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A_1 Adenosine Receptor Activation Modulates Central Nervous System Development and Repair

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Abstract Adenosine is an endogenous, autacoid purine nucleoside which performs many important biological roles, particularly during stressful events. Adenosine can signal through four adenosine receptor (AR) subtypes: A_1, A_{2A}, A_{2B}, and A_3. Of these, adenosine A_1 receptor (A_1AR) has a broad, wide distribution throughout different vertebrate cell types and the highest affinity to adenosine. The A_1AR-dependent action of adenosine is well documented in reports from numerous studies that have used different selective A_1AR agonists and antagonists as well as in animals that have a genetically manipulated A_1AR gene. Despite its wide distribution and function, A_1AR homo/hetero-oligomerization with other adenosine and non-adenosine receptors extends its biological role during developmental, physiological, and pathological situations. In this review, we initially discuss the A_1AR structure and most important signaling pathway triggered by its activation. Next, we summarize some of the most well-known biological effects of A_1AR in the central nervous system (CNS) during development and adulthood, in addition to its role in nervous system regeneration and repair.

Keywords Adenosine A_1 receptor · Receptor oligomerization · Neural development · Neural regeneration and repair · Multiple sclerosis

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Introduction

Adenosine is an endogenous, ubiquitous purine nucleoside which not only acts as a primary structural compound for a genetic material of the cells or a reserve source to provide energy in the form of ATP but also is an important signaling molecule that mediates a broad range of physiological effects. Under certain pathological conditions such as ischemia or hypoxia, adenosine produces a variety of responses known as the "signal of life" [1] or "body's natural defense" [2]. Primarily, adenosine is a metabolic by-product of ATP or its derivative (ADP, AMP, cAMP) in intra- or extracellular spaces. In addition, de novo adenosine production occurs via hydrolysis of *S*-adenosylhomocysteine [3, 4]. In addition, two main classes of nucleoside transporters mediate the influx or efflux of adenosine across the cell membrane [4].

Different physiological actions of adenosine are mediated by P1 receptors (or P1 purinoceptors) which are also known as adenosine receptors (ARs) (reviewed in [5]). The term adenosine receptor has been accepted by the International Union of Basic and Clinical Pharmacology (IUPHAR) [6]. Until now, there are four subtypes of AR cloned and described among vertebrates—the adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors [6]. Although adenosine is the full agonist of all four receptor subtypes [6], the adenosine A_1 receptor (A_1AR) has the highest affinity for adenosine [7]. Despite many similarities between ARs, they have distinct distribution, signal

transduction pathways, physiological effects, pharmacological properties, and therapeutic applications. Among these receptors, A_1AR has attracted a substantial interest because of its conserved expression in vertebrates, wide distribution throughout different cells, and higher affinity to the endogenous ligand adenosine. The concepts addressed in this paper include A_1AR structure, gene expression, and the most important findings that pertain to signaling through A_1AR function in central nervous system (CNS) development, regeneration, and repair.

A_1AR Structure and Its Expression Control

A_1AR is a 35–36-kDa glycoprotein [8, 9]; similar to other ARs, this glycoprotein belongs to class A of the G protein-coupled receptor (GPCR) superfamily [10]. In general, GPCRs share seven putative transmembrane α-helix structures connected by three extracellular and three intracellular loops. The extracellular domain, in particular the N-terminus half of these receptors, is involved in ligand binding. The cytoplasmic region that includes the C-terminus is responsible for the interaction with GTP-binding proteins in order to modulate other downstream effectors [10, 11]. A_1AR has a short N-terminus that lacks N-glycosylation sites. The A_1AR N-glycosylation acceptor site is located in the second extracellular loop [12].

Chimeric A_1/A_{2a} receptor experiments show that transmembrane 1–4 (TM1–4) domains of human A_1AR are involved in ligand binding. Although some authors believe that TM5–7 domains are important for ligand binding in humans and other species [13, 14], it has been reported that certain amino acid residues in these domains show a high degree of similarity between A_1AR and A_{2a}AR. Hence, they cannot distinguish between A_1AR- and A_{2a}AR-selective ligands. TM1–4 domains are also responsible for specificity of A_1-selective agonist and antagonist interactions [15].

