Adenosine receptors as potential therapeutic targets

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Recent studies indicate a widening role for adenosine receptors in many therapeutic areas. Adenosine receptors are involved in immunological and inflammatory responses, respiratory regulation, the cardiovascular system, the kidney, various CNS-mediated events including sleep and neuroprotection, as well as central and peripheral pain processes. In this review, the physiological role of adenosine receptors in these key areas is described with reference to the therapeutic potential of adenosine receptor agonists and antagonists.

There are increasing therapeutic applications for adenosine receptor agonists and antagonists that act at the four adenosine receptor subtypes A_1, A_{2A}, A_{2B} and A_3 (Ref. 1). Adenosine receptors transduce their signal by activation of heterotrimeric ($\alpha<\beta<\gamma$) G-proteins and subsequent interaction with several effector systems including adenylate cyclase, cGMP, phosphodiesterases, phosphoinositidase C, phospholipase A_2, and calcium and potassium ion channels[1,2]. Adenosine receptors are widely distributed throughout the body and studies on their structure–activity relationships have been recently reviewed[3,4]. The difficulty in separating therapeutic benefit from undesired side effects has dampened the development of drugs acting at these receptors. Greater understanding of the specific role of adenosine receptors in defined biological systems increases the potential for developing therapeutic drugs.

Inflammatory and immunological responses

Adenosine receptors play a modulatory role in inflammatory responses and there is increasing evidence for use of adenosine and its analogues in the treatment of severe inflammatory diseases such as arthritis[5,6] and asthma[7–9], as well as for more general inflammatory processes such as wound healing[10].

Endotoxaemia

Adenosine receptor ligands modulate the production of pro- and anti-inflammatory cytokines (Fig. 1)[5,11,12]. Tumour necrosis factor-α (TNF-α) production is inhibited at the level of transcription, predominantly by A_3-receptor activation suppressing steady-state mRNA levels[12]. Inhibition of TNF-α production by A_1- and A_2-receptor agonists has also been observed at higher agonist concentrations[11]. TNF-α promotes the production of lipopolysaccharide (LPS)-induced nitric oxide (NO) and both TNF-α and NO are negatively regulated by LPS-induced interleukin-10 (IL-10)[11]. Differences have been identified in adenosine receptor regulation of IL-10 production *in vivo* and *in vitro*. Contrary to that seen *in vitro* using RAW264.7 macrophage cell lines, in endotoxaemic mice, A_1-, A_2- and A_3-receptor selective agonists enhanced LPS-induced IL-10 production[11]. A_3-receptor and, to a lesser extent, A_2-receptor agonists also inhibited macrophage-induced macrophage inflammatory protein-1α (MIP-1α) at the level of transcription[5]. MIP-1α promotes neutrophil chemotaxis towards the inflammatory site and alteration in neutrophil function has been observed with adenosine receptor activation (Fig. 2)[5,6,13]. In human neutrophils, mRNA has been identified for both A_{2A} and A_{2B} receptors, the A_{2A} receptor subtype mediating adenosine-induced inhibition of neutrophil activation[13]. Anti-inflammatory therapies mediated by adenosine

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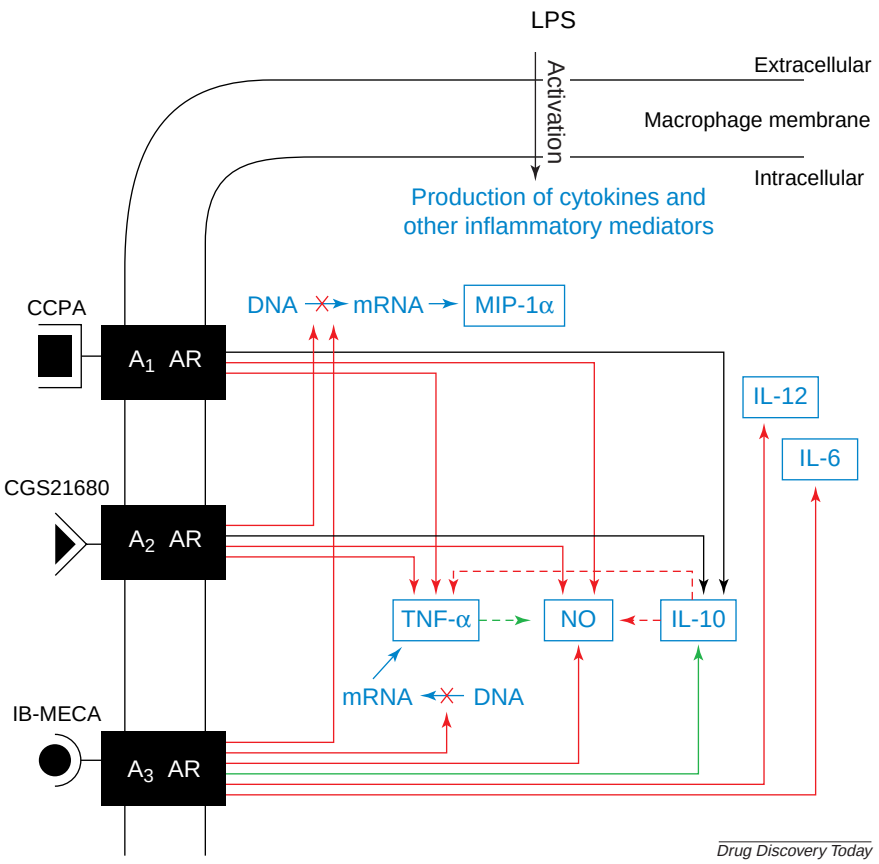

