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Review

Allosteric Modulation of Adenosine A_{2A} Receptors as a New Therapeutic Avenue

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Abstract: The therapeutic potential of targeting adenosine A_{2A} receptors (A_{2A}Rs) is immense due to their broad expression in the body and central nervous system. The role of A_{2A}Rs in cardiovascular function, inflammation, sleep/wake behaviors, cognition, and other primary nervous system functions has been extensively studied. Numerous A_{2A}R agonist and antagonist molecules are reported, many of which are currently in clinical trials or have already been approved for treatment. Allosteric modulators can selectively elicit a physiologic response only where and when the orthosteric ligand is released, which reduces the risk of an adverse effect resulting from A_{2A}R activation. Thus, these allosteric modulators have a potential therapeutic advantage over classical agonist and antagonist molecules. This review focuses on the recent developments regarding allosteric A_{2A}R modulation, which is a promising area for future pharmaceutical research because the list of existing allosteric A_{2A}R modulators and their physiologic effects is still short.

Keywords: adenosine A_{2A} receptors; allosteric modulator; insomnia; slow-wave sleep; inflammation; cardiovascular function; body temperature; drug development

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1. Introduction

Adenosine is a naturally occurring purine nucleoside that regulates various physiologic functions, including inflammation and wound healing, cardiac contraction, blood vessel formation, vasodilation, learning, memory, sleep, and arousal [1–7]. Adenosine is released by neurons and glial cells [8]. Extracellular adenosine modulates neuronal excitability, synaptic plasticity, and the release and reuptake of several neurotransmitters [9–12]. The effects of extracellular adenosine are modulated via four subtypes of G-protein coupled adenosine receptors (GPCRs), denoted A_1, A_{2A}, A_{2B}, and A_3 [13]. Adenosine A_{2A} receptors (A_{2A}Rs) are broadly expressed in the brain, cardiovascular system, blood vessels, spleen, thymus, leukocytes, and lung, making them an important drug target [14]. This review focuses on allosteric A_{2A}R modulation and the latest developments in this emerging field.

The therapeutic potential of targeting A_{2A}Rs has prompted the development of numerous antagonist and agonist molecules to selectively control A_{2A}R function. The myriad A_{2A}R agonists and antagonists are considered potential therapeutic agents for inflammation, sickle cell disease, ischemia-reperfusion injury, and central nervous system (CNS) diseases [15,16]. The A_{2A}R agonist regadenoson was approved by the US Food and Drug Administration to boost blood flow during cardiac stress tests [16]. Many other agonists and antagonists are undergoing clinical trials.

Medicinal chemists have made many efforts to develop small molecules as allosteric modulators in recent years. Unlike agonist and antagonist molecules, allosteric modulators evoke a selective physiologic response only where and when the orthosteric ligand is released [17]. GPCRs, including adenosine receptors, are allosterically regulated [17,18]. The list of existing allosteric $A_{2A}R$ modulators is short, however, and the physiologic opportunities for modulators are just emerging, making allosteric $A_{2A}R$ modulation a promising area for future research.

2. Adenosine and Its Receptors

Adenosine was initially recognized as a physiologic regulator of coronary vascular tone; since then, a growing body of reports indicates that adenosine regulates cellular functions through specific receptors present on the cell surface [19–21]. Adenosine is an endogenous purine nucleoside consisting of adenine and *D*-ribose, and is formed through hydrolysis of *S*-adenosylhomocysteine or adenosine monophosphate [22,23]. Adenosine formation from *S*-adenosylhomocysteine relies on the intracellular activity of the enzyme *S*-adenosylhomocysteine hydrolase, which bi-directionally assures the constant occupancy of a bound adenosine concentration in the cells [24]. Different enzymes mediate the formation of adenosine from adenosine monophosphate at both intracellular and extracellular levels.

Although adenosine does not exclusively act on synapses and is not stored in synaptic vesicles, it has a direct role in synaptic processes and the regulation of various neurotransmitters in the CNS. Nucleoside transporters mediate adenosine release and reuptake mechanisms through a concentration gradient between the intracellular and extracellular spaces. Therefore, adenosine is postulated as a modulator that affects neurotransmitter release and neuronal hyper- or depolarization and regulates glial cells [25]. Despite the modulatory role of adenosine, neurotransmitter properties are also observed for adenosine, which is due to the presence of the adenosine-producing enzyme in synapses. Extracellular adenosine acts on neurons through specific adenosine receptors [26].

Purinergic receptors are the natural target of purine molecules such as adenosine and adenosine triphosphate. These receptors were recognized for the first time in 1978 [27]. Two types of purinergic receptors, P1 and P2, were subsequently identified based on their pharmacologic profile [28]. P1 receptors recognize adenosine as a primary natural ligand and are therefore also called adenosine receptors. Each of the four types of adenosine receptors, A_1R, $A_{2A}R$, $A_{2B}R$, or A_3R, is characterized by a distinct pharmacologic profile. These receptors are members of the GPCR superfamily [17]. $A_{2A}Rs$ and $A_{2B}Rs$ are Gs-coupled receptors, and their activation increases the activity of adenylyl cyclase, the enzyme that initiates cyclic AMP (cAMP) synthesis in the cells. A_1Rs and A_3Rs are Gi/q coupled receptors, and their activation through adenosine or agonist molecules inhibits the activity of adenylyl cyclase, which suppresses cAMP synthesis in the cells.

3. $A_{2A}R$ and Its Physiologic Roles

The four types of adenosine receptors, A_1R, $A_{2A}R$, $A_{2B}R$, or A_3R, react with extracellular adenosine [13]. The activation of $A_{2B}Rs$ reportedly requires a high adenosine concentration. Unlike $A_{2B}Rs$, adenosine levels under basal physiologic conditions are adequate to activate A_1Rs, $A_{2A}Rs$, and A_3Rs with relatively equal potency. The pharmacologic strength of an endogenous ligand or agonist at its receptor, however, relies on the number of receptors on the cells. Higher concentrations of adenosine are needed to show an effect in the presence of only a few receptors. Local expression of the A_1Rs and $A_{2A}Rs$ in the brain is suggested to be relatively higher than that of the other two adenosine receptors [6,29].

$A_{2A}Rs$ were first identified by Libert and colleagues when they cloned several orphan GPCRs from the dog thyroid [30]. Afterward, $A_{2A}Rs$ were cloned from other species, including guinea pigs, mice, rats, and humans [31–34]. As with the other GPCRs, $A_{2A}Rs$ induce classical secondary messenger pathways. The $A_{2A}R$ signaling pathway may vary depending on the cell and tissue type in which the receptors occur. For example, Gs is the major G-protein associated with $A_{2A}Rs$ in the peripheral system. On the other hand, $A_{2A}Rs$ in the striatum, where they are highly expressed, mediate their effects mainly through Golf activation in the rat. Active Gs and Golf proteins stimulate adenylyl cyclase (Figure 1) which increases cellular cAMP levels and activates protein kinase A (PKA) which then phosphorylates and promotes cAMP-responsive element-binding protein 1 (CREB1) [16,35]. Activation of A_{2A}Rs also activates extracellular signal-regulated kinases (ERK) and several other kinases of the mitogen-activated protein kinase (MAPK) family which trigger specific cellular responses [36]. A_{2A}Rs form heterodimer structures with other GPCRs (e.g., metabotropic glutamate type 5 receptor (mGluR5)/A_{2A}R, cannabinoid receptor type 1 (CB_1)/A_{2A}R, dopamine D_2 receptor (D_2R)/A_{2A}R, dopamine D_3 receptor/A_{2A}R), and even CB_1/A_{2A}R/D_2R heterotrimers [37–41].

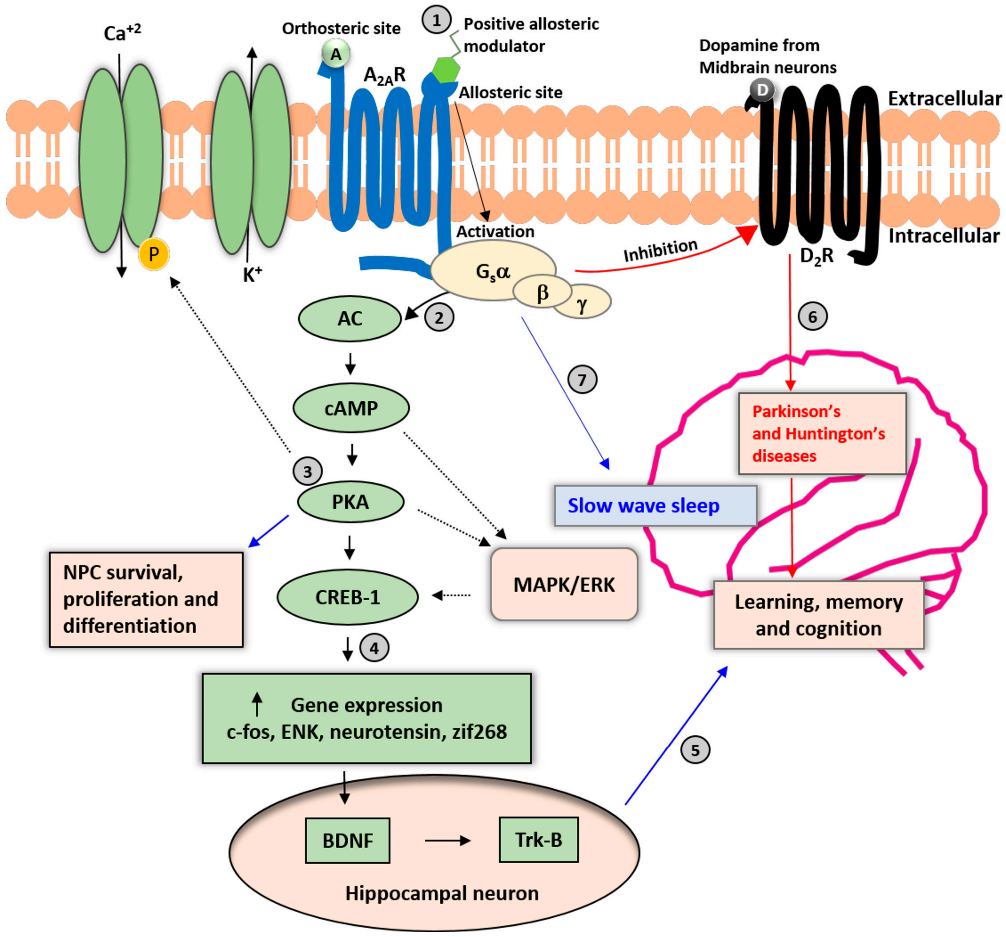


Figure 1. Neuronal A_{2A}R signaling cascades. A_{2A}R is a Gs(olf)-protein-coupled receptor involved in various physiologic processes. (1) The allosteric modulation sites may be pharmacologically relevant for avoiding adverse effects on the cardiovascular and other peripheral systems. (2) Binding of adenosine and an allosteric modulator to A_{2A}Rs enhances the activation of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA), resulting in the phosphorylation of calcium ion channels and increased influx of Ca^{+2} into the cytoplasm. (3) The PKA pathway also promotes neural progenitor cell (NPC) survival, proliferation, and differentiation; and activation of the mitogen-activated protein (MAP)-kinase pathway. (4) PKA-mediated phosphorylation of the cAMP-responsive element binding protein 1 (CREB-1) regulates the expression of genes such as c-fos, enkephalin (ENK), neurotensin, and zinc finger protein 268 (zif268). (5) The secretion of brain-derived neurotrophic factor (BDNF) and activation of tropomyosin receptor kinase B (TrkB) receptors in response to A_{2A}R activation in hippocampal neurons may be relevant for cognitive functions such as learning and memory. (6) A_{2A}R activation may be a counter mechanism to control the activation and expression of dopamine D_2 receptors (D_2Rs). Long-term imbalance of D_2R signaling leads to impairments in cognitive and motor functions and the development of Parkinson's and Huntington's diseases. (7) Activation of A_{2A}R in the nucleus accumbens increases slow-wave sleep in mice. Solid black arrows represent the primary signaling pathway of A_{2A}Rs, and dashed black arrows represent secondary signaling pathways. A: Adenosine; D: Dopamine.