The A_1AR gene is located on chromosome 22q11.2 [16]. A comparison study in rodents, bovines, dogs, and humans has shown an approximately 90% similarity in the coding region of the A_1AR gene [8, 17]. The results of genomic cloning of human A_1AR suggest that this gene contains six exons and five introns. The coding region encodes 326 amino acid residues of an integral protein. Expression of the A_1AR gene is complicated in which exons 1 and 2 are never expressed and an alternative splicing mechanism causes transcription of exon 3 or 4 along with exons 5 and 6 [8]. There are two different transcripts of the A_1AR gene: one that contains exons 3, 5, and 6 and the other includes exons 4, 5, and 6. Evidence has shown that different tissues contain both transcripts; however, A_1AR transcripts that contain exons 3, 5, and 6 are more prominent in tissues with high expression of this gene [8, 18]. Precise molecular studies show that human A_1AR gene expression is controlled by two distinct promoters, A and B. The distribution of transcriptional expression of these promoters is tissue specific. Expression of promoter A-mediated transcripts is limited to certain tissues, whereas the expression of transcripts mediated by promoter B is seen in all tissues that express A_1AR. The transcriptional activity of promoter A is much higher than that of promoter B [19]. The activity of these putative promoters is regulated by different factors; for example, dexamethasone drives promoter B expression in humans. This effect is highlighted more in tissues where promoter A is inactive [20]. The promoter region of A_1AR also includes consensus sequences for activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) transcriptional factors. AP-1 appears to mediate the basal level of A_1AR expression, but NF-κB promotes A_1AR expression following oxidative stress [21]. The expression levels of A_1AR in different tissues may change developmentally.

Signaling Through A_1AR Activation

Traditionally, A_1AR has been shown to couple downstream effectors via the Gi/Go group of G proteins and negatively regulate adenylyl cyclase activity. Of note, A_1AR is one of the GPCRs that can evoke other G proteins such as Gs and Gi, depending on the agonist structure. The level of [^{35}S]GTPγS binding to G proteins is immunoprecipitated with subtype-specific antibodies, and the level of effector activation has shown that small changes in agonist structures can alter the ability of A_1AR to activate multiple G proteins with diverse potency and efficacy due to different receptor conformations [22]. This is a clear form of allosteric modulation which is named "functional selectivity" or "biased signaling." Some authors also describe this as "agonist-dependent receptor signaling" [22].

Gi protein-dependent adenylyl cyclase inhibition is the most prominent signaling pathway which couples A_1AR with downstream effectors. However, A_1AR can also modify intracellular calcium $[Ca^{2+}]_i$ through coupling with Gi proteins. According to research, A_1AR activation in a hamster vas deferens smooth muscle-derived DDT_1MF-2 cell line is concentration dependent for both adenosine and its agonists, per se, or in synergism with ATP receptors. This can be coupled with phospholipase C (PLC) and can mobilize calcium from intracellular stores via enhancement of inositol 1,4,5-triphosphate (IP_3) formation [23]. Possibly, these interactions of A_1AR with ATP receptors can significantly increase and prolong the calcium response. The calcium response is pertussis toxin (PTX) sensitive, which can occur in the absence of extracellular calcium. A similar increase in $[Ca^{2+}]_i$ after agonist stimulation has been observed in CHO-K1 cells transfected with the human brain A_1AR sequence. The coupling of the receptor with phosphoinositide C directly or via βγ subunits

was suggested [24]. However, significant evidence indicates that a verity of plasma membrane Ca^{2+} influx channels can be activated in response to A_1AR stimulation and, in turn, lead to Ca^{2+} entry from the extracellular space [25]. In this situation, A_1AR activation often induces Ca^{2+} influx via a G protein-coupled PLC and DAG/protein kinase C (PKC) as downstream effectors [25]. These effectors are known as activators of voltage-dependent Ca^{2+} channels (VDCCs) that include L-type Ca^{2+} channels and voltage-independent Ca^{2+} channels such as receptor-operated channels (ROCs) in different cells [25, 26]. In contrast, A_1AR activation inhibits Ca^{2+} influx via VDCCs in neurons [27, 28]. Therefore, modulation of $[Ca^{2+}]_i$ can be an important mechanism that triggers downstream signaling pathway following A_1AR activation. A_1AR stimulation is thought to contribute to mitogen-activated protein kinase (MAPK) activity in Chinese ovary (CHO) cells independent of PKC [29]. This indicates that A_1AR has the capability to couple to more than one signal transducing pathway and, therefore, can influence different aspects of cell life.

Interaction Between A_1AR and Other Proteins

Interestingly, other protein interactions may bias A_1AR signal transduction and localization in cell membranes. Adenosine deaminase (ADA) is an intra/extracellular enzyme which catabolizes adenosine to inosine. Coexpression and functional interaction between A_1AR and ADA in the pig brain cortical membrane and DDT_1MF-2 cells has been reported [30, 31]. ADA increases the affinity of A_1AR to agonists and significantly augments the signaling via A_1AR [31]. There is concrete evidence that ADA increases A_1AR desensitization accompanied by significant disappearance of A_1AR from the cell surface [32, 33]. It has been considered that ADA expression on the cell surface not only acts as an ectoenzyme but also modulates A_1AR signaling and trafficking.

Heat-shock cognate (hsc) proteins, such as hsc73, act as chaperones, assist with correct folding of misfolding proteins, and play a central role during stressful conditions [34]. The physical association between the intracellular domain of A_1AR and that of hsc73 has been described in the cell body of rat cortical neurons, the rat cerebellum, and DDT_1MF-2 cells [35]. A_1AR interaction with ADA takes place outside of the cell membrane; however, hsc73 binding occurs inside the cell membrane. In contrast to ADA, significantly reduced ligand binding and agonist-mediated G protein coupling have been observed following cross-talk between A_1AR and hsc73. It was postulated that ADA brought A_1AR to a conformational structure more suitable for ligand binding. On the other hand, hsc73 might act in the opposite manner. However, in the presence of both proteins, the effect of ADA was shown to be stronger and could mask the effect of hsc73 [35].

