REVIEW ARTICLE

Adenosine signaling mediate pain transmission in the central nervous system

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Abstract

Pain is a common clinical symptom that seriously affects the quality of life in a variety of patient populations. In recent years, research on the role of adenosine signaling in pain modulation has made great progress. Adenosine is a purine nucleoside and a neuromodulator, and regulates multiple physiological and pathophysiological functions through the activation of four G protein–coupled receptors, which are classified as A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ARs). Adenosine and its receptors that are widespread in the central nervous system (CNS) play an important role in the processing of nociceptive sensory signals in different pain models. A_1Rs have the highest affinity to adenosine, and the role in analgesia has been well investigated. The roles of A_{2A}Rs and A_{2B}Rs in the modulation of pain are controversial because they have both analgesic and pronociceptive effects. The analgesic effects of A_3Rs are primarily manifested in neuropathic pain. In this article, we have reviewed the recent studies on ARs in the modulation of neuropathic pain, inflammatory pain, postoperative pain, and visceral pain in the CNS. Furthermore, we have outlined the pathways through which ARs contribute to pain regulation, thereby shedding light on how this mechanism can be targeted to provide effective pain relief.

Introduction

Pain is a distressing experience caused by tissue or nerve injury under different disease conditions, and it impacts human health globally. The latest research decision of the International Association for the Study of Pain (IASP) defines pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [1]. Peripheral neurons, spinal cord, and brain mechanisms contributed to the modulation of pain. Sensory neurons in the periphery are activated and project into the spinal cord dorsal horn, and projection neurons send information to the brainstem and thalamus, and then to several brain regions involved in sensory discrimination and emotional sensory perception [2, 3]. Loss of synaptic inhibition in the spinal dorsal horn is a major process underlying the development of several pain conditions [3]. The cortical and subcortical regions related to pain perception are the primary and secondary somatosensory cortices, insula, anterior cingulate, thalamus, and prefrontal cortex [4]. Hyperactivity of sensory neurons and activation of astrocytes and microglia are also major contributors of chronic central nervous pain [5].

At present, the research on pain has made substantial progress; however, there are still many painful conditions that cannot be alleviated. The mechanisms of action associated with pain are not fully understood. As a neurotransmitter, adenosine regulates pain by activating four G protein–coupled receptors, namely, adenosine A_1 receptors

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(A$_1$Rs), adenosine A$_{2A}$ receptors (A$_{2A}$Rs), adenosine A$_{2B}$ receptors (A$_{2B}$Rs), and adenosine A$_3$ receptors (A$_3$Rs) [6]. There are significant differences in the affinities of adenosine to these receptors; adenosine has the highest affinity for A$_1$Rs, followed by A$_3$Rs, A$_{2A}$Rs, and A$_{2B}$Rs subtypes (A$_1$Rs > A$_3$Rs > A$_{2A}$Rs > A$_{2B}$Rs) [7, 8]. Adenosine triphosphate (ATP) is the main source of adenosine, which is sequentially dephosphorylated inside the cell to form adenosine diphosphate (ADP), adenosine monophosphate (AMP), and adenosine. Outside the cell, ectonucleosidase triphosphate diphosphohydrolase (CD39) and ecto-5′-nucleotidase (CD73) promote adenosine formation. Adenosine acts on its cognate receptors (A$_1$Rs, A$_{2A}$Rs, A$_{2B}$Rs, and A$_3$Rs) to regulate a variety of pain conditions [9–11] (Fig. 1). Accordingly, adenosine signaling has been identified as a potential target for the regulation of pain. Although the role of ARs in pain regulation has been studied in both central and peripheral nervous system, the role of CNS is more widely studied [12, 13].

Since the 1970s, research on adenosine analgesia have mainly focused on the A$_1$Rs [14]. With the intensive research on adenosine and its receptors, many investigators have found that other receptors (A$_{2A}$Rs, A$_{2B}$Rs, and A$_3$Rs) also regulate pain. As per our present knowledge, adenosine signaling in the spinal cord involved in the analgesic process can be summarized in a relatively accurate manner as follows:

(1) presynaptic inhibition of excitatory neurotransmitter release and inhibits the release of certain neurotransmitters in cerebrospinal fluid (CSF) and (2) postsynaptic inhibition of the effects of excitatory neurotransmitters [15]. However, owing to the scarcity of studies on the brain, the specific and in-depth analgesic mechanism is yet to be elucidated.