Figure 1. *Adenosine receptor (AR) agonists modulate the production of inflammatory mediators. Lipopolysaccharide (LPS)-induced macrophage activation releases several cytokines and inflammatory mediators, some of which are shown here. Adenosine receptor agonists modulate the production of many inflammatory mediators by either enhancing (in green) or inhibiting (in red) production. Mixed responses (in black) have been observed with adenosine receptor-mediated modulation of interleukin 10 (IL-10) production depending on the system or model studied. Abbreviations: CCPA, 2-chloro-N^6-cyclopentyladenosine; IB-MECA, N^6-3-isodobenzyl-5′-N-methylcarboxamidoadenosine; MIP-1α, macrophage inflammatory protein-1α; NO, nitric oxide.*

receptors are not necessarily limited to the use of adenosine receptor-specific agonists. Some anti-inflammatory agents, such as methotrexate and sulfasalazine, elicit their effect by promoting release of endogenous adenosine[6].

The adhesive capacity of the vascular endothelium is crucial to recruiting leukocytes to inflamed sites[6], indicating that the endothelium could be a target for anti-inflammatory therapies. In stimulated human umbilical vein endothelial cells, adenosine inhibited the release of IL-6 and IL-8, and reduced the expression of E-selectin and vascular cell adhesion molecule-1 (VCAM-1), but not intercellular adhesion molecule-1 (ICAM-1)[14]. These effects of adenosine were enhanced by inhibition of endogenous adenosine deaminase. By contrast, adenosine acting through A_1 receptors can promote inflammation by enhancing the adhesive capacity of the vascular endothelium[6].

Diminished adenosine receptor function might contribute to autoimmune diseases or to where there is excessive inflammation[15]. Activation of A_{2A} receptors in peripheral human lymphocyte membranes decreased immune and inflammatory responses[15]. Inflammation is important in wound healing[10] and, *in vitro*, A_{2A}-receptor agonists promote fibroblast and endothelial cell migration into artificial wounds[10]. In rat and mice animal models, CGS21680 accelerates wound healing in both normal healthy animals and in diabetes-induced animals that display impaired wound healing[10].

Respiratory illnesses

In the airways, levels of adenosine are increased in inflammatory[7] and asthmatic[16] conditions, leading to the promotion of several effector functions. A_{2B} receptors trigger degranulation of canine and human mast cell lines[8,9] and recombinant A_{2B} receptors can be blocked by theophylline and enprofylline at therapeutic concentrations[8,9]. Development of potent, selective A_{2B}-receptor antagonists could provide effective anti-asthma agents[7]. The use of antisense oligodeoxynucleotides showed that desensitization on repeated challenge with adenosine or dust-mites in an allergic rabbit model is mediated by the A_1 receptor[16]. Meanwhile, A_3-receptor antagonists could be useful in the treatment of allergic disease by inhibiting infiltration or transmigration of eosinophils into tissues such as the airway lumen[17].

The adenosine analogue inosine, which is selective for the A_3-receptor subtype, facilitates mast cell degranulation through calcium ion mobilization[18]. Maximal degranulation requires the intracellular uptake of adenosine and its

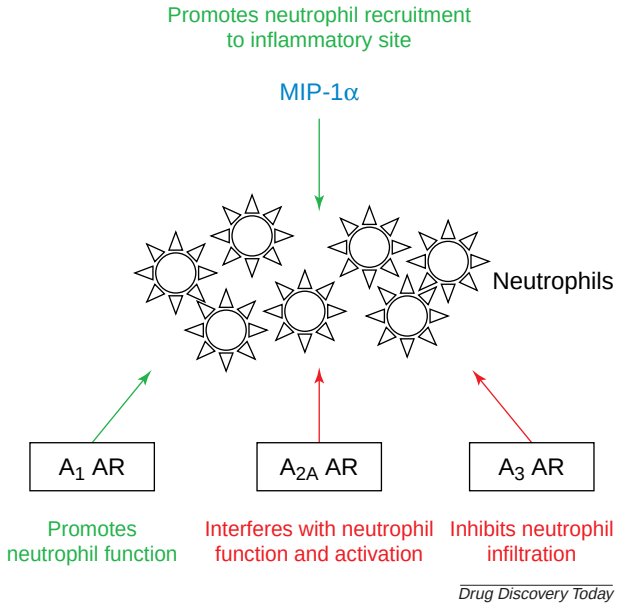

Figure 2. *Adenosine receptor actions at the inflammatory site. Adenosine receptor (AR) agonists either enhance (green) or inhibit (red) neutrophil actions. Abbreviations: MIP-1α, macrophage inflammatory protein-1α.*

subsequent deamination into inosine. Degranulation was inhibited by adenosine uptake blockers and adenosine deaminase inhibitors[18]. Inosine binds to recombinant rat and guinea pig lung A_3 receptors, stimulates RBL-2H3 rat mast-like cell degranulation and causes mast cell-dependent constriction of hamster cheek pouch arterioles that is attenuated by A_3-receptor blockade[18].