The interaction between A_1AR and 4.1G, a member of erythrocyte membrane cytoskeleton, is another example. Information obtained from the yeast two-hybrid method has shown that the third intracellular loop of A_1AR binds to 4.1G. This interaction alters the localization of A_1AR and attenuates the influence of A_1AR on cAMP reduction. Also, this interaction mainly reduces the A_1AR-mediated increase in $[Ca^{2+}]_i$ [36].

Homo/Hetero-Oligomerization of A_1AR with Other Receptors

The ability to form complexes with other membrane or cytosolic proteins provides an opportunity for cells to expand the complexity of signal transduction from certain receptors such as A_1AR. For numerous years, researchers have believed that GPCRs exist and mediate their relevant physiological effects as monomer units. However, extensive evidence from pharmacological and biological experiments show that GPCRs have the capability to assemble and form dimers or oligomers of identical receptors (homo-oligomerization) or different receptors (hetero-oligomerization). It has been suggested that oligomer formation in GPCRs might influence cell surface expression, affinity, coupling properties, or a downstream signaling pathway and offer a type of allosteric regulation of these receptors (for review, see [37–39]). This novel view, therefore, established another level of complexity in A_1AR signaling, which was similar to other GPCRs. Table 1 summarizes the background literature on the existence and biological significance of homo/hetero-oligomer formation between A_1AR and other receptors. Taken together, these data show that cross-talk between A_1AR and other receptors by altering signal transduction probably results in important physiological consequences. These data also support its wide-range developmental role and pivotal effects on neural diseases and regeneration.

Numerous lines of evidence indicate that the exact molecular mechanism which underlies A_1AR signaling may differ according to cell type and effector system, the chemical properties of agonists and antagonists, presence of other cell membrane-binding proteins or receptors, and acute versus chronic stimulation. We present additional detailed evidence about A_1AR-dependent signal transduction pathway and downstream effectors in the CNS.

A_1AR in the CNS

This section briefly reviews information that pertains to the current knowledge of the roles of A_1AR in the CNS. Because of genetically manipulated experimental animals as well as selective agonists and antagonists, the roles of this receptor in the CNS have attracted much interest and are the focus of different investigations.

Table 1 Oligomerization between the A_1 adenosine receptor (A_1AR) and other purinergic or non-purinergic receptors

Homo/hetero-oligomer	Cell/tissue system		Method(s)	Effect(s)	Refs.
	In vitro	In vivo			
A_1AR-A_1AR	HEK cells	Bovine/pig brains	IP; WB; BRET; PLA; RBA	↑ A_1AR agonist binding affinity; A_1AR antagonists induced biphasic response	[40, 41]
A_1AR-$A_{2A}AR$	HEK cells	Rat striatal glutamatergic neuron	EM; IP; WB; RBA; BRET	↓ A_1AR agonist binding affinity; modulation of glutamate release	[42]
A_1AR-$P2Y_1R$	HEK cells	Different regions of the rat brain	EM; IP; WB; IF; RBA; BRET	↓ A_1AR-Gi/Go protein coupling potency; ↓ A_1AR-induced cAMP inhibition; ↑ $P2Y_1R$-Gi/Go protein coupling via A_1AR; ↑ $P2Y_1R$-induced cAMP inhibition; modulation of glutamate release	[43–46]
A_1AR-$P2Y_2R$	HEK cells	Not determined	EM; IP; WB; RBA; IF	↓ A_1AR agonist binding affinity; ↓ A_1AR-induced cAMP inhibition; ↑ $P2Y_2R$-induced Ca^{2+} signaling	[47]
A_1AR-β_1AR	HEK cells	Adult human heart	IP; WB, IF; RBA	↑ β_1R agonist binding affinity; no change in β_1R-induced cAMP production; ↓ A_1AR-induced cAMP inhibition	[48]
A_1AR-β_2AR	HEK cells	Adult human heart	IP; WB, IF; RBA	↑ β_2R agonist binding affinity; ↓ β_2R-induced cAMP production; ↑ A_1AR coupling to ERK signaling; ↓ A_1AR-induced cAMP inhibition	[48]
A_1AR-$mGlu_{1\alpha}R$	HEK cells; Neura 2a cells; mouse Purkinje cells	Rat cerebellum	IP; WB; IF; RBA; FRET	Synergism in Ca^{2+} mobilization; ↑ neuroprotection; ↓ $mGlu_{1\alpha}R$ ligand sensitivity; ↓ $mGlu_{1\alpha}R$-mediated LTD; ↓ A_1AR-induced cAMP inhibition	[49–51]
A_1AR-D_1R	Mouse fibroblast Ltk^- cells; rat cortical neuron	Rat nucleus accumbens	IP; IF; RBA	↓ D_1R binding affinity; ↓ D_1R-induced cAMP production	[52–55]

