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Review

Adenosine-Related Mechanisms in Non-Adenosine Receptor Drugs

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Abstract: Many ligands directly target adenosine receptors (ARs). Here we review the effects on adenosinergic signaling of other drugs that are not typically identified as binding ARs. Non-AR mechanisms include raising adenosine levels by inhibiting adenosine transport (e.g. ticagrelor, ethanol, cannabidiol), affecting intracellular metabolic pathways (e.g. methotrexate, nicotinamide riboside, salicylate, AICA riboside), or undetermined means (e.g. acupuncture). Yet other compounds bind ARs, in addition to their canonical 'on-target' activity (e.g. mefloquine). The strength of experimental support varies widely. AR knockout mice are the 'gold standard' method for investigating an AR role, but few drugs have been tested in these mice. Given the interest in AR modulation for treatment of cancer, CNS, immune, metabolic, cardiovascular, and musculoskeletal conditions, it is informative to consider AR and non-AR adenosinergic effects of approved drugs and conventional treatments.

Keywords: adenosine receptor; nucleoside transport; CNS; inflammation; cardiovascular system; pain

1. Introduction

Drugs acting on adenosine signaling can do so in multiple ways. They can act directly as ligands on one or more of the four G protein-coupled receptors (GPCRs) for adenosine. Alternatively, they can stimulate or inhibit pathways of adenosine generation, degradation, or clearance. Some drugs that were not originally postulated to involve adenosine mechanisms are now suspected or confirmed to be doing so. Here, we review selected examples of adenosinergic mechanisms of action (MoA) proposed for diverse drugs and treatments, emphasizing examples where the compound does not bind directly to an adenosine receptor (AR). On one hand, clear characterization of adenosine-related mechanisms in non-adenosine receptor drugs can potentially lead to drug repurposing of compounds that are already approved for clinical use. On the other hand, the strength of current experimental support varies widely, and additional experiments are needed to validate many of the claims of an adenosinergic MoA.

1.1. Endogenous adenosine

Extracellular adenosine is considered a retaliatory metabolite in a protective feedback control pathway in response to excessive intracellular ATP consumption (Newby 1984). Intracellular AMP and adenosine rise when ATP is depleted in low energy states and function as metabolic stress signals, leading to activation of non-AR-dependent mechanisms such as AMP-dependent protein kinase (AMPK) (Camici et al., 2018) and, indirectly, to AR activation by extracellular adenosine. Extracellular adenosine can also be produced locally during injury or an inflammatory response (Haskó et al., 2008). Adenosine has been known as an anti-inflammatory mediator and an endogenous antiseizure substance in the brain (Boison, 2013). Some pathological states are associated with an imbalance in adenosinergic signaling. In cancer, local adenosine levels are elevated in the tumor

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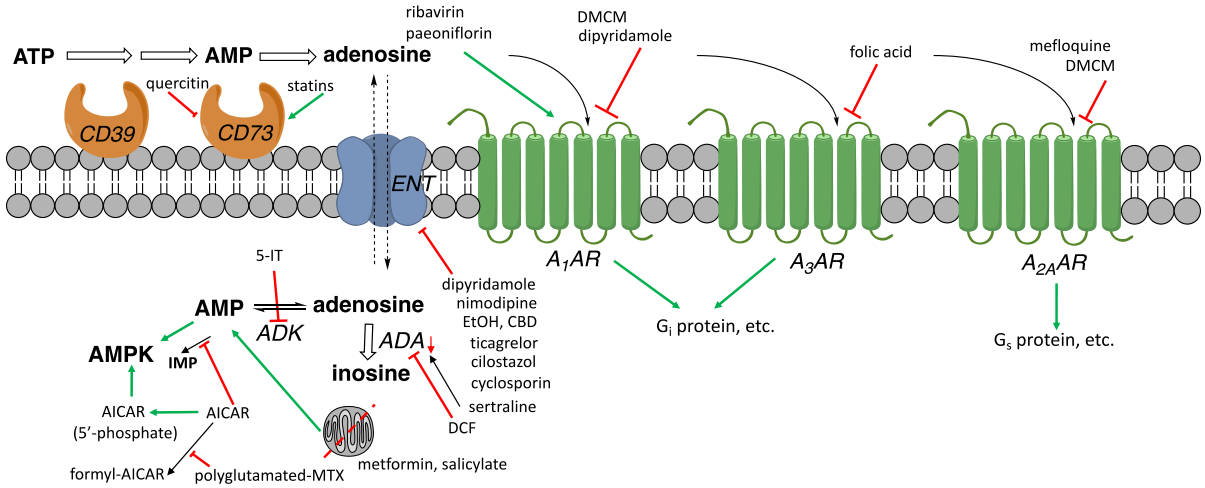
microenvironment and allow a tumor to evade immune attack, suggesting pharmacological blockade of adenosinergic signaling for use in cancer therapy (Allard et al., 2018). Conversely, in other disease states the removal of extracellular adenosine, for example, as an indirect result of the pathological upregulation of intracellular adenosine kinase in the brain can exacerbate epilepsy (Boison, 2013). In bone, reduced A_{2A}AR or A_{2B}AR signaling has a deleterious effect in models of osteo- and rheumatoid arthritis (Mediero et al., 2013; Knowles, 2019). The four AR subtypes (A_1, A_{2A}, A_{2B}, A_3, Figure 1) are important in the body's adaptation to stress (Haskó et al., 2008; Antonioli et al., 2018), often in the context of increasing local blood flow by vasodilation. However, a mouse line having knockout (KO) of all four ARs displayed no differences in growth, fertility, metabolism, and thermal regulation, although long-term survival was diminished (Xiao et al., 2019). This suggested that ARs have a more pronounced role to regulate allostasis than homeostasis. The interplay of generally anti-inflammatory adenosine acting at ARs with related adenine nucleotides acting at pro-inflammatory P2X and P2Y receptors is to be considered in the larger context of purinergic signaling (Burnstock, 2017).

A



B

A_1AR			$A_{2A}AR$	$A_{2B}AR$	A_3AR
d-opioids	sleep deprivation	tianeptine	ketamine	diazepam ⇓	glucocorticoids
k-opioids	gabapentin	ketogenic diet	dizocilpine		
sildenafil	amitriptyline	ECT	melatonin		
ketamine	acupuncture		sulfasalazine, salicylates		
creatine	tianeptine		$ZnCl_2$		
$ZnCl_2$	incarvillateine				

Figure 1. A. Adenosinergic pathways and their putative modulators. These interactions have been found in model systems *in vitro* or *in vivo*, and do not imply necessarily that a compound achieves the indicated effect when administered in humans. Intracellular processes resulting in elevated adenosine concentrations are shown. B. Drugs or treatments that influence adenosinergic signaling, including those having an undetermined or unclear mechanism (all are stimulatory, except as shown). Inhibitory and stimulatory effects on the production of cAMP, mediated by Gi and Gs proteins, are shown. Intracellular adenosine concentrations can also be raised through inhibition of ADK (e.g. by 5-iodotubercidin (5-IT, structure not shown), ABT-702 **18** or A-134974 **19**) or of adenosine deaminases (e.g. by deoxycoformycin, DCF **20**). Also, AICAR inhibits an enzyme that metabolizes AMP by deamination, to indirectly increase intracellular adenosine. Compounds that modulate adenosine signaling without a specific AR assigned are not shown.