Here, we reviewed the distribution of AR subtypes in pain pathways and the role of each subtype in pain regulation of CNS, then highlight the regulatory roles and molecular mechanisms of several receptors in different pain models.

Location of adenosine receptors in the pain circuitry

ARs are present in various parts of the human body and mediate a myriad of physiological processes. Moreover, ARs affect the functioning of numerous systems in the body, including the CNS, cardiovascular system, gastrointestinal system, respiratory system, immune system, renal system, and metabolic system as well as different organs and tissues such as the kidney, bones, joints, eyes, and skin [9]. A$_1$Rs are expressed in both central and peripheral nervous systems. In brain regions, A$_1$Rs are found in the hippocampus, cerebral cortex, cerebellum, caudate-putamen, globus pallidus, thalamic nucleus, brain stem nucleus, periaqueductal gray (PAG), and basal ganglia, mainly in the excitatory synapses [6, 9, 16]. In the spinal cord dorsal horn, they are located
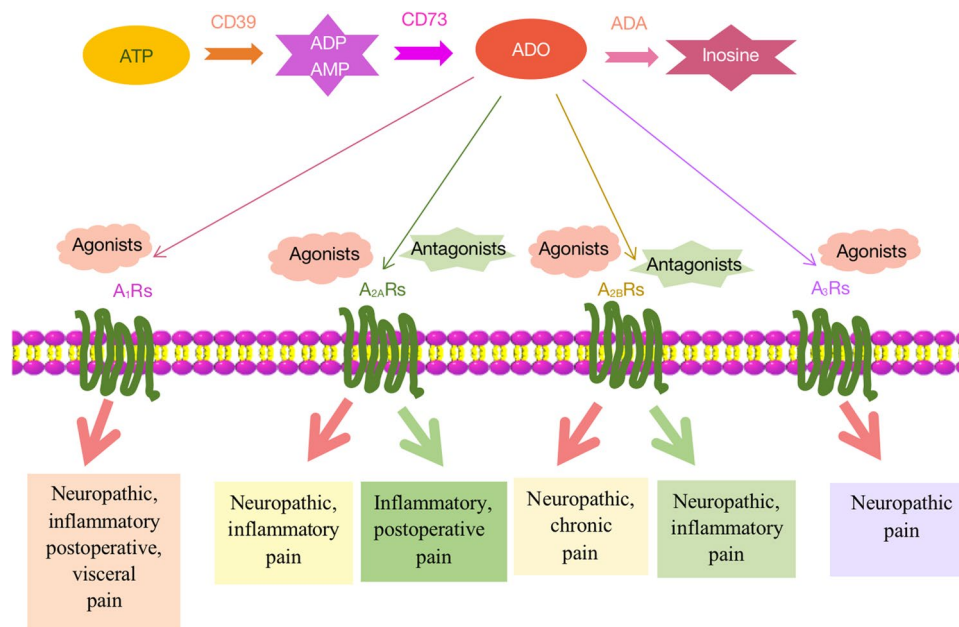

Fig. 1 A schematic view of adenosine metabolism and ARs participating in pain. The dephosphorylation of ATP, ADP, and AMP is the main mechanism for the extracellular production of adenosine. A$_1$R activation in CNS exerts analgesic effects in various types of pain such as inflammatory, neuropathic, postoperative, and visceral pain. The activation of A$_{2A}$Rs can play an analgesic role in neuropathic pain. On the other hand, the blockage of A$_{2A}$Rs exerts antinociceptive effects in postoperative pain. Both agonists and antagonists of A$_{2A}$Rs have been shown to relieve inflammatory pain. The stimulation of the A$_{2B}$Rs has an antinociceptive effect on chronic pain, while the blocking of A$_{2B}$Rs is helpful in the treatment of inflammatory pain. Besides, neuropathic pain can be relieved by agonists and antagonists of A$_{2B}$Rs. Finally, A$_3$R activation gives analgesic effects in neuropathic pain

in the inner lamina II postsynaptic neuronal cell bodies and processes [17]. Besides, the expression of A_1Rs is found in the peripheral sensory nerve endings [6].

$A_{2A}Rs$ are mainly located in the CNS and peripheral immune cells. Specifically, $A_{2A}Rs$ are widely expressed in both the pre- and postsynaptic neurons, which are found in the caudate nucleus, nucleus accumbens, olfactory tubercle, and putamen of the brain. Experimental studies have illustrated the distribution of $A_{2A}Rs$ in the lumbar spinal cord, the substantia gelatinosa, and in the lamina II neurons [18]. Immunochemical studies have revealed that $A_{2A}Rs$ are expressed in the dorsal horn neurons of the thoracic spinal cord [19]. $A_{2B}Rs$ are present on the presynaptic and postsynaptic neurons as well as on mature and precursor oligodendrocytes. They are widely expressed, but mostly in low abundance [10, 20, 21]. In the periphery, $A_{2B}Rs$ are found on immune cells and inflammatory cells [22].