The development of electron microscopy, selective radioligands, and antibodies has greatly contributed to $A_{2A}R$ distribution mapping. Furthermore, advancements in electron microscopy helped to determine the interactions of agonists and antagonists with their receptors and receptor density in particular regions. $A_{2A}Rs$ are concentrated on GABAergic medium-sized spiny neurons of the striatum, core and shell regions of the nucleus accumbens (NAc), olfactory tubercule, and dopamine-rich areas of the brain [16].

$A_{2A}Rs$ play a significant role in regulating the indirect pathways of the basal ganglia in the brain (Figure 2) [16]. The basal ganglia have an evolutionarily conserved essential role in learned habits, goal-directed movements, and locomotion [42]. The basal ganglia carry out their functions through direct and indirect circuits, originating in conspicuous populations of striatal medium spiny neurons that project to different output structures. Direct pathway neurons express excitatory dopamine D_1 receptors (D_1Rs) and inhibitory A_1Rs, whereas indirect pathway neurons express inhibitory D_2Rs and excitatory $A_{2A}Rs$ [43]. Studies in mice revealed that both direct- and indirect pathway medium spiny neurons are active during mouse locomotion but quiescent during inactive phases [44], and chemogenetic activation of direct and indirect pathways neurons increases and decreases locomotor activity, respectively [45]. Moreover, recent findings indicate that optogenetic activation of indirect pathway neurons in the NAc, a part of the brain that is associated with motivation and pleasure, induces slow-wave sleep, whereas inhibition suppresses slow-wave sleep [46]. Other observations show that when an action does not result in a reward, increased activity of indirect pathways occurs, suggesting a role of the indirect pathways in controlling goal-directed behavior [47].

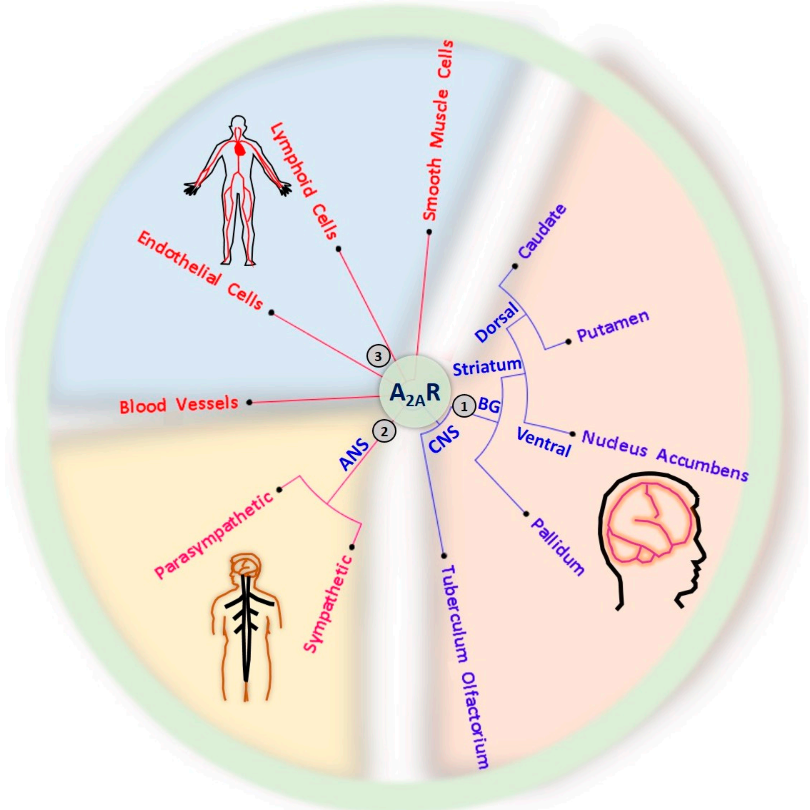


Figure 2. Expression of $A_{2A}Rs$ in the central nervous system (CNS), autonomic nervous system (ANS), circulatory system, and musculoskeletal system. (1) CNS $A_{2A}Rs$ are mainly expressed in the basal ganglia (BG), including the dorsal pallidum, the nucleus accumbens in the ventral part of the striatum, and the dorsal striatum comprising the caudate and putamen. (2) $A_{2A}Rs$ are also expressed in the sympathetic and parasympathetic ANS. (3) The distribution of $A_{2A}Rs$ is not limited to the nervous system; $A_{2A}Rs$ are also found in the circulation system, including heart, blood vessels, lymphoid cells (immune cells), and smooth muscle cells of the musculoskeletal system.

Apart from medium-sized spiny neurons in the basal ganglia, A_{2A}Rs are expressed in various other tissues, including smooth muscle cells, thymus, blood platelets, endothelial and lymphoid cells, leukocytes, spleen, blood vessels, lung, heart, and neurons in sympathetic and parasympathetic nervous systems [14,48] (Figure 2). A_{2A}Rs have a wide range of physiologic functions in the body, such as protecting tissues from inflammatory damage, mediating vasodilation, and supporting the formation of new blood vessels.

Several A_{2A}R agonists and antagonists are currently in clinical trials. The selective A_{2A}R agonist regadenoson was approved by the US Food and Drug Administration to increase blood flow in cardiac nuclear stress tests. On the other hand, the effects of A_{2A}R antagonists for the treatment of Parkinson's disease (PD) are promising. Other trials have been conducted with several agonists and antagonists aimed at treating infectious disease, ischemia-reperfusion injury, cancer, inflammation, sickle cell disease, diabetic nephropathy, and other CNS disorders. The increasing number of reports and patents demonstrates the growing interest in targeting the A_{2A}R [16].

4. The Concept of Allosteric Modulation

The most common method to stimulate receptors in pharmacology and biochemistry is to target orthosteric sites with their endogenous ligand, agonists, or antagonists. On the other hand, studies show that receptor activity can be altered by small molecules that bind to an allosteric site different from the site where the endogenous ligand, agonists, or antagonists would bind [49]. The small molecules that bind to the allosteric sites of the receptors are termed allosteric modulators. Unlike endogenous ligands, agonists, or antagonists, an allosteric modulator cannot itself activate or inactivate receptors but alters the receptor's response to substrates that bind to orthosteric sites in two ways: (1) increase or decrease affinity, i.e., the ability of orthosteric substances to bind receptors, and (2) increase/decrease efficacy, i.e., the ability of orthosteric substances to activate receptors [50]. Allosteric modulators reportedly change the receptor conformation, which alters the effect of the endogenous ligand, agonist, and antagonist binding [51]. The concept of receptor modulation is not straightforward with respect to practical implementation. Allosteric modulators do not necessarily equally alter the affinity and efficacy of endogenous ligands, agonists, or antagonists of the receptors. An allosteric modulator may alter the efficacy or affinity of the endogenous ligand, but not that of the agonist or antagonist of the receptors or vice versa [52].

The term 'allostery' was first used in enzymology studies in the early 1960s [53–55]. Subsequently, allosteric modulation has been identified for all receptor superfamilies, including GPCRs, nuclear hormone receptors [56,57], receptor tyrosine kinases [58,59], and ligand/voltage-gated ion channels [60–64]. The term "allosteric" began to be used increasingly in the literature, and a broad spectrum of allosteric modulators was described. Consequently, the classification of allosteric modulators was necessary to avoid possible confusion [65–67]. Three properties are considered in the classification of allosteric modulators: (1) affinity modulation of the orthosteric ligand, (2) modulation of the signaling effect of the orthosteric ligand, and (3) direct effects of the allosteric modulator in the absence of the orthosteric ligand. Moreover, allosteric modulators are classified in terms of their effects on orthosteric ligands as positive allosteric modulators (PAM), negative allosteric modulators (NAM), or silent allosteric modulators, also known as neutral allosteric ligands [68]. PAMs enhance the agonist/antagonist affinity and efficacy, whereas NAMs decrease orthosteric ligand affinity and efficacy. Unlike PAMs and NAMs, silent allosteric modulators do not affect the agonist or antagonist activity of orthosteric ligands, but bind to the allosteric site of the receptors and prevent PAMs or NAMs from binding to the same site, thereby inhibiting the activity of positive/negative allosteric modulators [52]. It is important to note that activities of allosteric modulators are therefore limited by where and when the orthosteric ligand is released. Thus, in contrast to agonists or antagonists, allosteric modulators promise greater safety and fewer side effects in therapeutic applications.

5. Allosteric $A_{2A}R$ Modulation

Adenosine receptors are among the first known allosterically regulated GPCRs. Early studies demonstrated that amiloride and its analogs are allosteric $A_{2A}R$ inhibitors [17,18,69]. Subsequent studies revealed that the amiloride analog 5-(N,N-hexamethylene)-amiloride (HMA) is a potent allosteric $A_{2A}R$ inhibitor. The other amiloride analogs, benzamil, 5-(N-methyl-N-isobutyl)amiloride (MIBA), 5-(N-methyl- N-guanidinocarbonyl-methyl)amiloride (MCGMA), and phenamil, were found to be more effective allosteric inhibitors than amiloride at rat $A_{2A}Rs$ [17,70]. Moreover, amiloride and its analogues do not affect the dissociation rate of the agonist [^{3}H]CGS21680 (3-{4-[2-({6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-9H-purin-2-yl}amino) ethyl]phenyl}propanoic acid), but increase the dissociation rate of the antagonist [^{3}H]ZM241385 (4-(2-{[7-amino-2-(furan-2-yl)[1,2,4]triazolo[1,5-a] [1,3,5] triazin-5-yl]amino}ethyl)phenol) from $A_{2A}Rs$ [71]. By contrast, sodium ions, for example, deteriorate the dissociation rate of the antagonist [^{3}H]ZM241385 from $A_{2A}Rs$ in a dose-dependent manner [17]. It is important to note that other adenosine receptor agonists and antagonists are differentially affected by amilorides [70]. A new approach specifically targeting the sodium ion pocket, known as fragment-screening based on affinity mass spectrometry, led to the discovery of fragment Fg754 as a new $A_{2A}R$ NAM carrying a novel azetidine moiety and exhibiting inhibitory potency comparable to HMA. Subsequent simulations of the molecular dynamics, structure-activity relationship studies of the ligand, and nuclear magnetic resonance analyses in solution revealed the unique binding mode and antagonistic properties of Fg754, which is distinctly different from HMA [72]. In addition, cholesterol is reported to be a weak PAM of $A_{2A}Rs$ [73].