$A_{2A}AR$ adenosine A_{2A} receptor, *$P2Y_1R$* $P2Y_1$ receptor, *$P2Y_2R$* $P2Y_2$ receptor, *$\beta1AR$* β1 adrenergic receptor, *$\beta2AR$* β2 adrenergic receptor, *$mGlu_{1\alpha}R$* metabotropic glutamate type 1α receptor, *D_1R* dopamine type 1 receptor, *HEK* human embryonic kidney, *IP* immunoprecipitation, *WB* Western blot, *BRET* bioluminescence resonance energy transfer, *PLA* proximity ligation assay, *RBA* radiobinding assay, *IF* immunofluorescence, *EM* electron microscopy, *FRET* Förster resonance energy transfer

Distribution of A_1AR in the CNS

The distribution pattern of A_1AR in the CNS shows that A_1AR is widely expressed throughout the CNS, particularly the brain, where it controls a variety of functions. The distribution and density of A_1AR based on positron emission tomography (PET) has been studied by the use of different radioligands in humans. These studies clearly localize a high density of this receptor in the putamen and thalamus, intermediate density in most cortical regions, and low density in the midbrain, brain stem, and cerebellum [56–58]. Studies show dissimilar distribution of A_1AR among different cortical regions [57–59]. The presence of A_1AR has been studied in the human hippocampus, too [59]. In situ and in vitro cellular localization studies show that neurons, astrocytes, oligodendrocytes, and microglia are equipped with A_1AR. However, its distribution is not homogenous in different types of neurons and/or other cell types of the CNS. The subcellular localization of A_1AR on neurons has been defined by radioligand autoradiography studies or receptor binding and Western blot analysis followed by cell fractioning experiments, particularly in the rat hippocampus. Research shows that A_1ARs are expressed on axons [60]; however, most direct observations clearly demonstrate high concentrations of this receptor in the plasma membrane of nerve terminals, mainly in the presynaptic components of the active zone and postsynaptic density. A_1AR is also found in the extrasynaptic part of nerve terminals [61, 62].

Neuromodulatory and Neuroprotective Effects of A_1AR Signaling

Previously, researchers have stated that adenosine had critical neuromodulatory and neuroprotective effects. However, these effects are mainly mediated by A_1AR occupancy and activation of downstream intracellular pathways in the CNS with possible involvement by $A_{2A}AR$ and other ARs [42]. Data suggest that A_1AR signaling plays potent inhibitory roles in synaptic transmission. Evidence to confirm this phenomenon has been obtained from pharmacological assays that used selective A_1AR agonists and antagonists along with genetically manipulated animal approaches. Evidence exists about how adenosine, through A_1AR stimulation, regulates a variety of different effector systems to play a role as a modulator. (i) A_1AR activation leads to the opening of different K^+ channels in the presynaptic or postsynaptic plasma membrane, outward

potassium current, and hyperpolarization of different neurons in various brain regions. In this way, A_1AR reduces neurotransmitter release or decreases neuronal excitability. The coupling of A_1AR to ATP-sensitive K^+ (K_{ATP}) channels [63], G protein-dependent inwardly rectifying potassium (GIRK) channels [64, 65], and small-conductance Ca^{2+}-activated K^+ (SK) channels [65] has been demonstrated in hippocampus neurons [63], stellate neurons of the entorhinal cortex [28], and retinal ganglion cells [65]. (ii) Ca^{2+} influx from the extracellular space into the presynaptic nerve terminals presumably plays an important role in spontaneous quantal release or action potential-dependent release of various neurotransmitters. A_1AR activation may inhibit Ca^{2+} influx through VDCCs in the presynaptic membrane [27, 28] and cause suppression of neurotransmitter release. (iii) Modulation of the synaptic secretory apparatus is another probable mechanism; however, no evidence currently exists. The release of different neurotransmitters such as glutamate [28, 66], GABA [27, 28, 67], serotonin [68], histamine [69], and dopamine [70, 71] may be inhibited by one or combination of these possible mechanisms. As previously mentioned, the analysis gives evidence about the coexistence of heteromeric complexes of A_1AR with different proteins or other receptors. In these conditions, it seems likely that the existence of an A_1AR partner can modify the functional consequences of A_1AR activation including effects on neurotransmitter release [42].

