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Adenosine Kinase is a New Therapeutic Target to Prevent Ischemic Neuronal Death

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Abstract: The brain has evolved several endogenous mechanisms to protect itself from the deleterious consequences of stroke. One of these endogenous neuroprotective systems is centered on the purine ribonucleoside adenosine, which exerts potent neuroprotective functions within the brain. One major goal in stroke research is to explore and utilize such endogenous neuroprotective mechanisms therapeutically. This review illustrates molecular approaches to study the role of the adenosine system within the context of stroke and highlights innovative therapeutic approaches aimed at increasing adenosinergic function. New research data suggest that the major adenosine regulating enzyme adenosine kinase (ADK) plays a prominent role in determining the brain's susceptibility to ischemic injury. Endogenous ADK is rapidly downregulated following a stroke, possibly an endogenous neuroprotective mechanism aimed at raising ambient levels of adenosine in the brain. Conversely, transgenic overexpression of ADK in the brain renders the brain more susceptible to stroke-induced neuronal cell loss. In the present review we will first summarize the physiological role of adenosine metabolism within the context of ischemic brain injury. Next, we will highlight the key role of ADK in determining the brain's susceptibility to ischemic injury, and finally we will discuss potential therapeutic applications of adenosine augmentation to provide neuroprotection in stroke.

Keywords: Adenosine, adenosine kinase, ischemia, reperfusion, neuroprotection, stroke, transgenic mice, gene knockout.

1. INTRODUCTION

Ischemic stroke has a mortality rate of around 30% and is the third leading cause of death in major industrialized countries. In addition, it is a major cause of long-lasting disabilities. Ischemic stroke can result from the occlusion of a major brain artery, either by an embolus or by local thrombosis, which leads to transient or permanent reduction in cerebral blood flow and extensive neuronal cell death [1, 2]. Despite intensive research and the development of neuroprotective drugs, to date, no adequate therapy exists to prevent neuronal injury after stroke. Previous strategies to prevent neuronal cell loss have mostly focused on the *N*-methyl-D-aspartate (NMDA) receptor, which is considered to be the main target responsible for excitotoxic Ca^{2+} overload in the ischemic brain that is linked to apoptotic signal transduction pathways [3, 4]. Based on this dogma, most experimental strategies to prevent ischemic neuronal cell death include caspase inhibitors [2] and blockade of calcium-permeable acid sensitive ion channels to prevent glutamate receptor-independent Ca^{2+} toxicity [3]. Although several neuroprotective drugs have been developed with demonstrated positive results in animal models of stroke, none of those has proven efficacious in human clinical trials [4-7].

2. MOLECULAR BASIS OF ADENOSINE SYSTEM

The brain has evolved several endogenous mechanisms to protect itself from the deleterious consequences of ischemic brain injury. The purine ribonucleoside adenosine is one of these endogenous mediators of neuroprotection.

2.1. Adenosine Receptors

Adenosine – present already in the pre-biotic world – has evolved as a potent homeostatic regulator in all known forms of life and in all mammalian organ systems. In the brain, adenosine is an important modulator of neuronal function and regulates transmitter release and neuronal excitability [8, 9]. Adenosine exerts potent neuroprotective functions through activation of four different subtypes of G-protein coupled adenosine receptors (Fig. **1**) which also influence a wide range of other brain functions in both physiological and pathophysiological situations. For instance, adenosine is critically involved in the regulation of sleep, locomotion, anxiety, cognition and memory [8-10], as well as in the pathophysiology of stroke [11], and epilepsy [12].

At the molecular level, adenosine modulates the release of many neurotransmitters, i.e. dopamine, glutamate, GABA, serotonin, noradrenaline and acetylcholine, with the inhibition of excitatory neurotransmitter release (e.g. glutamate) being the most pronounced [10, 13, 14]. These modulations are mediated by the interplay of A_1 and A_{2A}Rs that have opposing actions mediated by different sets of G-proteins (Fig. **1**), which explains the high degree of complexity in the overall effects of adenosine [15-18]. For example, the adenosine-dependent inhibitory modulation of neurotransmitter release is largely mediated through activation of $G_{i/o}$ - coupled A_1Rs, which reduce neurotransmitter release at pre-synaptic nerve terminals and depress neuronal firing at postsynaptic sites [13, 19, 20]. In contrast, adenosine activates $G_{s/olf}$ - coupled A_{2A}Rs to exert excitatory activity based on stimulating the release of

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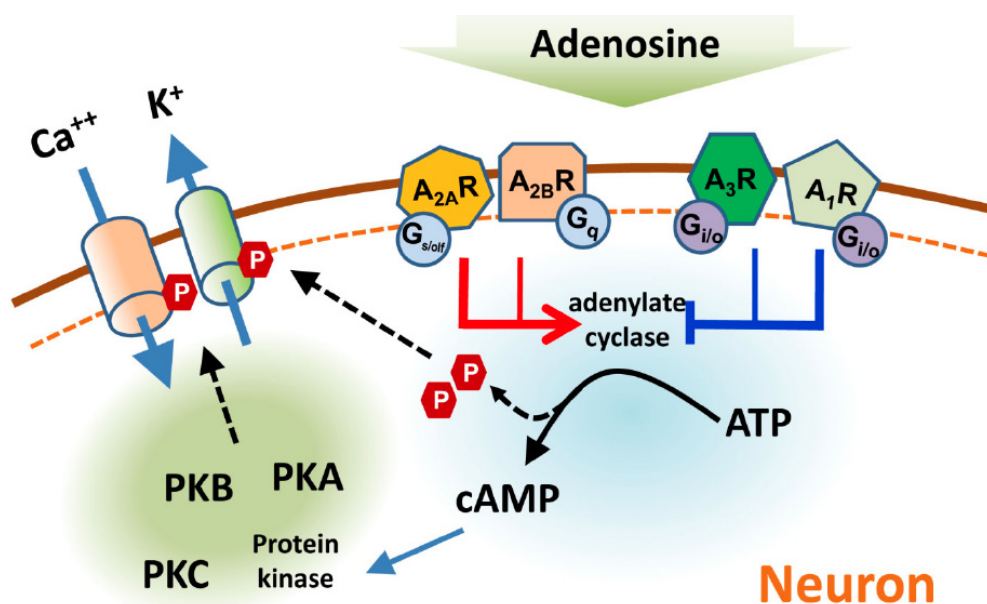

Fig. (1). Adenosine exerts its effects through its four subtypes of receptors, A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R. Adenosine activation of $G_{i/o}$-coupled A_1Rs and A_3Rs inhibits activity of cAMP cyclase. In contrast, adenosine-activation of $G_{s/olf}$-coupled $A_{2A}R$ and G_q-coupled $A_{2B}R$ enhances the activity of cAMP cyclase. Therefore, four subtypes of adenosine receptors manipulate cellular cAMP levels, and thus consequently affect downstream protein kinase pathways, i.e. PKA, PKC and PKB.

glutamate and acetylcholine, and also of GABA in the striatum and hippocampus [21-25].

2.2. Adenosine Regulation and ADK

The metabolism of adenosine in brain has extensively been reviewed elsewhere [8, 9, 14, 26]. Briefly, intra- and extra-cellular adenosine levels are in a dynamic exchange by equilibrative and concentrative nucleoside transporters [27, 28]. Intra- and extra-cellular enzymes of adenosine metabolism include adenosine kinase (ADK), adenosine deaminase (ADA), and S-adenosylhomocysteine- hydrolase (SAH-hydrolase), and ecto- and 5' nucleotidases (NT) (Fig. **2**).

Physiologically, adenosine is generated extracellularly as a product of the breakdown of adenine nucleotides, such as ATP, by a variety of ecto-nucleotidases (ecto-NT). Adenosine can also be formed intracellularly by hydrolysis of S-adenosylhomocysteine (SAH) *via* SAH-hydrolase [29]; thereby, adenosine is an obligatory end product of all transmethylation reactions [30]. A second intracellular source for adenosine is dephosphorylation of AMP by cytoplasmic 5'-nucleotidase (5'-NT) [31]. Adenosine levels are largely regulated by an astrocyte-based adenosine cycle (Fig. **2**) [32, 33] Due to the existence of 2 types of equilibrative adenosine transporters in the astrocyte membrane, the intracellular astrocyte-specific enzyme ADK fulfills the role of a metabolic re-uptake system for adenosine [32, 34]. The removal of adenosine is thus predominantly regulated by ADK *via* conversion of adenosine into AMP, whereas the degradation of adenosine into inosine by adenosine deaminase (ADA) plays only a minor role in regulating adenosinergic function [35, 36]. It is important to note that extracellular and intracellular levels of adenosine are usually maintained in the same range because most cells possess highly efficient equilibratory facilitated diffusion transporters for nucleosides [37].

3. ADENOSINE AND STROKE

3.1. Adenosine Responses to Stroke

Under physiological conditions, extracellular levels of adenosine (25-250 nM) remain within the affinity range of the A_1 and $A_{2A}Rs$. However, adenosine levels can rapidly rise to micromolar levels in response to metabolic stress situations, such as excessive energy consumption or lack of oxygen. Under those conditions, when an imbalance of energy supply and demand occurs, adenosine fulfils the role of a "retaliatory metabolite" to reset the energy balance [38].