Identification of binding sites for allosteric modulators on $A_{2A}Rs$ based on the crystal structure of the receptor is critical for the development of new allosteric modulators. Two tightly linked residues, histidine residue number 278 (His^{278}) in transmembrane domain 1 and glutamic $acid^{13}$ in transmembrane domain 7 of the human $A_{2A}R$, are reported to be the most crucial components for agonist recognition and play a partial role in the allosteric regulation by sodium ions [70,71,74–77]. Studies of the crystal structure of the antagonist-bound adenosine $A_{2A}R$ revealed that a highly conserved aspartate (Asp) residue in the second transmembrane domain is involved in sodium modulation of GPCRs [78]. Comparative studies of crystal structures in which a sodium ion bound in the allosteric site of human protease-activated receptor 1 [79], the β1-adrenergic receptor [80,81], the human δ-opioid receptor [82], and the human adenosine $A_{2A}R$ [78] show that sodium ions interact with the common residues $Asp^{2.50}$ (superscript numbers refer to the Ballesteros and Weinstein residue numbering system [83]) $serine^{3.39}$, $tryptophan^{6.48}$, asparagine $(Asn)^{7.45}$, and $Asn^{7.49}$, either directly or through water-mediated hydrogen bonding [83]. Pre-crystal structure studies revealed that the positively charged sodium ion forms a permanent salt bridge with the negatively charged amino acid $Asp^{2.50}$, suggesting that this residue represents the most conserved sodium ion binding site among GPCRs [84].

Subsequent studies on the crystal structure of the $A_{2A}R$ at 1.8 Å resolution provided sufficient resolution to confirm that $Asp^{2.50}$ interacts directly with sodium ions via the salt bridge [78]. The crystal structures of agonist complexes for two variants in the first sodium coordination shell of the human A_{2A} adenosine receptor have also been reported [85]. A fluorine-19 nuclear magnetic resonance spectroscopy study suggested that $A_{2A}Rs$ have four distinct activation states; a partial agonist that favors the population of an active state (S_3), an active state induced by full agonists ($S_{3'}$), and two inactive states ($S_{1–2}$); this study also demonstrated that sodium ions enhance the inactive states of $A_{2A}Rs$ [86]. In contrast, partial agonists and HMA induce active states, indicating that HMA competes with sodium ions for interaction with $A_{2A}Rs$ [84]. Moreover, all-atom simulations of molecular dynamics have shown that Fg754 can steadily enter the transmembrane domain core and form contacts with transmembrane helices 2, 3, 6, and 7, and extracellular loop 2. Particularly, the azetidine moiety of Fg754 may occupy the sodium ion-binding site by forming a salt bridge [72]. Another molecular dynamics simulation study described the allosteric effects of a mini-Gs protein on A_{2A}Rs [87].

In conclusion, the effects of amiloride and its derivatives on A_{2A}Rs are well studied. While the findings indicate that amiloride competes with sodium ions at the allosteric site of the A_{2A}R, with Asp being the crucial amino acid, the allosteric binding site(s) of other small molecules selective for A_{2A}Rs remain unknown.

6. Allosteric A_{2A}R Modulators and Their Potential Clinical Application

Allosteric A_{2A}R modulation could be a new target for drug discovery [88]. Allosteric modulators can selectively elicit a physiologic response where and when the orthosteric ligand is released, thereby reducing the risk of an adverse effect of A_{2A}R activation. Moreover, the possibility of saturating allosteric effects offers greater potential for fine-tuning the physiologic response in a positive or negative direction. As allosteric modulators have no pharmacologic effect beyond the saturation dose, these molecules are associated with a lower risk for adverse effects than orthosteric ligands, giving them a potential therapeutic advantage over classical agonists and antagonists [18,89].

Some compounds act as allosteric A_{2A}R modulators, such as sodium ions, amiloride, and potassium-sparing diuretics, that also modulate other GPCRs than A_{2A}Rs [90]. For example, PD120918 is reported to enhance the activity of A_{2A}R agonists in the rat striatum [91]. In contrast, thiadiazoles such as SCH-202676 alter the binding characteristics A_{2A}R agonists and antagonists [92]. Some studies, however, suggest that thiadiazoles act as binding or oxidizing agents for SH groups rather than as allosteric modulators [92]. To date, only a relatively small number of selective allosteric A_{2A}R modulators have been reported (Table 1) [93].

Table 1. Allosteric $A_{2A}R$ modulators and their functions.

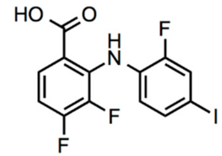
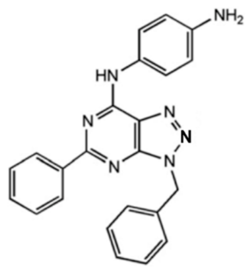
Name	Type	Pharmacology	Structure	Physiologic Effects
3,4-Difluoro-2-((2-fluoro-4-iodophenyl)amino)benzoic acid	Allosteric enhancer/modulator	Enhanced adenosine signaling at mouse $A_{2A}Rs$.		Induced slow wave sleep without affecting cardiovascular function or body temperature in wild-type male mice [94,95].
AEA061	Allosteric enhancer/modulator	Enhanced adenosine and inosine signaling and increased effect of the $A_{2A}R$ agonist CGS 21680.	Not disclosed	Inhibited the production of tumor necrosis factor-α, macrophage inflammatory protein-1α, 1β, and 2, interleukin-1α, keratinocyte chemokine, and RANTES (regulated upon activation, normal T cell expressed and presumably secreted) in macrophages and splenocytes, reduced circulating plasma tumor necrosis factor-α and monocyte chemoattractant protein-1 levels, and increased plasma interleukin-10 during lipopolysaccharide-induced endotoxemia [96,97].
N-(3-Benzyl-5-phenyl-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7yl-)-(4-aminophenyl)-amine	Allosteric modulator	Inhibited the binding of antagonists and agonists at the $A_{2A}R$ orthosteric site [93].		Unknown

Table 1. *Cont.*

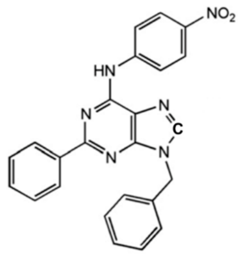
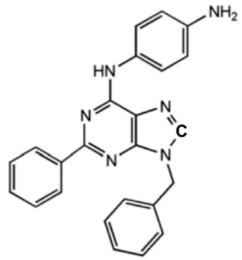
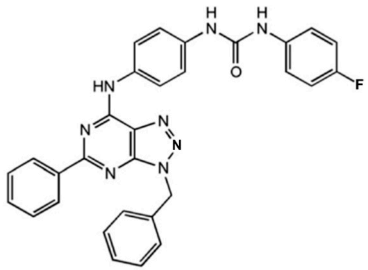
Name	Type	Pharmacology	Structure	Physiologic Effects
N^6-[(4-Nitro)-phenyl]-9-benzyl-2-phenyladenine	Allosteric modulator	Inhibited the binding of antagonists and agonists at the $A_{2A}R$ orthosteric site [93].		Unknown
N^6-[(4-Amino)-phenyl]-9-benzyl-2-phenyladenine	Allosteric modulator	Inhibited the binding of antagonists and agonists at the $A_{2A}R$ orthosteric site [93].		Unknown
1-[4-(3-Benzyl-5-phenyl-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7-ylamino)-phenyl]-3-(4-fluorophenyl)-urea	Allosteric modulator	Modulated the binding of antagonist and agonist at the $A_{2A}R$ orthosteric site [93].		Unknown

Table 1. *Cont.*

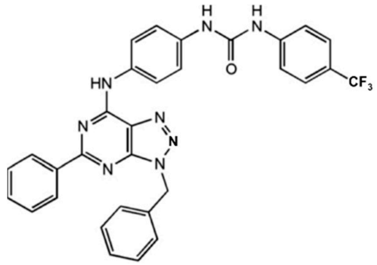
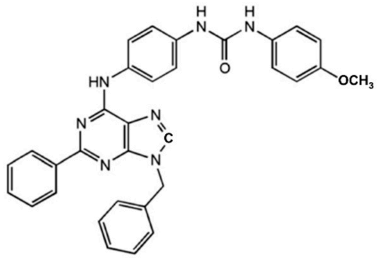
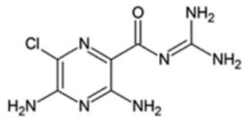
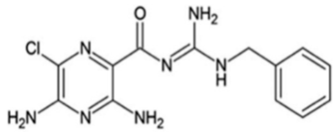
Name	Type	Pharmacology	Structure	Physiologic Effects
1-[4-(3-Benzyl-5-phenyl-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7-ylamino)-phenyl]-3-(4-trifluoromethylphenyl)-urea	Allosteric modulator	Modulated the binding of antagonist and agonist at the $A_{2A}R$ orthosteric site [93].		Unknown
1-[4-(9-Benzyl-2-phenyl-9H-purin-6-ylamino)-phenyl]-3-(4-methoxyphenyl-urea	Allosteric modulator	Modulated the binding of antagonist and agonist at the $A_{2A}R$ orthosteric site [93].		Unknown
Amiloride	Allosteric modulator	Increased the dissociation rate of the antagonist ZM-241,385 at rat $A_{2A}Rs$ [18,71].		Unknown
Benzamil	Allosteric modulator	Increased the dissociation rate of the antagonist ZM-241,385 at rat $A_{2A}Rs$ [71].		Unknown