The Effects of A_1AR Signaling on Sleep

Adenosine levels increase during wakefulness as a result of ATP metabolism; hence, adenosine is considered a hypnotic factor [72]. Evidence suggests that adenosine acts through A_1AR on different groups of neurons in various parts of the brain to regulate homeostatic sleep. In vivo studies have shown that deletion of A_1AR messenger RNA (mRNA) by an A_1AR antisense oligonucleotide in a rat cholinergic zone of the basal forebrain resulted in reduced non-rapid eye movement sleep and increased wakefulness [73]. A PET study showed that A_1AR levels in humans exposed to 24 h of sleep deprivation increased in the whole brain [74]. A_1AR signaling led to inhibition of discharge activity and generation of action potential or attenuated excitatory synaptic transmission in a subset of neurons that promote wakefulness. This mechanism has been found in cholinergic and some non-cholinergic neurons in the forebrain [75] considered responsible for cortical activity and in arousal, for promotion of hypocretin/ orexin neurons from the lateral hypothalamus [76]. A_1AR stimulation also inhibits histaminergic neurons in the tuberomammillary nucleus and promotes non-REM sleep [69]. Histaminergic neurons are involved in the arousal effects of histamine through H_1 receptors [77]. Possibly the abovementioned mechanisms contribute to inhibition of these neurons via A_1AR activation. On the other hand, it has been shown that administration of an A_1AR agonist into the lateral preoptic area induces waking [78]. Therefore A_1AR fulfills the criteria as a mediator of hypnogenic effects of adenosine in the brain. However, some authors state that the exact effects of A_1AR may be region dependent [69].

The Effects of A_1AR Signaling on Glial Cells

Glial cells are other CNS-resident cells that express A_1AR [79, 80]. However, there are few reports about the role of A_1AR signaling in regulating different functions of these cells in normal physiological or pathophysiological conditions. It has been shown that activation of A_1AR in cortical astrocytes downregulates sustained $[Ca^{2+}]_i$ response elicited by stimulation of P_2 purinoceptors, apparently through inhibition of adenylyl cyclase [80]. A_1AR activation also mediates reduction in Ca^{2+} mobilization induced by ATP into microglial cells [79]. This phenomenon may have significant physiological meaning considering the importance of Ca^{2+} signaling in different cellular functions. Inhibition of the hyperactive state of microglia under pathological conditions may explain AR-mediated neuroprotection. Oligodendrocytes display a high level of A_1AR expression, but the exact biological role of this receptor in oligodendrocytes remains to be described [81]. The role of this receptor in oligodendrocytes and other members of oligodendrocyte lineage cell development has been studied to a certain extent.

The Biological Roles of A_1AR Activation During Nervous System Development

An expanding body of data shows that adenosine through A_1AR exerts a potent biological role during prenatal development. These data have attracted profound interest because coffee, an ARs antagonist, is globally the most widely consumed beverage, even among pregnant women. Average caffeine consumption of more than 100 mg/day is associated with an increased risk of fetal growth restriction and reduction in birth weight [82]. On the other hand, A_1AR, among other known GPCRs, expresses early during embryogenesis and is involved in the development of the CNS and heart. The brain and heart are two main sites of A_1AR expression during embryogenesis. Here, we provide insights into the role of A_1AR expression and its activity during nervous system development.

Additional information about A_1AR expression has been obtained from studies of the fetal life of rats [83]. This study reported the presence of *A_1AR* mRNA in neural tissue as early as gestation day 11 according to in situ hybridization and a radioligand receptor assay. However, receptor-labeling autoradiography detected A_1AR protein levels in neural tissue no sooner than gestation day 14; after which, A_1AR expression

and concentration increased in the CNS with further development [83]. Several researchers believe that A_1ARs which exist in the brains of immature animals are not able to fully couple to G proteins. The [^{35}S]GTPγS binding assay indicated a developmental delay in A_1AR signaling in the brains of neonate rats. This delay might be due to poor A_1AR expression or coupling to G proteins in younger animals [84]. Therefore, another pronounced concern about A_1AR activity is the developmental changes in its expression and signaling.

It is important to note that functional A_1ARs also express on different neural lineage cells. RT-PCR analysis of oligodendrocyte progenitor cells (OPCs) shows that both embryonic and postnatal OPCs of mice express A_1AR [85]. Functional protein expression of A_1AR at different developmental stages of oligodendrocytes, from OPCs to mature oligodendrocytes, has been detected by immunocytochemistry and radioreceptor assays. Mature oligodendrocytes reportedly express higher levels of A_1AR compared to OPCs [81]. Expression of A_1AR in human embryonic stem cell-derived OPCs has been shown in the authors' laboratory (in press).

A_1AR expression is simultaneous with the time in which neural formation, migration, and differentiation are very active and local adenosine changes with metabolic states of cells or environmental conditions. A potential role is played by A_1AR signaling during the development of the nervous system. A_1AR signaling appears to inhibit neurite outgrowth by activation of Rho A kinase in PC12 cells that express A_1AR and a primary culture of cortical and hippocampal neurons [86]. Previously, it has been reported that small GTPase such as Rho A is involved in reorganization of the cytoskeleton and induces axon retraction through activation of Rho kinases [87, 88]. Converging lines of evidence therefore provide support for the concept that A_1AR activity may influence neuronal differentiation by modulating neurite outgrowth.

