in rat hippocampus in a receptor subtype-dependent manner. Eur J Neurosci 2004;19:2539–50.
[79] Borowicz KK, Świąder M, Kamiński R, Kuźniar H, Kleinrok Z, Czuczwar SJ. Two essential amino acids, L-lysine and L-histidine, in five types of experimental seizures. Pol J Pharmacol 2000;52:345–52.
[80] Borowicz KK, Świąder M, Wielosz M, Czuczwar SJ. Influence of the combined treatment of LY 300164 (an AMPA/kainate receptor antagonist) with adenosine receptor agonists on the electroconvulsive threshold in mice. Eur Neuropsychopharmacol 2004;14:407–12.
[81] von Lubitz DK, Carter MF, Deutsch SI, Lin RC, Mastropaolo J, Meshulam Y, et al. The effects of adenosine A3 receptor stimulation on seizures in mice. Eur J Pharmacol 1995;275:23–9.
[82] Noji T, Karasawa A, Kusaka H. Adenosine uptake inhibitors. Eur J Pharmacol 2004;495:1–16.
[83] Ugarkar BG, Castellino AJ, DaRe JM, Kopcho JJ, Wiesner JB, Schanzer JM, et al. Adenosine kinase inhibitors. 2. Synthesis, enzyme inhibition, and antiseizure activity of diaryltubercidin analogues. J Med Chem 2000;43:2894–2905.
[84] Wiesner JB, Ugarkar BG, Castellino AJ, Barankiewicz J, Dumas DP, Gruber HE, et al. Adenosine kinase inhibitors as a novel approach to anticonvulsant therapy. J Pharmacol Exp Ther 1999;289:1669–77.
[85] McGaraughty S, Cowart M, Jarvis MF. Recent developments in the discovery of novel adenosine kinase inhibitors: mechanism of action and therapeutic potential. CNS Drug Rev 2001;7:415–32.
[86] Kowaluk EA, Jarvis MF. Therapeutic potential of adenosine kinase inhibitors. Expert Opin Investig Drugs 2000;9:551–64.
[87] Czuczwar SJ, Szczepanik B, Wamil A, Janusz W, Kleinrok Z. Differential effects of agents enhancing purinergic transmission upon the antielectroshock efficacy of carbamazepine, diphenylhydantoin, diazepam, phenobarbital, and valproate in mice. J Neural Transm Gen Sect 1990;81:153–66.
[88] Gasior M, Borowicz K, Kleinrok Z, Starownik R, Czuczwar SJ. Anticonvulsant and adverse effects of MK-801, LY 235959, and GYKI 52466 in combination with Ca^{2+} channel inhibitors in mice. Pharmacol Biochem Behav 1997;56:629–35.
[89] Dragunow M, Robertson HA. 8-Cyclopentyl 1,3-dimethylxanthine prolongs epileptic seizures in rats. Brain Res 1987;417:377–9.