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Plasma adenosine is locally cleared in a few seconds (Moser et al., 1989); at pharmacologic doses in humans, intravenous adenosine has a plasma half-life of only ~1 min (Blardi et al., 1993). These properties mean that plasma adenosine levels (100-1000 nM, Moser et al., 1989), while important, are not the only relevant concentration. Adenosine is a local or paracrine modulator, so local levels dictate most of the physiologic effects.

1.2. Action of various drugs involves adenosine

While the strength of the data varies, adenosine is proposed to be involved in the effects of ethanol, anti-inflammatory drugs, vasodilators, and various drugs and natural products used for treating depression, anxiety, behavioral and sleep disorders, and pain. Possible actions include directly or indirectly modulating adenosine levels, acting directly on ARs or their binding partners (receptor or non-receptor).

There is a wide range of pharmacologically active substances that were not developed originally as AR ligands but have a principle or secondary MoA involving extracellular adenosine and ARs. Many substances alter adenosine signaling indirectly, by increasing or decreasing the level of extracellular adenosine (Phillis and Wu, 1982; Antonioli et al., 2007) or by affecting protein partners of the ARs. Often, this modulation of adenosinergic signaling does not involve a measurable, direct binding interaction with the ARs, as determined early on for anxiolytics that nevertheless interact with purinergic pathways (Williams et al., 1981).

1.3. Known AR ligands

Directly acting, potent agonists (**1** – **15**) and antagonists (**22** – **39**) of each of the ARs are available as pharmacological probes and, in several cases, agents approved for human use (Figure 2, Jacobson and Müller, 2011). AR agonists **1** and **9** are approved for clinical use in myocardial perfusion diagnostics and supraventricular tachycardia diagnosis and treatment. A_3AR agonists **13** and **14** are in clinical trials for autoimmune inflammatory and liver diseases, respectively, and A_{2A}AR antagonist **33** is used for Parkinson's disease. Selective A_{2A}AR antagonists **30** and **31** are in clinical trials for cancer. The binding affinities of selected AR ligands are shown in Table 1. XAC **23** can be considered a pan-antagonist of human ARs, and NECA **3** is a pan-agonist.

Table 1. Affinities of selected AR ligands (K_i, nM) that have been used to define adenosinergic activities of non-adenosine receptor drugs (refer to Figure 2 for some structures). Species is human, unless noted (m, mouse; r, rat). Values from Jacobson and Müller, 2011; Carlin et al., 2017; Wan et al., 2004; Tosh et al, 2019. Values that represent >100-fold selectivity are shown in bold.

Compound	A_1AR	A_{2A}AR	A_{2B}AR	A_3AR
Agonists				
Adenosine **1**	~100	310	15,000	290
	73 (r)	150 (r)	5100 (r)	6500 (r)
NECA **3**	6.8	2.2	140	16
	63 (r), 0.45 (m)	12 (r)	120 (m)	113 (r), 14.1 (m)
R-PIA **4**	2.04	220 (r)	150,000	33
	1.2 (r)			158 (r)
CPA **5**	2.3	794	18,600	72
	0.22 (m)	808 (m)		534 (m)
CCPA **6**	0.83	2270	ND	43
	0.27 (m)	988 (m)		16 (m)
Cl-ENBA	**0.51**	1340	ND	1290
	0.20 (m)	3985 (m)		2414 (m)

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CGS21680 **8**	289	**27**	>10,000	67
	193 (m)	**10 (m)**		48 (m)
Regadenoson **9**	>16,000	**290**	>10,000	>10,000
	7.75 (m)	77.2 (m)	>100,000 (m)	>10,000 (m)
Bay60-6583[a] **12**	>10,000	>10,000	**3 – 10**	>10,000
	351 (m)	>10,000 (m)	136 (m)	3920 (m)
IB-MECA **13**	51	2900	11,000	1.8
	5.9 (m)	~1000 (m)		0.087 (m)
Cl-IB-MECA **14**	220	5360	>10,000	61.4
	35 (m)	~10,000 (m)		**0.18 (m)**
Antagonists				
Caffeine **23**	10,700	24,300	33,800	13,300
				>100,000 (r)
8-SPT **24**	537 (m)	12,400 (m)	4990 (m)	>10,000 (m)
XAC **25**	6.8	18.4	7.75	25.6
	1.2 (r), 2.2 (m)	63 (r), 83 (m)	63 (r), 4.5 (m)	29,000 (r), ~10,000 (m)
DPCPX **26**	3.0	129	51	795
	1.5 (m)	598 (m)	86.2 (m)	>10,000 (r, m)
CSC[b]	28,000 (r)	54 (r)	8200 (r)	>10,000 (r)
SCH442416 **28**	1110	**4.1**	>10,000	>10,000
	765 (m)	**1.27 (m)**		>10,000 (m)
ZM241385 **30**	774	1.6	75	743
	249 (m)	0.72 (m)	31 (m)	10,000 (m)
MRS1754 **32**	403	503	**1.97**	570
	16.8 (r)	621 (r)	12.8 (r)	
PSB-603 **33**	>10,000	>10,000	**0.553**	>10,000
	>10,000 (r)	>10,000 (r)	**0.351 (m)**	
MRS1220 **36**	81 (m)	9.1 (m)	ND	>10,000 (m)
MRS1523 **37**	>10,000	3660	>10,000	18.9
	15,600 (r)	2050 (r)	>10,000 (m)	113 (r)
	5330 (m)	>10,000 (m)		702 (m)

a – may be antagonist at A_1AR and A_3AR, partial agonist at $A_{2B}AR$. b – K_i MAO-B, 80.6 nM. ND, not determined.