In vitro, it has been demonstrated that adenosine levels rise as an acute consequence of ischemia and increased neuronal activity [39], as well as under external stimuli such as high K^+, electrical stimulation, glutamate receptor agonists, hypoxia, and hypoglycemia [26]. Sustained enhancement of the tone of adenosine was found in hippocampal slices after the induction of ischemia [40] or seizures [41]. In line with these findings, adenosine levels were found to rise after cerebral ischemia *in vivo* [42]. The following mechanisms have been suggested to account for the increase of extracellular adenosine levels following ischemic events: *(i)* increased ATP release from cells and accelerated extracellular breakdown of ATP by ecto-nucleotidases [43]; *(ii)* inhibition of intracellular adenosine removal, *via* decreased activity of ADK [44]; and *(iii)* inhibition of equilibrative nucleoside transporters between cell membranes [10].

3.2. Adenosine Receptors and Stroke

Within the context of stroke, the adenosine receptor-mediated net effect of adenosine can best be summarized by the following findings, which have recently been reviewed [34, 45-47]: Activation of the adenosine A_1 receptor (A_1R) provides presynaptic inhibition, neuronal hyperpolarization and is involved in ischemic preconditioning. Consequently,

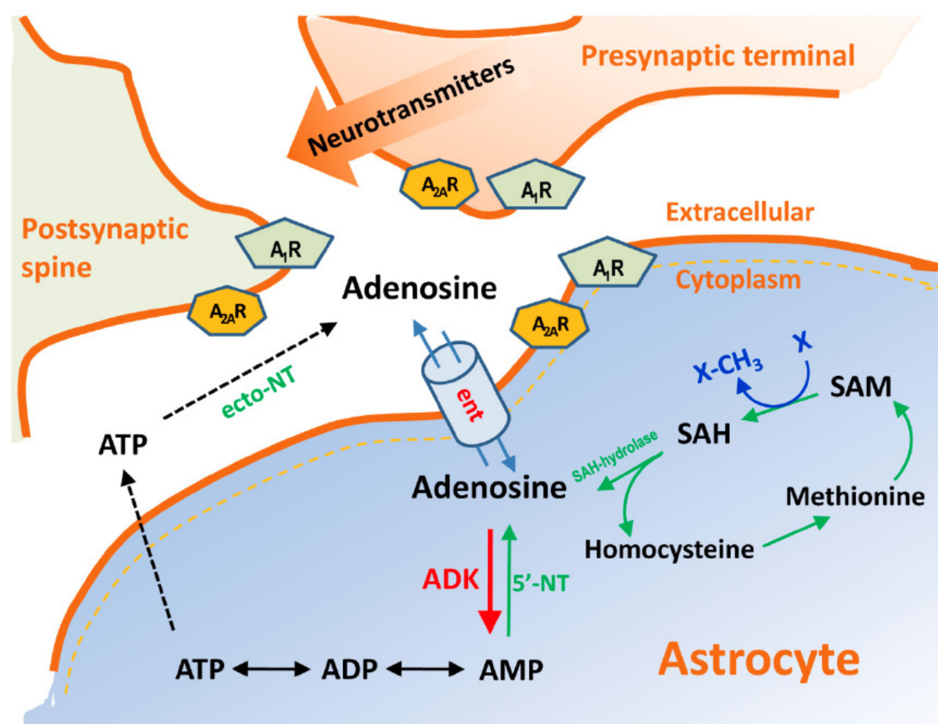

Fig. (2). Adenosine metabolic pathways and major enzymes involved: ecto - nucleotidase (ecto-NT), 5'- nucleotidase (5'-NT), adenosine kinase (ADK), S-adenosylhomocysteine-hydrolase (SAH-hydrolase), S-adenosylmethionine (SAM), equilibrative nucleoside transporter (ent).

mice with a genetic deletion of the A_1R exhibit decreased tolerance to hypoxia and loss of preconditioning in several tissues [48, 49]. However, in A_1R knockout mice the damage resultant from global ischemia was not different from wild-type (WT) mice, although pretreatment with an A_1R antagonist aggravated ischemic damage in WT mice [50].

The role of the $A_{2A}R$ in stroke appears to be more complex; depending on the context, activation of this receptor can either have beneficial or detrimental effects [51, 52]. Thus, both activation and inactivation of the $A_{2A}R$ can provide protection against ischemic damage [53]. Beneficial neuroprotective effects of $A_{2A}R$ activation can be attributed to peripheral effects such as inhibition of platelet aggregation, vasodilatation, and anti-inflammatory actions [54, 55]. However, it has been demonstrated that ischemic brain injury was aggravated in neonatal mice with genetic $A_{2A}R$ deletion compared to WT [51, 56]. Detrimental effects of $A_{2A}R$ activation can be attributed to increased glutamate outflow [57-59], mitogen activated protein kinase (MAPK) activation [60], as well as activation of pro-inflammatory cytokines *via* activation of $A_{2A}Rs$ on bone marrow cells [61]. Thus, a knockout of $A_{2A}Rs$ led to increased tolerance to ischemia [51, 56] and significantly attenuated infarct volumes and improved neurological behavioral deficit scores following ischemia when compared to WT mice [62]; this effect was further demonstrated to be largely dependent on $A_{2A}Rs$ present on bone marrow-derived cells (BMDCs) [61]. Based on these findings, the net effect of inhibition of central $A_{2A}Rs$ is generally thought to be neuroprotective.

The effects of $A_{2B}R$ during ischemia are not as well characterized as other adenosine receptors, as this is perhaps the least studied member of the adenosine receptor family. The $A_{2B}R$ seems to be involved in the regulation of inflammatory processes that play an important role in long-term outcome after stroke [63], whereas the A_3R is involved in preconditioning in some species [64]. In addition, genetic deletion of the A_3R led to an increase in ischemic injury after stroke, indicating A_3Rs are endogeneous neuroprotectors against ischemia [65].

3.3. The Role of Adenosine in Ischemic Preconditioning

3.3.1. Ischemic Preconditioning

In the treatment of cerebral ischemia two basic principles are used: *(i)* Limitation of the acute injury-induced damage, and *(ii)* prophylactic approaches to afford tolerance or resistance to injurious processes that follow a cerebral stroke [66]. Preconditioning of the brain to afford tolerance to the effects of stroke has emerged as an attractive therapeutic strategy.

Ischemic preconditioning, tolerance, and endogenous neuroprotection of the brain have been reviewed extensively [1, 67-69]. It is generally known that certain antecedent treatments or events can protect individuals from injury due to an ischemic episode. For the induction of ischemic preconditioning in the brain, a variety of stimuli, including short periods of ischemia or hypoxia, cortical spreading depression, brief seizures, exposure to inhaled anesthetic, or low dose of bacterial endotoxin [1, 70, 71] have been shown to be effective. Due to the protective time window, ischemic preconditioning in the brain is either based on rapid (classical) preconditioning, induced almost immediately after stimulation, or delayed preconditioning, induced one to three days after injury, a process that requires protein synthesis [72, 73].

3.3.2. Involvement of the Adenosine System in Ischemic Preconditioning

It has been suggested that a local rapid increase of central adenosine is one of the mechanisms involved in acute neuronal ischemic preconditioning [11, 74]. In fact, the increase of adenosine during stroke is not limited to the CNS, adenosine increases have also been documented in serum following a transient ischemic attack (TIA) or after stroke in humans [75]. Moreover, the involvement of adenosine in ischemic preconditioning has been confirmed in the heart [76, 77], indicating that adenosine-based preconditioning might be a general protective mechanism. In addition, adenosine-based mechanisms might contribute in delayed preconditioning. Long-term effects of adenosine include modulation of the brain's immune response, induction of microglial proliferation, and phagocytosis of dead cells and debris in the affected tissue [78]. Adenosine may also be involved in tissue remodeling after injury by promoting angiogenesis and, thus, facilitating the replacement of dysfunctional blood vessels [79].

Apart from the involvement of adenosine in ischemic preconditioning, adenosine receptors are also implicated. The neuroprotective A_1R has been shown to be upregulated rapidly in the brain as a consequence of an ischemic episode [80]. The involvement of the A_1R in ischemic preconditioning is also supported by pharmacological studies showing that DPCPX, a selective A_1 receptor antagonist, attenuates the protective effects of preconditioning when administrated during a brief conditioning treatment [81].

4. ADK AS THERAPEUTIC TARGET AGAINST ISCHEMIC BRAIN INJURY

Adenosine kinase (ADK), the major adenosine regulating enzyme, has recently been identified as a novel therapeutic target to prevent ischemic neuronal cell death [35, 44], seizures and epileptogenesis [32, 82], as well as other neurological conditions such as chronic pain [83]. In the following overview, we will first describe the pathological responses of ADK within the context of stroke and then highlight the key regulatory role of ADK to determine the susceptibility to ischemic brain injury and possible mechanisms involved. Finally, we will discuss potential therapeutic applications of focal adenosine augmentation therapies against stroke.

4.1. ADK-Based Genetic Tools to Study Preconditioning

Given the crucial role of ADK as key regulator of adenosine and the demonstration that pharmacological inhibition of ADK potentiates adenosine-based neuroprotection in cerebral ischemia [83, 84], several ADK-based molecular tools have been developed during the past several years to facilitate research on adenosine-based mechanisms in stroke and ischemic preconditioning:

First, *Adk* cDNA has successfully been cloned and part of the *Adk* gene has been incorporated into gene expression and targeting vectors [30, 85, 86]. Second, genetically engineered mouse lines have been generated and characterized, which include a systemic knockout of the *Adk*-gene that is lethal [30], a line with brain-wide overexpression of ADK (Adk-tg) [86], and a line with reduced ADK expression in the cortex and hippocampus (fb-Adk-def) [82]. Third, an antibody against ADK was generated and validated in ADK knockout mice [30]. Fourth, therapeutic cell lines, including embryonic stem cells, have been engineered to release adenosine based on genetic disruption of ADK [87-89]. Fifth, micro-RNAs directed against ADK have been expressed in a lentiviral expression system and shown to effectively downregulate ADK [90]. These molecular tools are now available and are expected to move the adenosine field forward in our attempts to understand and to exploit adenosinergic signalling therapeutically within the context of stroke therapy and prevention.

