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Review

Adenosine Type A_{2A} Receptor in Peripheral Cell from Patients with Alzheimer's Disease, Vascular Dementia, and Idiopathic Normal Pressure Hydrocephalus: A New/Old Potential Target

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Abstract. As the European population gets older, the incidence of neurological disorders increases with significant impact on social costs. Despite differences in disease etiology, several brain disorders in the elderly (e.g., Alzheimer's disease, vascular dementia, normal pressure hydrocephalus) share dementia as a common clinical feature. The current treatment for the majority of these diseases is merely symptomatic and does not modify the course of the illness. Symptoms of normal pressure hydrocephalus are the only ones that can be modified if they are recognized in time and treated appropriately. Therefore, an important clinical strategy may be disclosed by pathogenic pathways that can be modified and to find drugs that can slow down or even arrest disease progression. Possibly a way to answer this question could be by re-examining all the molecules which have so far succeeded in improving many aspects of cognitive deterioration in some neurodegenerative conditions, that were not considered because of controversial opinions. The main purpose of this summary is to further substantiate the hypothesis that the pathway of adenosine type A_{2A} receptor could be used as a potential target to develop new/old therapeutic strategies.

Keywords: Adenosine, adenosine receptors, elderly, neurodegeneration

Up to one billion people worldwide suffer from brain diseases, which include neurological disorders. In Europe, brain disease represents 35% of all diseases affecting 179 million patients and costing more than 800 billion euro per annum, which is more

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cardiovascular disease and cancer combined [1]. As the European population gets older, the incidence of neurological disorders increases creating an enormous problem for social costs. Despite differences in disease etiology, several brain disorders in the elderly [e.g., Alzheimer's disease (AD), vascular dementia (VaD), normal pressure hydrocephalus (NPH)] share dementia as common clinical symptom [2]. This leads us to believe that there could be a common pathological pathway in different brain diseases.

The current treatment for the majority of these diseases is merely symptomatic and does not modify the course of the illness. NPH symptoms are the only ones that we can modify if they are recognized in time and treated appropriately [3].

Therefore, it is crucial to find pathogenic pathways that can be modified and to be able to test drugs that can slow down or even arrest disease progression.

Possibly a way to answer this question could be by re-examining all the molecules which have so far succeeded in improving many aspects of cognitive deterioration in some neuropathological conditions that were not considered because of controversial opinions.

ADENOSINE

Many epidemiological studies showed that the usual consumption of moderate quantities of caffeine produces long-lasting benefits to memory function in healthy brains [4]. Such benefits include the reduction of both memory decline caused by aging and the risk of developing dementia and AD, suggesting a potential therapeutic use of caffeine. But where do the beneficial effects of caffeine come from?

Currently, the beneficial effects triggered on the brain by methylxanthine caffeine (1,3,7-trimethylxanthine) seems to be related to structural similarities between the compound itself and an endogenously produced molecule known as adenosine.

ATP (adenosine triphosphate) is not only the principal and universal cellular energetic compound, but it can also be released into the extracellular medium where it acts as a signaling molecule [5]. Almost every synaptic and secretory vesicle contains ATP, which can be stored with other classic neurotransmitters such as GABA or glutamate, or alone in ATP-only vesicles. ATP levels are usually very low in extracellular medium, but they rapidly increase during pathological conditions such as inflammation or cell death. ATP can act as either sole transmitter or as co-transmitter.

ATP can be released from neurons and glial cells in an uncontrolled manner or via vesicular release. After its release, ATP is rapidly degraded into adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), and adenosine (Ado) [5, 6].

ADENOSINE RECEPTORS

The physiological responses to Ado take place through the binding and the activation of one or more of the trans-membrane high-affinity A_1 (A_1R) or A_{2A} ($A_{2A}R$), low-affinity A_{2B}, or low-abundance A_3 receptors [7].

These G-protein coupled receptors regulate the second messenger cAMP in opposite directions; while A_1 and A_3 receptors are inhibitory G_i-coupled, A_{2A} and A_{2B} receptors are excitatory G_s-coupled, thereby decreasing and increasing cAMP levels, respectively [8, 9]. The activation of these receptors can also modulate Ca^{2+} channels and the phospholipase C pathway.

Through these actions and by modulating both the release and the uptake of different neurotransmitters, the balance between the activation of adenosine A_1R and $A_{2A}R$ allows the fine-tuning of synaptic transmission and plasticity in the hippocampus [9].

