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Visions & Reflections

Heterodimeric adenosine receptors: a device to regulate neurotransmitter release

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Abstract. Since 1990 it has been known that dimers are the basic functional form of nearly all G-protein-coupled receptors (GPCRs) and that homo- and heterodimerization may play a key role in correct receptor maturation and trafficking to the plasma membrane. Nevertheless, homo- and heterodimerization of GPCR has become a matter of debate especially in the search for the precise physiological meaning of this phenomenon. This article focuses on how heterodimerization of adenosine A_1 and A_{2A} receptors, which are coupled to apparently opposite signalling pathways, allows adenosine to exert a fine-tuning modulation of striatal glutamatergic neurotransmission, providing a switch mechanism by which low and high concentrations of adenosine inhibit and stimulate, respectively, glutamate release.

Keywords. Adenosine A_1 receptor, adenosine A_{2A} receptor, heteromeric receptor, glutamatergic neurotransmission.

The pharmacological study of membrane receptors largely relies on the study of individual receptors that are thought to signal in a largely independent manner. However, this simplistic view is being challenged by the increasing recognition that several receptors do not signal as individual units, but rather as part of more complex networks that are based on the physical association of different receptor proteins. These 'receptosomes' confer novel regulatory properties to signal reception units, which may be accompanied by novel and still largely unexplored pharmacological and physiopathological properties.

The first proposal for the formation of receptor dimers was made in 1979–1980. When searching for an explanation of where all the recently discovered neuropeptides in the brain could integrate their messages with those of classical transmitters such as the monoamines, it was postulated that an intramembrane interaction between neuropeptide and monoamine receptors could be involved. The first observations were published in the early 1980s and showed that substance P could modulate the high-affinity serotonin (5HT) binding sites in spinal cord membrane preparations using biochemical binding techniques [1]. Since then, the existence of heterodimers of several G-protein-coupled receptors (GPCRs) has been reported [2], using an increasing number of biochemical and biophysical techniques. These have revealed the possibility that GPCR for different ligands may be physically associated, forming heterodimers that allow a tight mutual control of the signalling mediated by the two receptors [2]. Interestingly, it has also been noted that GPCRs which are activated by the same ligand could also form dimers. In some cases, this dimerization is essential for receptor function, as occurs for the $GABA_B$ receptor [3] and for the amino acid taste receptors [4]. Heterodimers formed by recep-

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tors activated by the same ligand have also been described for members of the family of muscarinic acetylcholine receptors [5], adrenergic receptors [6], dopamine receptors [7] and opioid receptors [8]. However, apart from the heterodimerization of the delta and kappa opioid peptide receptors that occurs in a tissue-specific manner (namely in the spinal cord) and was proposed to be a spinal-selective target for tissue-selective analgesics [8], the possible physiopathological role of these heterodimers of GPCRs of the same family remains largely undefined. We will now review the evidence we have gathered for the formation of heterodimers composed of adenosine A_1 and A_{2A} receptors and discuss the relevance of this dimer for the control that adenosine exerts on glutamate release from corticostriatal afferent fibres.