The A_3R subtypes are widely expressed in different kinds of primary cells, tissues, and cell lines. In the CNS, they are distributed in the thalamus, hypothalamus, cortex, and hippocampus. A_3Rs are also present in the lumbar spinal cord and in the supraspinal region, including the rostral ventromedial medulla (RVM) [23]. In the periphery, A_3Rs are present in various cells such as immune cells and inflammatory cells [24]. All four ARs are found in the microglia and astrocytes [9, 20, 25].

Adenosine signaling and neuropathic pain

Neuropathic pain is pain caused by a lesion or disease of the somatosensory nervous system [1]. Mechanisms underlying the generation of neuralgia are associated with peripheral and central sensitization, including alterations in ion channels (sodium, calcium, and potassium), activation of immune cells such as neutrophils, mast cells, and macrophages, excessive activation of glial cells such as astrocytes and microglia, and epigenetic modulation [26, 27]. The alleviation of neuropathic pain by A_1Rs has been confirmed by many studies [28–31]. A_1Rs are present in pre- and postsynaptic membranes. Presynaptic A_1Rs can inhibit the release of neurotransmitters and the transmission of pain signals, while postsynaptic A_1Rs can induce neuronal membrane hyperpolarization. Research has demonstrated that A_1Rs can activate Ca^{2+} channels, cause intracellular K^+ outflow, promote neuronal hyperpolarization, and inhibit the excitability of postsynaptic neurons [32]. Mice lacking A_1Rs exhibited a high degree of neuropathic pain-like behavior. In mice with partial sciatic nerve injury, $A_1R^{-/-}$ mice displayed a significantly increased neuropathic pain-like behavioral response to heat or cold stimulation. Intrathecal administration of A_1R agonist effectively reduces this pain [33]. This analgesic effect was also demonstrated in another study involving mice with partial sciatic nerve ligation. Paeoniflorin significantly

improved the mechanical threshold and extended the thermal latent period, while A_1R antagonist eliminated the analgesic effect of paeoniflorin. Their finding suggests that paeoniflorin exerts its analgesic and hypnotic effects by activating A_1Rs to inhibit the neuronal hyperactivity in the anterior cingulate cortex and ventrolateral PAG [34]. Hyperalgesia and mechanical static allodynia in a rat model of vincristine induced peripheral neuropathy effectively alleviated by intrathecal injection of the A_1R agonist R-PIA. Intrathecal administration of R-PIA significantly reversed the increase of tumor necrosis factor-α (TNF-α) level and myeloperoxidase activity in a dose-dependent manner. This suggests that intrathecal injection of R-PIA appears to exert analgesic effects through its anti-inflammatory effects, particularly inhibition of TNF-α level and myeloperoxidase activity [35]. Furthermore, in a mice model of diabetes induced by streptozotocin, intrathecal injection of adenosine and A_1R agonist significantly improved the mechanical threshold [36]. On the other hand, in naive rats, it only reduced thermal hypersensitivity and had no effect on the threshold of mechanical stimulation. Electrophysiological studies have shown that CPA suppresses C fibers, but not the baseline of A fibers. The sensory nerve C fiber mediates hyperalgesia caused by nociceptive thermal stimulation, while the sensory nerve A fiber is mainly involved in hypersensitivity to pain caused by mechanical stimulation. Therefore, the authors hypothesize that A_1Rs may be expressed more in the nerve endings of C fiber than in A fiber [37]. Mitogen-activated protein kinase (MAPK) family, which consists of three independent signaling pathways, ERK, p38, and c-Jun N-terminal kinase, plays a key role in regulating nociceptive signaling. In different animal models, inhibition of all three MAPK pathways has been shown to reduce inflammatory and neuropathic pain [38]. Cobra neurotoxin (CNT), a short-chain peptide isolated from the venom of *Naja naja atra*, showed both a central antinociceptive effect and a hyperalgesic reaction in mice models. In the hot plate assay and spinal cord injury (SCI) model, a small dose (25 µg/kg) of CNT produced central analgesic effects by inhibiting the phosphorylation of ERK1 (p44 MAPK) and ERK2 (p42 MAPK) via activation of A_1R in the spinal cord [39]. The above research have demonstrated that A_1Rs could alleviate neuropathic pain. However, in a recent study, Metzner et al. found that although A_1Rs are expressed in dorsal root ganglion (DRG) neurons and dorsal horn neurons involved in pain processing, administration of the A_1R agonist capadenoson at established doses in vivo (0.03–1.0 mg/kg) did not alter mechanical hypersensitivity in mice models of spared nerve injury and paclitaxel-induced neuropathic pain. This suggests a partial A_1R agonist failed to relieve neuropathic pain in mice [40].