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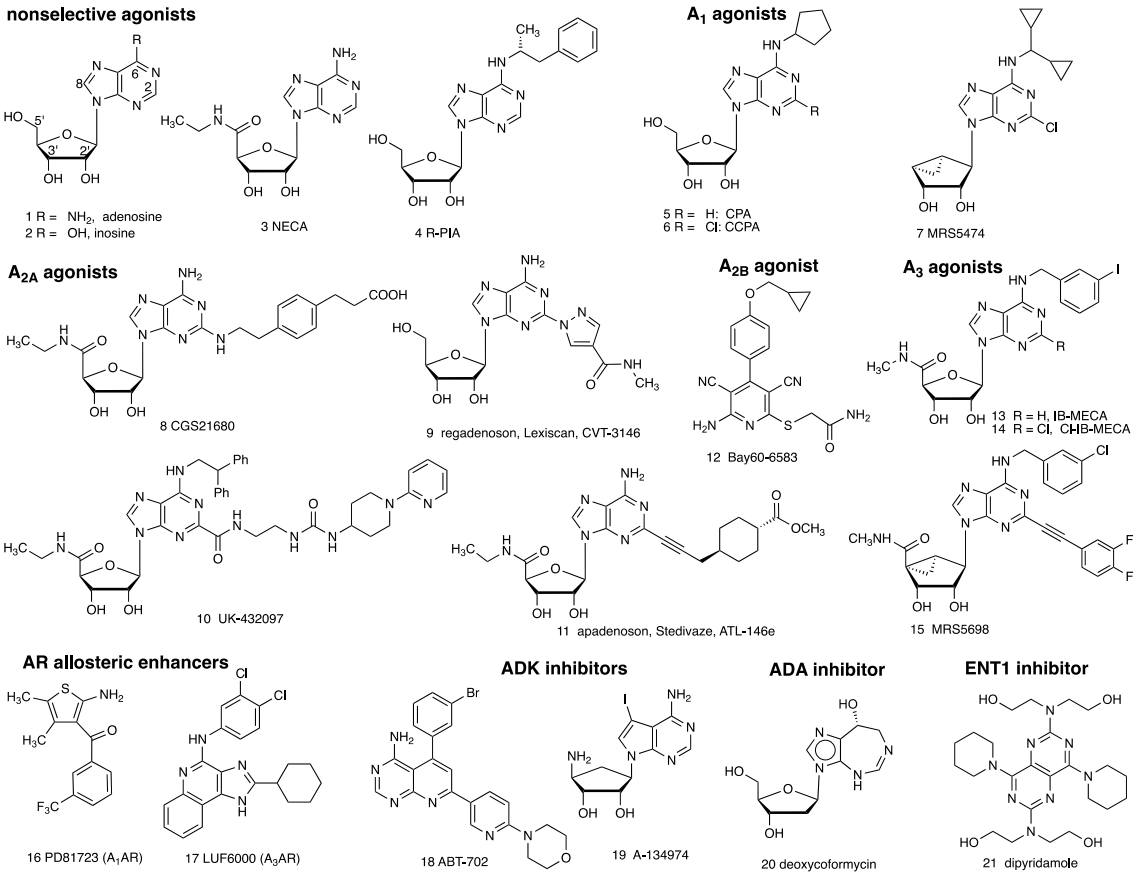

Figure 2. A. Direct (orthosteric agonists **1** – **15**) and indirect modulators (**16** – **21**) of ARs. Compounds **1**, **9**, **20** and **21** are in human use. Compounds **13** and **14** are in clinical trials.

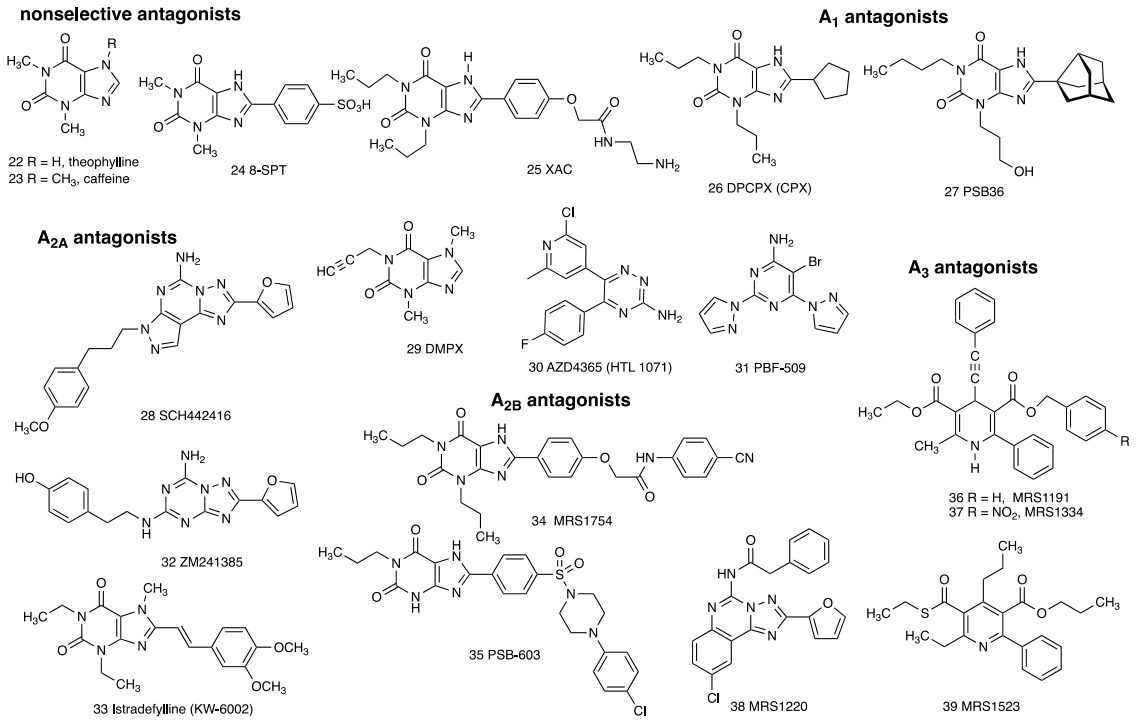

Figure 2. B. Competitive AR antagonists (**22** – **39**). Compounds **22** and **33** are in human use. Compounds **30** and **31** are in clinical trials for cancer.

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There are caveats with the use of many AR pharmacological probes. Marked species differences in the affinities and selectivities of agonists/antagonists have been documented, including between species as similar as rat and mouse (Jacobson and Müller, 2011; Alnouri et al., 2015). In some cases, insufficient attention is given to species differences and differences between binding selectivities and in vivo selectivities, which can lead to misinterpretation of in vivo data. For example, A_1AR antagonist DPCPX **26** also antagonizes the A_{2B}AR. A_{2A}AR antagonist DMPX **29** is of low A_{2A} selectivity. A_3AR antagonist MRS1220 **38** is A_3AR-selective in human but not in rat or mouse, where it is A_{2A}AR-selective. MRS1523 **39** is a preferred antagonist for use at rat or mouse A_3AR, but nevertheless displays only moderate selectivity. Also, AR ligands are sometimes used at concentrations or doses that exceed the limit of their selectivity and without considering biodistribution. For example, peripheral doses high enough to achieve desired brain levels may have off-target peripheral effects. Thus, there is a need to reexamine some of the earlier findings summarized here using AR knockout (KO) mice or at least more recently reported selective AR ligands.