Recent evidence has suggested that treatment of adult respiratory distress syndrome associated with septicaemia might be possible with the use of A_1-receptor antagonists[19]. In spontaneously breathing cats, antagonism of A_1 receptors inhibits acute lung injury caused by intralobar arterial infusion of endotoxin, as characterized by the presence of neutrophils, macrophages and red blood cells in alveoli, and by alveolar oedema and necrosis[19]. Further evidence for the role of A_1 receptors in airways is demonstrated by adenosine-induced bronchoconstriction in allergic rabbits[20]. *N*-6-(cyclopentyl)adenosine (CPA) produced a dose-dependent fall in dynamic compliance and a dose-dependent increase in airway resistance in antigen-immunized rabbits compared with sham-immunized rabbits. By contrast, the A_3-receptor agonist *N*-6-(2-(4-aminophenyl)ethyl)adenosine (APNEA) did not alter either dynamic compliance or airway resistance.

In the respiratory network, adenosine acts tonically at A_1-receptors[21] eliciting respiratory inhibition through the stimulation of pulmonary C fibres[22]. Increased activation of A_1 receptors contributes to hypoxic depression of synaptic transmission, whereas systemic 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) administration increases respiratory activity[21]. Endogenous adenosine release in the pulmonary circulation under hypoxic conditions can cause vasoconstriction and might contribute to the pulmonary hypertension associated with acute respiratory failure[23]. Hence, the adenosine receptor antagonist theophylline might be useful in the treatment of spinal cord injured patients with respiratory deficits[24]. Intravenous theophylline injections induced respiratory-related activity in the previously quiescent left phrenic nerve and hemidiaphragm. By contrast, this effect was not observed with enprofylline, another methylxanthine compound, or with the adenosine analogue *N*-6-(L-2-phenylisopropyl)adenosine (L-PIA) when administered alone.

Cardiovascular system

Adenosine is effective as an antiarrhythmic agent in the treatment of tachycardia, or for unmasking atrial tachyarrhythmias or ventricular pre-excitation. By slowing down the electrical conductance in the sinus and atrioventricular nodes, adenosine slows down or terminates abnormal cardiac rhythms[25–27]. Because of the short half-life of adenosine, potentially serious side effects, such as atrial or ventricular fibrillation, apnea and acceleration of ventricular tachycardia, are usually transient. Recognition of adenosine's infrequent proarrhythmic risks and observed early recurrence of tachycardia is important in maintaining appropriate safe and effective treatments[25–27]. Adenosine-regulating agents that either potentiate the activity of, or increase the interstitial concentration of, adenosine are potential long-term treatments for supraventricular arrhythmia and control of ventricular rate during atrial fibrillation or flutter[28].

Arrhythmias

Adenosine has been implicated in ischaemic-related cardiac arrhythmia. Adenosine receptor antagonists might have a role in the management of atrial and ventricular fibrillation occurring soon after the acute phase of a myocardial infarct[29]. Exogenous adenosine has been reported to induce atrial fibrillation in up to 7% of patients suffering from a myocardial infarct. Patients suffering sustained atrial fibrillation following acute inferior myocardial infarction, responded with a normal sinus rhythm and no adverse outcome to a slow intravenous injection of the adenosine receptor antagonist theophylline[29]. The use of

adenosine receptor antagonists in heart transplant patients with early bradyarrhythmias might be indicated as endogenous adenosine has been implicated in post-transplantation bradyarrhythmia[30]. Theophylline increases heart rate and facilitates withdrawal of chronotropic support in patients suffering from this condition, reducing the need for implantation of a permanent pacemaker.

Cardiac ischaemia

Exposure to brief cardiac ischaemia protects cardiac cells against injury during subsequent exposure to prolonged ischaemia. This ischaemic preconditioning involves adenosine receptor activation and can be pharmacologically mimicked by adenosine receptor agonists and blocked by adenosine receptor antagonists (Table 1). Cardiac protection in open-chest rabbits, produced either by activation of adrenergic receptors using a tyramine infusion, or by ischaemic preconditioning, was inhibited by adenosine receptor blockade. However, blockade of α_1-adrenoceptors with prazosin failed to inhibit ischaemic preconditioning, indicating that this process does not require α_1-adrenoceptor activation[31]. Cardiopulmonary bypass surgery alone, without ischaemic episodes, induces cardiac preconditioning through the activation of adenosine receptors and α_1-adrenoceptors in sheep[32]. Irreversible A_1-receptor agonists might also extend cardiac protection[33]. For example, *m*-DITC ADAC (*m*-diisothiocyanate-adenosine amine congener), an irreversible A_1-receptor agonist, mimics ischaemic preconditioning that has been achieved either by pre-exposure to the selective A_1-receptor agonist CPA or by brief pre-exposure to ischaemia. Furthermore, agents that regulate adenosine levels by either inhibition of adenosine uptake[34] or increased production using 5′-nucleotidase[35] also play a role in cardioprotection. Cardioprotection by preconditioning can be facilitated with 5′-nucleotidase that hydrolyze 5′-AMP, resulting in a subsequent increase in interstitial levels of adenosine[35].