In the CNS, the $A_{2A}Rs$ have been shown to be potential targets for alleviating neuropathic pain [41, 42]. Mice lacking $A_{2A}Rs$ ($A_{2A}Rs^{-/-}$) display hypoalgesia [43]. Loram et al.

found that a single intrathecal injection of $A_{2A}R$ agonist can relieve neuropathic pain constantly. In chronic constrictive injury (CCI) rat models, a single intrathecal injection of an $A_{2A}R$ agonist (ATL313) or CGS21680 produced a prolonged reversal of mechanical hyperalgesia and hyperthermia for at least 4 weeks. The combined intrathecal administration of $A_{2A}R$ antagonist and ATL313 eliminated the effect of ATL313 on neuropathic pain in rats. ATL313 was able to effectively attenuate the CCI-induced upregulation of microglia and astrocyte activation marks in spinal cord segments L4–L6 at 1 and 4 weeks after a single intrathecal injection of ATL313. The effect of ATL313 on neuropathic pain was temporarily abolished by the intrathecal administration of neutralizing interleukin (IL)-10 antibodies. In addition, IL-10 mRNA was significantly elevated in the CSF cells collected from the lumbar region. Therefore, $A_{2A}Rs$ may alleviate neuropathic pain by enhancing IL-10 in the immune-active cells of the CNS [44]. The researchers further explored the potential mechanism of the long-term effect of $A_{2A}R$ agonists. Intrathecal injection of ATL313 reverses neuropathic pain by significantly attenuating TNF-α production in microglia and astrocytes via PKA/protein kinase C (PKC) signaling. Therefore, intrathecal injection of adenosine $A_{2A}R$ agonist can reverse neuropathic hyperalgesia through PKA/PKC signal transduction [42]. This effect has also been demonstrated in another study. A single intrathecal injection of CGS21680 reversed SCI-induced tenderness for at least 6 weeks. To some extent, this reversal may be mediated by interleukin IL-10 [41]. Besides, many studies have shown that $A_{2A}Rs$ mediate analgesia by altering the transduction of signals in the relevant neurotransmitter system. The activation of N-methyl D-aspartate (NMDA) receptors can induce hyperalgesia; patch-clamp recordings showed that NMDA currents were inhibited during the application of selective $A_{2A}R$ agonists. These results allude that $A_{2A}Rs$ play a pivotal role in the regulation of NMDA receptors activity through a postsynaptic mechanism [18].

$A_{2B}R$ involvement has also been investigated in relation to neuropathic pain. Nevertheless, both agonists and antagonists have been shown to relieve neuralgia in different experiments. Studies supporting the pronociceptive effect of $A_{2B}Rs$ suggest that these receptors may be involved in pain. Electrophysiological studies have shown that the continuous increase in $A_{2B}Rs$ signaling enhances the excitability of primary sensory neurons, leading to chronic pain in the $Ada^{-/-}$ mice [7]. The prolonged accumulation of circulating adenosine may cause pain in three animal models of chronic pain, including $Ada^{-/-}$, sickle cell disease, and CFA-injected mice. Persistent elevation of plasma adenosine mediates the activation of $A_{2B}Rs$ in myeloid cells and induces TRPV1 gene expression in the DRG neurons through gp130-dependent IL-6/sIL-6R signal transduction, thereby promoting chronic pain [45]. On the contrary, studies supporting the

analgesic effect of $A_{2B}Rs$ suggest that they may alleviate neuropathic pain. In the CCI experiment, the long-term reversal of neuropathic hyperalgesia extended to $A_{2B}Rs$, while A_1Rs only produced a short-term reversal of mechanical hyperalgesia, which lasted < 3 days. [42].