Axon navigation to appropriate targets is also a critical stage in nervous system development in which a precise topographic pattern of neuron connections is formed. In this process, navigating neurons send out axons to reach the correct target. Axon guidance responds to a balance of molecular cues which attract them to a special target and repel them from inappropriate targets [89]. Engrailed homeoproteins, which are transcriptional and translational regulators, participate in axonal guidance [90, 91]. According to research, these proteins sensitize temporal growth cones of retinal ganglionic cells (RGCs) to a low concentration of ephrin A5 and induce temporal RGC collapse in the anterior portion of the optic tectum [92]. More importantly, sensitization has been reported to occur through A_1AR signaling during chick development. According to this observation, engrailed induces ATP synthesis and release from growth cones. Released ATP is subsequently degraded to adenosine which, in turn, stimulates A_1AR and enhances ephrin A5 activity. Temporal RGCs have higher concentrations of A_1AR than nasal ganglionic cells; hence, it is not surprising that engrailed is differentially involved in axon guidance activity of RGCs [92].

A study of other aspects of nervous system formation shows that A_1AR stimulation can effectively increase OPC migration, with no effect on OPC viability, proliferation, or differentiation. This study suggests that A_1AR signaling reduces cAMP accumulation in oligodendrocytes [81]; however, there is no data about the relationship between cAMP reduction and OPC migration. OPC migration is more prominent during white matter formation and after neural tissue injury [93]. A_1AR agonists may have a promising role in nervous system regeneration. However, in vivo evidence to ascertain the contribution of A_1AR on OPCs remains to be elucidated.

A literature survey revealed that exaggerated A_1AR activation exerted adverse effects on the developing brain rather than the protective roles observed in adults. A_1AR may have a role in the pathogenesis of some developmental brain injuries even in postnatal life. This is the time when the brain is yet vulnerable to hypoxia-ischemia conditions and brain development is not completed. According to research, A_1AR stimulation could not prevent ischemic brain damage in 7-day-old rats. It was suggested that A_1AR, compared to periphery, is poorly developed in the brain at this stage and this phenomenon hampers the ability of A_1AR to exert functional responses [84]. On the other hand, an A_1AR-mediated ventriculomegaly (expansion of the brain ventricle) has been shown in early postnatal life of mice exposed to hypoxia from P3 to P14. Mice with loss of A_1AR expression attenuated ventriculomegaly and retained myelin basic protein (MBP) expression and white matter although they were kept in hypoxic conditions [94]. Ventricle enlargement can be caused by overproduction of cerebrospinal fluid (CSF). In the choroid plexus, the main site of CSF production, Na^+-K^+ ATPase pump activity is closely associated with CSF secretion [95]. Increased expression of Na^+-K^+ ATPase has been observed in A_1AR overexpression in transgenic mice [96] and in the choroid plexus of rats treated by an A_1AR agonist for 2 weeks or long-term exposure to caffeine [97]. Therefore, it is thought that increased expression of Na^+-K^+ ATPase and overproduction of CSF are a possible underlying mechanism on the effect of A_1AR stimulation on ventriculomegaly [97]. The effects of A_1AR signaling on Na^+-K^+ ATPase expression during earlier developmental time points remain to be clarified. Instead, in perinatal mice exposed to hypoxia, ventriculomegaly is secondary to a reduction in the preoligodendrocyte process arborization and development, which, in turn, affects white matter formation. It is suggested that abnormal oligodendrocyte development may have been a consequence of A_1AR activation since caffeine can prevent ventriculomegaly and hypomyelination [98]. Such studies have also confirmed the potential role of A_1AR action to mediate the immediate adverse effects of hypoxia-ischemia on the developing brain.

Additional, direct evidence by using selective A_1AR agonists is needed. Chronic administration of caffeine to pregnant rats has been reported to cause a significant decrease in the total number of A_1ARs in the fetus as well as the maternal brain [87].

In the past decade, in vitro and in vivo studies on rodent models have shown that the developing nervous system is also sensitive to the harmful effects of metabolic disorders such as hypoglycemia. Administration of A_1AR antagonists or A_1AR ablation leads to protection against hypoglycemia [99]. This data provides a link between the prominent roles of A_1AR in signal transduction of the consequences of environmental changes such as metabolic disorders to nervous system development.

In summary, it seems signaling through A_1AR has a potentially dual feature during nervous system development. Although we know more about the long-lasting trophic effects of A_1AR activation during this process, data about the effects of A_1AR signaling on correct formation of the nervous system is limited. Considering today's knowledge, pharmacological inhibition of the central A_1AR may have beneficial effects against some environmental disturbances that occur during nervous system development [98].

A_1AR Signaling Might Be Effective in Adult Nervous System Repair

It has been clearly shown that adenosine through A_1AR stimulation can attenuate spontaneous or evoked release of neurotransmitter release [28, 66] and/or fine-tune regulation of neuronal activity, in basal physiological conditions [28, 65, 100]. These neuromodulatory effects may play a role in neuroprotection and have a physiological implication which is critically highlighted in stressful situations.