Adenosine receptor activation improves functional recovery and reduces cardiac injury during myocardial ischaemic-reperfusion following brief ischaemia (Table 1). Intracoronary platelet adhesion can reduce post-ischaemic recovery of heart function[36]. Endogenous adenosine prevents platelet adhesion and hence, prevents loss of myocardial function during reperfusion, but not during low-flow ischaemia. In the coronary arteries of isolated guinea pig hearts infused with homologous platelets, both DPCPX and 1,3-dimethyl-7-propylxanthine (DMPX) increased platelet retention during the first stages of reperfusion, indicating that this effect is mediated through A_1 and A_2 receptors[36].

Uncoupling of adenosine receptors from their signalling mechanisms produces injury after prolonged ischaemia and reperfusion[37]. In a regional infarct model in open-chest cats, inhibition of ischaemia-reperfusion injury of the heart was observed with the selective A_1-receptor antagonists, xanthine amine congener (XAC), bamifylline (BAM) and DPCPX. These adenosine receptor antagonists might therefore have implications in the prevention or early treatment of ischaemic-reperfusion injury of the heart after coronary artery bypass graft surgery or cardiac transplant surgery. Because of the potential recoupling effects of these antagonists, they might also be useful during or after angioplasty or thrombolytic therapy of the heart.

A_{2A} receptors have been immunologically identified and characterized in human cardiovascular tissues, specifically the ventricle and the atria[38]. In the rat, the A_{2A}-receptor antagonist 5-amino-7-(2-phenylethyl)-2-(d-furyl)-pyrazolo[4,3-e]-1,2,4-trizolo[1,5-c]pyrimidine (SCH58261) slightly increased both systolic and diastolic blood pressure and heart rate when administered alone, suggesting that the A_{2A} receptor mediates tonic vascular regulation[39], while selective A_{2A}-receptor agonists are potent vasodilators[40,41]. A reduction in blood pressure and an increase in heart rate and plasma renin activity are caused by a reflex

Table 1. Adenosine receptor activation produces cardioprotection against ischaemic injury

Adenosine receptor subtype	Tissues in which cardioprotection is produced by preconditioning	Tissues in which cardioprotection is produced during myocardial ischaemic-reperfusion
A_1	Cultured chicken ventricular myocytes[88], human atrium[89], transgenic mice with increased A_1-receptor number[90], rabbit heart[91]	Isolated rabbit heart[92], transgenic mice with increased A_1-receptor number[90]
A_2	–	Pig myocardial infarct[93]
A_3	Cultured chicken ventricular myocytes[88], rat atrial cells transfected with human A_3 receptors[88], rabbit heart[91], human atrium[89]	–

increase in sympathetic activity, triggered by a decrease in blood pressure rather than by a direct stimulating effect on cardiac and renal A_{2A} receptors[41]. The reduction in infarct size caused by activation of A_2 receptors has been related to inhibition of neutrophil activity[13,42], leukocyte inhibitory effects and observed coronary vasodilatation[43]. Meanwhile, A_{2B} receptors might play a cardioprotective role in cardiac fibrosis as in rat left ventricular cardiac fibroblasts, adenosine inhibited fetal calf serum-induced collagen and total protein synthesis through the activation of A_{2B} receptors[44].

Renal system

Adenosine receptors are widely distributed throughout the nephron, mediating several effects in the renal system, therefore reducing the risk of cardiovascular disease in hypertensive patients[45]. Inhibition of adenosine deaminase has been shown to lower blood pressure in older but not younger spontaneous hypertensive rats. Hence, cardiovascular protection in older hypertensive patients might be achieved by using adenosine deaminase inhibitors[45]. Meanwhile, blockade of renal adenosine receptors might improve renal function in patients with chronic congestive heart failure, as adenosine reduces renal blood flow via vasoconstriction[46].

