A2B Adenosine Receptors

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Adenosine is a signalling molecule which, by activating specific membrane receptors, acts as an important player during brain insults such as ischemia. or demyelinating injuries. Here we review data in the literature describing A2B receptor-mediated effects in preclinical in vitro and in vivo models of cerebral ischemia and myelination that point to A2B receptor ligands as putative therapeutic targets for the still unmet treatment of stroke or demyelinating diseases.

Keywords: adenosine ; A2B receptor ; brain ischemia ; demyelination ; multiple sclerosis ; oligodendrocyte maturation ; oxygen and glucose deprivation ; hippocampus ; synaptic transmission ; voltage-dependent K+ current

1. Introduction

Adenosine acts through the activation of four different purinergic P1 receptors: A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (A_1Rs, A_{2A}Rs, A_{2B}Rs, and A_3Rs, respectively), all belonging to the G-protein coupled, metabotropic receptor family [1].

The most widely recognized adenosine signaling is through the activation of A_1Rs, which inhibits adenylyl cyclase (AC) through $G_{i/o}$ protein activation [2]. A_1Rs are dominant in the central nervous system (CNS), where they inhibit neurotransmission and mediate sedative, anticonvulsant, anxiolytic, and locomotor depressant effects [3].

The A_{2A}R subtype is known to stimulate AC [2] being coupled to G_s proteins [1]. At central level, the functional effect of A_{2A}R activation is at variance from A_1Rs, as they are reported to enhance glutamate release [4][5]. In the periphery, A_{2A}Rs are highly expressed in inflammatory cells including lymphocytes, granulocytes, and monocytes/macrophages, where their activation reduces pro-inflammatory cytokine production, i.e., tumor necrosis factor-alpha (TNFα), interleukin-1β (IL-1 β), and IL-6 [6] and enhances the release of anti-inflammatory mediators, such as IL-10 [7].

The relatively new A_3R subtype is coupled to $G_{i/o}$ proteins and inhibits AC but, under particular conditions or in different cell types, activation of $G_{q/11}$ by A_3R agonists has also been reported [1]. Most of the cell types of the immune system express functional A_3Rs on their surface [8] and its activation is one of the most powerful stimuli for mast cell degranulation.

2. A_{2B} Adenosine Receptors (A_{2B}Rs)

This adenosine receptor subtype is somewhat the most enigmatic and less studied among the four P1 receptors. Although it was cloned in 1995 [9], a pharmacological and physiological characterization of A_{2B}Rs has long been precluded by the lack of suitable ligands able to discriminate among the other adenosine receptor subtypes [10].

The distribution of A_{2B}Rs in the CNS on neurons and glia is scarce but widespread, whereas in the periphery, abundant expression of A_{2B}Rs is observed in the bronchial epithelium, vascular beds, smooth muscles, mast cells, monocytes, and digestive tracts such as ileum and colon [1]. The activation of A_{2B}Rs stimulates G_s and, in some cases, $G_{q/11}$ proteins, thus enhancing intracellular [cAMP] or [IP_3], respectively [1]. As mentioned above for the cognate A_{2A}R subtype, in addition to brain cells and endothelial cells, A_{2B}Rs are present on hematic cells, such as lymphocytes and neutrophils, with the highest expression levels on macrophages [11]. Here, A_{2B} receptors in most cases are coexpressed with A_{2A}Rs and their activation exerts anti-inflammatory effects, inhibiting vascular adhesion and migration of inflammatory cells [12].

Differently from the high affinity A_1Rs, A_{2A}Rs and A_3Rs, which are activated by physiological levels of extracellular adenosine (low nM and high nM, respectively [13]), the A_{2B}R needs much higher adenosine concentrations (in the μM range) reached only in conditions of tissue damage or injury. Such a low affinity of A_{2B}Rs for the endogenous agonist implies that they represent a good therapeutic target, since they are activated only by high adenosine efflux reached under pathological conditions or injury, when a massive release of adenosine occurs [14].

3. A_{2B}Rs and Oligodendrogliogenesis

We recently and originally demonstrated that A_{2B}Rs are crucially involved in oligodendrocyte progenitor cell (OPC) maturation. We found that the selective A_{2B}R agonists BAY60-6582 (10 μM) and P453 (500 nM) inhibited the differentiation of purified primary OPC cultures, as demonstrated by the reduced expression of myelin basic protein (MBP) and myelin associated glycoprotein (MAG). We also demonstrated that A_{2B}R activation reversibly inhibits tetraethylammonium- (TEA-) sensitive, sustained I_K, and 4-amynopyridine- (4-AP) sensitive, transient I_A, conductances [15]. As I_K are known to be necessary to OPC maturation [16], this could be one of the mechanisms by which A_{2B}Rs inhibit myelin production. These results are similar to what was observed in cultured OPCs exposed to the A_{2A}R agonist CGS21680, as demonstrated by us in a previous work [17][18].