Table 1. *Cont.*

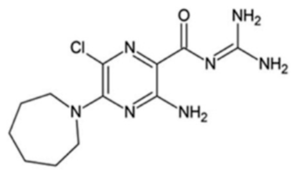
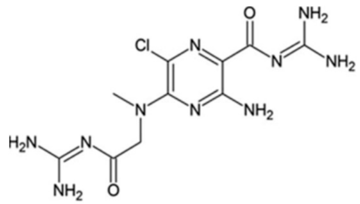
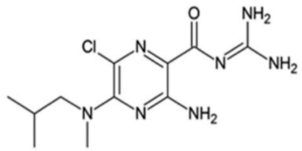
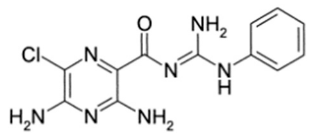
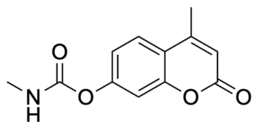
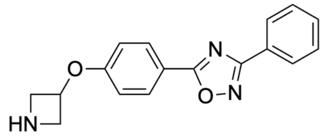
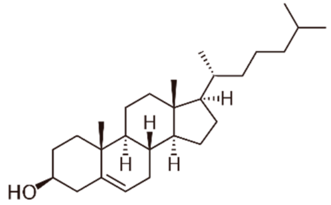
Name	Type	Pharmacology	Structure	Physiologic Effects
HMA; 5-(N,N-hexamethylene)amiloride	Allosteric modulator	Increased the dissociation rate of the antagonist ZM-241,385 at rat A_{2A}Rs [71].		Unknown
MGCMA; 5-(N-methyl-N-guanidinocarbonyl-methyl)amiloride	Allosteric modulator	Increased the dissociation rate of the antagonist ZM-241,385 at rat A_{2A}Rs [71].		Unknown
MIBA; 5-(N-methyl-N-isobutyl)amiloride	Allosteric modulator	Increased the dissociation rate of the antagonist ZM-241,385 at rat A_{2A}Rs [71].		Unknown
Phenamil	Allosteric modulator	Increased the dissociation rate of the antagonist ZM-241,385 at rat A_{2A}Rs [71].		Unknown
Sodium Ion	Allosteric modulator	Positively modulated A_{2A}Rs [71].	Na^+	Unknown
PD120918 {4-methyl-7-[(methyl-amino)carbonyl]oxy}-2H-1-benzopyran-2-one}	Allosteric modulator	Enhanced agonist radioligand binding to rat striatal A_{2A}Rs without functional enhancement [18,91].		Unknown

Table 1. *Cont.*

Name	Type	Pharmacology	Structure	Physiologic Effects
Fg754	Allosteric modulator	Increased the dissociation rate of the agonist CGS21680 at A_{2A}Rs expressing HEK-293 cells [72].		Unknown
Cholesterol	Allosteric modulator	Decreased the dissociation rate of the agonist NECA at A_{2A}Rs-embedded nanodiscs [73].		Unknown

6.1. Allosteric $A_{2A}R$ Modulation Related to Inflammation

Adenosine is present in high concentrations in inflamed areas due to cell activation and breakdown [98–100]. The intracellular concentration of cAMP has a regulatory role in immune and inflammatory cells [101] and specifically, A_{2A}Rs are responsible for the anti-inflammatory effects of adenosine [102,103]. The anti-inflammatory effects of A_{2A}R agonists are well known. Their therapeutic benefit, however, is not a given due to the potential adverse effects of A_{2A}R agonists following systemic administration [7].

AEA061, which has an undisclosed structure, promotes the anti-inflammatory effects of adenosine by allosterically enhancing the activity of endogenous adenosine at A_{2A}Rs [96]. AEA061, which has no activity at a rat or human A_{2A}Rs in the absence of adenosine, inhibits the production of cytokines such as interleukin-1α, macrophage inflammatory protein-1α, 1β, and 2, keratinocyte chemokine, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), and tumor necrosis factor-α in monocytes and splenocytes in a mouse model of lipopolysaccharide-induced inflammation. Therefore, positive allosteric modulators of A_{2A}Rs may represent a potential therapeutic approach to inflammation.

Inosine and inosine analog 6-S-[(4-nitrophenyl)methyl]-6-thioinosine (NBMPR) selectively and dose-dependently activate human A_{2A}Rs. NBMPR and inosine inhibit the production of pro-inflammatory cytokines and chemokines in splenic monocytes of wild-type mice, but not A_{2A}R knockout mice. The positive allosteric A_{2A}R modulator AEA061 enhances inosine-mediated A_{2A}R activation, inosine-mediated inhibition of pro-inflammatory cytokines, and chemokine production by splenic monocytes [97].

6.2. Allosteric $A_{2A}R$ Modulation Related to Sleep and Neurologic Disorders

A_{2A}Rs are also expressed in the CNS, with the highest levels in the ventral and dorsal striatum [104]. A_{2A}Rs are present in the pre/postsynaptic compartment of neurons and microglia, oligodendrocytes, astrocytes, and capillary endothelial cells [12,105–110]. A growing number of reports illustrate that A_{2A}Rs play a critical role in emotional and cognitive processes, motivation, and voluntary movements [111]. Moreover, A_{2A}R-expressing neurons in the NAc regulate sleep [8,47,112]. Therefore, A_{2A}R stimulation should be considered a potential treatment approach for insomnia. Insomnia is a sleep disorder that affects millions of people worldwide and frequently co-occurs with a wide range of psychiatric disorders [113–115] Although A_{2A}R agonists have strong sleep-inducing effects [116–119], they also have adverse cardiovascular effects and thus cannot be used clinically to treat sleep disorders. Moreover, the development of adenosine analogs to treat CNS disorders, including insomnia, is hampered by the poor transport of these drugs across the blood–brain barrier. In mice, a small blood–brain barrier-permeable monocarboxylate (3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino) benzoic acid, denoted as A_{2A}R PAM-1, was recently found to induce sleep by enhancing A_{2A}R signaling in the brain (Figure 3) but, surprisingly, did not exhibit the typical unwanted cardiovascular and body temperature effects of A_{2A}R agonists [94,95]. More specifically, A_{2A}R PAM-1 dose-dependently enhanced A_{2A}R signaling in A_{2A}R-expressing Chinese hamster ovary (CHO) cells but not in CHO cells lacking A_{2A}R expression or in the absence of adenosine (Figure 3). The A_{2A}R PAM-1 did not alter the activity of the A_{2A}R agonist CGS 21680 [120]. Intracerebroventricular infusion and intraperitoneal injection of A_{2A}R PAM-1 induced prolonged slow-wave sleep, but not rapid-eye-movement sleep, in wild-type mice, but not A_{2A}R knockout mice. Further testing revealed that A_{2A}R PAM-1, unlike A_{2A}R agonists, had no effects on blood pressure, cardiac function, or body temperature, suggesting that adenosine or A_{2A}R expression levels in the cardiovascular system are insufficient to elicit an A_{2A}R PAM-1 response under normal physiologic conditions. Therefore, molecules that allosterically enhance A_{2A}R signaling may be developed to help people with insomnia fall asleep more easily. Moreover, A_1Rs play a crucial role in the resolution of sleep need by modulating slow-wave activity, a slow, oscillatory neocortical activity that intensifies in correlation with wake duration and declines during sleep [121]. Slow-wave activity is widely used as a marker of mammalian sleep homeostasis and is necessary for sleep function. Therefore, dual allosteric $A_1R/A_{2A}R$ modulators may be useful for improving not only the maintenance of sleep but also its function.

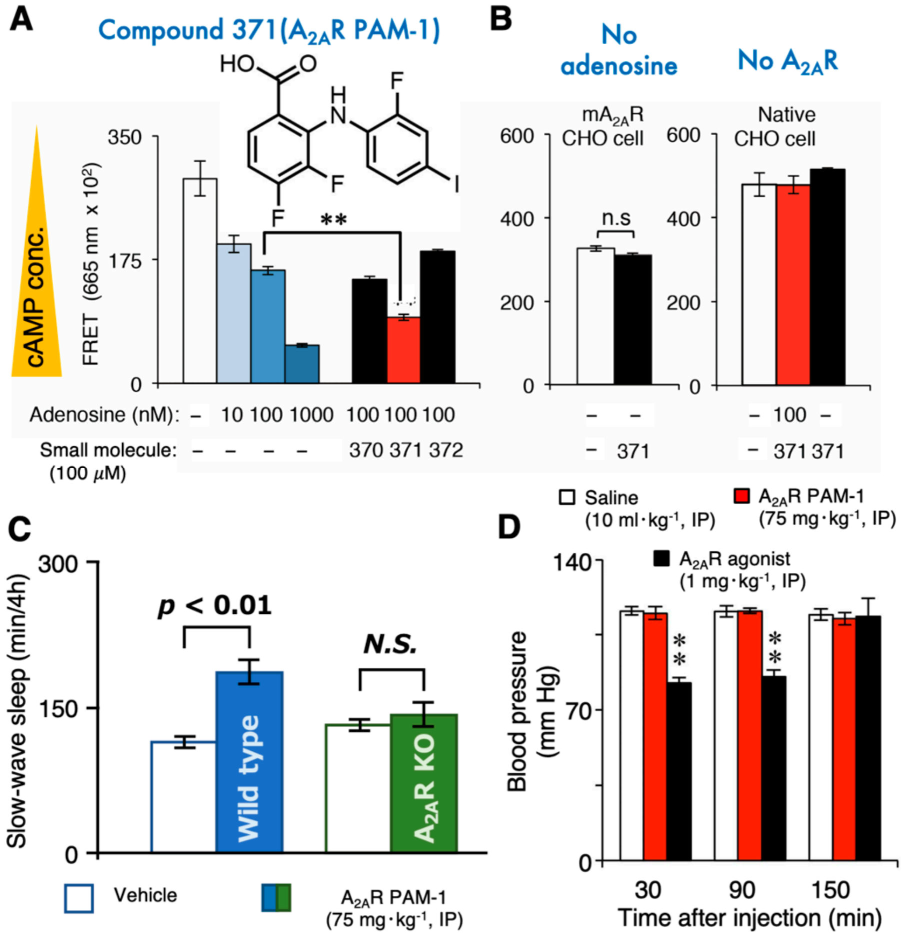


Figure 3. The $A_{2A}R$ positive allosteric modulator (PAM)-1 induces sleep without cardiovascular effects. (**A**,**B**) $A_{2A}R$ PAM-1 enhanced the activity of adenosine on $A_{2A}R$-expressing Chinese Hamster Ovary (CHO) cells when cAMP was measured by a fluorescence energy transfer (FRET) immunoassay (**A**), whereas $A_{2A}R$ PAM-1 did not enhance cAMP production without adenosine or in native CHO cells without $A_{2A}R$ expression (**B**). (**C**) Intraperitoneal (IP) injection of $A_{2A}R$ PAM-1 increased slow-wave sleep in wild-type mice, but not in $A_{2A}R$-knockout (KO) mice. (**D**) $A_{2A}R$ PAM-1 did not affect cardiovascular functions (e.g., blood pressure), unlike a classic $A_{2A}R$ agonist (CGS 21680) [94,95]. ** $p < 0.01$.