4.2. Genetic Upregulation of ADK Increases Vulnerability to Ischemic Injury

Whereas pharmacological inhibition of ADK does not allow for discrimination between systemic and brain-specific effects, transgenic mice with regionally altered brain expression levels of ADK afford the unique possibility to study the roles of increased or decreased *endogenous* ADK on the brain's susceptibility to stroke-induced neuronal cell loss [91]. First, we demonstrated that brain-wide transgenic overexpression of ADK (Adk-tg mice; 141% ADK of control) renders the brain more susceptible to ischemic cell death [91]. Remarkably, Adk-tg mice showed a 3-fold increase in infarct volume after 15 min of MCAO and 24 hour of reperfusion, compared to the infarct volume observed in wild-type mice with normal ADK expression. More strikingly, 60 min of MCAO was lethal in all Adk-tg mice within 24 hours of reperfusion, whereas all wild-type mice survived this procedure with 50% infarct volumes. In an attempt to reproduce an injury in Adk-tg mice that matches the injury in wild-type mice observed after 60 min of MCAO, we successively decreased the occlusion time of the middle cerebral artery in Adk-tg mice. Our findings demonstrate that 30 min of MCAO in Adk-tg mice leads to an infarct volume that is comparable to the infarct volume obtained after 60 min of MCAO in wild-type mice (Fig. **3**).

Conversely, injury levels corresponding to wild-type mice were recreated by pre-treatment of Adk-tg mice with the ADK inhibitor 5-ITU prior to MCAO. These experiments demonstrate that low levels of ADK are essential to maintain adenosine-mediated neuroprotection [91]. In line with the above findings, a subsequent study suggested that the expression levels of *endogenous* ADK might be critically involved in the brain's susceptibility to neuronal cell loss after MCAO [44]. In this study it was demonstrated that the hippocampus of wild-type mice was consistently spared from ischemic injury after 60 min of MCAO followed by 24 hours of reperfusion, a time span during which the endogenous hippocampal ADK was significantly reduced, leading to a 2.2-fold increase of adenosine with a maximum after 3 hours of reperfusion. In contrast, hippocampal injury in Adk-tg mice became evident after only 15 mins of MCAO. The high susceptibility to stroke-induced hippocampal cell loss in Adk-tg mice can best be explained by *constitutive* transgenic overexpression of ADK and the resulting inability to raise adenosine levels by downregulating the enzyme. Therefore, increased activity of ADK and consequent dysregulation of the adenosine system promotes cell death in stroke.

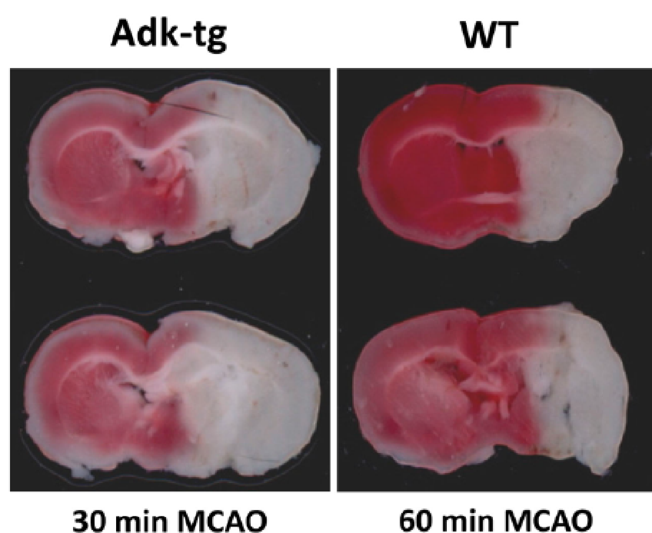

Fig. (3). Triphenyltetrazolium chloride (TTC) staining of two brain slices from Adk-tg (left) and wild-type (WT) mice (right) subjected to MCAO and 24 h of reperfusion. 30 min of MCAO in Adk-tg mice leads to an infarct volume that is comparable to the infarct volume obtained after 60 min of MCAO in wild-type mice.

4.3. Downregulation of ADK as an Endogenous Protective Mechanism

The key adenosine-regulating enzyme ADK is highly conserved in evolution [92] and features ubiquitous species and tissue distribution in all eukaryotes [93]. It catalyzes the reaction: adenosine + ATP → AMP + ADP. ADK is a remarkable enzyme as it is regulated by all of its four substrates and metabolites (adenosine, AMP, ADP, and ATP). It is therefore ideally positioned to function both as a sensor for the energy state of a cell as well as a switch to adjust adenosine levels according to metabolic needs. While ADK shows a dominant neuronal expression pattern during neonatal brain development [94], in adult brain the enzyme is highly specific for astrocytes and has been demonstrated to play a crucial role in maintaining a protective tone of adenosine [36, 85].

Endogenous astrocytic ADK is rapidly downregulated as an acute response to stroke or other brain insults that are associated with lack of oxygen and/or glucose, or that are associated with high levels of oxygen consumption and resulting pH changes [32, 44, 85]. Downregulation of ADK as an acute response to stroke appears to be an efficient endogenous neuroprotective mechanism aimed at raising ambient levels of adenosine in brain. Following stroke, the extracellular amounts of adenosine can increase rapidly and excessively to micromolar and up to millimolar levels [26] and are thus critically positioned to act as an endogenous neuroprotectant during cerebral ischemia and other neuronal insults [8, 9]. *In vitro,* oxygen-glucose deprivation in cultured cortical neurons led to the downregulation of ADK and a rapid rise in extracellular adenosine that was maintained for at least one hour [95]. Importantly, the rapid elevation of the level of ambient adenosine was demonstrated to be due to the release of adenosine – as a consequence of changes in intracellular ADK-dependent metabolism – rather than by extracellular cleavage of ATP [96]. The notion of downregulation of ADK as a general protective response to brain injury was corroborated in pharmacological studies using an ADK inhibitor in the rat MCAO model of transient focal cerebral ischemia [84]. In a recent *in vivo* study, ADK was shown to be transiently downregulated in ischemic mouse brain suggesting that acute downregulation of ADK following stroke causally participates in the endogenous adenosine-dependent protective mechanism of the brain [44]. Similarly, protective downregulation of ADK has also been observed within 2 hours following acute seizures in mice [85].

Due to the existence of a highly active substrate cycle between adenosine and AMP that is catalyzed by ADK and 5'-nucleotidase, minor changes in ADK activity can rapidly translate into major changes of adenosine [97, 98]. ADK is encoded by one of the largest known genes with a size of about 500,000 base pairs in rodents and human; it is not yet known, whether ADK is regulated at the genomic or transcriptional level. Protein phosphorylation as a mechanism for the regulation of ADK activity has been excluded [99]. In order to be effective and efficient as a rapid modulator of ambient levels of adenosine, a more rapid mechanism to regulate ADK activity is needed. Pioneering work from Datta and colleagues performed on *Leishmania* ADK suggests the following mechanism for ADK regulation [100-102]: ADK is active as a monomer that is stabilized by a cyclophilin. Dissociation from the cyclophilin and aggregation leads to rapid enzyme inactivation. Interestingly, the aggregate is stabilized by ADP. Thus, when energy reserves run low, the inactive form of ADK is stabilized by an increase in ambient ADP. Inhibition of ADK leads to an increase in adenosine, which due to its inhibitory role on metabolism resets the energy balance of the cell. This is a feasible and highly likely mechanism for how ADK could fulfill the role of a rapid "neuroprotective switch" under ischemic conditions. The neuroprotective effects of ADK inactivation in stroke can further be potentiated by reduced clearance of endogenously released adenosine. In sum, direct increases in adenosine in combination with reduced clearance combine to exert sustained suppression of excitatory neurotransmission. Thus, under conditions of ischemic or oxidative stress increased activation of A_1Rs leads to the suppression of overstimulation of glutamate receptors and thereby to suppression of neuronal excitation and excitotoxicity [103-105].

4.4. Stem Cell-Based Focal Adenosine Augmentation Prevents Stroke-Induced Injury

Focal stem cell therapies can provide regeneration and repair combined with the sustained delivery of therapeutic compounds. Consequently, several cell therapies are currently under development for the treatment of neurological disorders. We have recently used focal stem cell-based adenosine delivery to demonstrate that providing adenosine to the brain by stem cell-derived brain implants can decrease ischemic neuronal damage [106]. In this study, the intrastriatal transplantation of embryonic stem cell-derived neural precursor (NP) or glial precursor (GP) cells (transplanted 7 days prior to ischemia) demonstrated significant protection against ischemic brain injury. This implies that stem cell derived implants have endogenous mechanisms to protect the brain from ischemic insults.

However, when the same cells were engineered to release adenosine by bi-allelic disruption of the *Adk*-gene, their therapeutic efficacy was potentiated about two-fold. *Adk*-deficient GPs transplanted into mouse brain (again 7 days prior to MCAO) protected almost the entire brain from injury related to 60 min of MCAO followed by 24 hours of reperfusion. These findings clearly demonstrate that focal augmentation of adenosine is sufficient to provide extensive protection in cortex and striatum.