1.4. Known modulators of adenosine pharmacokinetics

Several enzymes that are responsible for the production or metabolism of adenosine also regulate its location and concentration. Intracellular adenosine kinase (ADK) and adenosine deaminase (ADA, two forms, one intracellular and one extracellular) remove adenosine, while extracellular CD73 (5′-nucleotidase; and indirectly CD39) and intracellular S-adenosyl-L-homocysteine hydrolase produce adenosine. In addition, there are equilibrative (ENT1-3, SLC29 family) and concentrative (CNT1-3, SLC28 family) transporters with various levels of specificity (Li et al., 2012, Boswell-Casteel and Hays, 2107), which, when inhibited, often lead to an increase of extracellular nucleosides, including adenosine. The ADA inhibitor deoxycoformycin **20**, an anticancer drug, and ENT1/2 inhibitors dipyridamole **21** and dilazep (structure not shown), with cardiovascular indications, are in human use. Inhibitors of ADK (Kowaluk et al., 2000) and ADA (Terasaka et al., 2000) have anti-inflammatory effects. Increasing the level of extracellular adenosine by ADA inhibition ameliorates experiment colitis (Antonioli et al., 2007). Furthermore low-dose methotrexate (MTX), which is widely used for treating chronic inflammatory disorders, e.g. rheumatoid arthritis and psoriasis, increases adenosine levels (Tian and Cronstein, 2007).

Enzyme and transport inhibitors and AR allosteric enhancers that indirectly modulate the levels of AR activation are shown (**16** – **21**, Figure 2A). The widely used anti-platelet and vasodilator drug, dipyridamole **21**, which was initially shown to inhibit cyclic nucleotide phosphodiesterases (PDEs), probably has its main effects by ENT1 inhibition, leading to activation of ARs by increasing local extracellular adenosine concentrations (Kim and Liao et al., 2008). In the brain, ENT1 and ADK are principally expressed in astrocytes, which are a major source of extracellular adenosine via ATP (Ruby et al., 2010; Boison, 2013).

2. Proposed adenosinergic mechanism of diverse drugs and treatments

Reports dating back nearly four decades implicate adenosinergic mechanisms in the MoA of non-AR drugs (e. g. **40** – **69**, Figure 3). However, the pharmacological tool compounds used in these studies were often ambiguous. Initially, the AR agonists and antagonists available were of marginal AR subtype selectivity, and other pharmacological tools such as CD73 inhibitors were also suboptimal. Pharmacokinetic considerations are also important. For example, in mice the highly A_1AR selective agonist CPA **5**, will activate peripheral A_3AR at lower intraperitoneal (i.p.) doses than those that activate central A_1ARs (Carlin et al., 2017). The majority of these reports do not utilize AR KO mice, so there is a need for re-evaluation of many of these conclusions using more modern methods. Nevertheless, we present here the evidence as published, acknowledging that some conclusions might require reinterpretation.

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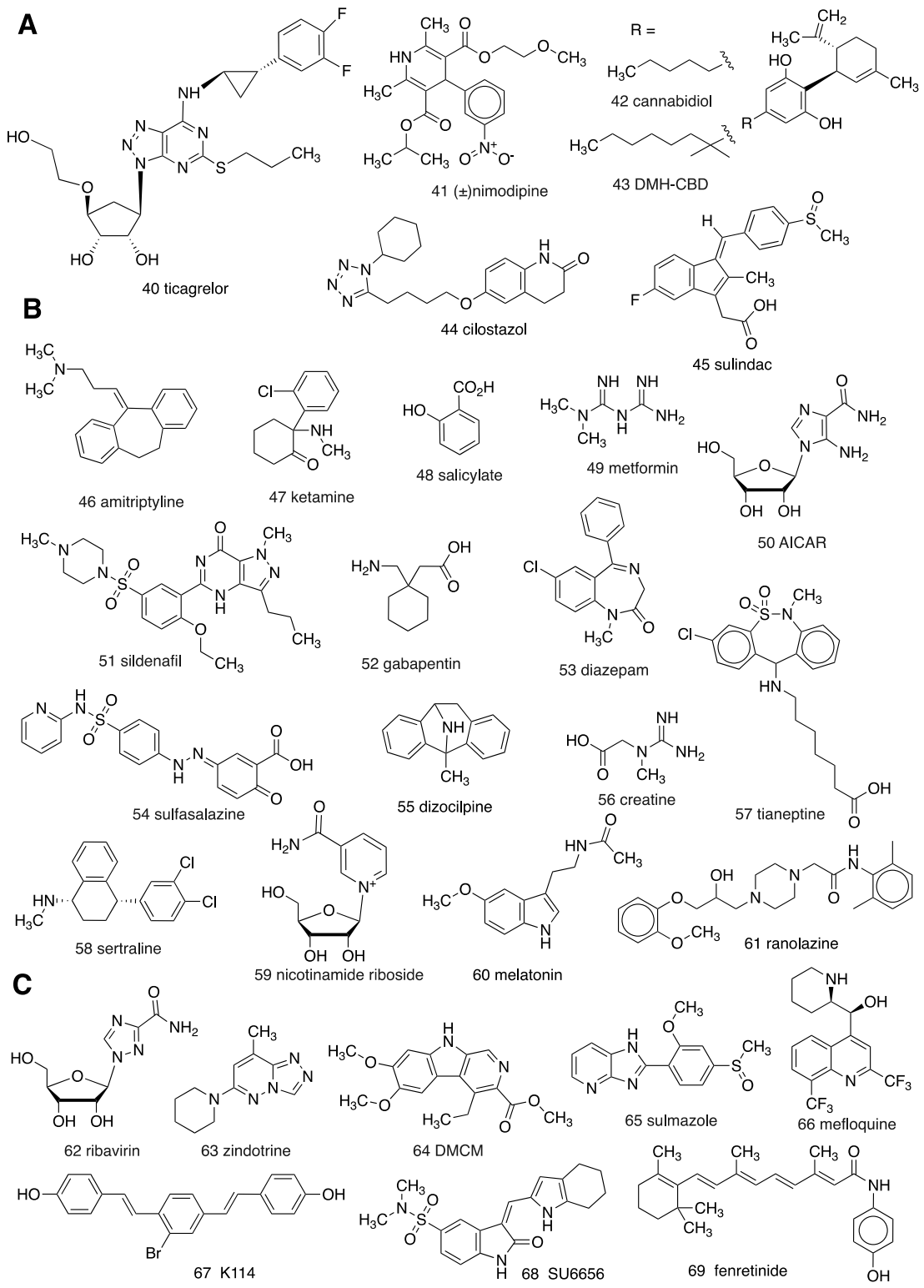

Figure 3. Structures of diverse drugs suggested to have an unanticipated involvement of adenosinergic signaling in their MoA: A. By modulating adenosine transport; B. By unclear mechanisms; C. By direct AR interaction.