$A_{2A}Rs$ have roles in neurodegenerative, neurodevelopmental, and psychiatric diseases. The potential therapeutic use of $A_{2A}R$ agonists and antagonists for specific conditions such as Niemann Pick disease, schizophrenia, autism-spectrum disorders, depression, anxiety, Alzheimer's disease, attention-deficit hyperactivity disorder, PD, and fragile X syndrome is comprehensively discussed in the literature [122]. Allosteric $A_{2A}R$ modulators may provide alternative therapeutic options for neurologic disorders to circumvent the complexity of central and peripheral adenosine signaling. For example, dopamine-replacement therapy in PD is potentiated by blocking $A_{2A}Rs$ due to the adenosine-dopamine antagonism in the striatum [123]. Decade-long preclinical studies of $A_{2A}R$ antagonists in PD models led to clinical trials of the $A_{2A}R$ antagonist istradefylline, which confirmed its clinically significant motor benefit in advanced PD patients and resulted in the approval of istradefylline for the treatment of PD patients in Japan and the US. The complexity of adenosine signaling contributed at least partially to the debilitating side effects and suspension of the clinical phase III trial of the $A_{2A}R$ antagonist tozadenant for PD, which resulted in the death of five patients due to inflammatory complications. Thus, there is also a critical need to develop safer and more effective means of suppressing $A_{2A}R$ signaling; for example, by negative allosteric modulation. Whereas the most potent PD medication is levodopa (L-3,4-dihydroxyphenylalanine), clinicians try to limit levodopa doses to the extent possible to avoid various adverse effects occurring with chronic use, such as dyskinesia and dopamine dysregulation. $A_{2A}R$ PAM, when administered concomitantly with levodopa, may mitigate some of these side effects, but strong evidence is currently lacking.

In addition, positive allosteric modulators of $A_{2A}Rs$ may also alleviate various symptoms in neuropsychologic disorders. For example, psychotic symptoms such as delusions are caused by impaired discrimination of environmental stimuli. Recent evidence shows that D_2Rs mediate discrimination learning in the NAc, but $A_{2A}Rs$ expressed together with D_2Rs in the NAc are required for discrimination learning. While normal mice can discriminate between reward-predictive and non-reward-predictive tones several days after generalized reward conditioning (when any tone is reward-predictive), mice in which $A_{2A}Rs$ are blocked in the NAc do not show this ability [124]. In addition, hypofunction of NMDA-type glutamate receptors is thought to be involved in schizophrenia, as NMDA receptor antagonists such as phencyclidine and dizocilpine (MK-801) cause psychotic and cognitive disorders in humans and animals [125]. Deleting $A_{2A}Rs$ in NAc astrocytes leads to motor and memory impairments relevant to schizophrenia, namely exacerbation of the MK-801-induced psychomotor response and impaired working memory [126]. Thus, the enhancement of $A_{2A}R$ signaling may be helpful to treat sleep disorders as well as schizophrenia and other psychotic disorders by overcoming dopaminergic hyperactivity or glutamatergic hypoactivity.

7. Concluding Remarks

Here, we discussed recent developments regarding allosteric $A_{2A}R$ modulation. Although numerous allosteric modulators of $A_{2A}Rs$ have been identified, the physiologic functions of only a few of them have been established. The sleep-promoting effects and inflammatory process-modulating roles of allosteric $A_{2A}R$ modulators open the doors for the potential therapeutic use of these molecules for treating diseases. Allosteric modulators exert their effects only where and when the orthosteric ligand is released, conferring a potential therapeutic advantage over classical antagonists and agonist molecules. Thus, allosteric $A_{2A}R$ modulation could provide patients with an effective and safe treatment for various diseases.

Finally, $A_{2A}Rs$ form heterodimer structures with other receptors such as D_2Rs and mGluR5 in the CNS. Receptor heterodimers may be an applicable target for developing $A_{2A}R$ PAMs with high specificity for the heterodimer and thus limited adverse effects [38,127–131].

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References

1. Adair, T.H. Growth Regulation of the Vascular System: An Emerging Role for Adenosine. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2005**, *289*, R283–R296. [CrossRef] [PubMed]
2. Chen, J.-F. Adenosine Receptor Control of Cognition in Normal and Disease. *Int. Rev. Neurobiol.* **2014**, *119*, 257–307. [CrossRef] [PubMed]
3. Feoktistov, I.; Biaggioni, I.; Cronstein, B.N. Adenosine Receptors in Wound Healing, Fibrosis and Angiogenesis. *Handb. Exp. Pharmacol.* **2009**, 383–397. [CrossRef]
4. Headrick, J.P.; Ashton, K.J.; Rose'meyer, R.B.; Peart, J.N. Cardiovascular Adenosine Receptors: Expression, Actions and Interactions. *Pharmacol. Ther.* **2013**, *140*, 92–111. [CrossRef] [PubMed]
5. Hein, T.W.; Wang, W.; Zoghi, B.; Muthuchamy, M.; Kuo, L. Functional and Molecular Characterization of Receptor Subtypes Mediating Coronary Microvascular Dilation to Adenosine. *J. Mol. Cell. Cardiol.* **2001**, *33*, 271–282. [CrossRef] [PubMed]
6. Lazarus, M.; Chen, J.-F.; Huang, Z.-L.; Urade, Y.; Fredholm, B.B. Adenosine and Sleep. *Handb. Exp. Pharmacol.* **2019**, *253*, 359–381. [CrossRef]
7. Ohta, A.; Sitkovsky, M. Role of G-Protein-Coupled Adenosine Receptors in Downregulation of Inflammation and Protection from Tissue Damage. *Nature* **2001**, *414*, 916–920. [CrossRef]
8. Zhou, X.; Oishi, Y.; Cherasse, Y.; Korkutata, M.; Fujii, S.; Lee, C.-Y.; Lazarus, M. Extracellular Adenosine and Slow-Wave Sleep Are Increased after Ablation of Nucleus Accumbens Core Astrocytes and Neurons in Mice. *Neurochem. Int.* **2019**, *124*, 256–263. [CrossRef]
9. Chin, J.H. Adenosine Receptors in Brain: Neuromodulation and Role in Epilepsy. *Ann. Neurol.* **1989**, *26*, 695–698. [CrossRef]
10. Ciruela, F.; Casadó, V.; Rodrigues, R.J.; Luján, R.; Burgueño, J.; Canals, M.; Borycz, J.; Rebola, N.; Goldberg, S.R.; Mallol, J.; et al. Presynaptic Control of Striatal Glutamatergic Neurotransmission by Adenosine A1-A2A Receptor Heteromers. *J. Neurosci.* **2006**, *26*, 2080–2087. [CrossRef]
11. Kamikubo, Y.; Shimomura, T.; Fujita, Y.; Tabata, T.; Kashiyama, T.; Sakurai, T.; Fukurotani, K.; Kano, M. Functional Cooperation of Metabotropic Adenosine and Glutamate Receptors Regulates Postsynaptic Plasticity in the Cerebellum. *J. Neurosci.* **2013**, *33*, 18661–18671. [CrossRef] [PubMed]
12. Matos, M.; Augusto, E.; Santos-Rodrigues, A.D.; Schwarzschild, M.A.; Chen, J.-F.; Cunha, R.A.; Agostinho, P. Adenosine A2A Receptors Modulate Glutamate Uptake in Cultured Astrocytes and Gliosomes. *Glia* **2012**, *60*, 702–716. [CrossRef] [PubMed]
13. Fredholm, B.B.; Ijzerman, A.P.; Jacobson, K.A.; Linden, J.; Müller, C.E. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and Classification of Adenosine Receptors—An Update. *Pharmacol. Rev.* **2011**, *63*, 1–34. [CrossRef] [PubMed]
14. Fredholm, B.B.; Ijzerman, A.P.; Jacobson, K.A.; Klotz, K.N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and Classification of Adenosine Receptors. *Pharmacol. Rev.* **2001**, *53*, 527–552.
15. Chen, J.-F.; Eltzschig, H.K.; Fredholm, B.B. Adenosine Receptors as Drug Targets—What Are the Challenges? *Nat. Rev. Drug Discov.* **2013**, *12*, 265–286. [CrossRef]
16. de Lera Ruiz, M.; Lim, Y.-H.; Zheng, J. Adenosine A2A Receptor as a Drug Discovery Target. *J. Med. Chem.* **2014**, *57*, 3623–3650. [CrossRef]
17. Göblyös, A.; Ijzerman, A.P. Allosteric Modulation of Adenosine Receptors. *Purinergic Signal.* **2009**, *5*, 51–61. [CrossRef]
18. Gao, Z.-G.; Kim, S.-K.; Ijzerman, A.P.; Jacobson, K.A. Allosteric Modulation of the Adenosine Family of Receptors. *Mini Rev. Med. Chem.* **2005**, *5*, 545–553. [CrossRef]
19. Drury, A.N.; Szent-Györgyi, A. The Physiological Activity of Adenine Compounds with Especial Reference to their Action upon the Mammalian Heart. *J. Physiol.* **1929**, *68*, 213–237. [CrossRef]
20. Haskó, G.; Pacher, P.; Deitch, E.A.; Vizi, E.S. Shaping of Monocyte and Macrophage Function by Adenosine Receptors. *Pharmacol. Ther.* **2007**, *113*, 264–275. [CrossRef] [PubMed]
21. Sattin, A.; Rall, T.W. The Effect of Adenosine and Adenine Nucleotides on the Cyclic Adenosine 3′, 5′-Phosphate Content of Guinea Pig Cerebral Cortex Slices. *Mol. Pharmacol.* **1970**, *6*, 13–23.
22. Fredholm, B.B. Adenosine, an Endogenous Distress Signal, Modulates Tissue Damage and Repair. *Cell Death Differ.* **2007**, *14*, 1315–1323. [CrossRef] [PubMed]
23. Schrader, J. Metabolism of Adenosine and Sites of Production in the Heart. In *Regulatory Function of Adenosine, Proceedings of the International Symposium on Adenosine, Charlottesville, Virginia, 7–11 June 1982*; Berne, R.M., Rall, T.W., Rubio, R., Eds.; Developments in Pharmacology 2; Springer: Boston, MA, USA, 1983; pp. 133–156, ISBN 978-1-4613-3909-0.
24. Ballarín, M.; Fredholm, B.B.; Ambrosio, S.; Mahy, N. Extracellular Levels of Adenosine and its Metabolites in the Striatum of Awake Rats: Inhibition of Uptake and Metabolism. *Acta Physiol. Scand.* **1991**, *142*, 97–103. [CrossRef] [PubMed]
25. Boison, D.; Singer, P.; Shen, H.-Y.; Feldon, J.; Yee, B.K. Adenosine Hypothesis of Schizophrenia—Opportunities for Pharmacotherapy. *Neuropharmacology* **2012**, *62*, 1527–1543. [CrossRef] [PubMed]
26. Cacciari, B.; Pastorin, G.; Bolcato, C.; Spalluto, G.; Bacilieri, M.; Moro, S. A2B Adenosine Receptor Antagonists: Recent Developments. *Mini Rev. Med. Chem.* **2005**, *5*, 1053–1060. [CrossRef] [PubMed]
27. Burnstock, G.; Cocks, T.; Crowe, R.; Kasakov, L. Purinergic Innervation of the Guinea-Pig Urinary Bladder. *Br. J. Pharmacol.* **1978**, *63*, 125–138. [CrossRef]