4.5. ADK: A Rational Therapeutic Target

The neurochemical rationale outlined above clearly defines ADK as a potential therapeutic target for ischemic brain injury. ADK inhibition is expected to exert powerful neuroprotection through the following mechanisms:

Activation of A_1Rs can *(i)* inhibit the presynaptic release of excitatory neurotransmitters, such as dopamine and glutamate [107-109]; *(ii)* reduce Ca^{2+} influx and enhance the K^+ and Cl^- conductance postsynaptically in neurons leading to membrane hyperpolarization [110]; *(iii)* hyperpolarize astrocyte cell membranes and improve the astrocytic uptake of excessive extracellular K^+ and glutamate [111]; *(iv)* attenuate basal and NMDA - induced production of nitric oxide [112]. In addition, increased A_{2A}R activation by increased levels of adenosine (caused by ADK-inhibition), has neuroprotective properties that are context dependent. For instance, the activation of A_{2A}Rs may *(i)* inhibit platelet aggregation, thus reducing the potential for vessel obstruction [55]; *(ii)* exert anti-inflammatory actions during the post-stroke period [52, 80, 113]. Finally, stimulation of the adenosine A_3 receptor may also be beneficial in brain ischemia, although the mechanisms underlying this effect are less clear [114].

5. ADENOSINE-BASED PHARMACOLOGICAL APPROACHES

5.1. Neuro-Protection *via* Acute A_1R Agonism or Chronic A_1R Antagonism

Since A_1R activation is known to mediate the neuroprotective activity of adenosine, the majority of pharmacological studies have focused on acute A_1R activation. The majority of A_1R agonists exert potent neuroprotective properties in a wide range of animal models of either global or focal ischemia. For instance, either acute systemic or intracerebroventricular administration of the A_1R agonist, N6-cyclohexyl-adenosine (CHA) attenuated hippocampal neuronal cell loss and improved neurological deficits in gerbils and rats subjected to global forebrain ischemia [115-117]. In line with these findings, the local administration of an adenosine analogue, 2-chloroadenosine (CADO), attenuated hippocampal cell loss in rats subjected to transient global forebrain ischemia [118]. Similarly, other A_1R agonists, N6-cyclopentyladenosine (CPA) and 2-chloro-N6-cyclopentyladenosine (CCPA), reduced neuronal loss and mortality in gerbils after global forebrain ischemia [119, 120]. Moreover, the A_1R agonist adenosine amine congener (ADAC) increased survival in the gerbil when administrated after an ischemic event [121]. All of the above confirm that the stimulation of A_1Rs exerts neuroprotective effects both *in vitro* and in animal models of hypoxia/ischemia. However, the therapeutic use of selective A_1R agonists is severely hampered by unwanted peripheral side effects, i.e. sedation, bradycardia and hypotension [122].

A_1R *antagonists* have also been shown to have protective effects after chronic administration (effect inversion after chronic dosage). For example, both 2-chloro-N6-cyclopentyladenosine (DPCPX) and caffeine given chronically for 2 to 3 weeks before an ischemic insult reduced neuronal injury in rats and gerbils [120, 123]. Similar neuroprotective effects were also observed in gerbils subjected to global forebrain ischemia after caffeine was given for 4 weeks [119, 124]. The above findings suggest the beneficial effects of chronic administration of A_1R antagonists, most likely mediated by up-regulation of A_1Rs.

5.2. Neuroprotection *via* A_{2A}R Antagonists

The neuroprotective properties of A_{2A}R antagonists have also been demonstrated recently [125-128]. For instance, pharmacological data shows that both the selective A_{2A}R antagonists, ZM241385 and 8-(3-chlorostyryl) caffeine (CSC), as well as the less selective ones, CGS15943 and CP66,713, are able to attenuate global forebrain ischemia-induced hippocampal neuronal cell loss in gerbils or rats [53, 125, 128, 129]. Similarly, the selective A_{2A}R antagonist SCH58261 was shown to reduce cortical infarct volume in permanent middle cerebral artery occlusion [127]. In addition, SCH58261 reduced brain damage in neonatal rats with unilateral carotid artery occlusion [130]. In contrast to the central effects of A_1R agonists, neuroprotection mediated by A_{2A}R antagonism is mainly due to peripheral mechanisms including vasodilation, inhibition of platelet aggregation and the suppression of neutrophil superoxide generation [51, 131, 132]. However, it needs to be mentioned that in several studies, A_{2A}R *agonists* also exhibit neuroprotective activity, for example in the global ischemia model in the gerbil [53]

5.3. Neuroprotection by A_3R Manipulation

In addition to the pharmacological manipulation of A_1 and A_{2A}Rs, the protective effects of A_3R agonists have also been evaluated recently. For instance, the selective A_3R agonist, chloro-N(6)- (3-iodo-benzyl)-adenosine-5'-N-methyluronamide (Cl-IB-MECA) when given intracerebroventricularly or intravenously by repeated administration not only protected primary cortical cultures against hypoxia-mediated decrease in cell viability *in vitro*, but also decreased cerebral infarction in mice subjected to MCAO [133]. However, paradoxical effects of A_3R agonists in the brain have also been demonstrated: high concentration of Cl-IB-MECA have lethal effects, whereas low, nanomolar concentrations of A_3R agonists protect against apoptosis or ischemic damage [134].

5.4. ADK Inhibition Protects Against Ischemia

As a result of efforts in medicinal chemistry, adenosine-potentiating agents have been developed that elevate endogenous adenosine levels by inhibition of adenosine metabolism. Thus, inhibitors of ADK, ADA, or adenosine transporters have been developed and have been shown to protect from ischemic brain injury in different *in vivo* models [83, 84, 135-137]. Based on these findings, the pharmacological inhibition of ADK has been proposed to be the most effective strategy to provide neuroprotection in stroke.

Since Miller *et al.* (1996) first demonstrated a dose-dependent neuroprotection by the ADK inhibitor 5'-deoxyiodotubercidin (5-lTU) in a rat MCAO model afforded [136], it was realized that ADK inhibition might be used therapeutically as a neuroprotectant during the pre-and peri-stroke period. Thus, adenosine augmentation *via* ADK-inhibition was seen as a viable strategy to overcome the limited therapeutic time-window of systemic adenosine administration. Later on, a study from Tatlisumak *et al.* (1998) demonstrated that a different ADK inhibitor - GP683 [4-(N-Phenylamino) - 5-phenyl-7- (5'-deoxy ß-D-ribofurasonyl) pyrrolo [2, 3-d] pyrimidine] - was effective in reducing the infarction volume in a rat model of focal cerebral ischemia. Similar to 5-ITU, GP683 was also demonstrated to provide effective neuroprotection when given 30 min after the induction of ischemia [137].

These pharmacological studies using ADK-inhibition suggested the following: *(i)* In contrast to A_1R agonists, ADK inhibition, either by 5-ITU or by GP683, did not induce hypothermia, suggesting that its anti-ischemic effects are mediated by mechanisms other than brain hypothermia. Importantly, ADK inhibition avoided systematic side-effects of hypothermia. *(ii)* The delayed administration of ADK inhibitors, both 5-ITU (0.33 mg/kg, i.v.) and GP683 (1.0 mg/kg, i.p.), at 30 min after the induction of ischemia resulted in a significant reduction in infarct volume, 32% and 44% respectively. These unique features of an ADK-based therapeutic approach suggest a wider therapeutic index of ADK inhibitors in stroke. Based on the concept that ADK inhibition may potentiate an event-specific surge in adenosine and thereby provide event-specific benefits, avoiding systemic side effects [83, 138-140]. ADK inhibitors were further developed for the potential treatment of stroke, epilepsy, and chronic pain [141]. However, due to liver toxicity of ADK inhibition [30], and due to brain hemorrhage in preclinical models in dogs [139, 140], ADK-inhibitors might not be a therapeutic option and drug development efforts have largely been stalled.

6. CONCLUDING REMARKS AND OUTLOOK

Therapeutic strategies aimed at enhancing adenosinergic function have widely been explored for their capability to protect the brain from ischemic injury. However, although the use of adenosine as a neuroprotective agent against stroke has been evaluated extensively in previous studies [142, 143], currently no adenosine-based therapeutics exist for the treatment of cerebral ischemia and reperfusion-induced brain damage.

Two major limitations have accounted for the delayed development of adenosine-based therapeutic strategies:

- Potent peripheral and central side effects of adenosine [144], which include suppression of cardiac functions, sedation, and renal impairment, preclude the systemic use of adenosine or its receptor agonists [12].
- The short physiological half-life of adenosine limits drug delivery and sustained therapeutic activity. Therefore, a novel generation of adenosine-based therapeutics is needed. The development of therapeutics for stroke therapy requires *(i)* local application/action combined with sustained delivery and *(ii)* the identification of novel therapeutic targets.

The identification of ADK as key regulator for ambient levels of adenosine and thus as a control point for adenosine-mediated downstream effects unravels a promising therapeutic target. Thus, inhibition of ADK as a strategy to increase adenosine levels represents a logical neurochemical rationale for therapeutic intervention. Importantly, ADK-inhibition was demonstrated to have several key advantages in terms of site and event selectivity compared to alternative adenosine-based approaches. The rationale for ADK-based therapeutics is based on the following arguments:

- ADK inhibitors can selectively enhance adenosine availability at traumatized tissue sites [145]; therefore ADK inhibitors may selectively amplify local endogenous adenosine responses.
- Local ADK inhibition may be of prophylactic value in stroke.
- Compared to direct-acting adenosine agonists, ADK inhibition offers the possibility of providing sustained augmentation of protective adenosine, which may extend the therapeutic window available for stroke therapy [83] (Fig. **4**).

In conclusion, the development of ADK-based pharmacotherapeutics still need to overcome limitations in *in vivo* half-life, cellular penetrability and oral bioavailability.