Both A_1- and A_2-receptor activation produce hypertensive effects. The activation of A_1 receptors by renal infusion of 2-chloro-*N*^{6}-cyclopentyladenosine (CCPA) and of A_2 receptors by renal infusion of 2-hesinyl-5′-*N*-ethyl-carboxamidoadenosine (2HE-NECA) both produced decreases in arterial blood pressure, glomerular filtration rate, and urinary water and sodium excretion[47]. A_1-receptor activation was also associated with a decrease in heart rate, whereas A_2-receptor activation was associated with an increase in heart rate. The renal response to A_2-receptor activation was enhanced in the presence of intact renal nerves, whereas the renal response caused by A_1-receptor activation was not. Animal studies using ocular normotensive cynomolgus monkeys also identified differences in the involvement of A_1 and A_2 receptors in peripheral hypertension[48]. Early ocular hypertension, followed by ocular hypotension, was produced by application of *N*-6-(*R*-phenylisopropyl)adenosine (*R*-PIA) and *N*-6-(cyclohexyl)adenosine (CHA). This model of hypertension was associated with A_2-receptor activation, as shown by inhibition of the response by the A_2-receptor antagonist DMPX. The secondary hypotension, which was unaffected by DMPX, is seemingly mediated by A_1 receptors. A rise in intraocular pressure has also been observed in rabbits and cats[49], where the response to the A_2-receptor agonist 2-phenylaminoadenosine (CV1808) response (which was much larger than that to the A_1-receptor agonist *R*-PIA) was blocked by DMPX pretreatment.

Compounds with antagonistic properties to the adenosine receptor, such as the *Microtea debilis* flavanoid cirsimarin, show potential as therapies for acute renal failure[50]. High concentrations of cirsimaritin, which are produced in the urine after consumption of cirsimarin, inhibits the urinary actions of adenosine. Urinary adenosine might be involved in regulatory feedback mechanisms of sodium channels in the nephron, where sodium reabsorption is stimulated by low (nanomolar) urinary adenosine levels, and sodium excretion is increased by high (micromolar) urinary adenosine concentrations[51]. An increased renal A_1-receptor density might explain the enhanced renal vasoconstriction of adenosine in glycerol-induced acute renal failure[52].

Renal infusion of an A_2-receptor agonist renal infusion significantly reduced renal blood flow while significantly increasing plasma renin activity in innervated but not denervated kidneys. By contrast, intact renal nerves were not necessary in the A_1-receptor-mediated decrease in renal blood flow and decrease in plasma renin activity[53]. The antihypertensive effects mediated by A_{2A}-receptor activation have been associated with a reflex increase in sympathetic activity caused by a decrease in blood pressure[41], rather than caused by a direct effect of the agonist on renal and cardiac A_2 receptors.

Central nervous system

Parkinson's disease

Several studies have recently suggested that the A_2-receptor subtype is physiologically relevant in cerebral ischaemia[54], neurological disorders[55,56] and neurodegenerative processes such as Parkinson's disease[54,57–59].

The modulatory role of adenosine on dopamine and *N*-methyl-D-aspartate (NMDA)-receptor neurotransmission indicates a possible therapeutic potential for adenosine receptors in CNS disorders such as schizophrenia, dementia and depression (Fig. 3). Human studies with non-selective and selective A_{2A}-receptor antagonists have indicated that the motor stimulant activity of A_{2A} receptors might reduce hypokinesia and tremor associated with Parkinson's disease[60].

Figure 3 describes the anticataleptic actions of A_{2A}-receptor antagonists and the interactions of the A_2 receptor with dopamine and NMDA pathways[56,57,59,61]. This involvement of A_2 receptors in the dopamine pathway has been shown in dopamine-denervated animal models[58]. This interaction[56–59,62] might be at the level of cholinergic neurons[59], as after stimulation with an adenosine agonist, the centrally acting anticholinergic agent scopolamine not only blocked the cataleptic activity, but also reduced the potentiation of haloperidol-induced catalepsy. The

involvement of the A_{2A} receptor in the dopamine pathway is selectively mediated through the dopamine D2 receptor in intact (non-lesioned) animals[56]. By contrast, in lesioned animals, A_{2A} receptors interact with D1 receptors. This discrepancy is possibly caused by 6-hydroxydopamine-induced lesions in the substantia nigra causing functional supersensitivity of dopamine receptors and second messenger systems resulting in altered receptor interactions[56].

Several novel A_{2A}-receptor antagonists have been suggested as potential therapeutics for Parkinson's disease[62–64]. SCH58261, a selective A_{2A}-receptor antagonist, not only enhanced locomotor activity and increased wakefulness, but also potentiated the activity of L-DOPA and dopamine receptor agonists in the dopamine-denervated rat model[62]. These antagonists potentiate contralateral turning behaviour caused by the D1-receptor agonist SK&F38393 in unilateral dopamine-denervated rats, and increase SK&F38393-induced c-fos expression in the lesioned dopaminergic nigrostriatal. The A_1-receptor antagonist DPCPX induced small potentiation in contralateral turning with no change in c-fos expression[58].

CPA, at doses devoid of intrinsic behavioural effects, inhibits the electroencephalographic (EEG) effects of the non-competitive NMDA-receptor antagonist MK801 (Ref. 55). The A_2-receptor agonist CGS216880 also inhibits MK801-induced EEG, but only at depressant doses. A_2 and NMDA receptors in haloperidol-treated rats interact through indirect monoamine-dependent mechanisms, rather than via a direct relationship between the two receptors[57]. Potentiation of the anticataleptic actions of NMDA-receptor antagonists, by coadministration with the adenosine receptor antagonist, theophylline, was only elicited in haloperidol-pretreated animals and not in reserpine/α-methyl-p-tyrosine-pretreated animals[57].