28. Matsumoto, T.; Tostes, R.C.; Webb, R.C. Alterations in Vasoconstrictor Responses to the Endothelium-Derived Contracting Factor Uridine Adenosine Tetraphosphate Are Region Specific in DOCA-Salt Hypertensive Rats. *Pharmacol. Res.* **2012**, *65*, 81–90. [CrossRef]
29. Fredholm, B.B.; Chen, J.-F.; Cunha, R.A.; Svenningsson, P.; Vaugeois, J.-M. Adenosine and Brain Function. *Int. Rev. Neurobiol.* **2005**, *63*, 191–270. [CrossRef]
30. Maenhaut, C.; Van Sande, J.; Libert, F.; Abramowicz, M.; Parmentier, M.; Vanderhaegen, J.J.; Dumont, J.E.; Vassart, G.; Schiffmann, S. RDC8 Codes for an Adenosine A2 Receptor with Physiological Constitutive Activity. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 1169–1178. [CrossRef]
31. Chern, Y.; King, K.; Lai, H.L.; Lai, H.T. Molecular Cloning of a Novel Adenosine Receptor Gene from Rat Brain. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 304–309. [CrossRef]
32. Furlong, T.J.; Pierce, K.D.; Selbie, L.A.; Shine, J. Molecular Characterization of a Human Brain Adenosine A2 Receptor. *Brain Res. Mol. Brain Res.* **1992**, *15*, 62–66. [CrossRef]
33. Ledent, C.; Vaugeois, J.M.; Schiffmann, S.N.; Pedrazzini, T.; El Yacoubi, M.; Vanderhaeghen, J.J.; Costentin, J.; Heath, J.K.; Vassart, G.; Parmentier, M. Aggressiveness, Hypoalgesia and High Blood Pressure in Mice Lacking the Adenosine A2a Receptor. *Nature* **1997**, *388*, 674–678. [CrossRef] [PubMed]
34. Meng, F.; Xie, G.X.; Chalmers, D.; Morgan, C.; Watson, S.J.; Akil, H. Cloning and Expression of the A2a Adenosine Receptor from Guinea Pig Brain. *Neurochem. Res.* **1994**, *19*, 613–621. [CrossRef] [PubMed]
35. Kull, B.; Svenningsson, P.; Fredholm, B.B. Adenosine A2A Receptors Are Colocalized with and Activate Golf in Rat Striatum. *Mol. Pharmacol.* **2000**, *58*, 771–777. [CrossRef] [PubMed]
36. Schulte, G.; Fredholm, B.B. Human Adenosine A(1), A(2A), A(2B), and A(3) Receptors Expressed in Chinese Hamster Ovary Cells All Mediate the Phosphorylation of Extracellular-Regulated Kinase 1/2. *Mol. Pharmacol.* **2000**, *58*, 477–482. [CrossRef] [PubMed]
37. Ferré, S.; Goldberg, S.R.; Lluis, C.; Franco, R. Looking for the Role of Cannabinoid Receptor Heteromers in Striatal Function. *Neuropharmacology* **2009**, *56* (Suppl. S1), 226–234. [CrossRef] [PubMed]
38. Ferré, S.; Karcz-Kubicha, M.; Hope, B.T.; Popoli, P.; Burgueño, J.; Gutiérrez, M.A.; Casadó, V.; Fuxe, K.; Goldberg, S.R.; Lluis, C.; et al. Synergistic Interaction between Adenosine A2A and Glutamate MGlu5 Receptors: Implications for Striatal Neuronal Function. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11940–11945. [CrossRef]
39. Fuxe, K.; Ferré, S.; Canals, M.; Torvinen, M.; Terasmaa, A.; Marcellino, D.; Goldberg, S.R.; Staines, W.; Jacobsen, K.X.; Lluis, C.; et al. Adenosine A2A and Dopamine D2 Heteromeric Receptor Complexes and Their Function. *J. Mol. Neurosci.* **2005**, *26*, 209–220. [CrossRef]
40. Navarro, G.; Carriba, P.; Gandía, J.; Ciruela, F.; Casadó, V.; Cortés, A.; Mallol, J.; Canela, E.I.; Lluis, C.; Franco, R. Detection of Heteromers Formed by Cannabinoid CB1, Dopamine D2, and Adenosine A2A G-Protein-Coupled Receptors by Combining Bimolecular Fluorescence Complementation and Bioluminescence Energy Transfer. *Sci. World J.* **2008**, *8*, 1088–1097. [CrossRef]
41. Torvinen, M.; Marcellino, D.; Canals, M.; Agnati, L.F.; Lluis, C.; Franco, R.; Fuxe, K. Adenosine A2A Receptor and Dopamine D3 Receptor Interactions: Evidence of Functional A2A/D3 Heteromeric Complexes. *Mol. Pharmacol.* **2005**, *67*, 400–407. [CrossRef]
42. Grillner, S.; Robertson, B. The Basal Ganglia Over 500 Million Years. *Curr. Biol.* **2016**, *26*, R1088–R1100. [CrossRef] [PubMed]
43. Oishi, Y.; Lazarus, M. The Control of Sleep and Wakefulness by Mesolimbic Dopamine Systems. *Neurosci. Res.* **2017**, *118*, 66–73. [CrossRef] [PubMed]
44. Cui, G.; Jun, S.B.; Jin, X.; Pham, M.D.; Vogel, S.S.; Lovinger, D.M.; Costa, R.M. Concurrent Activation of Striatal Direct and Indirect Pathways during Action Initiation. *Nature* **2013**, *494*, 238–242. [CrossRef] [PubMed]
45. Zhu, X.; Ottenheimer, D.; DiLeone, R.J. Activity of D1/2 Receptor Expressing Neurons in the Nucleus Accumbens Regulates Running, Locomotion, and Food Intake. *Front. Behav. Neurosci.* **2016**, *10*, 66. [CrossRef]
46. Oishi, Y.; Xu, Q.; Wang, L.; Zhang, B.-J.; Takahashi, K.; Takata, Y.; Luo, Y.-J.; Cherasse, Y.; Schiffmann, S.N.; de Kerchove d'Exaerde, A.; et al. Slow-Wave Sleep Is Controlled by a Subset of Nucleus Accumbens Core Neurons in Mice. *Nat. Commun.* **2017**, *8*, 734. [CrossRef]
47. Nonomura, S.; Nishizawa, K.; Sakai, Y.; Kawaguchi, Y.; Kato, S.; Uchigashima, M.; Watanabe, M.; Yamanaka, K.; Enomoto, K.; Chiken, S.; et al. Monitoring and Updating of Action Selection for Goal-Directed Behavior through the Striatal Direct and Indirect Pathways. *Neuron* **2018**, *99*, 1302–1314. [CrossRef]
48. Fredholm, B.B.; Cunha, R.A.; Svenningsson, P. Pharmacology of Adenosine A2A Receptors and Therapeutic Applications. *Curr. Top. Med. Chem.* **2003**, *3*, 413–426. [CrossRef]
49. Ritter, J.; Flower, R.; Henderson, G.; Loke, Y.K.; MacEwan, D.; Rang, H. *Rang & Dale's Pharmacology*, 9th ed.; Elsevier: Amsterdam, The Netherlands, 2018.
50. Neubig, R.R.; Spedding, M.; Kenakin, T.; Christopoulos, A. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on Terms and Symbols in Quantitative Pharmacology. *Pharmacol. Rev.* **2003**, *55*, 597–606. [CrossRef]
51. Hu, J. Allosteric Modulators of the Human Calcium-Sensing Receptor: Structures, Sites of Action, and Therapeutic Potentials. *Endocr. Metab. Immune Disord. Drug Targets* **2008**, *8*, 192–197. [CrossRef]
52. Kenakin, T. *Pharmacology in Drug Discovery and Development*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2016.