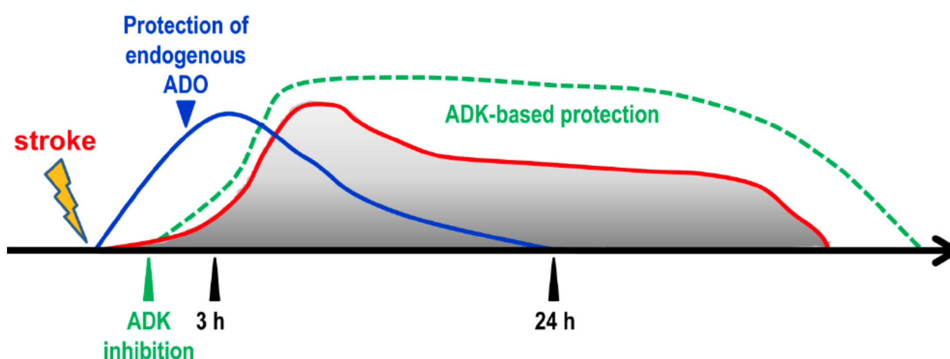

Fig. (4). Model for adenosine-based neuroprotection after stroke. The endogenous surge of adenosine (ADO, blue) initiated after ischemia onset reaches a peak at 3 h post-stroke and is partially responsible for delayed neuronal cell loss (red) after stroke. Adenosine levels reach baseline values within 24 hours; this rapid decrease in adenosine levels may explain the concurrent neuronal cell loss. Therapeutic inhibition of ADK (green) may extend the duration of the endogenous adenosine-surge and therefore might provide extended neuroprotection.

Limitations of systemic ADK inhibition may also include liver toxicity and the risk of brain hemorrhage. Eventually, local cell or gene-based approaches might become a future therapeutic option.

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REFERENCES

[1] Dirnagl, U.; Simon, R.P.; Hallenbeck, J.M. Ischemic tolerance and endogenous neuroprotection. *Trends Neurosci.,* **2003**, *26(5)*, 248-254.

[2] Liou, A.K.; Clark, R.S.; Henshall, D.C.; Yin, X.M.; Chen, J. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. *Prog. Neurobiol.,* **2003**, *69(2),* 103-142.

[3] Xiong, Z.G.; Zhu, X.M.; Chu, X.P.; Minami, M.; Hey, J.; Wei, W.L.; MacDonald, J.F.; Wemmie, J.A.; Price, M.P.; Welsh, M.J.; Simon, R.P. Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. *Cell,* **2004**, *118(6)*, 687-698.

[4] De Keyser, J.; Schaaf, M.; Teelken, A. Peptidylarginine deiminase activity in postmortem white matter of patients with multiple sclerosis. *Neurosci. Lett.,* **1999**, *260(1)*, 74-76.

[5] Hoyte, L.; Barber, P.A.; Buchan, A.M.; Hill, M.D. The rise and fall of NMDA antagonists for ischemic stroke. *Curr. Mol. Med.,* **2004**, *4(2)*, 131-136.

[6] Hoyte, L.; Kaur, J.; Buchan, A.M. Lost in translation: taking neuroprotection from animal models to clinical trials. *Exp. Neurol.,* **2004**, *188(2)*, 200-204.

[7] Wahlgren, N.G.; Ahmed, N. Neuroprotection in cerebral ischaemia: facts and fancies--the need for new approaches. *Cerebrovasc. Dis.,* **2004**, 17 Suppl 1, 153-166.

[8] Fredholm, B.B.; Chen, J.F.; Cunha, R.A.; Svenningsson, P.; Vaugeois, J.M. Adenosine and brain function. *Int. Rev. Neurobiol.,* **2005**, *63*, 191-270.

[9] Fredholm, B.B.; Chen, J.F.; Masino, S.A.; Vaugeois, J.M. Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. *Annu. Rev. Pharmacol. Toxicol.,* **2005**, *45*, 385-412.

[10] Dunwiddie, T.V.; Masino, S.A. The role and regulation of adenosine in the central nervous system. *Annu. Rev. Neurosci.,* **2001**, *24*, 31-55.

[11] Cunha, R.A. Neuroprotection by adenosine in the brain: from A1 receptor activation to A2A receptor blockade. *Purinergic Signaling,* **2005**, *1*, 111-134.

[12] Boison, D. Adenosine and epilepsy: from therapeutic rationale to new therapeutic strategies. *Neuroscientist,* **2005**, *11(1)*, 25-36.

[13] Fredholm, B.B.; Dunwiddie, T.V. How does adenosine inhibit transmitter release? *Trends Pharmacol. Sci.,* **1988**, *9*, 130-134.

[14] Cunha, R.A. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. *Neurochem. Int.,* **2001**, *38(2)*, 107-125.

[15] Dixon, A.K.; Widdowson, L.; Richardson, P.J. Desensitisation of the adenosine A1 receptor by the A2A receptor in the rat striatum. *J. Neurochem.,* **1997**, *69(1)*, 315-321.

[16] O'Kane, E.M.; Stone, T.W. Interaction between adenosine A1 and A2 receptor-mediated responses in the rat hippocampus *in vitro*. *Eur. J. Pharmacol.,* **1998**, *362(1)*, 17-25.

[17] Lopes, L.V.; Cunha, R.A.; Ribeiro, J.A. Cross talk between A(1) and A(2A) adenosine receptors in the hippocampus and cortex of young adult and old rats. *J. Neurophysiol.,* **1999**, *82(6)*, 3196-3203.

[18] Gorter, J.A.; van Vliet, E.A.; Lopes da Silva, F.H.; Isom, L.L.; Aronica, E. Sodium channel beta1-subunit expression is increased in reactive astrocytes in a rat model for mesial temporal lobe epilepsy. *Eur. J. Neurosci.,* **2002**, *16(2)*, 360-364.

[19] Snyder, S.H. Adenosine as a neuromodulator. *Annu. Rev. Neurosci.,* **1985**, *8*, 103-124.

[20] Latini, S.; Pazzagli, M.; Pepeu, G.; Pedata, F. A2 adenosine receptors: their presence and neuromodulatory role in the central nervous system. *Gen. Pharmacol.,* **1996**, *27(6)*, 925-933.

[21] Kirkpatrick, K.A.; Richardson, P.J. Adenosine receptor-mediated modulation of acetylcholine release from rat striatal synaptosomes. *Br. J. Pharmacol.,* **1993**, *110(3)*, 949-954.

[22] Cunha, R.A.; Constantino, M.C.; Sebastiao, A.M.; Ribeiro, J.A. Modification of A1 and A2a adenosine receptor binding in aged striatum, hippocampus and cortex of the rat. *Neuroreport,* **1995**, *6(11)*, 1583-1588.

[23] Cunha, R.A.; Johansson, B.; Fredholm, B.B.; Ribeiro, J.A.; Sebastiao, A.M. Adenosine A2A receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus. *Neurosci. Lett.,* **1995**, *196(1-2)*, 41-44.

[24] Kirk, I.P.; Richardson, P.J. Adenosine A2a receptor-mediated modulation of striatal [3H]GABA and [3H]acetylcholine release. *J. Neurochem.,* **1994**, *62(3)*, 960-966.

[25] Sebastiao, A.M.; Ribeiro, J.A. Adenosine A2 receptor-mediated excitatory actions on the nervous system. *Prog. Neurobiol.,* **1996**, *48(3)*, 167-189.

[26] Latini, S.; Pedata, F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J. Neurochem.,* **2001**, *79(3)*, 463-484.

[27] Gray, J.H.; Owen, R.P.; Giacomini, K.M. The concentrative nucleoside transporter family, SLC28. *Pflugers Arch.,* **2004**, *447(5)*, 728-734.

[28] Baldwin, S.A.; Beal, P.R.; Yao, S.Y.; King, A.E.; Cass, C.E.; Young, J.D. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch.,* **2004**, *447(5)*, 735-743.

[29] Broch, O.J.; Ueland, P.M. Regional and subcellular distribution of S-adenosylhomocysteine hydrolase in the adult rat brain. *J. Neurochem.,* **1980**, *35(2)*, 484-488.

[30] Boison, D.; Scheurer, L.; Zumsteg, V.; Rülicke, T.; Litynski, P.; Fowler, B.; Brandner, S.; Mohler, H. Neonatal hepatic steatosis by disruption of the adenosine kinase gene. *Proc. Natl. Acad. Sci. USA,* **2002**, *99(10)*, 6985-6990.

[31] Schubert, P.; Komp, W.; Kreutzberg, G.W. Correlation of 5'-nucleotidase activity and selective transneuronal transfer of adenosine in the hippocampus. *Brain Res.,* **1979**, *168(2)*, 419-424.

[32] Boison, D. The adenosine kinase hypothesis of epileptogenesis. *Prog. Neurobiol*., **2008**, *84(3)*, 249-262.

[33] Boison, D.; Stewart, K.-A. Therapeutic epilepsy research: from pharmacological rationale to focal adenosine augmentation. *Biochem. Pharmacol.,* **2009**, *78*(12), 1428-1437.

[34] Boison, D.; Chen, J.F.; Fredholm, B.B. Adenosine signalling and function in glial cells. *Cell Death Differ*., **2010**, *17*(7), 1071-1082.

[35] Boison, D. Adenosine kinase, epilepsy and stroke: mechanisms and therapies. *Trends Pharmacol. Sci.,* **2006**, *27(12)*, 652-658.

[36] Pak, M.A.; Haas, H.L.; Decking, U.K.M.; Schrader, J. Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacol.,* **1994**, *33*, 1049-1053.