Adenosine is a molecule that plays a key regulatory role in the nervous system since its extracellular concentration rises as a function of neuronal activity, acting as a workload or energy-dependent neuromodulator through combined presynaptic, postsynaptic and non-synaptic actions [9, 10]. Extracellular adenosine regulates several central functions including neuronal viability, neuronal membrane potential, propagation of action potentials, astrocytic functions, microglia reactivity, primary metabolism in both neurons and astrocytes and blood flow [10]. Adenosine mediates its actions through activation of specific GPCRs, for which four subtypes have been identified (A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R) [11]. The most abundant adenosine receptor in many regions is the inhibitory A_1R which is functionally coupled to members of the pertussis-toxin-sensitive family of G proteins (G_{i1}, G_{i2}, G_{i3} and G_o) and whose activation regulates the activity of membrane and intracellular proteins such as adenylate cyclase, Ca^{2+} channels, K^+ channels and phospholipase C [12]. In central neuronal tissue, adenosine can also signal through the activation of $A_{2A}R$, which is coupled mostly to G_S proteins [10], thus being mainly linked to adenylyl cyclase activation and the protein kinase A (PKA)-dependent signalling cascades. As the A_1R and $A_{2A}R$ operate through opposite signal transduction pathways, they have always been considered as isolated entities in the context of adenosine neuromodulation. However, there is already good evidence to conceive a role for dimers involving adenosine receptors in the context of neuromodulation. In fact, adenosine receptors have been shown to form homodimers [13, 14], which are believed to be the functional receptor species placed at the cell surface [14], and also heterodimers with other GPCRs. For example, A_1R has been found to heterodimerize with the dopamine D_1 receptor (D_1R), this phenomenon being essential for differential desensitization mechanisms and for receptor trafficking [15]. Heterodimers of A_1R with metabotropic glutamate receptor 1α ($mGlu_{1\alpha}$) seem to play a key role in preventing glutamate excitotoxicity [16], whereas the physiological role for the proven heterodimerization of A_1R and purinergic $P2Y_1$ receptor ($P2Y_1R$) has not yet been elucidated [17]. On the other hand, $A_{2A}R$ also has the ability to heterodimerize with the dopamine D_2 receptor (D_2R) [18, 19], this heterodimerization being the molecular basis for the antagonistic interactions that regulate the function of the GABAergic enkephalinergic neurons [20]. $A_{2A}R$ also heterodimerizes with the metabotropic glutamate receptor 5 ($mGlu_5$) [21], giving rise to a synergistic functional interaction demonstrated both at the biochemical and behavioural levels [20].

The existence of these dimeric forms involving either A_1Rs or $A_{2A}Rs$ has not altered the widespread acceptance that these two adenosine receptors with opposite physiological functions signal independently. Unexpectedly, however, A_1R and $A_{2A}R$ are coexpressed in a variety of cells, as in glutamatergic neurons of the hippocampus [22] and striatum [23]. Furthermore, some evidence indicates a potential interaction between A_1Rs and $A_{2A}Rs$ [24–26], suggesting the possibility that these receptors establish a molecular and/or functional cross-talk, i.e. as if they were part of the same molecular transduction complex or signalosome. The formation of A_1R-$A_{2A}R$ heterodimers in mammalian cells and in striatal glutamatergic nerve terminals was recently demonstrated using a combination of approaches, including radioligand-binding experiments, coimmunoprecipitation, bioluminescence resonance energy transfer and time-resolved fluorescence resonance energy transfer techniques [23]. Exploration of the signalling properties of this novel heterodimer suggests that it may constitute the molecular basis implicated in the functional cross-talk between these two receptors, affording a logical and economical device to fine-tune adenosine neuromodulation.

Radioligand-binding experiments reveal that the formation of the A_1R-$A_{2A}R$ heteromer in a heterologous expression system does not modify the affinity of the A_1Rs or $A_{2A}Rs$ for their respective agonists. Interestingly, the affinity of the A_1R for its agonist decreases when the $A_{2A}R$ is activated, but the activation of the A_1R does not alter $A_{2A}R$ binding characteristics. Thus, in the heterodimer, A_1R responsiveness to agonist is modulated by the $A_{2A}R$, whereas the latter preserves its own binding signature, indicating that $A_{2A}Rs$ can control A_1R functionality, but not vice versa. This ability of $A_{2A}Rs$ to control A_1Rs in the A_1R-$A_{2A}R$ heterodimer was further confirmed functionally. In fact, upon formation of the A_1R-$A_{2A}R$ heterodimer, activation of the $A_{2A}R$ decreases A_1R-mediated effects, as shown by the significant reduction in the A_1R-induced intracellular calcium peak obtained by preincubation with a selective $A_{2A}R$ agonist [23].

The question still remained as to whether the formation of this A_1R-$A_{2A}R$ heterodimer might play a physiological role in native brain preparations. This question is of major relevance since the involvement of GPCR heterodimers in pathophysiological processes remains poorly explored

despite substantial evidence for their presence in native brain preparations. We will first review the evidence indicating the existence of A_1R-$A_{2A}R$ dimers in brain tissue and then critically review their possible functional role.