The role of A_3Rs in the CNS is mainly reflected in the reversal of neuropathic pain. Many studies have shown that glial cells can release nitroxidative species and pro-inflammatory cytokines to sensitize the dorsal horn neurons and cause pain, whereas A_3Rs can attenuate nociception by modulating the glial cells [23]. The systemic administration of A_3R agonist (IB MECA) can reverse neuropathic pain after peripheral tibial nerve injury by inhibiting the activation of microglia and phosphorylation of p38 MAPK nerve in the spinal dorsal horn [46]. In rat models of oxaliplatin [47]- and paclitaxel [48]-induced peripheral neuropathy, intraperitoneal injection of the agonist IB-MECA effectively blocked the development of neuropathic pain by suppressing the redox-sensitive transcription factor (NFκB) and mitogen-activated protein kinases (ERK and p38), reducing the astrocyte overactivation, inhibiting the generation of pro-inflammatory and neuroexcitatory cytokines (TNF, IL-1β), and increasing the levels of the anti-inflammatory/neuro-protective cytokines (IL-10, IL-4). Furthermore, A_3Rs can also achieve analgesic effect by regulating the MAPK/ERK pathway. In a rat model of neuropathic pain induced by spinal nerve ligation (SNL), amitriptyline inhibits MAPK/ERK and cyclic AMP response element-binding protein pathways and proinflammatory cytokines, including TNF-α, intercellular adhesion molecule 1 (ICAM-1), macrophage inflammatory protein 2 (MIP-2), and monocyte chemoattractant protein 1 (MCP-1), by activating A_3Rs to exert antinociceptive effects [49]. In a CCI-induced neuropathic pain model in mice, intraperitoneal injection of the highly selective A_3R agonist MRS5980, which releases IL-10 by activating A_3Rs expressed on CD4 $^+$ T cells, reduces the excitability of neuronal DRG and thereby reverses established hypersensitivity [50]. In addition, the regulation of neurotransmitter imbalance is one major pathway by which A_3Rs mediate antinociception. Electrophysiological studies have shown that voltage-dependent Ca^{2+} channels (VDCCs) play a key role in neuropathic pain. These channels are activated at the presynaptic level and induce the release of neurotransmitters through the sensory neurons of the central and peripheral nervous systems, including the DRG. Selective A_3R activation inhibits N-type VDCC opening, which curtails the neurotransmitter release and decreases the action potential firing in isolated rat DRG neurons [51]. Little et al. demonstrated that augmenting the endogenous adenosine level through selective adenosine kinase inhibition can produce strong analgesic effects in models of neuropathic pain through the A_3R signaling pathway. Similar results were achieved by spinal or RVM injection of the A_3R agonist IB-MECA. The

antinociceptive functions of A_3R agonists are independent of endogenous opioid or endocannabinoid pathways. These agonists reverse allodynia by activating the 5-HT and norepinephrine circuits and reducing the excitability of a wide and dynamic range of spinal neurons. This finding suggests that the activation of A_3Rs by adenosine is an endogenous antinociceptive pathway [52]. Restoration of the GABAergic inhibition system contributes to the reversal of neuropathic pain after A_3R activation. The activated A_3Rs can act on the neurons and promote the release of GABAergic, thereby inhibiting nerve excitability and reducing nociceptive signals and producing an analgesic effect [23]. Intrathecal administration of the A_3R agonist MRS5698 dose-dependently reversed CCI-induced mechanical allodynia, and spinal administration of the A_3R antagonist MRS1523 blocked the analgesia produced by MRS5698. Their study suggests MRS5698 contributed to restore K^+–Cl^- cotransporter 2 (KCC2)–dependent Cl^- transport in CNS neurons, which underlies the increased capacity for GABAergic neurotransmission [53]. In addition, A_3R agonists can reduce the activation of astrocytes and restore the normal function of the GABAergic inhibitory system by lowering the brain-derived neurotrophic factor (BDNF) signaling [23, 46]. A single dose of IB-MECA was more effective in analgesia in the neuralgia model than in inflammatory pain, and IB-MECA reversed the neuralgia model–induced increase in brainstem BDNF levels to attenuate pain [54].

To summarize, among the four subtypes of ARs, A_1Rs, $A_{2A}Rs$, and A_3Rs are mainly involved in neuropathic pain, while there are relatively few studies on $A_{2B}Rs$. A_1Rs exert their effects by inhibiting TNF-α expression through the MAPK/ERK pathway. $A_{2A}Rs$ alleviate neuropathic pain by inhibiting microglia, astrocytes, and TNF-α through the PKA/PKC signaling pathway, and enhancing IL-10. In neuropathic pain, A_3Rs play an analgesic role by inhibiting TNF, IL-1β, and BDNF, increasing IL-10 and IL-4 level signals, and restoring the normal function of the GABAergic inhibitory system by regulating the MAPK/ERK pathway.