4. A_{2B}Rs and brain ischemia

Brain ischemia results from a permanent or transient reduction in cerebral blood flow mostly due to the occlusion of a brain artery. The consequent reduction of blood and/or oxygen supply to the brain leads to neuronal death caused by excessive glutamate release [19]. This early excitotoxic damage is followed by a secondary chronic phase of neuroinflammation that develops hours and days after ischemia. During stroke, adenosine is released in massive amounts [13][20]. The block of A_{2B}Rs is neuroprotective as it counteracts glutamate overload by preserving the inhibitory role of A_1Rs on neurotransmission [21][22][23], as demonstrated by us in an in vitro model of brain ischemia reproduced in rat hippocampal slices by oxygen and glucose deprivation (OGD)[22]. The selective A2BR antagonists PSB-603 (50 nM) and by MRS1754 (200 nM) prevents irreversible synaptic failure and anoxic depolarization (AD) appearance produced by a severe, 7 min, OGD event in CA1 hippocampal slices [22].

However, beyond neuroprotection exerted by A_{2B}R antagonists acting at the neuro-glial level, evidence in the literature points to a beneficial role exerted by A_{2B}R agonists acting on the same receptor subtype expressed on blood vessels and inflammatory cells [11][24]. Indeed, post-treatment with intravenous BAY60-6583 (1 mg/kg) reduces lesion volume and attenuates brain swelling and blood–brain barrier disruption at 24 h after ischemia induced by transient (2 h) middle cerebral artery occlusion (tMCAo) [25]. Additionally, in the same work, BAY60-6583 mitigates sensorimotor deficits in the presence of tPA and inhibits tPA-enhanced matrix metalloprotease-9 activation, thus decreasing BBB permeability 24 h after ischemia [25].

Our group of research contributed to the field by demonstrating that the chronic treatment with BAY60-6583, administered intraperitoneally twice/day for 7 days at the dose of 0.1 mg/kg, from 4 h after focal ischemia induced by tMCAo, since one day after ischemia protects from neurological deficit. Seven days after ischemia it protects from ischemic brain damage, neuronal death, microglia activation, and astrocyte alteration [26]. Interestingly, in the same paper, it was demonstrated that, 7 days after ischemia, the A_{2B} agonist decreases TNF-α and increases IL-10 levels in the blood.

5. A_{2B}Rs and demyelinating diseases

Demyelination occurs in a variety of pathological conditions affecting central or peripheral nervous systems. As an example, myelin disorganization in caudate/putamen striatal nuclei have been reported by us [27] and others [28][29]. Furthermore, chronic demyelinating diseases, such as multiple sclerosis (MS), are highly invalidating pathologies with elevated incidence among the “under 40” population worldwide [30], but an efficacious therapy is still lacking.

crucial role of adenosine, and in particular of A_{2A}R and/or A_{2B}R subtypes, in demyelinating pathologies have been postulated.

Under these conditions, excessive signaling by excitatory neurotransmitters like glutamate may be deleterious to neurons and oligodendroglia by exacerbating excitotoxicity and contributing to brain injury. For this reason, the inhibitory effect on glutamate release described above for antagonists at both A_2R subtypes could prove protective. This was indeed the case, as demonstrated by Chen and colleagues [31] and by Wei and co-workers [32] who reported that A_{2A}R and A_{2B}R antagonists, respectively, alleviated the clinical symptoms of EAE and prevented demyelination and CNS damage. Recent data by Liu and co-workers [33] confirmed that A_{2B}R activation seems to participate in EAE-induced damage as BAY60-6583 reverted the protective effects, i.e., reduced inflammatory cell infiltration and demyelination, exerted by mesenchymal stem cell therapy in EAE mice. Of note, the above results demonstrating a deleterious role of A_{2B}Rs in demyelinating diseases are in agreement with our in vitro data demonstrating that A_{2B}R blockade [15], as well as A_{2A}R antagonism [17], facilitates OPC differentiation in vitro.

However, things are probably more complicated as suggested by the fact that, again, A_2R-mediated actions are mainly anti-inflammatory when observed in a longer time-span. Indeed, genetically modified $A_{2A}R^{-/-}$ EAE mice are more prone to EAE-induced damage [34], and the $A_{2A}R$ agonist CGS61680 ameliorates EAE by reducing Th1 lymphocyte activation and cytokine-induced BBB dysfunction [35].

6. Conclusions

In conclusion, results underlie that after hypoxia/ischemia, brain injury results from a complex sequence of pathophysiological events that evolve over time—a primary acute mechanism of excitotoxicity and periinfarct depolarizations followed by a secondary brain injury activation triggered by protracted neuroinflammation. Information so far acquired indicates that adenosine $A_{2B}Rs$ located on any cell type of the brain and on vascular and blood cells partake in either salvage or demise of the tissue after a stroke, including protracted demyelination.

Thus, they all represent important targets for drugs having different therapeutic time-windows after stroke. The final protective outcome for an agonist versus antagonist compound depends on time of administration and district of activation of the receptor targeted by the drug.

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