The distribution of A_1R and $A_{2A}R$ overlaps in some brain areas, namely in dopamine-rich regions [27, 28]. We have recently demonstrated the presence of both A_1R and $A_{2A}R$ in the same glutamatergic nerve terminals, both in the hipppocampus [22] and striatum [23]. Ultrastructural analysis by means of immunolelectron microscopy of A_1R and $A_{2A}R$ distribution in striatum reveals that these receptors codistribute and colocalize in striatal glutamatergic synapses [23]. Glutamatergic terminals in the striatum establish synaptic contacts with the dendritic spines of the GABAergic efferent neurons, which are subdivided into enkephalinergic and dynorphinergic neurons. The $A_{2A}R$ is known to be abundantly expressed by the GABAergic enkephalinergic neurons [2]. Thus, at the postsynaptic site, $A_{2A}Rs$ are located along the extrasynaptic plasma membrane and in a perisynaptic ring surrounding the postsynaptic density [23]. A_1Rs, which are known to be expressed by both GABAergic enkephalinergic and dynorphinergic neurons [10], were also localized postsynaptically at glutamatergic synapses. A_1R and $A_{2A}R$ colocalize at the postsynaptic and at the presynaptic level, often being found intrasynaptically, in the active zone [23]. Despite this heterogenous distribution of both A_1Rs and $A_{2A}Rs$ intra- and extrasynaptically in synapses between glutamatergic corticostriatal projections, the immunocytochemistry results together with the coimmunoprecipitation experiments clearly showed that the striatal A_1R-$A_{2A}R$ heteromer is preferentially localized in the striatal glutamatergic terminals at both presypnatic and postsynaptic levels.

In excitatory glutamatergic synapses, the most accepted role for adenosine is the inhibition of synaptic transmission through activation of presynaptic A_1Rs, the prototypical inhibitory $G_{i/o}$-protein-coupled receptor whose stimulation decreases the probability of neurotransmitter release [29]. As previously discussed [30], this predominant ability to record A_1R-mediated inhibition of excitatory transmission mainly results from the experimental design requiring the attainment of robust signals hardly prone to be further facilitated, together with pharmacological agents largely selective for A_1Rs rather than for $A_{2A}Rs$. In fact, the use of particular stimulating conditions together with pharmacological tools with adequate selectivity to interfere with $A_{2A}Rs$ has made possible the identification of a parallel ability of adenosine to facilitate the evoked glutamate release via activation of presynaptic $A_{2A}Rs$ [20]. The obvious question is: how does adenosine choose between A_1R and $A_{2A}Rs$ in the glutamatergic nerve terminal to control the glutamate release? To answer this question we need first to understand several important assumptions concerning A_1R and $A_{2A}Rs$. First, these receptors have different affinities for endogenous adenosine, with the A_1R showing a higher affinity than the $A_{2A}R$ [31]. Thus, at moderate adenosine concentrations preferential A_1R stimulation occurs and glutamate release is inhibited. In contrast, when adenosine reaches concen-