In particular, we can find $A_{2A}R$ in a wide variety of tissues, including the nervous system and the peripheral immune system, where they are expressed at different levels: from significantly high levels in neurons and peripheral cells (lymphocytes and neutrophils) to lower levels in glial cells [7].

The different levels of expression of $A_{2A}R$ in different tissues are consistent with the sophisticated, multifaceted neurochemical, and molecular effects of the Ado system. On the basis of *in vitro* [10, 11] and *in vivo* [12] studies, it has become clear that $A_{2A}R$, through complex mechanisms which are still poorly understood [13–15], plays a critical role in the modulation of inflammatory reactions, influencing functional outcome in a wide spectrum of pathologies including brain diseases [16, 17].

Considering data of gene expression and receptor densities obtained by our group [18–20], the main purpose of this summary is to further substantiate the hypothesis that the pathway of $A_{2A}R$ could be used to help stratify elderly patients and as a potential target to develop new/old therapeutic strategies.

A_{2A}R in brain diseases

A_{2A}R could play a key role in different pathological conditions; in particular we decided to focus on AD, VaD, and idiopathic NPH (iNPH) because these are the most frequently encountered in our clinical practice.

a) Alzheimer's disease

Alzheimer's disease (AD) is the most common age related progressive neurodegenerative disorder and the primary cause of dementia in the elderly [21].

The characteristic clinical presentation of AD is a progressive loss of memory and specific cognitive function, ultimately leading to the loss of independence and death. The hallmark neuropathological changes in AD are neuritic plaques (amyloid-β (Aβ) deposition), neurofibrillary tangles (tauopathy), and neuronal loss most prominent in specific temporal, parietal, and frontal regions of the brain. Numerous studies support the hypothesis that AD pathology is more complex than Aβ and tau accumulation, indicating the involvement of inflammation [22, 23], prionopathy [24], oxidative stress [25], and metabolic abnormalities [26, 27] in the brain.

The blockade of adenosine A_{2A}R affords neuroprotection against chronic noxious brain insults [28].

It was also recently shown that A_{2A} receptor antagonists can prevent memory impairment in animal models of aging [29] and AD [30, 31].

Indeed, several longitudinal studies support the inverse relationship between caffeine consumption and both decreased memory impairment associated with aging [32] as well as reduced risk of developing AD [33, 34] and generally dementia [35], showing also an improvement in psychomotor speed and verbal memory performance in non demented elderly population [36, 37], less decline in verbal retrieval and visuospatial memory [38], and less neuropathological lesions at death [39].

Interestingly subjects with plasma caffeine level greater than 1200 ng/ml at onset were associated with stable MCI and no conversion to dementia during the 2–4 years follow-up examination [35].

Several studies suggest that adenosine receptors change their pattern of localization and density in affected brain regions. Postmortem analyses of the frontal cortex of AD patients showed that the total number and levels of A_{2A}R, but also A_1R, are significantly increased in either early or advanced stages of the disease.

In particular, the blockade of adenosine A_{2A}R receptors, which have a synaptic localization in the hippocampus [40], prevents Aβ induced amnesia, as well as A_{2A}R antagonists prevent Aβ-induced toxicity in cultured neurons [41]. In addition, oral administration of a selective A_{2A}R antagonist improves spatial memory and reduces tau hyperphosphorylation in tau mice. These findings support the concept of direct effects of A_{2A}R on neurons to control their susceptibility to neurotoxic stimuli.

Alternatively, A_{2A}R might control the apoptotic machinery in neurons and other types of cells in the brain, in a manner similar to the control by A_{2A}R of apoptosis in PC12 cells or in neutrophils [28].

Of interest, there are currently five concurring hypothesis to explain the robust neuroprotective effects afforded by A_{2A}R in noxious brain conditions in adult animals: (1) presynaptic control of glutamate release; (2) control of astroglyosis and of glutamate uptake and release by astrocytes; (3) direct control of neuronal viability by interference with pathways of cell death; (4) control of microglia reactivity; (5) control of the reactivity of infiltrating lymphoid cells [28].