A_1AR Activation Effects on Trauma and Seizure

Synaptic transmission or neuronal firing rate is suppressed by A_1AR during ischemia and trauma [101] or metabolic disorders [63], thereby preventing excessive neurotoxicity [27]. These conditions are accompanied by elevated concentrations of extracellular adenosine [102]. Central activation of A_1AR suppresses seizures and exerts antiepileptic effects. Many findings support the observation that this finding is related to the neuroprotective effects of adenosine acting at A_1AR [103–106]. It has been reported that the presence of some special single nucleotide polymorphism (SNP) in the A_1AR human gene is accompanied by more severe posttraumatic seizures after a traumatic brain injury (TBI) [107]. This report provides more evidence for the importance of A_1AR signaling in reducing the adverse effects of TBI.

A_1AR Activation Effects on Multiple Sclerosis

Of note, A_1AR signaling plays a prominent neuroprotective role in chronic neurodegenerative disorders such as multiple sclerosis (MS). A_1AR-induced neuroprotective responses in this situation, however, are supported by several in vivo studies on A_1AR knockdown animals or pharmacological drugs [108, 109]. Lack of A_1AR in a mice model of MS has resulted in increased demyelination and axonal injury, whereas A_1AR activation maintained myelin sheath integrity and improved neurobehavioral responses in $A_1AR^{+/+}$ animals [108]. Reduced demyelination and significantly enhanced remyelination after treatment with an A_1AR agonist were reported in another lysophosphatidylcholine-induced demyelination rat model [109]. Researchers observed downregulation of A_1AR expression on blood-derived monocytic cells and microglia in the brains of MS patients [110, 111] as well as in the spinal cords of an experimental autoimmune encephalomyelitis (EAE) model of MS in mice [108]. Interestingly, double application of an A_1AR agonist with a common AR antagonist such as caffeine has potentiated the ability of A_1AR to ameliorate the severity of MS symptoms, which was most likely due to enhanced A_1AR gene expression [108]. Upregulation of A_1AR transcription has been observed in a guinea pig spinal cord homogenate-induced EAE model in rats after chronic caffeine application, which was accompanied with significant reduction in EAE symptoms [112]. In agreement with these results, a case-control study on the relationship between the type of routine daily diet and MS risk in 75 case and 75 matched control women indicated a reverse association between coffee consumption and the risk of MS [113]. Recently, a large-scale retrospective case-control study of 2779 cases and 3960 matched controls further strengthened the advantage of drinking coffee in MS. These results indicated consumption of high amounts of coffee significantly reduced the risk of MS [114]. However, in these studies, there was no data about the exact factor in coffee that determined the benefits of this beverage or probable mechanisms. Based on previous studies on experimental animals, the authors in the aforementioned study postulated that caffeine-dependent A_1AR mRNA upregulation and/or consequence effects of A_1AR activation on the immune system were the most probable mechanisms for these observations [114]. Glucocorticoids such as dexamethasone, which is used for immunomodulatory therapy in MS patients, upregulate A_1AR gene expression in the brain tissue [20, 115]. These observations may raise the usefulness of caffeine consumption or some other routine medications to treat MS symptoms through alterations in A1AR signaling.

A_1AR activation may exert beneficial effects on MS through triggering the following mechanisms. (i) It has been suggested that A_1AR inhibition is accompanied by increased macrophage/microglia activation which enhances expression

and release of pro-inflammatory cytokine including interleukin-1β (IL-1β) and matrix metalloproteinase-12 (MMP-12). Such cytokines induce demyelination and axonal injury upon oligodendrocyte degeneration. A_1AR signaling activation attenuates pro-inflammatory responses and maintains myelin sheath integrity by augmenting anti-inflammatory responses [108]. An obvious shift from Th1 to Th2 immune response has been shown in a model of rat EAE which is possibly related to A_1AR activation [112]. (ii) Neural stem cells (NSCs) also express A_1AR. Activation of this receptor induces proliferation of NSCs through direct activation of ERK/MEK and Akt signaling pathways [116]. NSCs constitute a well-defined self-renewal multipotent cell population in neurogenic niches that have the capability to differentiate to all types of CNS cells. These cells participate in normal CNS development and are responsible for neurogenesis during adulthood or improved recovery after diseases or insults (reviewed in [117, 118]). They can also produce oligodendrocyte progenitors and participate in remyelination. Therefore, it has been suggested that an enhanced level of adenosine during CNS injury stimulates neuroregeneration through A_1AR-induced NSC proliferation in a paracrine/autocrine manner, although there is no direct evidence for this assumption [116]. (iii) A_1AR agonists stimulate OPC migration [81]. OPC recruitment to damaged white matter area as well as their differentiation is frequently severely inhibited in MS disease that leads to reduced remyelination [119]. (iv) It has been suggested that A_1AR activation may promote OPC differentiation. Recently, it was reported that sustained, outward rectifier I_k current increased after in vitro A_1AR stimulation in OPCs (unpublished data in [120]). On the other hand, it was demonstrated that modulation of the I_k current had a direct association with oligodendrocyte development, in which inhibition of I_k prevented OPC differentiation and vice versa [120–122]. Direct evidence is needed for clarification. Figure 1 summarizes the most important proposed mechanisms of the beneficial effects of A_1AR stimulation on MS.