53. Koshland, D.E.; Némethy, G.; Filmer, D. Comparison of Experimental Binding Data and Theoretical Models in Proteins Containing Subunits. *Biochemistry* **1966**, *5*, 365–385. [CrossRef]
54. Monod, J.; Wyman, J.; Changeux, J.-P. On the Nature of Allosteric Transitions: A Plausible Model. *J. Mol. Biol.* **1965**, *12*, 88–118. [CrossRef]
55. Monod, J.; Changeux, J.-P.; Jacob, F. Allosteric Proteins and Cellular Control Systems. *J. Mol. Biol.* **1963**, *6*, 306–329. [CrossRef]
56. Estébanez-Perpiñá, E.; Arnold, L.A.; Nguyen, P.; Rodrigues, E.D.; Mar, E.; Bateman, R.; Pallai, P.; Shokat, K.M.; Baxter, J.D.; Guy, R.K.; et al. A Surface on the Androgen Receptor That Allosterically Regulates Coactivator Binding. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16074–16079. [CrossRef] [PubMed]
57. Hughes, T.S.; Giri, P.K.; de Vera, I.M.S.; Marciano, D.P.; Kuruvilla, D.S.; Shin, Y.; Blayo, A.-L.; Kamenecka, T.M.; Burris, T.P.; Griffin, P.R.; et al. An Alternate Binding Site for PPARγ Ligands. *Nat. Commun.* **2014**, *5*, 3571. [CrossRef] [PubMed]
58. Bono, F.; De Smet, F.; Herbert, C.; De Bock, K.; Georgiadou, M.; Fons, P.; Tjwa, M.; Alcouffe, C.; Ny, A.; Bianciotto, M.; et al. Inhibition of Tumor Angiogenesis and Growth by a Small-Molecule Multi-FGF Receptor Blocker with Allosteric Properties. *Cancer Cell* **2013**, *23*, 477–488. [CrossRef]
59. De Smet, F.; Christopoulos, A.; Carmeliet, P. Allosteric Targeting of Receptor Tyrosine Kinases. *Nat. Biotechnol.* **2014**, *32*, 1113–1120. [CrossRef]
60. Catterall, W.A.; Cestèle, S.; Yarov-Yarovoy, V.; Yu, F.H.; Konoki, K.; Scheuer, T. Voltage-Gated Ion Channels and Gating Modifier Toxins. *Toxicon* **2007**, *49*, 124–141. [CrossRef]
61. Olsen, R.W.; Chang, C.-S.S.; Li, G.; Hanchar, H.J.; Wallner, M. Fishing for Allosteric Sites on GABA(A) Receptors. *Biochem. Pharmacol.* **2004**, *68*, 1675–1684. [CrossRef]
62. Spedding, M.; Kenny, B.; Chatelain, P. New Drug Binding Sites in Ca2+ Channels. *Trends Pharmacol. Sci.* **1995**, *16*, 139–142. [CrossRef]
63. Taly, A.; Corringer, P.-J.; Guedin, D.; Lestage, P.; Changeux, J.-P. Nicotinic Receptors: Allosteric Transitions and Therapeutic Targets in the Nervous System. *Nat. Rev. Drug Discov.* **2009**, *8*, 733–750. [CrossRef]
64. Traynelis, S.F.; Wollmuth, L.P.; McBain, C.J.; Menniti, F.S.; Vance, K.M.; Ogden, K.K.; Hansen, K.B.; Yuan, H.; Myers, S.J.; Dingledine, R. Glutamate Receptor Ion Channels: Structure, Regulation, and Function. *Pharmacol. Rev.* **2010**, *62*, 405–496. [CrossRef] [PubMed]
65. Colquhoun, D. Binding, Gating, Affinity and Efficacy: The Interpretation of Structure-Activity Relationships for Agonists and of the Effects of Mutating Receptors. *Br. J. Pharmacol.* **1998**, *125*, 924–947. [CrossRef] [PubMed]
66. Fenton, A.W. Allostery: An Illustrated Definition for the "Second Secret of Life". *Trends Biochem. Sci.* **2008**, *33*, 420–425. [CrossRef] [PubMed]
67. Nussinov, R.; Tsai, C.-J. Allostery in Disease and in Drug Discovery. *Cell* **2013**, *153*, 293–305. [CrossRef] [PubMed]
68. Christopoulos, A.; Changeux, J.-P.; Catterall, W.A.; Fabbro, D.; Burris, T.P.; Cidlowski, J.A.; Olsen, R.W.; Peters, J.A.; Neubig, R.R.; Pin, J.-P.; et al. International Union of Basic and Clinical Pharmacology. XC. Multisite Pharmacology: Recommendations for the Nomenclature of Receptor Allosterism and Allosteric Ligands. *Pharmacol. Rev.* **2014**, *66*, 918–947. [CrossRef]
69. Jacobson, K.A.; Gao, Z.-G. Adenosine Receptors as Therapeutic Targets. *Nat. Rev. Drug Discov.* **2006**, *5*, 247–264. [CrossRef]
70. Gao, Z.-G.; Melman, N.; Erdmann, A.; Kim, S.G.; Müller, C.E.; IJzerman, A.P.; Jacobson, K.A. Differential Allosteric Modulation by Amiloride Analogues of Agonist and Antagonist Binding at A(1) and A(3) Adenosine Receptors. *Biochem. Pharmacol.* **2003**, *65*, 525–534. [CrossRef]
71. Gao, Z.G.; Ijzerman, A.P. Allosteric Modulation of A(2A) Adenosine Receptors by Amiloride Analogues and Sodium Ions. *Biochem. Pharmacol.* **2000**, *60*, 669–676. [CrossRef]
72. Lu, Y.; Liu, H.; Yang, D.; Zhong, L.; Xin, Y.; Zhao, S.; Wang, M.-W.; Zhou, Q.; Shui, W. Affinity Mass Spectrometry-Based Fragment Screening Identified a New Negative Allosteric Modulator of the Adenosine A2A Receptor Targeting the Sodium Ion Pocket. *ACS Chem. Biol.* **2021**, *16*, 991–1002. [CrossRef]
73. Huang, S.K.; Almurad, O.; Pejana, R.J.; Morrison, Z.A.; Pandey, A.; Picard, L.-P.; Nitz, M.; Sljoka, A.; Prosser, R.S. Allosteric Modulation of the Adenosine A2A Receptor by Cholesterol. *eLife* **2022**, *11*, e73901. [CrossRef]
74. Bhattacharya, S.; Youkey, R.L.; Ghartey, K.; Leonard, M.; Linden, J.; Tucker, A.L. The Allosteric Enhancer PD81,723 Increases Chimaeric A1/A2A Adenosine Receptor Coupling with Gs. *Biochem. J.* **2006**, *396*, 139–146. [CrossRef] [PubMed]
75. Gao, Z.-G.; Kim, S.G.; Soltysiak, K.A.; Melman, N.; IJzerman, A.P.; Jacobson, K.A. Selective Allosteric Enhancement of Agonist Binding and Function at Human A3 Adenosine Receptors by a Series of Imidazoquinoline Derivatives. *Mol. Pharmacol.* **2002**, *62*, 81–89. [CrossRef] [PubMed]
76. Gao, Z.G.; Van Muijlwijk-Koezen, J.E.; Chen, A.; Müller, C.E.; Ijzerman, A.P.; Jacobson, K.A. Allosteric Modulation of A(3) Adenosine Receptors by a Series of 3-(2-Pyridinyl)Isoquinoline Derivatives. *Mol. Pharmacol.* **2001**, *60*, 1057–1063. [CrossRef] [PubMed]
77. van Galen, P.J.; Nissen, P.; van Wijngaarden, I.; IJzerman, A.P.; Soudijn, W. 1H-Imidazo[4,5-c]Quinolin-4-Amines: Novel Non-Xanthine Adenosine Antagonists. *J. Med. Chem.* **1991**, *34*, 1202–1206. [CrossRef]
78. Liu, W.; Chun, E.; Thompson, A.A.; Chubukov, P.; Xu, F.; Katritch, V.; Han, G.W.; Roth, C.B.; Heitman, L.H.; IJzerman, A.P.; et al. Structural Basis for Allosteric Regulation of GPCRs by Sodium Ions. *Science* **2012**, *337*, 232–236. [CrossRef]
79. Zhang, C.; Srinivasan, Y.; Arlow, D.H.; Fung, J.J.; Palmer, D.; Zheng, Y.; Green, H.F.; Pandey, A.; Dror, R.O.; Shaw, D.E.; et al. High-Resolution Crystal Structure of Human Protease-Activated Receptor 1. *Nature* **2012**, *492*, 387–392. [CrossRef]

80. Christopher, J.A.; Brown, J.; Doré, A.S.; Errey, J.C.; Koglin, M.; Marshall, F.H.; Myszka, D.G.; Rich, R.L.; Tate, C.G.; Tehan, B.; et al. Biophysical Fragment Screening of the B1-Adrenergic Receptor: Identification of High Affinity Arylpiperazine Leads Using Structure-Based Drug Design. *J. Med. Chem.* **2013**, *56*, 3446–3455. [CrossRef]
81. Miller-Gallacher, J.L.; Nehmé, R.; Warne, T.; Edwards, P.C.; Schertler, G.F.X.; Leslie, A.G.W.; Tate, C.G. The 2.1 Å Resolution Structure of Cyanopindolol-Bound B1-Adrenoceptor Identifies an Intramembrane Na+ Ion That Stabilises the Ligand-Free Receptor. *PLoS ONE* **2014**, *9*, e92727. [CrossRef]
82. Fenalti, G.; Giguere, P.M.; Katritch, V.; Huang, X.-P.; Thompson, A.A.; Cherezov, V.; Roth, B.L.; Stevens, R.C. Molecular Control of δ-Opioid Receptor Signalling. *Nature* **2014**, *506*, 191–196. [CrossRef]
83. Ballesteros, J.A.; Weinstein, H. Integrated Methods for the Construction of Three-Dimensional Models and Computational Probing of Structure-Function Relations in G Protein-Coupled Receptors. In *Receptor Molecular Biology*; Methods in Neurosciences 25; Sealfon, S.C., Ed.; Academic Press: Cambridge, MA, USA, 1995; pp. 366–428.
84. Horstman, D.A.; Brandon, S.; Wilson, A.L.; Guyer, C.A.; Cragoe, E.J.; Limbird, L.E. An Aspartate Conserved among G-Protein Receptors Confers Allosteric Regulation of Alpha 2-Adrenergic Receptors by Sodium. *J. Biol. Chem.* **1990**, *265*, 21590–21595. [CrossRef]
85. White, K.L.; Eddy, M.T.; Gao, Z.-G.; Han, G.W.; Lian, T.; Deary, A.; Patel, N.; Jacobson, K.A.; Katritch, V.; Stevens, R.C. Structural Connection between Activation Microswitch and Allosteric Sodium Site in GPCR Signaling. *Structure* **2018**, *26*, 259–269. [CrossRef] [PubMed]
86. Ye, L.; Van Eps, N.; Zimmer, M.; Ernst, O.P.; Prosser, R.S. Activation of the A2A Adenosine G-Protein-Coupled Receptor by Conformational Selection. *Nature* **2016**, *533*, 265–268. [CrossRef] [PubMed]
87. Renault, P.; Louet, M.; Marie, J.; Labesse, G.; Floquet, N. Molecular Dynamics Simulations of the Allosteric Modulation of the Adenosine A2A Receptor by a Mini-G Protein. *Sci. Rep.* **2019**, *9*, 5495. [CrossRef] [PubMed]
88. Christopoulos, A. Allosteric Binding Sites on Cell-Surface Receptors: Novel Targets for Drug Discovery. *Nat. Rev. Drug Discov.* **2002**, *1*, 198–210. [CrossRef] [PubMed]
89. Christopoulos, A. Advances in G Protein-Coupled Receptor Allostery: From Function to Structure. *Mol. Pharmacol.* **2014**, *86*, 463–478. [CrossRef]
90. Jiang, Q.; Lee, B.X.; Glashofer, M.; van Rhee, A.M.; Jacobson, K.A. Mutagenesis Reveals Structure-Activity Parallels between Human A2A Adenosine Receptors and Biogenic Amine G Protein-Coupled Receptors. *J. Med. Chem.* **1997**, *40*, 2588–2595. [CrossRef]
91. Bruns, R.F.; Fergus, J.H. Allosteric Enhancement of Adenosine A1 Receptor Binding and Function by 2-Amino-3-Benzoylthiophenes. *Mol. Pharmacol.* **1990**, *38*, 939–949.
92. Göblyös, A.; de Vries, H.; Brussee, J.; Ijzerman, A.P. Synthesis and Biological Evaluation of a New Series of 2,3,5-Substituted [1,2,4]-Thiadiazoles as Modulators of Adenosine A1 Receptors and Their Molecular Mechanism of Action. *J. Med. Chem.* **2005**, *48*, 1145–1151. [CrossRef]
93. Giorgi, I.; Biagi, G.; Bianucci, A.M.; Borghini, A.; Livi, O.; Leonardi, M.; Pietra, D.; Calderone, V.; Martelli, A. N6-1,3-Diphenylurea Derivatives of 2-Phenyl-9-Benzyladenines and 8-Azaadenines: Synthesis and Biological Evaluation as Allosteric Modulators of A2A Adenosine Receptors. *Eur. J. Med. Chem.* **2008**, *43*, 1639–1647. [CrossRef]
94. Korkutata, M.; Saitoh, T.; Cherasse, Y.; Ioka, S.; Duo, F.; Qin, R.; Murakoshi, N.; Fujii, S.; Zhou, X.; Sugiyama, F.; et al. Enhancing Endogenous Adenosine A2A Receptor Signaling Induces Slow-Wave Sleep without Affecting Body Temperature and Cardiovascular Function. *Neuropharmacology* **2019**, *144*, 122–132. [CrossRef]
95. Korkutata, M.; Saitoh, T.; Feng, D.; Murakoshi, N.; Sugiyama, F.; Cherasse, Y.; Nagase, H.; Lazarus, M. Allosteric Modulation of Adenosine A2A Receptors in Mice Induces Slow-Wave Sleep without Cardiovascular Effects. *Sleep Med.* **2017**, *40*, e181. [CrossRef]
96. Welihinda, A.A.; Amento, E.P. Positive Allosteric Modulation of the Adenosine A2a Receptor Attenuates Inflammation. *J. Inflamm.* **2014**, *11*, 37. [CrossRef] [PubMed]
97. Welihinda, A.A.; Kaur, M.; Raveendran, K.S.; Amento, E.P. Enhancement of Inosine-Mediated A2AR Signaling through Positive Allosteric Modulation. *Cell. Signal.* **2018**, *42*, 227–235. [CrossRef] [PubMed]
98. Martin, C.; Leone, M.; Viviand, X.; Ayem, M.L.; Guieu, R. High Adenosine Plasma Concentration as a Prognostic Index for Outcome in Patients with Septic Shock. *Crit. Care Med.* **2000**, *28*, 3198–3202. [CrossRef] [PubMed]
99. Sottofattori, E.; Anzaldi, M.; Ottonello, L. HPLC Determination of Adenosine in Human Synovial Fluid. *J. Pharm. Biomed. Anal.* **2001**, *24*, 1143–1146. [CrossRef]
100. Sperlágh, B.; Dóda, M.; Baranyi, M.; Haskó, G. Ischemic-like Condition Releases Norepinephrine and Purines from Different Sources in Superfused Rat Spleen Strips. *J. Neuroimmunol.* **2000**, *111*, 45–54. [CrossRef]
101. Livingston, M.; Heaney, L.G.; Ennis, M. Adenosine, Inflammation and Asthma—A Review. *Inflamm. Res.* **2004**, *53*, 171–178. [CrossRef]
102. Haskó, G.; Cronstein, B.N. Adenosine: An Endogenous Regulator of Innate Immunity. *Trends Immunol.* **2004**, *25*, 33–39. [CrossRef]
103. Sitkovsky, M.V. Use of the A(2A) Adenosine Receptor as a Physiological Immunosuppressor and to Engineer Inflammation In Vivo. *Biochem. Pharmacol.* **2003**, *65*, 493–501. [CrossRef]
104. Rosin, D.L.; Hettinger, B.D.; Lee, A.; Linden, J. Anatomy of Adenosine A2A Receptors in Brain: Morphological Substrates for Integration of Striatal Function. *Neurology* **2003**, *61*, S12–S18. [CrossRef]