It is now well established that especially during the earliest phases of AD, inflammation is a predominant event, and that activation of the adenosine system through A_{2A}R agonism can lead to the downregulation of the inflammatory response [42] as well as the prevention of Aβ-induced synaptotoxicity by promoting the release of interleukin-10 (IL-10), the major anti-inflammatory cytokine, by resident cells. Another critical aspect pointing to the use of adenosine receptor agonists is that AD patients show impaired signaling by the neurotrophin molecule brain derived neutrophic factor (BDNF), and that A_{2A}R activation is critical for both BDNF-dependent and independent hippocampal synaptic transmission, plasticity, and long term potentiation. Based on the "bidirectional effect" of A_{2A}R activation and inhibition proposed by Dai and Zhou [43], different stages of the pathological process as well as the route of administration may significantly impact the efficacy of treatment with either agonists or antagonists for adenosine receptors. The apparently paradoxical use of two oppositely acting ligands to treat the same neurodegenerative condition suggests that factors such as dosage, drug delivery method, state of disease progression, and extracellular concentrations of potential excitotoxic transmitters might determine similar cellular responses to opposite pharmacological treatments. More specifically, it seems that the

protection afforded by $A_{2A}R$ agonists against AD is transient but effective during the earliest phases of the disease, and it is mainly achieved through a stimulatory effect on the release and production of anti-inflammatory cytokines by resident glial and peripheral immune cells. Conversely, both prophylactic and long-term neuroprotective effects of caffeine and/or $A_{2A}R$ antagonists are for the most attributable to inhibition of reactive oxygen species activity, tau pathology and Aβ production by neuronal cells [44].

Moreover, the role of $A_{2A}R$ as a neuromodulator as well as homeostatic control in the brain to integrate dopamine, glutamate and BDNF signaling and to modulate synaptic plasticity in brain regions relevant to learning and memory, provides the molecular and cellular bases for $A_{2A}R$ control of cognition [45].

b) Vascular dementia

In elderly patients there is an increased likelihood of other neuropathological abnormalities including cerebrovascular lesions [46–48]. Over the last years, there has been increasing evidence that the previously held sharp distinction between AD and VaD may not be so clear-cut, especially in old age [2].

VaD is the second most common cause of dementia after AD. The diagnosis of VaD is based on a number of criteria: cognitive deficits, history of stroke and/or focal vascular neurological deficits, and temporal association between stroke and onset of dementia [49]. VaD arises as a consequence of ischemic insults such as hemorrhage and hypoperfusion that trigger neurodegeneration by depriving nerve cells of oxygen and glucose [50, 51]. Such deprivation results in the depletion of nerve cell energy supplies, leading to membrane depolarization, followed by an excessive release of glutamate which activates the N-methyl-D-aspartate receptor (NMDAR). This allows the influx of toxic levels of Ca^{2+} into nerve cells, which, in turn, activates intracellular calcium-dependent enzymes [52, 53].

One of the main adaptive mechanisms in response to hypoxia/ischemia is the cellular activation of adenosine A_1R which inhibits excessive excitatory synaptic transmission. On the contrary, adenosine $A_{2A}R$ contributes to excessive excitotoxicity.

$A_{2A}R$ antagonists are protective against ischemic damage in different animal model of ischemia administered both preischemia and after hypoxia/ischemia.

This ability is largely attributed to the control of excessive glutamatergic transmission and of the ensuing acute excitoxicity after ischemia [54].

A further mechanism by which $A_{2A}R$ antagonism is protective may be due to the capability of increasing GABA extracellular concentration during ischemia. The major part of excitatory glutamatergic innervation is modulated by inhibitory GABA releasing interneurons. Potentiation of GABAergic synaptic transmission has neuroprotective effects in several experimental models of cerebral ischemia [55] and evidence shows that selective $A_{2A}R$ stimulation decreases ischemia-evoked GABA outflow [56, 57] and enhances GABA transport into nerve terminals.

Neuroprotective strategies with antagonists of adenosine $A_{2A}R$ are aimed at targeting the brain parenchyma to antagonize excitotoxicity and ensuing production of harmful molecular events responsible for acute brain damage. The limit of such strategies is that these drugs are effective if administered in the first 4 hours after ischemia (in about the same time-window offered by clot removing therapies) [54].

In an apparent paradoxical manner, also adenosine $A_{2A}R$ agonists were found protective under hypoxia/ischemia.

In the hours and days after ischemia, adenosine $A_{2A}R$ located on vascular and blood cells may be the targets of agonist drugs aimed at dampening vascular adhesion signals and neuroinflammation [54].