2.1. Vasoactive and other cardiovascular effects

Adenosine **1** was first purified in 1929, after it was found to cause bradycardia (now known to be an effect of A_1AR activation in the sinoatrial node) as a bioassay (Drury and Szent-Györgyi). The smooth muscle relaxant and vasodilatory properties of adenosine, by activation of the A_{2A} and

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A_{2B}ARs, led to the early suggestion for the use of adenosine agonists as anti-hypertensive agents (Hutchison, 1989). Although there were problems with this approach (e.g. side effects and desensitization), other cardiovascular applications of AR agonists and antagonists have been considered. Furthermore, various cardiovascular physiological effects ascribed to presumed non-AR modulators, e. g. ticagrelor **40** (see below), are in fact mediated by adenosine.

Ischemic preconditioning of the heart, when a pretreatment improves the outcome from an ischemia event, is thought to involve ARs, consistent with the highly elevated levels of adenosine that occur in hypoxia. Preconditioning can also be induced by exogenous AR agonists, potentially through activation of each of the four AR subtypes, which are present on cardiac myocytes (Cohen et al., 2010; Singh et al., 2018; Wan et al., 2019). Adenosine pretreatment also conditions against ischemic cardiac damage ~24-72 hours later (Baxter, 2002). The cardioprotection by selective opioid receptor agonists, such as natrindole (δ-opioid) or GNTI (κ-opioid), in remote preconditioning was found to be dependent on AR signaling, possibly through a direct interaction between the opioid receptors and the A_1AR (Surendra et al., 2013). Sildenafil **51**, an inhibitor of PDE5 which was originally developed as an antihypertensive agent and is used for treating erectile dysfunction, induces a delayed cardioprotective effect. This protection in the mouse heart is absent in A_1AR KO mice or upon treatment with the potent A_1AR antagonist DPCPX **26** (Salloum et al., 2007), suggesting that sildenafil activates adenosinergic signaling by an unclear mechanism. The anti-ischemic effect in the heart of anti-angina drug ranolazine, a sodium and potassium channel blocker, appears be dependent on its causing elevated adenosine levels (Le et al., 2019).

Metformin **49** is a first-line drug for treating type 2 diabetes mellitus (T2D) and is being considered for other indications. Metformin likely affects the cellular energy state, but the specific mechanism(s) involved are debated. Elucidating metformin's molecular target(s) has been hampered by the high doses and tissue/cell concentrations required for clinical efficacy. Proposed mechanisms, which are not mutually exclusive, include inhibition of respiratory chain complex 1, inhibition of mitochondrial glycerophosphate dehydrogenase, activation of AMPK, and effects in the intestine (both via the microbiome and independent of it) (Rena et al., 2017). Metformin also inhibits AMP deaminase (Lanaspa et al., 2012; Ouyang et al., 2011), but at concentrations higher than are achieved clinically. The proposal that metformin can increase extracellular adenosine by inhibiting adenosine kinase was not supported by direct experiments (Paiva et al., 2009).

Nicotinamide riboside (NR) **53**, a metabolic precursor of NAD^+, has anti-aging effects and provides health benefits that mimic dietary calorie restriction (Martens et al., 2018). NR increases ATP, which leads to a higher level of adenosine. Adenosine content in peripheral blood mononuclear cells from human subjects was raised by 50% following dietary NR supplementation, compared to a placebo.

The antiplatelet drug ticagrelor **40** is principally a reversible inhibitor of the ADP-activated $P2Y_{12}$ receptor. It has a secondary action that causes an increase in extracellular adenosine by blocking the ENT1 in erythrocytes in whole blood and endothelial cells (Nylander et al., 2013). The adenosine is thought to act on the A_{2A}AR on platelets to contribute to ticagrelor's desired antiaggregatory effect and is possibly implicated in the drug's side effect of dyspnea (Cattaneo, 2012). Nimodipine **41**, an L-type Ca^{2+} channel blocker used for treating vasospasm, at pharmacological doses inhibits ENT1 binding and adenosine uptake (Streissnig et al., 1985). [^{3}H](±)Nimodipine bound to the nucleoside transporter in human red blood cell ghosts with a K_d of 52 nM. The (+)-isomer of nimodipine was preferred at the transporter, in contrast to Ca^{2+} channels, which prefer the (-)-isomer. Binding affinities of several other dihydropyridine Ca^{2+} channel blockers were weaker, yet measurable. The anti-angina drug ranolazine **61** increases extracellular adenosine concentration in the heart, which would account for its anti-adrenergic and cardioprotective effects, possibly by inhibition of cytosolic 5'-nucleotidase (Le et al., 2020). Mineralocorticoid receptor antagonist canrenone (structure not shown) has been proposed to exert cardioprotective effects through A_{2B}AR activation as a result of increased extracellular adenosine (Schmidt et al., 2010).

The anti-claudication drug cilostazol **44** acts as a dual inhibitor of PDE3 and adenosine uptake (Liu et al., 2006; Fong et al., 2010), and cilostazol-induced cardioprotection is antagonized by a non-

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selective AR antagonist 8-sulfophenyl-theophyline **24** (Bai et al, 2011). Chronic administration of the immunosuppressive drug cyclosporin A blocks ENT uptake of adenosine to increase its plasma levels (Guieu et al., 1998). The binding K_i values of known ENT1 inhibitors, such as the potent draflazine, and their IC_{50} values in adenosine uptake were compared (Rosenbrier Ribeiro, et al., 2017), and the ENT1 potency correlated with bronchospasm and dyspnea in patients with respiratory conditions. In screening 1625 diverse drug molecules, 22% displayed K_i values < 1 μM and 58% had K_i values < 10 μM, indicating that ENT1 is a relatively common off-target activity and requires careful evaluation in the drug development process. Statins enhance CD73 expression in cardiomyocytes and endothelial cells; administration of rosuvastatin (structure not shown) in humans augmented adenosine produced an additive vasodilation with dipyridamole and protection against ischemia-reperfusion injury, which were antagonized by caffeine (Meijer et al., 2009).

2.2. Treatment of inflammation

The adenosinergic mechanisms of three classes of anti-inflammatory drugs have been extensively explored by Cronstein and colleagues (Cronstein et al., 1999). Low doses of MTX, a folate antimetabolite used in cancer treatment at 100-fold higher doses, are a classic treatment of rheumatoid arthritis. Cronstein and coworkers found that its MoA involves increasing adenosine levels, which results in the activation of the A_{2A}AR on immune cells (Tian and Cronstein, 2007). Human polymorphisms of the adenosine monophosphate deaminase 1 (AMPD1), inosine monophosphate synthase (ATIC), and inosine triphosphate pyrophosphohydrolase (ITPA) genes that are involved in adenosine release correlate with favorable responses to MTX treatment for rheumatoid arthritis (Wessels et al., 2006). The polyglutamated form of MTX indirectly increases levels of 5-aminoimidazole-4-carboxamide riboside (**50**, AICA riboside, AICAR), by inhibiting its metabolism by AICAR transformylase (Tian and Cronstein, 2007). Increased intracellular AICAR results in higher adenosine levels (see below). In the in vivo mouse air-pouch model of inflammation, methotrexate increased levels of AICAR in spleen cells, and in exudates increased adenosine to inhibit leukocyte accumulation. The AR subtype involved in this MoA of MTX is the A_{2A}AR.