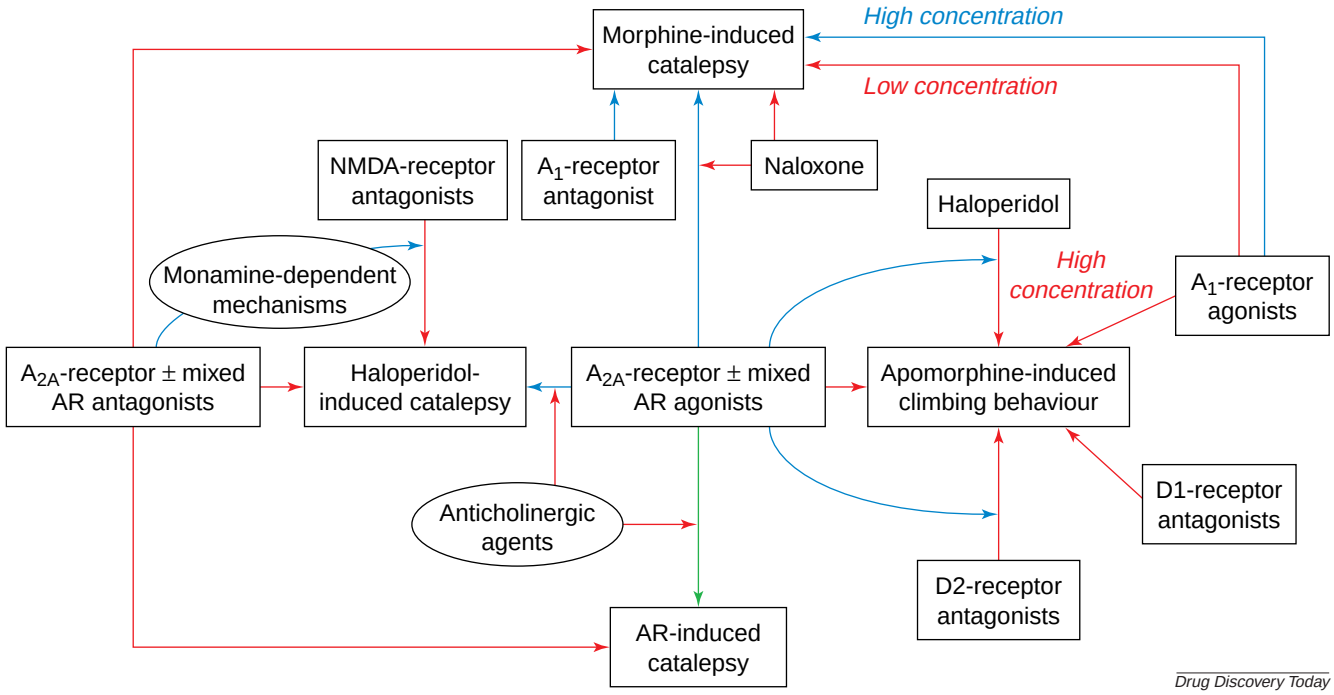

Figure 3. *Adenosine A_{2A}-receptor antagonists block catalepsy induced by adenosine receptor (AR) agonists, the antipsychotic agent haloperidol (dopamine D2-receptor antagonist) and morphine (opioid-receptor agonist), while potentiating the anticataleptic activity of N-methyl-D-aspartate (NMDA)-receptor antagonists. The interaction between adenosine receptors and the dopaminergic pathway is at the level of cholinergic neurons. By contrast, the relationship between A_2 and NMDA receptors in haloperidol-induced catalepsy is by indirect monamine-dependent mechanisms. A_{2A}-receptor agonists inhibit apomorphine (dopamine agonist)-induced climbing behaviour in mice and potentiate the effects of dopamine receptor antagonists and morphine. Differences between the interactions of the A_{2A} receptor and either the D1 or the D2 receptor have been associated with experimental models studied. Inhibitory effects are shown in red, potentiating effects shown in green, induction shown in blue.*

Neurodegenerative diseases

Microglial activation, which might have a causative role in neurodegenerative diseases such as Alzheimer's disease[65], could be increased by simultaneous stimulation of A_1 and A_2 receptors[66]. Rat microglial cells express A_1-, A_{2A}- and A_3-receptor mRNA but not A_{2B}-receptor mRNA[65]. Only combinations of CPA and CGS21680 or the mixed A_1/A_2-receptor agonist 5′-*N*-ethylcarboxamidoadenosine (NECA) increased microglial activation determined by incorporation of [^{3}H]-thymidine into microglial DNA[66]. The effect of NECA on microglial proliferation was antagonized by DPCPX. Neurodegenerative treatment might be possible with sufficient reduction in microglial proliferation by direct alteration of A_1-receptor activation[66], or inhibition of microglial activity through A_{2A}-receptor-mediated cAMP second messenger systems with either A_{2A}-receptor antagonists or cyclooxygenase inhibitors[65].