Indeed, adenosine acting on $A_{2A}R$ on endothelial cells of brain vessels is implicated in cerebral blood flow regulation as a vasodilator agent, thus adenosine $A_{2A}R$ agonists might favor brain reperfusion after ischemia [58].

Moreover, a bulk of evidences indicates that peripheral effects on $A_{2A}R$ located on blood cells greatly account for protective effects of adenosine $A_{2A}R$ agonists after ischemia. In fact, the $A_{2A}R$ is expressed both on cells of innate (microglia, macrophages, mast cells, monocytes, dendritic cells, neutrophils) and on adaptive (lymphocytes) immunity [59].

Finally, $A_{2A}R$ activation is known to reduce ischemia-induced rolling, adhesion, and transmigration of various peripheral inflammatory cells (such as lymphocytes, neutrophils) [59].

c) Idiopathic normal pressure hydrocephalus

iNPH may be considered a treatable neurodegenerative disease, affecting predominately elderly people. It is caused by altered cerebrospinal fluid (CSF) reabsorption and metabolism affecting brain homeostasis. Increased CSF volume can result in the damage of brain tissue and several brain disturbances. iNPH is

manifested clinically as gait instability, urinary incontinence, and dementia [60]. It is important to mention that iNPH is the reason of about 5% of all dementia cases [60].

The degenerative changes accompanying iNPH may be reversible if they are recognized early and treated properly. The early diagnosis of iNPH is difficult because of various disease manifestations and overlap with other neurological disorders, which may also present the above-mentioned symptoms common in elderly. It could be easily mistaken for other neurodegenerative disorders, which makes iNPH one of the important misdiagnosed diseases worldwide [61, 62].

The most frequent therapeutic approach to iNPH is the ventriculoperitoneal shunt insertion, connecting the brain ventricles to abdominal cavity, where the excessive CSF volume can be absorbed [63, 64]. CSF shunting can lead to partial or complete amelioration of the patient's state with full or partial return to premorbid social and health condition. Unfortunately, the effect of the shunt is not durable. Recent data showed that nearly half of the initially well treated iNPH patients eventually developed iNPH-related dementia within a 4.7 years median follow up. iNPH-related degenerative changes of the brain appear usually early in the course of the disease, stressing the role of timely diagnostics [65]. Diagnosis at the early stage gives patients high probability of all symptoms disappearing after shunt insertion [64, 66].

It has been hypothesized that cerebrovascular diseases could have a role in etiology of chronic hydrocephalus [67]. Moreover, many studies show a significantly increased prevalence of cardiovascular diseases and risk factors for vascular diseases in iNPH compared to healthy subjects [68]. So far contrasting data have been reported on inflammatory involvement in iNPH patients. Some studies suggest an alteration of immune system in this pathology [69], but other authors deny it [70].

Since Ado system plays an important role both in vascular protection and in modulation of inflammatory reactions and neuroinflammation, it could be involved in the pathophysiology of iNPH disease.

It is interesting to note that the levels of many inflammatory molecules are different in iNPH than healthy subjects. Altered CSF levels of IL-4 and IL-10 [69], transforming growth factor (TGF)–β1, TGF-β type II receptor, leucine-rich α-2-glycoprotein [70], monocyte chemotactic protein-1 [69] and tumor necrosis factor-α were reported in iNPH patients.

In particular, elevated levels of CSF IL-10 were found in patients with iNPH [71], contrasting published lower levels in AD [72].

IL-10 level decreases after shunt insertion and stabilizes at levels lower than 0.5 pg/ml for two years [71].

Interestingly, activation of $A_{2A}R$ on both glial cells [73] and neurons [74] has been shown to increase IL-10 production.

PERIPHERAL CELLS TO STUDY BRAIN DISORDERS

Nowadays we need to find a reliable, minimally invasive, and inexpensive biomarker for dementia. Given the limited availability of brain tissue, we need to find putative dementia biomarkers and genetic risk alleles from blood tests and CSF samples [75, 76].

In particular peripheral blood mononuclear cells (PBMCs) reflect inflammatory mechanisms in a more specific way compared to the serum/plasma since these blood cells are a critical component of the immune system which provide defense against infection and respond to intruders. The lymphocyte population consists of CD4+ and CD8+ T cells, B cells and natural killer cells, CD14+ monocytes, basophils, neutrophils, eosinophils, and dendritic cells. PBMCs offer the advantage to study the molecular events associated with dementia development in the different stages of the disease, while studies on post-mortem brain samples offer a picture of the end results of these processes, which do not necessarily reflect the mechanisms underlying disease development. Moreover, PBMCs share much of the non-synaptic biochemical environment of neurons and contain the full complement of epigenetic enzymes and machinery, which are found in both neurons and peripheral nucleated cells, as in most other tissues.