The MoA of acetylsalicylic acid (ASA), a non-steroidal anti-inflammatory drug (NSAID) and a prodrug of salicylic acid **48**, is thought to involve in part ARs (Cronstein et al., 1999). Previously, high doses of ASA (3-5 g/day) administered to rheumatoid arthritis patients (Fries et al., 1993) was thought to elevate adenosine signaling. However, in the CNS, the expression of proinflammatory COX-2 was upregulated by A_{2A}AR activation, suggesting the use of an antagonist for reducing neuroinflammation (Fiebich et al., 1996). Although the principal MoA of acetylsalicylic acid is to inhibit cyclooxygenase (COX1 and 2) enzymes, its chemical precursor salicylate acts via with multiple pathways. It decreases leukocyte counts likely through effects on nuclear factor-κB, while it also inhibits various dehydrogenases and kinases and stimulates AMPK and improves glycemia in T2D patients (Goldfine et al., 2013). Salicylate also uncouples oxidative phosphorylation leading to increased intracellular AMP and, indirectly, extracellular adenosine (Cronstein et al., 1999). The anti-inflammatory drug sulfasalazine **54** is a prodrug of 5-amino-salicylic acid and is used in rheumatoid arthritis patients that do not respond to NSAIDs. Unlike salicylic acid, it is considered a disease modifying antirheumatic drug (DMARD) by suppressing immune system function. There is evidence that the MoA of sulfasalazine also involves adenosinergic signaling by interfering with AICAR metabolism (Gadangi et al., 1996, Cronstein et al., 1999). The anti-inflammatory drug sulindac **45** competitively inhibits ENT1 (Li et al., 2012).

Glucocorticoids are foundational anti-inflammatory drugs. Cronstein and colleagues did not find an adenosine-dependent MoA for the action of glucocorticoids on leukocytes (Cronstein et al., 1999). However, there is a report that use of glucocorticoids in treating inflammation involves ARs as a secondary mechanism, i.e. activation of the A_3AR to promote survival of anti-inflammatory monocytes (Barczyk et al., 2010). The anti-inflammatory effect of ketamine **47**, an N-methyl-D-aspartatic acid (NMDA) receptor antagonist, has been ascribed to ARs (Mazar et al., 2005). However, the A_{2A}AR antagonist DMPX **29** used in that study is weak and marginally selective. Macrocyclic derivatives related to the immunosuppressant drug rapamycin were found to block adenosine

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uptake by binding to ENT1 and consequently increase AR signaling (Guo et al., 2018). Eplerenone, an antagonist of the cytosolic mineralocorticoid receptor, is cardioprotective and its benefit was lost when adenosine signaling was blocked in mice and rats (van den Berg et al., 2014). However, administration of eplerenone in healthy human subjects did not elevate circulating adenosine levels (van den Berg et al., 2014).

There may be a connection between ARs and the anti-inflammatory effects of the cannabinoid system. The cannabinoid metabolite cannabidiol (**42**, CBD) was recently approved by the FDA for rare forms of childhood epilepsy. CDB does not bind potently to cannabinoid receptors (CBRs) or to ARs, but it inhibits adenosine uptake by binding to ENT1 with a K_i value of <250 nM (Carrier et al., 2006), which may contribute to its anti-inflammatory effect by indirectly activating the A_{2A}AR (Burstein, 2015). The anti-ischemic and anti-stroke activity of CBD partly results from AR activation (Castillo et al., 2010). In newborn mouse brain slices subjected to deprivation of oxygen and glucose, CDB (100 μM) reduced hypoxic ischemic brain damage by lowering glutamate release (associated with A_1AR activation, Boison, 2013) and expression of IL-6, TNFα, COX-2 and iNOS (all reversed by A_{2A}AR or CB_2R antagonists at high concentrations). A_1AR in the brain suppresses the release of excitatory neurotransmitters (Ruby et al., 2010). CBD's decrease of inflammation in acute lung injury is also dependent on the A_{2A}AR (Ribeiro et al., 2012). Pretreatment of mice with 20 mg/kg CBD (i.p.) 60 min prior to lipopolysaccharide-induced lung injury reduced neutrophil migration and induction of pro-inflammatory cytokines, and these effects were antagonized by the A_{2A}AR antagonist ZM241385 **32** (5 mg/kg, i.p.) administered 30 min prior to CBD. DMH-CBD **43**, dimethyl, chain-extended analogue of CBD was found to reduce NFκB activity by indirectly activating the A_{2A}AR (Silva et al., 2019). The adenosinergic mechanisms proposed for CBD and its analogues merit further validation using AR KO mice.

2.3. Pain, antidepressant, sleep and other behavioral intervention

Activation of each of the AR subtypes, except A_{2B}AR (Hu et al., 2016), has been found to relieve pain in various animal models (Janes et al., 2017). For decades, a connection between the opioid system and the adenosine system has been known (Stone et al., 1979). Morphine induces the release of adenosine to activate the A_1AR in the spinal cord (Sweeney et al., 1987), an effect that is attenuated in chronic neuropathic pain (Sandner-Kiesling et al., 2001).

A_1AR activation has been shown to produce an antidepressant effect that mimics the effect of sleep deprivation, which increases brain adenosine (Hines et al., 2013). The antidepressant effect of A_1AR activation is mediated by the transcription factor homer1a in the brain, which was demonstrated using both AR agonists and A_1AR KO mice (Serchov et al., 2015).

The anesthetic drug and NMDA receptor uncompetitive inhibitor ketamine **47** is a novel antidepressant medication having a sustained effect with rapid onset. Its antidepressant action, as evident in the mouse tail suspension assay, is dependent on activation of A_1AR and A_{2A}AR (Cunha et al., 2015). The antidepressant effects of ketamine and creatine **56**, a metabolite that facilitates ATP recycling, were reduced by caffeine **23**, DPCPX **26** or ZM241385 **32** and produced synergistic antidepressant effects with co-administered AR agonists. The anxiolytic effect of another NMDA receptor antagonist, dizocilpine (**55**, MK801, 50 μg/kg), on mice in the elevated plus-maze depends on A_1AR activation by endogenous adenosine, as determined by coadministration of DPCPX **26** (Fraser et al., 1997).