Neuroprotection from kainic acid-induced neurotoxicity in rats has been observed with systemic coadministration of the A_1-receptor agonist *R*-PIA and blocked by DPCPX[67]. Peripheral A_{2A}-receptor involvement has also been implicated in neuroprotection against kainate excitotoxicity[68]. A therapeutic time window for treatment of cerebral ischaemia with the A_1-receptor agonist, adenosine amine congener (ADAC), has been demonstrated in gerbils[69]. Brain damage caused by cerebral ischaemia and the associated release of excitatory amino acids, was also counteracted by A_{2A}-receptor antagonists[54]. The novel A_{2A}-receptor antagonist SCH58261, which has shown potential in treatment of Parkinson's disease, has also demonstrated neuroprotective properties by reducing brain infarct size in cerebral ischaemia in the rat[62].

Behavioural animal models, such as the elevated plus-maze[70,71], have linked A_1 receptors to anxiolytic activity. The NMDA-receptor antagonist, dizocilpine, showing anxiolytic activity through NMDA-channel blockage, has been associated with the subsequent release of endogenous adenosine[70]. This appears to be through the A_1 receptor, as the A_1-receptor antagonist DPCPX reversed the anxiolytic effects of dizocilpine while the A_2-receptor antagonist DMPX had no effect[70]. The effects of ifenprodil, an NMDA receptor polyamine-site antagonist, which also produces anxiolytic activity in the plus-maze model, were reduced by CPA and DPCPX but were not reversed[71].

Sleep

Adenosine receptors have a modulatory role in sleep and wakefulness in the mammalian nervous system, with a major involvement of the A_{2A} receptor[72]. Maximum levels of extracellular adenosine in the rat hippocampus but not in the neostriatum corresponded to sleep-like behaviour and times of decreased overall movement and eating behaviour[73].

Pain

Adenosine influences pain transmission both centrally and peripherally. In an inflammatory model of thermal hyperalgesia, spinally administered adenosine receptor agonists produce antinociception through the activation of A_1 receptors[74]. The A_1- and A_2-receptor agonists, N^6-cyclohexyladenosine (CHA) and CGS21680, and the adenosine kinase inhibitors, 5′-amino-5′deoxyadenosine (NH_2dADO) and 5-iodotubercidin (ITU), produced antinociception in unilateral hind paw carrageenan-induced thermal hyperalgesia in rats. Adenosine receptor-mediated antinociception was reversed by caffeine and 8-cyclopentyltheophylline (CPT) but not by DMPX, indicating involvement of the A_1-receptor subtype. A_1 receptors are also involved in peripherally mediated antinociception[75,76]. In the rat formalin test, antinociception (as shown by an inhibition of flinching behaviour) was observed with ipsilateral (same side) coadministration of formalin and NH_2dADO but not contralateral (opposite side) coadministration, indicating a peripheral rather than a systemic response[76]. This peripheral antinociception, caused by accumulation of adenosine at its receptors, was inhibited by caffeine while intrinsic tone was blocked by CPT (Ref. 76). Peripheral and central antinociception produced by NH_2dADO was enhanced by coadministration of the adenosine deaminase inhibitor 2′-deoxycoformycin (dCF)[74,76]. The combined actions of NH_2dADO and dCF could then be blocked by caffeine[76].

In humans, peripheral administration of adenosine produces pain responses similar to that observed under ischaemic conditions[75]. The adenosine receptor antagonist, caffeine, has produced hypoalgesic effects against experimental ischaemic skeletal muscle pain in humans[77]. Pain ratings in individuals receiving caffeine prior to experimental trials were significantly reduced for up to 30 minutes compared with that of individuals receiving placebo[77]. In patients suffering from syndrome X and displaying an altered perception of cardiac pain possibly caused by adenosine hypersensitivity, intravenous administration of the adenosine receptor antagonist aminophylline improves exercise tolerance[78]. Oral administration of aminophylline increased the time taken to induce angina during exercise compared with placebo administration and, in some cases, reduced the total number of episodes of chest pain[78].

In the human CNS, activation of A_1 receptors produces antinociception against acute nociceptive, inflammatory

and neuropathic pain tests[75]. There is suggestion of the involvement of spinal A_2 receptors in pain processes but no data on A_3 receptors[75]. A_3-receptor agonists produce peripheral nociception and increase oedema mediated by 5-hydroxytryptamine (5-HT, serotonin) and histamine release[79]. Activation of the A_3 receptor by subcutaneous administration of *N*-6-benzyl-NECA increases intrinsic flinching behaviour in the presence of formalin and increases paw oedema when administered alone or in the presence of formalin. The effects of *N*-6-benzyl-NECA are blocked by histamine H_1- and 5-HT_2-receptor antagonists, but not by other 5-HT or adenosine receptor antagonists[79].