Several differences have been shown in PBMCs from patients affected by dementia compared to sex- and age-matched PBMCs from normal individuals. The differences include immunophenotype combined with pro-inflammatory cytokine production [77], transcriptional and epigenetic mechanisms [78, 79], and global DNA methylation [80]. These substantial evidences are in favor of the notion that PBMCs seem to directly participate to neuropathological processes and provide a window into the central nervous system [76].

A_{2A}R IN PBMCS FROM PATIENTS WITH DIFFERENT BRAIN DISEASES

In light of these considerations, our group has investigated the A_{2A}R pathway in PBMCs of AD, VaD, iNPH, and mild cognitive impairment (MCI), a stage in which patients have a greater cognitive decline than expected for their age and educational level [81]. MCI could be amnestic (aMCI), considered the preclinical state of AD, and multiple cognitive domain (mcdMCI) types.

Indeed, we analyzed the gene expression and receptor density of A_{2A}R in PBMCs from above mentioned patients comparing to non-demented age- and gender-matched healthy controls with similar educational levels.

Interestingly, in PBMCs we found a significant linear increase in A_{2A}R gene expression from iNPH, which showed the lowest values, to aMCI, which showed the highest values. Similarly, protein density was lower in mcdMCI, VaD, and iNPH than controls while a similar density was showed in aMCI, AD, and controls.

The lack of a strict correlation between mRNA levels and receptor densities could be due to the fact that we measured the steady state level of A_{2A}R mRNA as well as the steady state level of receptor densities. We did not take into consideration the mechanisms that regulate these processes.

There could be some miRNAs-dependent mechanisms that could lead to a reduction of the translational levels of mRNAs [82]. At post-translational level the discrepancy observed between gene and protein expression could be explained by a quick protein degradation, preceded by some post-translational modifications, in order to have a transitory effect in response to a certain stimulus [83].

Moreover, A_{2A}R is upregulated in the preclinical stage and in overt AD than controls. In particular we found higher A_{2A}R levels in aMCI than AD supporting an involvement of the Ado system mainly in the early stages of this disease. These results seem to fit in with a previous demonstration that the increased expressions of A_{2A}R in the brain cortex are mainly an early event in AD [84]. In aMCI, the highest A_{2A}R levels could counterbalance the existing inflammation. Indeed, the activation of A_{2A}R by agonists can lead to the downregulation of the inflammatory response [42], to reduce production of pro-inflammatory cytokines and chemokines and to increase production of the anti-inflammatory cytokines [85].

We also showed that A_{2A}R expression is lower in VaD, mcdMCI, and in particular in iNPH than controls [19, 20]. This downregulation of A_{2A}R may depend on the brain vascular alterations occurred in VaD and iNPH patients [20]. Indeed, the inhibition of A_{2A}R by antagonists is protective against ischemic damage in different animal model of ischemia [54] and decreases infarct volumes after cerebral ischemia [86–90]. However, contrasting data have been reported so far on the beneficial/detrimental effects of A_{2A}R on brain cells, indeed also the agonists of A_{2A}R can protect the central nervous system against ischemia [17, 91].

Ultimately determining A_{2A}R expression in PBMCs could contribute to the recognition of cases of aMCI among the heterogeneous group of MCI patients [18] and to the identification of VaD patients with moderate degree of sensitivity and specificity from a heterogeneous group composed of VaD and AD patients.

These results highlight the possible role of A_{2A}R in differentiating a particular preclinical state of dementia and in distinguishing AD and VaD pathologies that are often closely associated in the elderly [19].

It can be concluded that A_{2A}R may play an important and distinctive role in the onset of dementia in the elderly especially if similar differences will be confirmed in other neurological diseases.

Considering that there are already drugs active on adenosine receptors both in use in clinical practice and under development, we could speculate that A_{2A}R may be a suitable target to study novel compounds with higher selectivity, oral bioavailability, stability *in vivo*, longer half-life, and better capability to cross the blood-brain barrier.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://www.j-alz.com/manuscript-disclosures/16-0324r1).

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