Amitriptyline **46** is an inhibitor of norepinephrine and other neurotransmitter transporters that is used to treat depression and chronic neuropathic pain. Increased A_1AR signaling in the spine and periphery has been proposed as a MoA of amitriptyline, along with $5HT_7$ receptor agonism secondarily (Liu et al., 2013). Amitriptyline (10 mg/kg, i.p.) was also found to increase the pain threshold of neuropathic rats following sciatic nerve ligation and reduce the associated proinflammatory signaling to ERK1/2 and CREB (Kim et al., 2018). Both effects of amitriptyline were antagonized by A_3AR antagonist MRS1191 **36** (1 mg/kg subcutaneously immediately before amitriptyline). However, whether the A_1AR- and A_3AR-dependent effects of amitriptyline were the result of increased adenosine levels or the mechanisms involved was not probed. Also, amitriptyline

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binds with moderate affinity to human A_{2A}AR (K_i 4.8±0.11 μM), but not to A_1AR, which might contribute to its side effects (Kalkan et al., 2018).

Gabapentin **52,** an inhibitor of certain voltage-dependent calcium channels is widely used for treating seizures and chronic neuropathic pain. Its anti-hyperalgesic affect is antagonized by the nonselective AR antagonist caffeine and by intrathecal administration of the selective A_1AR antagonist DPCPX **26** (Martins et al., 2015). Thus, its beneficial action in pain may be partly dependent on A_1AR activation. Gabapentin is also used to treat restless leg syndrome, which was recently reported to have a MoA involving the A_1AR (Ferré et al., 2018). One of the more unusual proposals is a peripheral adenosinergic MoA of warm water immersion therapy for treating persistent inflammatory pain, based on the antagonism by locally administered DPCPX (Martins et al., 2014).

The experimental anti-ischemic and anticancer drug AICAR enters the cell through nucleoside transporters ENT1 and CNT3 (Boß et al., 2016), and, as its 5′-monophosphorylated product ZMP, activates AMPK. AICAR also competes with adenosine in nucleoside transport, which contributes to elevated extracellular adenosine, as shown in the rat hippocampus (Gadalla et al., 2004). AICAR also raises coronary blood levels of adenosine during heart ischemia (Gruber et al., 1989).

The neuropeptide orexin, which binds to two orexin receptor GPCRs, induces an antinociceptive effect that is measurable in a rat model of colonic distension. Unlike other models in which adenosine has an antinociceptive effect, in this model A_1AR signaling increases nociception (Okamura et al., 2016). Thus, an A_1AR antagonist is predicted to be beneficial in this context. Note that non-selective AR antagonist caffeine is used in combination with over-the-counter pain medications to boost their effectiveness, but the reasons are unclear. Orexin also has a dual role as enhancer and suppressive compensator in central A_1AR-induced hypothermia (Futatsuki et al., 2018).

Various herbal pain treatments are thought to involve AR signaling (*Haematostaphis barteri*, Ameyaw et al., 2016; *Clinacanthus nutans*, Zakaria et al., 2018). An antinociceptive and neuroprotective traditional Chinese medicine containing paeoniflorin, a monoterpene glucoside isolated from peony root, has allosteric A_1AR activation as its putative mechanism of action (Gao et al., 2018). Uliginosin B is a naturally occurring acylphloroglucinol that reduces pain, and its MoA appears to involve adenosine signaling (Stolz et al., 2016). Incarvillateine is a complex monoterpene alkaloid that induces an antinociceptive effect, which is associated with AR signaling, but not opioid receptor activation (Wang et al., 2015).

The pain-suppressing effect of acupuncture was found to be dependent on A_1AR signaling. The proposed mechanism is that an acupuncture needle increases transient, local levels of ATP, itself a pronociceptive agent, which indirectly raises adenosine levels to activate the A_1AR on peripheral sensory nerves (Goldman et al., 2010). ATP acting at the P2X3 receptor is known to mediate the painful effects of distension (Burnstock, 2017), and its hydrolysis product adenosine would reduce pain. Furthermore, electroacupuncture is effective in suppressing inflammation in part by A_1AR signaling (Liao et al., 2017).

The main MoA of the benzodiazepines as anti-anxiety drugs is as allosteric activators of the $GABA_A$ receptor, but other factors influence its action. Benzodiazepine anxiolytics were reported by Phillis and Wu (1982) as among many diverse, centrally active drugs to inhibit adenosine uptake, usually at micromolar concentrations, along with antipsychotics trifluoperazine, spiroperidol and sulpiride. Like gabapentin, benzodiazepines are also used to treat restless leg syndrome, which is now associated with the A_1AR (Ferré et al., 2018). Curiously, some indirect effects of the benzodiazepine diazepam **53** on this receptor have been demonstrated, consistent with diazepam reversing some of the stimulant effects of caffeine. However, diazepam decreases A_{2B}AR signaling, though diazepam does not bind directly to the receptor (Hoffmann et al., 2013). Chronic diazepam treatment lowered the density of hippocampal A_1AR and striatal A_{2A}AR radioligand binding in the brain by 13% and 46%, respectively (Hawkins et al., 1988). However, in vivo brain binding of [^{3}H]DPCPX **26** was decreased by chronic exposure of mice to two other benzodiazepines (Kaplan et al., 1992).

The antidepressant drug sertraline **58**, a selective serotonin reuptake inhibitor (SSRI), reduced the level of ADA activity in rats (Azimzadeh et al., 2017). Tianeptine **57**, an atypical tricyclic

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antidepressant, delayed the onset time of seizures induced by pentylenetetrazole in mice. A mechanism of action of tianeptine might be indirect A_1AR activation, based on inhibition of the protection by DPCPX **26** (Uzbay et al., 2006). Zinc chloride (30 mg/kg, i.p.) induced an antidepressant effect in the mouse forced swimming test that was blocked by caffeine or antagonists of A_1AR (DPCPX **26**, 2 mg/kg i.p.) or A_{2A}AR (ZM241385 **32**, 1 mg/kg i.p.) (Lobato et al., 2008). Somewhat selective agonists of those AR subtypes and dipyridamole **21** potentiated the effect of a subthreshold dose of Zn^{2+}.

Adenosine acts through the A_1AR and A_{2A}AR in the brain to promote sleep. Melatonin receptors are involved in regulation of circadian rhythm and the sleep cycle (Posadzki et al., 2018). Gandhi et al. (2015) found that melatonin **60** promotes sleep in part through ARs using A_{2A}AR KO mice and ADK inhibition with ABT-702 **18**. Melatonin promotes the local release of forebrain adenosine in mammals. The involvement of adenosine could provide a mechanistic link between circadian and homeostatic sleep control.

One of the effects of ethanol is the inhibition of adenosine uptake through transporters (ENT1), which raises the level of extracellular adenosine (Nagy et al., 1990). With acute exposure (but not chronic exposure) to clinically relevant ethanol concentrations of 50 – 200 mM, the uptake of adenosine in lymphoma cells was decreased by 30-40%, leading to increased extracellular adenosine. Using A_{2A}AR KO mice or following pretreatment of WT mice with nonselective antagonist caffeine, this receptor was found to mediate the hypnotic effects of ethanol indicated by NREM sleep (Fang et al., 2017). Acute ethanol exposure has a dual effect on adenosine signaling in rat hippocampal slices (Hughes et al., 2018). Depressant concentrations of ethanol rapidly increased the basal release of adenosine (leading to A_1AR activation), but its release was inhibited during electrographic seizure activity that was induced using 4-aminopyridine. The known hypothermic effect of ethanol was partly blunted in A_{2A}AR KO mice, in both males and females (Naasila et al., 2002). A_1AR activation is also involved in the sedative/hypnotic effects of ethanol and are a primary mechanism of ethanol-induced ataxia (Ruby et al., 2010). ENT1-null mice have a decreased adenosine tone, a reduced response to ethanol, and greater ethanol consumption (Choi et al., 2004).