[37] Cass, C.E.; Young, J.D.; Baldwin, S.A.; Cabrita, M.A.; Graham, K.A.; Griffiths, M.; Jennings, L.L.; Mackey, J.R.; Ng, A.M.; Ritzel, M.W.; Vickers, M.F.; Yao, S.Y. Nucleoside transporters of mammalian cells. *Pharm. Biotechnol.,* **1999**, *12*, 313-352.

[38] Newby, A.C. Adenosine: origin and clinical roles. *Adv. Exp. Med. Biol.,* **1991**, *309A*, 265-270.

[39] Masino, S.A.; Latini, S.; Bordoni, F.; Pedata, F.; Dunwiddie, T.V. Changes in hippocampal adenosine efflux, ATP levels, and synaptic transmission induced by increased temperature. *Synapse,* **2001**, *41(1)*, 58-64.

[40] Pearson, T.; Damian, K.; Lynas, R.E.; Frenguelli, B.G. Sustained elevation of extracellular adenosine and activation of A (1) receptors underlie the post-ischaemic inhibition of neuronal function in rat hippocampus *in vitro*. *J. Neurochem.,* **2006**, *97(5)*, 1357-1368.

[41] Etherington, L.A.; Patterson, G.E.; Meechan, L.; Boison, D.; Irving, A.J.; Dale, N.; Frenguelli, B. Astrocytic adenosine kinase regulates basal synaptic adenosine levels and seizure activity but not activity-dependent adenosine release in the hippocampus. *Neuropharmacology,* **2009**, *56(2)*, 429-437.

[42] Kobayashi, T.; Yamada, T.; Okada, Y. The levels of adenosine and its metabolites in the guinea pig and rat brain during complete ischemia-*in vivo* study. *Brain Res.,* **1998**, *787(2)*, 211-219.

[43] Colgan, S.P.; Eltzschig, H.K.; Eckle, T.; Thompson, L.F. Physiological roles for ecto-5'-nucleotidase (CD73). *Purinergic Signal,* **2006**, *2(2)*, 351-360.

[44] Pignataro, G.; Maysami, S.; Studer, F.E.; Wilz, A.; Simon, R. P.; Boison, D. Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. *J. Cereb. Blood Flow Metab.,* **2008**, *28*, 17-23.

[45] Fredholm, B.B. Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ* **2007**, *14(7)*, 1315-1323.

[46] Fredholm, B.B.; Chern, Y.; Franco, R.; Sitkovsky, M. Aspects of the general biology of adenosine A2A signaling. *Prog. Neurobiol.,* **2007**, *83(5)*, 263-276.

[47] Boison, D. Adenosine-based modulation of brain activity. *Curr. Neuropharmacol.,* **2009**, *7(3)*, 158-159.

[48] Morrison, R.R.; Teng, B.; Oldenburg, P.J.; Katwa, L.C.; Schnermann, J.B.; Mustafa, S.J. Effects of targeted deletion of A1 adenosine receptors on postischemic cardiac function and expression of adenosine receptor subtypes. *Am. J. Physiol. Heart Circ. Physiol.,* **2006**, *291(4)*, H1875-1882.

[49] Reichelt, M.E.; Willems, L.; Molina, J.G.; Sun, C.X.; Noble, J.C.; Ashton, K.J.; Schnermann, J.; Blackburn, M.R.; Headrick, J.P. Genetic deletion of the A1 adenosine receptor limits myocardial ischemic tolerance. *Circ. Res.,* **2005**, *96(3)*, 363-367.

[50] Olsson, T.; Cronberg, T.; Rytter, A.; Asztely, F.; Fredholm, B.B.; Smith, M.L.; Wieloch, T. Deletion of the adenosine A1 receptor gene does not alter neuronal damage following ischaemia *in vivo* or *in vitro*. *Eur. J. Neurosci.,* **2004**, *20(5)*, 1197-1204.

[51] Chen, J.F.; Pedata, F. Modulation of ischemic brain injury and neuroinflammation by adenosine A(2A) receptors. *Curr. Pharm. Des.,* **2008**, *14(15)*, 1490-1499.

[52] Chen, J.F.; Sonsalla, P.K.; Pedata, F.; Melani, A.; Domenici, M.R.; Popoli, P.; Geiger, J.; Lopes, L.V.; de Mendonca, A. Adenosine A2A receptors and brain injury: broad spectrum of neuroprotection, multifaceted actions and "fine tuning" modulation. *Prog. Neurobiol.,* **2007**, *83(5)*, 310-331.

[53] von Lubitz, D.K.; Carter, M.F.; Beenhakker, M.; Lin, R.C.; Jacobson, K.A. Adenosine: a prototherapeutic concept in neurodegeneration. *Ann N Y Acad Sci.,* **1995***, 765,* 163-78*;* discussion 196-7.

[54] Phillis, J.W.; O'Regan, M.H. Deoxycoformycin antagonizes ischemia-induced neuronal degeneration. *Brain Res. Bull.,* **1989**, *22(3)*, 537-540.

[55] Phillis, J.W. Adenosine and adenine nucleotides as regulators of cerebral blood flow: roles of acidosis, cell swelling, and KATP channels. *Crit. Rev. Neurobiol.,* **2004**, *16(4)*, 237-270.

[56] Cunha, R.A.; Ferre, S.; Vaugeois, J.M.; Chen, J.F. Potential therapeutic interest of adenosine A(2A) receptors in psychiatric disorders. *Curr. Pharm. Des.,* **2008**, *14(15)*, 1512-1524.

[57] Pedata, F.; Gianfriddo, M.; Turchi, D.; Melani, A. The protective effect of adenosine A2A receptor antagonism in cerebral ischemia. *Neurol. Res.,* **2005**, *27(2)*, 169-174.

[58] Pedata, F.; Corsi, C.; Melani, A.; Bordoni, F.; Latini, S. Adenosine extracellular brain concentrations and role of A2A receptors in ischemia. *Ann. N.Y. Acad. Sci.,* **2001**, *939*, 74-84.

[59] Marcoli, M.; Raiteri, L.; Bonfanti, A.; Monopoli, A.; Ongini, E.; Raiteri, M.; Maura, G. Sensitivity to selective adenosine A1 and A2A receptor antagonists of the release of glutamate induced by ischemia in rat cerebrocortical slices. *Neuropharmacology,* **2003**, *45(2)*, 201-210.

[60] Melani, A.; Gianfriddo, M.; Vannucchi, M.G.; Cipriani, S.; Baraldi, P.G.; Giovannini, M.G.; Pedata, F. The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. *Brain Res.,* **2006**, 1073-1074, 470-480.

[61] Yu, L.; Huang, Z.; Mariani, J.; Wang, Y.; Moskowitz, M.; Chen, J.F. Selective inactivation or reconstitution of adenosine A2A receptors in bone marrow cells reveals their significant contribution to the development of ischemic brain injury. *Nat. Med.,* **2004**, *10(10)*, 1081-1087.

[62] Chen, J.F.; Huang, Z.; Ma, J.; Zhu, J.; Moratalla, R.; Standaert, D.; Moskowitz, M.A.; Fink, J.S.; Schwarzschild, M.A. A(2A) adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J. Neurosci.,* **1999**, *19(21)*, 9192-9200.

[63] Stone, T.W.; Ceruti, S.; Abbracchio, M.P. Adenosine receptors and neurological disease: neuroprotection and neurodegeneration. *Handb. Exp. Pharmacol.,* **2009**, *193*, 535-587.

[64] von Arnim, C.A.; Timmler, M.; Ludolph, A.C.; Riepe, M.W. Adenosine receptor up-regulation: initiated upon preconditioning but not upheld. *Neuroreport,* **2000**, *11(6)*, 1223-1226.

[65] Chen, Y.; Corriden, R.; Inoue, Y.; Yip, L.; Hashiguchi, N.; Zinkernagel, A.; Nizet, V.; Insel, P.A.; Junger, W.G. ATP release guides neutrophil chemotaxis *via* P2Y2 and A3 receptors. *Science,* **2006**, *314(5806)*, 1792-1795.

[66] Dirnagl, U.; Becker, K.; Meisel, A. Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. *Lancet Neurol.,* **2009**, *8(4)*, 398-412.

[67] Nakatomi, H.; Kuriu, T.; Okabe, S.; Yamamoto, S.; Hatano, O.; Kawahara, N.; Tamura, A.; Kirino, T.; Nakafuku, M. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell,* **2002**, *110(4)*, 429-441.

[68] Gidday, J.M. Cerebral preconditioning and ischaemic tolerance. *Nat. Rev. Neurosci.,* **2006**, *7(6)*, 437-448.

[69] Bowen, K.K.; Naylor, M.; Vemuganti, R. Prevention of inflammation is a mechanism of preconditioning-induced neuroprotection against focal cerebral ischemia. *Neurochem. Int.,* **2006**, *49(2)*, 127-135.

[70] Dhodda, V.K.; Sailor, K.A.; Bowen, K.K.; Vemuganti, R. Putative endogenous mediators of preconditioning-induced ischemic tolerance in rat brain identified by genomic and proteomic analysis. *J. Neurochem.,* **2004**, *89(1)*, 73-89.

[71] Plamondon, H.; Blondeau, N.; Heurteaux, C.; Lazdunski, M. Mutually protective actions of kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: involvement of adenosine A1 receptors and K(ATP) channels. *J. Cereb. Blood Flow Metab.,* **1999**, *19(12)*, 1296-1308.

[72] Stenzel-Poore, M.P.; Stevens, S.L.; King, J.S.; Simon, R.P. Preconditioning reprograms the response to ischemic injury and primes the emergence of unique endogenous neuroprotective phenotypes: a speculative synthesis. *Stroke,* **2007**, *38*(2 Suppl), 680-685.