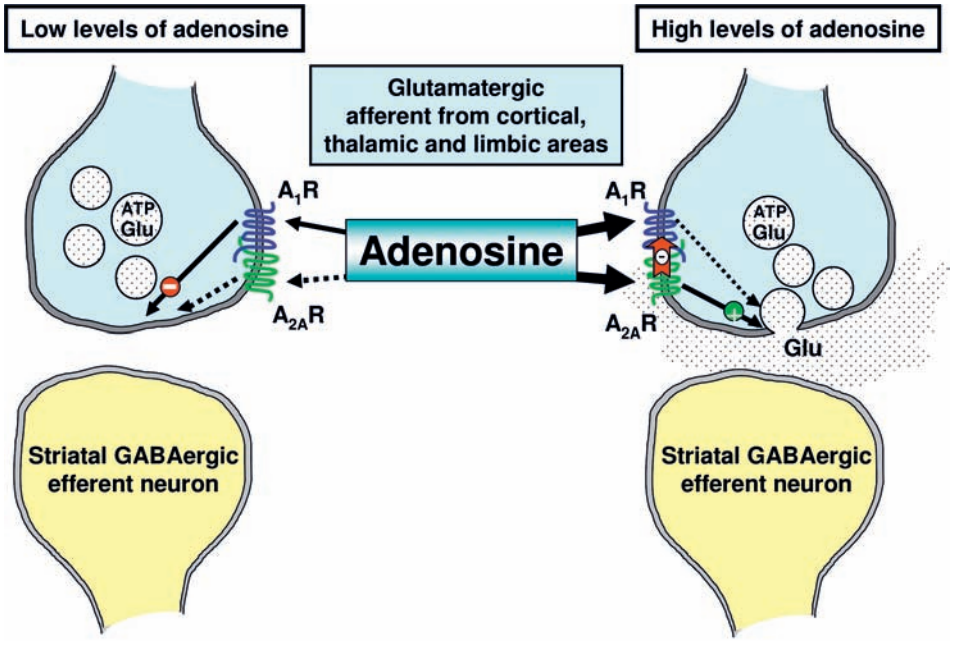

Figure 1. Scheme showing differences in the efficacy of adenosine to stimulate A_1Rs and $A_{2A}Rs$ in the A_1R-$A_{2A}R$ heterodimers. Low concentrations of adenosine activate predominantly A_1Rs, inhibiting glutamate release. High concentrations of adenosine also activate $A_{2A}Rs$, which, by means of the A_1R-$A_{2A}R$ intramembrane interaction (red arrow), antagonizes A_1R function, therefore facilitating glutamate release.

trations high enough to stimulate the $A_{2A}R$, glutamate release is enhanced [20]. However, in this scenario, the A_1R would be maximally effective and should effectively counteract any attempts of $A_{2A}Rs$ to facilitate glutamate release. How can this conflict be eliminated? The data we obtained measuring the evoked release of glutamate from striatal nerve terminals indicate that the functional properties of the A_1R-$A_{2A}R$ heteromer allow resolution of this issue since A_1R responsiveness in the heterodimer is under the control of $A_{2A}Rs$. Thus, we showed that the predominant A_1R-mediated inhibition of glutamate release can be abrogated by preincubation with an $A_{2A}R$ agonist, and simultaneous activation of A_1Rs and $A_{2A}Rs$ always leads to predominant facilitation of the evoked release of glutamate. Furthermore, increasing concentrations of the natural ligand, adenosine, first trigger an inhibitory effect at lower concentrations, which is reverted to a facilitatory effect with increasing concentrations. This leads to the conclusion that the A_1R-$A_{2A}R$ heteromerization provides a basic mechanism to select the pattern of adenosine neuromodulation in striatum based on the intramembrane receptor-receptor communication allowing $A_{2A}Rs$ to block the A_1R inhibitory function. Thus, the regulation of glutamate release by adenosine switches from inhibition to facilitation thanks to the A_1R-$A_{2A}R$ heterodimer, in a manner dependent on extracellular adenosine levels [23].

Concluding remarks

At a relatively low concentration, extracellular adenosine preferentially stimulates A_1Rs, which display a higher affinity for adenosine than $A_{2A}Rs$. A preferential A_1R stimulation in glutamatergic nerve terminals inhibits glutamatergic neurotransmission. On the other hand, under conditions of robust production of extracellular adenosine in striatum, adenosine A_{2A} receptor activation in the existing A_1R-$A_{2A}R$ heterodimers would limit A_1R-mediated function, the outcome being a facilitation of the evoked release of glutamate (Fig. 1). Thus, the A_1R-$A_{2A}R$ heterodimer exerts a fine-tuning modulation of glutamatergic neurotransmission in striatum, providing a switch mechanism by which low and high concentrations of adenosine inhibit and stimulate, respectively, glutamate release. Heterodimerization of different receptors for the same neurotransmitter constitutes a rationale to understand how, depending on its concentration, a given neurotransmitter may facilitate or inhibit neural transmission.

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