2.4. Anticancer drugs

Anti-cancer drug deoxycoformycin (pentostatin, DCF **20**) inhibits intracellular ADA to interfere with DNA synthesis. A secondary consequence of ADA inhibition is a rise in extracellular adenosine, which can activate ARs. Several ADA inhibitors including DCF were shown to moderately raise the concentration of extracellular adenosine in guinea pig atria to augment exogenous agonist-induced myocardial A_1AR activation (Kemeny-Beke et al., 2007). Similar effects of DCF to raise endogenous adenosine levels in vivo were reported earlier.

The cell entry and cytotoxicity of anticancer nucleosides such as cytarabine, fludarabine and gemcitabine is dependent on nucleoside transporters, and transporter levels in cancer cells can be a determinant of drug efficacy and toxicity (Boswell-Casteel and Hays, 2017). However, the transporters can be downregulated to create drug resistance (Mackey et al., 2000). Non-nucleoside anticancer drug gefitinib and other tyrosine kinase inhibitors also inhibit nucleoside transporters.

2.5. AR interaction with other GPCRs

ARs can heterodimerize with other GPCRs, as shown for A_1AR and the dopamine D_1R (Ginés et al., 2000). These direct GPCR interactions enable allosteric pharmacological interactions to occur. For example, disruption of the A_{2A}AR–D_2R complex by an A_{2A}AR agonist blocks the inhibition of cocaine self-administration (Borroto-Escuela et al., 2018). Thus, there can potentially be MoA interactions between ARs and any of the numerous other GPCRs that participate in their heterodimerization and oligomerization (reviewed in Vecchio et al., 2018). Interaction of A_{2A}AR–$mGlu_5$R (metabotropic glutamate receptor) are thought to be important in Parkinson's disease. Thus, non-adenosinergic agents can interact with purinergic signaling pathways through the allosteric interactions within GPCR heterodimers.

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Adenosine is also a partial agonist of the ghrelin receptor, GHSR1a, without a documented requirement for heterodimerization (Smith, 2005).

2.6. Other unanticipated interactions with ARs

Off-target binding at one or more of the ARs has been detected for various known drugs, suggesting a direct interaction, typically as antagonist. In some cases, an off-target interaction might either reinforce or attenuate the benefit from the drug treatment. Mefloquine **66**, an antimalarial drug that displays significant CNS side effects, was found to be a moderately potent A_1AR and A_{2A}AR antagonist, with K_i values of the (-)-(*R*,*S*)-isomer of 255 and 61 nM, respectively, and this led to the design of additional analogues with increased A_{2A}AR affinity as potential anti-Parkinson's drugs (Gillespie et al., 2008). The AR antagonism and other off-target GPCR activity might contribute to mefloquine's side effects. Dipyridamole **21** (K_i = 19 μM), proadifen (49 μM), a cytochrome P450 monooxygenase inhibitor, and folic acid (28 μM) weakly inhibit radioligand binding at the rat A_3AR (Siddiqi et al., 1996). Cardiotonic drug sulmazole **65** (23 μM), anti-asthmatic zindotrine **63** (0.9 μM), and proconvulsant beta-carboline DMCM **64** (3.3 μM) inhibit radioligand binding at the rat A_{2A}AR (Siddiqi et al., 1996). Sulmazole **65** (11 μM) and DMCM (1.6 μM) also inhibit rat A_1AR binding (Parsons et al., 1988; Siddiqi et al., 1996). A_3AR binding is inhibited by other drug substances, identified using high-throughput fluorescent screening of a chemical library: K114 (**67**, pK_i 6.43), Src inhibitor SU6656 (**64**, pK_i 6.17) and retinoic acid p-hydroxyanilide (**69**, pK_i 6.13, fenretinide) (Arruda et al., 2017).

Some flavonoids are known to bind to A_1AR and A_3AR (Jacobson et al., 2002). In addition, the flavone quercitin inhibits both the activity and expression of CD73, leading to a reduction of adenosinergic signaling (Braganhol et al., 2007). *In silico* screening for binding at ARs, based on receptor X-ray structures, has identified numerous diverse chemotypes as candidate ligands, including some with known biological activity (Rodríguez et al., 2016). The antiviral drug ribavirin binds to the A_1AR as a partial agonist (Tosh et al., 2019).

With broad screening of new drug candidates now standard, more compounds have been found to interact with purinergic signaling pathways. For example, AMG 337, an experimental kinase inhibitor for cancer, was found to inhibit ENT1 at < 1 μM and this was suggested to cause cerebral vasorelaxation and thus the dose-limiting headaches in patients (Amouzadeh et al., 2019).

Other nonpharmacological treatments are reported to interact with the adenosine system. Refractory seizure activity can be treated with a ketogenic diet, which works in part by increasing A_1AR-mediated inhibition of seizure activity (Masino et al., 2011) Electroconvulsive therapy as a treatment for mood disorders has been reported to act through the release of ATP to indirectly raise adenosine concentrations and activate the brain A_1AR leading to its antidepressant effect (van Calker and Biber, 2005). Curiously, pulsating electromagnetic fields (PEMFs) were found to upregulate adenosinergic signaling in cancer and inflammation models (Varani et al., 2017; Vincenzi et al., 2018).

3. Conclusions

We have collected reports on the unanticipated involvement of adenosinergic signaling in the action of diverse drugs. Some of the compounds or treatments are thought to raise endogenous adenosine levels by inhibiting adenosine transport, e.g. ticagrelor, ethanol, and CBD. Others presumably activate one or more of the ARs by raising adenosine levels or by undetermined or unclear mechanisms, e.g. PEMFs, or through intracellular pathways, e.g. methotrexate, salicylate, NR and AICAR. Yet other compounds directly bind one or more of the ARs, e.g. mefloquine.

Most of the detected interactions have not yet been validated as a MoA in humans. Some of these reports might represent epiphenomena, and we have commented on the strength of the evidence in some cases. Some of the effects may constitute one aspect of a broader cluster of actions. Therefore, it is essential that these observations be validated and characterized using modern tools, such as more specific pharmacological agents and genetically modified mouse lines. Given the interest in AR modulation for treatment of cancer, CNS, immune, metabolic, cardiovascular, and musculoskeletal

conditions, it is informative to consider AR and non-AR adenosinergic effects of approved drugs and conventional treatments.

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