Spinal sensory pain transmission associated with A_1 receptors is also related to spinal motor responses[80]. Electrically evoked potentials recorded from a neonatal rat spinal cord (indicated as slow ventral root potentials) are associated with nociceptive information and monosynaptic reflex, and reflect non-nociceptive transmission related to motor function. These potentials can be inhibited by CHA, *R*-PIA, NECA and CGS21680 (Ref. 80), the inhibitory actions of NECA being dose-dependently blocked by CPT. Dorsal root potential, reflecting $GABA_A$ (γ-aminobutyric acid) receptor-mediated presynaptic inhibition associated with analgesia, was also depressed by these agonists, but in a different potency order. CHA inhibition of dorsal root potential was antagonized by 8-CPT (Ref. 80).

Interaction of adenosine with GABA receptors

Potential for the enhancement of spinal cord stimulation (SCS) therapy to suppress pain transmission might involve $GABA_B$- and A_1-receptor-dependent mechanisms[81,82]. Intrathecal injection of *R*-PIA reduced mechanical and cold allodynia-like symptoms (high pain sensitivity to stimulus observed by abnormally low withdrawal thresholds) in the rat model of chronic central pain, induced photochemically via laser irradiation of the spinal cord[81,83]. Furthermore, this injection suppressed tactile allodynia in a rat model of mononeuropathy produced by sciatic chronic constriction injury[82]. The *R*-PIA effects against chronic allodynia-like behaviour were reversed by theophylline[83] and DPCPX (Ref. 82). The $GABA_B$-receptor agonist baclofen also suppressed allodynia when given alone[81]. Meanwhile, combined treatment of baclofen and *R*-PIA at low doses, ineffective when given alone, transformed rats in which SCS does not suppress allodynia into SCS-responders[81]. The SCS-responsiveness was then abolished on coadministration of low doses, ineffective when given alone, of the $GABA_B$- and A_1-receptor antagonists, CGP55845 and CPT, respectively[81]. SCS was also able to suppress tactile hypersensitivity in the presence of *R*-PIA (Ref. 81).

Adenosine receptors are also involved in central sensitization[84]. Extracellular single-unit recordings of spinal dorsal horn neuronal activity showed that *R*-PIA suppressed noxiously evoked activity and inhibited neuronal activity sensitized by topical application of mustard oil to a region of skin adjacent to the receptive field on the hind paw, and prevented mustard oil-induced sensitization of dorsal horn neurons. These responses to *R*-PIA were reversed by theophylline[84].

Interaction of adenosine with opioid receptors

Opioid–adenosine interactions have been demonstrated both at cellular and behavioural levels[85]. Opiate tolerance and dependence might be mediated by adenosine release and adenosine receptor activation. Short-term morphine treatment enhances adenosine release in brain and spinal tissues[85]. In mice, chronic morphine treatment with morphine pellet implantation significantly increased adenosine transporter-binding-site concentrations localized in the striatum and the hypothalamus, compared with that of chronic vehicle pellet implantation animals. This effect of chronic morphine treatment was not observed in the cortex, hippocampus, brainstem or cerebellum. In behavioural studies, acute morphine injections significantly stimulated ambulatory activity in chronic vehicle-treated mice compared with those injected chronically with morphine, this being indicative of opiate tolerance[85]. Intrathecal administration of the A_2-receptor antagonist, DMPX, attenuated inhibition of the tail-flick response produced by the intracerebroventricularly (ICV) administered opioid receptor agonists, morphine and β-endorphin[86]. By contrast, DMPX alone did not affect latency of tail-flick. However, the A_1-receptor antagonist, 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (PACPX) only attenuated the effect of ICV administration of β-endorphin and not morphine, indicating that A_2 but not A_1 receptors influence the antinociceptive effects of supraspinally administered morphine[86]. In rats, intrathecal administration of NECA and *R*-PIA reduces substance P-like immunoreactivity in cerebrospinal fluid (CSF), which can be reversed by systemic administration of theophylline[87]. The adenosine analogues also increased tail immersion latency with motor impairment at higher doses[87].

Conclusions

Adenosine receptors are widely distributed throughout the body. Recent studies demonstrate that adenosine receptors are involved in complex regulatory systems. A clear understanding of the specific interactions in the cardiovascular, respiratory, renal, central and peripheral

nervous systems and in immunological and inflammatory processes are likely to lead to new approaches for the use of adenosine receptor agonists and antagonists in a wide range of diseases.

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In short…

Biacore (Uppsala, Sweden) are spending $8 million over the next two years on developing products to improve the pharmaceutical industry's clinical candidate hit rate. The company will be collaborating with several pharmaceutical companies to develop instrumentation for applications downstream of high-throughput drug screening, such as lead characterization, lead optimization, and early absorption, distribution, metabolism and excretion (ADME) studies. By using their proprietary biosensor technology, the company aims to significantly reduce the time to market for new therapies by providing more comprehensive biological data on the compound earlier in the drug discovery process. Julian Abery, who is leading this project, said '…the new system will aim to increase understanding of lead compound optimization and generate more informative data in a phase of drug development where the savings for getting it right and the costs of getting it wrong are dramatically increased'.