A_1AR Activation Effects on Glioblastoma

Recently, researchers reported that A_1AR signaling was implicated in cancer stem cells (CSCs) derived from glioblastoma multiforme, the most malignant brain tumor. A_1AR stimulation significantly inhibited CSC proliferation. The A_1AR agonist initially affected CSC fate because of reduction in their stemness as well as increased differentiation. Continued treatment with an A_1AR agonist could promote cell apoptosis in differentiated cells. A_1AR stimulation also has been shown to sensitize CSCs to chemotherapy. According these observations, it was suggested that sequential application of an A_1AR agonist with chemotherapy might more efficiently inhibit tumor growth [123].

A_1AR Activation Effects on Pain

A_1AR stimulation reduces pain, another interesting finding which highlights the potential role of this receptor at both the central and peripheral nervous system levels. Several studies that have researched selective A_1AR agonists and antagonists report that activation of A_1AR is responsible for the antinociceptive effects of adenosine in neuropathic [124, 125] or inflammatory [126–128] models of pain. Increased hyperalgesia has been also reported in mice that lack A_1AR [129, 130]. However, the exact molecular

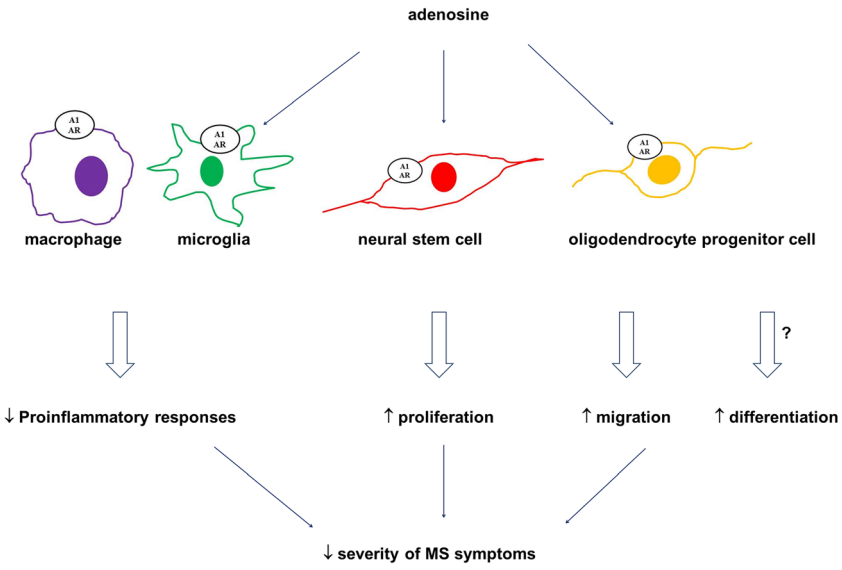

Fig. 1 Schematic diagram illustrates the main theories about the advantages of A_1AR activation on ameliorating MS symptoms

mechanisms triggered by A_1AR activation have not been fully elucidated. The analgesic effects of A_1AR stimulation reportedly depend on prevention of nociceptive neuron sensitization. This conclusion has been drawn from the following observations. (i) A_1AR activation led to activation of the nitric oxide/cGMP/protein kinase G/K_{ATP} channel signaling pathway [131]. It was demonstrated that activation of this signaling pathway by enhancing K^+ currents from K_{ATP} channels led to hyperpolarization of nociceptive neurons [132], which directly reduced neuronal sensitization. (ii) The analgesic effect of A_1AR activation, at least on inflammatory pain, has been shown to depend on inhibition of the hyperactive state of microglia by suppressing Ca^{2+} entry into these cells. Non-active microglia has a decreased ability to facilitate nociceptive neuron firing [79, 125]. Thereby, A_1AR activation can also indirectly block nociceptive neurons. Attempts to produce antinociceptive drugs based on this physiological effect of A_1AR are underway [133].

Conclusion

A_1AR is one of the four subtypes of the AR. It represents the common features of G protein-coupled receptors and usually directs signal transduction through the Gi/Go protein to different downstream effectors. Numerous evidences show that signaling through A_1AR plays a prominent role in mediating the potent effects of adenosine in neuromodulation. Because A_1AR has the highest affinity to adenosine, it is fully stimulated by a slight increase in adenosine levels. A_1AR particularly exerts its critical neuroprotective roles in stressful events that are more emphasized in adults. A_1AR is the earliest, dominant AR subtype expressed in embryos that has a significant involvement in nervous system development. Partial agonists and allosteric ligands of A_1AR will be promising to increase beneficial effects and simultaneously avoid undesirable effects of adenosine [133, 134]. The modulatory effect of A_1AR on neuroinflammation and neuroprotection, neural development and axogenesis, oligodendrocyte progenitor migration, as well as NSC-mediated repair are possible targets of this group of AR for therapeutic purposes.

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