105. Carman, A.J.; Mills, J.H.; Krenz, A.; Kim, D.-G.; Bynoe, M.S. Adenosine Receptor Signaling Modulates Permeability of the Blood–Brain Barrier. *J. Neurosci.* **2011**, *31*, 13272–13280. [CrossRef] [PubMed]
106. Melani, A.; Cipriani, S.; Vannucchi, M.G.; Nosi, D.; Donati, C.; Bruni, P.; Giovannini, M.G.; Pedata, F. Selective Adenosine A2a Receptor Antagonism Reduces JNK Activation in Oligodendrocytes after Cerebral Ischaemia. *Brain J. Neurol.* **2009**, *132*, 1480–1495. [CrossRef] [PubMed]
107. Mills, J.H.; Alabanza, L.; Weksler, B.B.; Couraud, P.-O.; Romero, I.A.; Bynoe, M.S. Human Brain Endothelial Cells Are Responsive to Adenosine Receptor Activation. *Purinergic Signal.* **2011**, *7*, 265–273. [CrossRef] [PubMed]
108. Nishizaki, T.; Nagai, K.; Nomura, T.; Tada, H.; Kanno, T.; Tozaki, H.; Li, X.X.; Kondoh, T.; Kodama, N.; Takahashi, E.; et al. A New Neuromodulatory Pathway with a Glial Contribution Mediated via A(2a) Adenosine Receptors. *Glia* **2002**, *39*, 133–147. [CrossRef]
109. Saura, J.; Angulo, E.; Ejarque, A.; Casadó, V.; Tusell, J.M.; Moratalla, R.; Chen, J.-F.; Schwarzschild, M.A.; Lluis, C.; Franco, R.; et al. Adenosine A2A Receptor Stimulation Potentiates Nitric Oxide Release by Activated Microglia. *J. Neurochem.* **2005**, *95*, 919–929. [CrossRef]
110. Yu, L.; Shen, H.-Y.; Coelho, J.E.; Araújo, I.M.; Huang, Q.-Y.; Day, Y.-J.; Rebola, N.; Canas, P.M.; Rapp, E.K.; Ferrara, J.; et al. Adenosine A2A Receptor Antagonists Exert Motor and Neuroprotective Effects by Distinct Cellular Mechanisms. *Ann. Neurol.* **2008**, *63*, 338–346. [CrossRef]
111. Chen, J.-F.; Lee, C.; Chern, Y. Adenosine Receptor Neurobiology: Overview. *Int. Rev. Neurobiol.* **2014**, *119*, 1–49. [CrossRef]
112. Lazarus, M.; Shen, H.-Y.; Cherasse, Y.; Qu, W.-M.; Huang, Z.-L.; Bass, C.E.; Winsky-Sommerer, R.; Semba, K.; Fredholm, B.B.; Boison, D.; et al. Arousal Effect of Caffeine Depends on Adenosine A2A Receptors in the Shell of the Nucleus Accumbens. *J. Neurosci.* **2011**, *31*, 10067–10075. [CrossRef]
113. Roth, T. Insomnia: Definition, Prevalence, Etiology, and Consequences. *J. Clin. Sleep Med.* **2007**, *3*, S7–S10. [CrossRef]
114. de Zambotti, M.; Goldstone, A.; Colrain, I.M.; Baker, F.C. Insomnia Disorder in Adolescence: Diagnosis, Impact, and Treatment. *Sleep Med. Rev.* **2018**, *39*, 12–24. [CrossRef]
115. Seow, L.S.E.; Abdin, E.; Chang, S.; Chong, S.A.; Subramaniam, M. Identifying the Best Sleep Measure to Screen Clinical Insomnia in a Psychiatric Population. *Sleep Med.* **2018**, *41*, 86–93. [CrossRef] [PubMed]
116. Methippara, M.M.; Kumar, S.; Alam, M.N.; Szymusiak, R.; McGinty, D. Effects on Sleep of Microdialysis of Adenosine A1 and A2a Receptor Analogs into the Lateral Preoptic Area of Rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2005**, *289*, R1715–R1723. [CrossRef] [PubMed]
117. Satoh, S.; Matsumura, H.; Koike, N.; Tokunaga, Y.; Maeda, T.; Hayaishi, O. Region-Dependent Difference in the Sleep-Promoting Potency of an Adenosine A2A Receptor Agonist. *Eur. J. Neurosci.* **1999**, *11*, 1587–1597. [CrossRef] [PubMed]
118. Scammell, T.E.; Gerashchenko, D.Y.; Mochizuki, T.; McCarthy, M.T.; Estabrooke, I.V.; Sears, C.A.; Saper, C.B.; Urade, Y.; Hayaishi, O. An Adenosine A2a Agonist Increases Sleep and Induces Fos in Ventrolateral Preoptic Neurons. *Neuroscience* **2001**, *107*, 653–663. [CrossRef]
119. Urade, Y.; Eguchi, N.; Qu, W.-M.; Sakata, M.; Huang, Z.-L.; Chen, J.-F.; Schwarzschild, M.A.; Fink, J.S.; Hayaishi, O. Sleep Regulation in Adenosine A2A Receptor-Deficient Mice. *Neurology* **2003**, *61*, S94–S96. [CrossRef]
120. Mustafa, K. A Potential Treatment for Insomnia by Positive Allosteric Modulation of Adenosine A2A Receptors. Ph.D. Thesis, University of Tsukuba, Tsukuba, Japan, 2019.
121. Lazarus, M.; Oishi, Y.; Bjorness, T.E.; Greene, R.W. Gating and the Need for Sleep: Dissociable Effects of Adenosine A1 and A2A Receptors. *Front. Neurosci.* **2019**, *13*, 740. [CrossRef]
122. Domenici, M.R.; Ferrante, A.; Martire, A.; Chiodi, V.; Pepponi, R.; Tebano, M.T.; Popoli, P. Adenosine A2A Receptor as Potential Therapeutic Target in Neuropsychiatric Disorders. *Pharmacol. Res.* **2019**, *147*, 104338. [CrossRef]
123. Franco, R.; Navarro, G. Adenosine A2A Receptor Antagonists in Neurodegenerative Diseases: Huge Potential and Huge Challenges. *Front. Psychiatry* **2018**, *9*, 68. [CrossRef]
124. Iino, Y.; Sawada, T.; Yamaguchi, K.; Tajiri, M.; Ishii, S.; Kasai, H.; Yagishita, S. Dopamine D2 Receptors in Discrimination Learning and Spine Enlargement. *Nature* **2020**, *579*, 555–560. [CrossRef]
125. Field, J.R.; Walker, A.G.; Conn, P.J. Targeting Glutamate Synapses in Schizophrenia. *Trends Mol. Med.* **2011**, *17*, 689–698. [CrossRef]
126. Matos, M.; Shen, H.-Y.; Augusto, E.; Wang, Y.; Wei, C.J.; Wang, Y.T.; Agostinho, P.; Boison, D.; Cunha, R.A.; Chen, J.-F. Deletion of Adenosine A2A Receptors from Astrocytes Disrupts Glutamate Homeostasis Leading to Psychomotor and Cognitive Impairment: Relevance to Schizophrenia. *Biol. Psychiatry* **2015**, *78*, 763–774. [CrossRef] [PubMed]
127. Ferre, S.; Ciruela, F.; Borycz, J.; Solinas, M.; Quarta, D.; Antoniou, K.; Quiroz, C.; Justinova, Z.; Lluis, C.; Franco, R.; et al. Adenosine A1-A2A Receptor Heteromers: New Targets for Caffeine in the Brain. *Front. Biosci. J. Virtual Libr.* **2008**, *13*, 2391–2399. [CrossRef] [PubMed]
128. Ferré, S.; Díaz-Ríos, M.; Salamone, J.D.; Prediger, R.D. New Developments on the Adenosine Mechanisms of the Central Effects of Caffeine and Their Implications for Neuropsychiatric Disorders. *J. Caffeine Adenosine Res.* **2018**, *8*, 121–131. [CrossRef] [PubMed]
129. Ferré, S.; Bonaventura, J.; Tomasi, D.; Navarro, G.; Moreno, E.; Cortés, A.; Lluís, C.; Casadó, V.; Volkow, N.D. Allosteric Mechanisms within the Adenosine A2A-Dopamine D2 Receptor Heterotetramer. *Neuropharmacology* **2016**, *104*, 154–160. [CrossRef] [PubMed]

130. Fuxe, K.; Marcellino, D.; Leo, G.; Agnati, L.F. Molecular Integration via Allosteric Interactions in Receptor Heteromers. A Working Hypothesis. *Curr. Opin. Pharmacol.* **2010**, *10*, 14–22. [CrossRef]
131. Moreno, E.; Chiarlone, A.; Medrano, M.; Puigdellívol, M.; Bibic, L.; Howell, L.A.; Resel, E.; Puente, N.; Casarejos, M.J.; Perucho, J.; et al. Singular Location and Signaling Profile of Adenosine A2A-Cannabinoid CB 1 Receptor Heteromers in the Dorsal Striatum. *Neuropsychopharmacology* **2018**, *43*, 964–977. [CrossRef]