[73] Barone, F.C.; White, R.F.; Spera, P.A.; Ellison, J.; Currie, R. W.; Wang, X.; Feuerstein, G.Z. Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. *Stroke,* **1998**, *29(9)*, 1937-1950; discussion 1950-1.

[74] Das, M.; Das, D.K. Molecular mechanism of preconditioning. *IUBMB Life,* **2008**, *60(4)*, 199-203.

[75] Pasini, F.L.; Capecchi, P.L.; Colafati, M.; Randisi, P.; Puccetti, L.; Di Perri, T. Systemic adenosine increase during cold pressor test is dependent on sympathetic activation. *Clin. Exp. Pharmacol. Physiol.,* **1999**, *26(10)*, 774-778.

[76] Mubagwa, K.; Flameng, W. Adenosine, adenosine receptors and myocardial protection: an updated overview. *Cardiovasc. Res.,* **2001**, *52(1)*, 25-39.

[77] Sommerschild, H.T.; Kirkeboen, K.A. Adenosine and cardioprotection during ischaemia and reperfusion--an overview. *Acta Anaesthesiol. Scand.,* **2000**, *44*(9), 1038-55.

[78] Hasko, G.; Pacher, P.; Vizi, E.S.; Illes, P. Adenosine receptor signaling in the brain immune system. *Trends Pharmacol. Sci.,* **2005**, *26(10)*, 511-516.

[79] Fischer, S.; Sharma, H.S.; Karliczek, G.F.; Schaper, W. Expression of vascular permeability factor/vascular endothelial growth factor in pig cerebral microvascular endothelial cells and its upregulation by adenosine. *Brain Res. Mol. Brain Res.,* **1995**, *28(1)*, 141-148.

[80] Fredholm, B.B. Adenosine and neuroprotection. *Int. Rev. Neurobiol.,* **1997**, *40*, 259-280.

[81] Akaiwa, K.; Akashi, H.; Harada, H.; Sakashita, H.; Hiromatsu, S.; Kano, T.; Aoyagi, S. Moderate cerebral venous congestion induces rapid cerebral protection *via* adenosine A1 receptor activation. *Brain Res.,* **2006**, *1122(1)*, 47-55.

[82] Li, T.; Ren, G.; Lusardi, T.; Wilz, A.; Lan, J.Q.; Iwasato, T.; Itohara, S.; Simon, R.P.; Boison, D. Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J. Clin. Inv.,* **2008**, *118(2)*, 571-582.

[83] Kowaluk, E.A.; Jarvis, M.F. Therapeutic potential of adenosine kinase inhibitors. *Exp. Opin. Investig. Drugs,* **2000**, *9(3)*, 551-564.

[84] Jiang, N.; Kowaluk, E.A.; Lee, C.H.; Mazdiyasni, H.; Chopp, M. Adenosine kinase inhibition protects brain against transient focal ischemia in rats. *Eur. J. Pharmacol.,* **1997**, *320(2-3)*, 131-137.

[85] Gouder, N.; Scheurer, L.; Fritschy, J.-M.; Boison, D. Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. *J. Neurosci.,* **2004**, *24(3)*, 692-701.

[86] Fedele, D.E.; Gouder, N.; Güttinger, M.; Gabernet, L.; Scheurer, L.; Rulicke, T.; Crestani, F.; Boison, D. Astrogliosis in epilepsy

leads to overexpression of adenosine kinase resulting in seizure aggravation. *Brain,* **2005**, *128*, 2383-2395.

[87] 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. USA* **2001**, *98(13)*, 7611-7616.

[88] Güttinger, M.; Padrun, V.; Pralong, W.; Boison, D. Seizure suppression and lack of adenosine A_1 receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. *Exp. Neurol.,* **2005**, *193*, 53-64.

[89] Fedele, D.E.; Koch, P.; Brüstle, O.; Scheurer, L.; Simpson, E. M.; Mohler, H.; Boison, D. Engineering embryonic stem cell derived glia for adenosine delivery. *Neurosci. Lett.,* **2004**, *370(2-3)*, 160-165.

[90] Ren, G.; Li, T.; Lan, J.Q.; Wilz, A.; Simon, R.P.; Boison, D. Lentiviral RNAi-induced downregulation of adenosine kinase in human mesenchymal stem cell grafts: a novel perspective for seizure control. *Exp. Neurol.,* **2007**, *208*, 26-37.

[91] Pignataro, G.; Simon, R.P.; Boison, D. Transgenic overexpression of adenosine kinase aggravates cell death in ischemia. *J. Cereb. Blood Flow Metab.,* **2007**, *27(1)*, 1-5.

[92] Gupta, Y.K.; Malhotra, J. Adenosinergic system as an endogenous anticonvulsant mechanism. *Indian J. Physiol. Pharmacol.,* **1997**, *41(4)*, 329-343.

[93] Andres, C.M.; Fox, I.H. Purification and properties of human placental adenosine kinase. *J. Biol. Chem.,* **1979**, *254*(22), 11388-11393.

[94] Studer, F.E.; Fedele, D.E.; Marowsky, A.; Schwerdel, C.; Wernli, K.; Vogt, K.; Fritschy, J.-M.; Boison, D. Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. *Neuroscience,* **2006**, *142*, 125-137.

[95] Lynch, J.J. 3rd; Alexander, K.M.; Jarvis, M.F.; Kowaluk, E.A. Inhibition of adenosine kinase during oxygen-glucose deprivation in rat cortical neuronal cultures. *Neurosci. Lett.,* **1998**, *252(3)*, 207-210.

[96] Frenguelli, B.G.; Wigmore, G.; Llaudet, E.; Dale, N. Temporal and mechanistic dissociation of ATP and adenosine release during ischaemia in the mammalian hippocampus. *J. Neurochem.,* **2007**, *101(5)*, 1400-1413.

[97] Arch, J.R.; Newsholme, E.A. Activities and some properties of 5'-nucleotidase, adenosine kinase and adenosine deaminase in tissues from vertebrates and invertebrates in relation to the control of the concentration and the physiological role of adenosine. *Biochem. J.,* **1978**, *174(3)*, 965-977.

[98] Bontemps, F.; Vincent, M.F.; Van den Berge, G. Mechanisms of elevation of adenosine levels in anoxic hepatocytes. *Biochem. J.,* **1993**, *290*, 671-677.

[99] Sahin, B.; Kansy, J.W.; Nairn, A.C.; Spychala, J.; Ealick, S.E.; Fienberg, A.A.; Greene, R.W.; Bibb, J.A. Molecular characterization of recombinant mouse adenosine kinase and evaluation as a target for protein phosphorylation. *Eur. J. Biochem.,* **2004**, *271(17)*, 3547-3555.

[100] Datta, A.K.; Bhaumik, D.; Chatterjee, R. Isolation and characterization of adenosine kinase from Leishmania donovani. *J. Biol. Chem.,* **1987**, *262(12)*, 5515-5521.

[101] Datta, K.; Franke, T.F.; Chan, T.O.; Makris, A.; Yang, S.I.; Kaplan, D.R.; Morrison, D.K.; Golemis, E.A.; Tsichlis, P.N. AH/PH domain-mediated interaction between Akt molecules and its potential role in Akt regulation. *Mol. Cell Biol.,* **1995**, *15(4)*, 2304-2310.

[102] Datta, S.K.; Guicherit, O.M.; Kellems, R.E. Adenylosuccinate synthetase - a dominant amplifiable genetic marker in mammalian cells. *Somat. Cell Mol. Genet.,* **1994**, *20(5)*, 381-389.

[103] Hagberg, H.; Andersson, P.; Lacarewicz, J.; Jacobson, I.; Butcher, S.; Sandberg, M. Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. *J. Neurochem.,* **1987**, *49*, 227-231.

[104] Kopf, S.R.; Melani, A.; Pedata, F.; Pepeu, G. Adenosine and memory storage: effect of A(1) and A(2) receptor antagonists. *Psychopharmacology (Berl),* **1999**, *146(2)*, 214-219.

[105] Phillis, J.W.; O'Regan, M.H. Effects of adenosine receptor antagonists on pial arteriolar dilation during carbon dioxide inhalation. *Eur. J. Pharmacol.,* **2003**, *476(3)*, 211-219.

[106] Pignataro, G.; Studer, F.E.; Wilz, A.; Simon, R.P.; Boison, D. Neuroprotection in ischemic mouse brain induced by stem cell-derived brain implants. *J. Cereb. Blood Flow Metab.,* **2007**, *27(5)*, 919-927.

[107] Zetterstrom, T.; Fillenz, M. Adenosine agonists can both inhibit and enhance *in vivo* striatal dopamine release. *Eur. J. Pharmacol.,* **1990**, *180(1)*, 137-143.

[108] Corradetti, R.; Lo Conte, G.; Moroni, F.; Passani, M.B.; Pepeu, G. Adenosine decreases aspartate and glutamate release from rat hippocampal slices. *Eur. J. Pharmacol.,* **1984**, *104*, 19-26.

[109] Schubert, P.; Rudolphi, K.A.; Fredholm, B.B.; Nakamura, Y. Modulation of nerve and glial function by adenosine--role in the development of ischemic damage. *Int. J. Biochem.,* **1994**, *26(10-11)*, 1227-1236.

[110] Greene, R.W.; Haas, H.L. The electrophysiology of adenosine in the mammalian central nervous system. *Progr. Neurobiol.,* **1991**, 36, 329-341.

[111] Kauppinen, R.A.; Enkvist, K.; Holopainen, I.; Akerman, K.E. Glucose deprivation depolarizes plasma membrane of cultured astrocytes and collapses transmembrane potassium and glutamate gradients. *Neuroscience,* **1988**, *26(1)*, 283-289.

[112] Bhardwaj, A.; Northington, F.J.; Koehler, R.C.; Stiefel, T.; Hanley, D.F.; Traystman, R.J. Adenosine modulates N-methyl-D-aspartate-stimulated hippocampal nitric oxide production *in vivo*. *Stroke,* **1995**, *26(9)*, 1627-1633.

[113] Ribeiro, J.A. What can adenosine neuromodulation do for neuroprotection? *Curr. Drug Targets CNS Neurol. Disord.,* **2005**, *4(4)*, 325-329.

[114] von Lubitz, D.K.; Lin, R.C.; Popik, P.; Carter, M.F.; Jacobson, K.A. Adenosine A3 receptor stimulation and cerebral ischemia. *Eur. J. Pharmacol.,* **1994**, *263(1-2)*, 59-67.

[115] von Lubitz, D.K.; Dambrosia, J.M.; Kempski, O.; Redmond, D.J. Cyclohexyl adenosine protects against neuronal death following ischemia in the CA1 region of gerbil hippocampus. *Stroke,* **1988**, *19(9)*, 1133-1139.

[116] von Lubitz, D.K.; Marangos, P.J. Cerebral ischemia in gerbils: postischemic administration of cyclohexyl adenosine and 8-sulfophenyl-theophylline. *J. Mol. Neurosci.,* **1990**, *2(1)*, 53-59.

[117] Zhou, J.G.; Meno, J.R.; Hsu, S.S.; Winn, H.R. Effects of theophylline and cyclohexyladenosine on brain injury following normo- and hyperglycemic ischemia: a histopathologic study in the rat. *J. Cereb. Blood Flow Metab.,* **1994**, *14(1)*, 166-173.

[118] Evans, M.C.; Swan, J.H.; Meldrum, B.S. An adenosine analogue, 2-chloroadenosine, protects against long term development of ischaemic cell loss in the rat hippocampus. *Neurosci. Lett.,* **1987**, *83(3)*, 287-292.

[119] Rudolphi, K.A.; Schubert, P. Modulation of neuronal and glial cell function by adenosine and neuroprotection in vascular dementia. *Behav. Brain Res.,* **1997**, *83(1-2)*, 123-128.

[120] von Lubitz, D.K.J.E.; Paul, I.A.; Ji, X.-d.; Carter, M.; Jacobson, K.A. Chronic adenosine A_1 receptor agonist and antagonist: effect on receptor density and *N*-methyl-D-aspartate induced seizures in mice. *Eur. J. Pharmacol.,* **1994**, *253*, 95-99.

[121] Von Lubitz, D.K.; Beenhakker, M.; Lin, R.C.; Carter, M.F.; Paul, I.A.; Bischofberger, N.; Jacobson, K.A. Reduction of postischemic brain damage and memory deficits following treatment with the selective adenosine A1 receptor agonist. *Eur. J. Pharmacol.,* **1996**, *302(1-3)*, 43-48.

[122] White, W.B. Noctural dosing of a novel delivery system of verapamil for systemic hypertension. *Am. J. Cardiol.,* **1996**, *77(11)*, 1030.

[123] Sutherland, G.R.; Peeling, J.; Lesiuk, H.J.; Brownstone, R. M.; Rydzy, M.; Saunders, J.K.; Geiger, J.D. The effects of caffeine on ischemic neuronal injury as determined by magnetic resonance imaging and histopathology. *Neuroscience,* **1991**, *42(1)*, 171-182.

[124] Rudolphi, K.A.; Keil, M.; Fastbom, J.; Fredholm, B.B. Ischaemic damage in gerbil hippocampus is reduced following upregulation of adenosine (A1) receptors by caffeine treatment. *Neurosci. Lett.,* **1989**, *103(3)*, 275-280.

[125] Gao, Y.; Phillis, J.W. CGS 15943, an adenosine A2 receptor antagonist, reduces cerebral ischemic injury in the Mongolian gerbil. *Life Sci.,* **1994**, *55(3)*, PL61-5.

[126] Melani, A.; Pantoni, L.; Bordoni, F.; Gianfriddo, M.; Bianchi, L.; Vannucchi, M.G.; Bertorelli, R.; Monopoli, A.; Pedata, F. The selective A2A receptor antagonist SCH 58261 reduces striatal transmitter outflow, turning behavior and ischemic brain damage

induced by permanent focal ischemia in the rat. *Brain Res.,* **2003**, *959(2)*, 243-250.

[127] Monopoli, A.; Lozza, G.; Forlani, A.; Mattavelli, A.; Ongini, E. Blockade of adenosine A2A receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. *Neuroreport,* **1998**, *9(17)*, 3955-3959.

[128] Phillis, J.W. The effects of selective A1 and A2a adenosine receptor antagonists on cerebral ischemic injury in the gerbil. *Brain Res.,* **1995**, *705(1-2)*, 79-84.

[129] Higashi, H.; Meno, J.R.; Marwaha, A.S.; Winn, H.R. Hippocampal injury and neurobehavioral deficits following hyperglycemic cerebral ischemia: effect of theophylline and ZM 241385. *J. Neurosurg.,* **2002**, *96(1)*, 117-126.

[130] Bona, E.; Aden, U.; Gilland, E.; Fredholm, B.B.; Hagberg, H. Neonatal cerebral hypoxia-ischemia: the effect of adenosine receptor antagonists. *Neuropharmacology,* **1997**, *36(9)*, 1327-1338.

[131] de Mendonca, A.; Sebastiao, A.M.; Ribeiro, J.A. Adenosine: does it have a neuroprotective role after all? *Brain Res. Rev.,* **2000**, *33(2-3)*, 258-274.

[132] Ongini, E.; Adami, M.; Ferri, C.; Bertorelli, R. Adenosine A2A receptors and neuroprotection. *Ann. N.Y. Acad. Sci.,* **1997**, *825*, 30-48.

[133] Chen, Y.; Fenoglio, K.A.; Dube, C.M.; Grigoriadis, D.E.; Baram, T.Z. Cellular and molecular mechanisms of hippocampal activation by acute stress are age-dependent. *Mol. Psychiatry,* **2006**, *11(11)*, 992-1002.

[134] Jacobson, K.A. Adenosine A3 receptors: novel ligands and paradoxical effects. *Trends Pharmacol. Sci.,* **1998**, *19(5)*, 184-91.

[135] Phillis, J.W.; Walter, G.A.; Simpson, R.E. Brain adenosine and transmitter amino acid release from the ischemic rat cerebral cortex: effects of the adenosine deaminase inhibitor deoxycoformycin. *J. Neurochem.,* **1991**, *56*, 644-650.

[136] Miller, L.P.; Jelovich, L.A.; Yao, L.P.; Dare, J.; Ugarkar, B.; Foster, A.C. Pre-and peristroke treatment with the adenosine kinase inhibitor, 5'-deoxyiodotubercidin, significantly reduces infarct volume after temporary occlusion of the middle cerebral artery in rats. *Neurosci. Lett.,* **1996**, *220(2)*, 73-76.

[137] Tatlisumak, T.; Takano, K.; Carano, R.A.; Miller, L.P.; Foster, A.C.; Fisher, M. Delayed treatment with an adenosine kinase inhibitor, GP683, attenuates infarct size in rats with temporary middle cerebral artery occlusion. *Stroke,* **1998**, *29(9)*, 1952-8.

[138] Wiesner, J.B.; Ugarkar, B.G.; Castellino, A.J.; Barankiewicz, J.; Dumas, D.P.; Gruber, H.E.; Foster, A.C.; Erion, M.D. Adenosine kinase inhibitors as a novel approach to anticonvulsant therapy. *J. Pharmacol. Exp. Ther.,* **1999**, *289(3)*, 1669-1677.

[139] McGaraughty, S.; Cowart, M.; Jarvis, M.F. Recent developments in the discovery of novel adenosine kinase inhibitors: mechanism of action and therapeutic potential. *CNS Drug Rev.,* **2001**, *7(4)*, 415-432.

[140] Erion, M.D.; Wiesner, J.B.; Rosengren, S.; Ugarkar, B.G.; Boyer, S.H.; Tsuchiya, M. Therapeutic potential of adenosine kinase inhibitors as analgesic agents. *Drug Dev. Res.,* **2000**, *50*, S14-06.

[141] Jarvis, M.F.; Mikusa, J.; Chu, K.L.; Wismer, C.T.; Honore, P.; Kowaluk, E.A.; McGaraughty, S. Comparison of the ability of adenosine kinase inhibitors and adenosine receptor agonists to attenuate thermal hyperalgesia and reduce motor performance in rats. *Pharmacol. Biochem. Behav.,* **2002**, *73(3)*, 573-581.

[142] Rudolphi, K. A.; Schubert, P.; Parkinson, F. E.; Fredholm, B. B. Neuroprotective role of adenosine in cerebral ischemia. *Trends Pharmacol. Sci.,* **1992**, *13*, 439-445.

[143] Kitagawa, H.; Mori, A.; Shimada, J.; Mitsumoto, Y.; Kikuchi, T. Intracerebral adenosine infusion improves neurological outcome after transient focal ischemia in rats. *Neurol. Res.,* **2002**, *24(3)*, 317-323.

[144] Williams, M.; Jarvis, M. F. Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem. Pharmacol.,* **2000**, *59*(10), 1173-1185.

[145] Britton, D.R.; Mikusa, J.; Lee, C.H.; Jarvis, M.F.; Williams, M.; Kowaluk, E.A. Site and event specific increase of striatal adenosine release by adenosine kinase inhibition in rats. *Neurosci. Lett.,* **1999**, *266(2)*, 93-96.

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