Adenosine signaling and inflammatory pain

Inflammatory pain is caused by thermal, chemical, or mechanical damage to nociceptors of the nervous system, as well as increased mechanical and thermal sensitivity due to inflammation-related changes [55]. The central underlying mechanisms of inflammatory pain remain unclear. The antinociceptive effect of adenosine signaling has been primarily attributed to the activation of A_1Rs. In an inflammatory model of thermal hyperalgesia, agents that act directly or indirectly on adenosine, when administered through the spinal cord, were found to produce antinociception via activation of the spinal A_1Rs [56]. Inhibitory glycinergic neurotransmission is an important target for adenosine regulation.

Activation of A_1Rs may increase the inhibitory postsynaptic currents mediated by glycine receptors in the spinal cord sections of rats injected with complete Freund's adjuvant (CFA) through the Gαi/PKAα3 and Gβγ/α1^{ins} pathways. Thus, nociceptive information is transmitted by inhibiting the excitability of neurons and weakening the spinal cord neural circuits. This enhanced spinal cord inhibition may be an important pathway by which A_1Rs reduce hyperalgesia [57]. The α1^{ins} phosphorylation at Ser380 by ERK has been proven to lower the glycinergic synaptic currents and contribute to spinal disinhibition. CFA-induced peripheral inflammation increased the phosphorylation of Ser380 in the spinal dorsal horn of mice and was inhibited by the activation of specific A_1Rs. This finding further supports the above analgesic mechanism of A_1Rs [58]. In addition, T35 (Zusanli) at 10.6-μM laser irradiation exerts analgesic effects by upregulating spinal A_1Rs to inhibit nociceptive sensation and NMDA receptor 1 phosphorylation in the monosodium iodoacetate–induced knee osteoarthritis model [59]. Moreover, the anti-inflammatory and analgesic effects of some drugs are also mediated through A_1Rs in the spinal cord, such as tramadol and Norisoboldine [60, 61]. We also compared the analgesic effects of A_1Rs with morphine. To the best of our knowledge, the intrathecal administration of both A_1R agonist and morphine can alleviate formalin-induced pain behaviors. However, while morphine acts on the presynaptic and postsynaptic mechanisms of the CSF glutamate release system, the analgesic mechanism of A_1Rs may be related to the reduction of postsynaptic excitability by inhibiting the glutamate terminal [62].

The role of adenosine in the regulation of inflammatory pain is also dependent on the $A_{2A}Rs$ [42, 44]. The relationship between $A_{2A}Rs$ and pain has long been controversial; both $A_{2A}R$ antagonists and agonists exhibit analgesic effects. Studies supporting the analgesic effect of $A_{2A}Rs$ indicate that the central application of $A_{2A}R$ agonists can produce an analgesic effect. The intrathecal injection of neuropeptide S (NPS) can alleviate the nociception caused by formalin. The function of adenosine in the regulation of inflammatory pain is mainly dependent on the $A_{2A}Rs$. Intrathecal injection of the $A_{2A}R$ antagonist ZM241385 blocked the antinociceptive effect of NPS throughout the experiment. In addition, the A_1R antagonist DPCPX blocked the effects of NPS during the first 5 min of the experiment. This observation demonstrates the involvement of adenosine in NPS analgesia, especially $A_{2A}R$, plays a more lasting role [63]. Such effects have also been observed in the brain. Intracerebroventricular injection of Adonis, an agonist-like monoclonal antibody with high specificity for the $A_{2A}Rs$, resulted in a significant dose-dependent increase in hot-plate and tail-flick latencies in mice, and this effect was prevented by $A_{2A}R$ antagonist [64]. These trials establish that $A_{2A}Rs$ have antinociceptive effects on the CNS. However, studies supporting the

pronociceptive effect of A_{2A}Rs have suggested that the central administration of A_{2A}R antagonist can produce an analgesic effect. Intrathecal injection of A_{2A}R antagonist SCH58261 produced antinociception in formalin test, suggesting that A_{2A}Rs are involved in the antinociceptive effect of the spinal cord [65]. The A_{2A}R antagonist ZM241385 blocked bladder hyperactivity and hyperalgesia from cyclophosphamide-induced cystitis. A_{2A}Rs and transient receptor potential vanilloid 1 (TRPV1) are co-expressed in DRG neurons. Inhibition of A_{2A}Rs decreased the sensitivity of TRPV1 in DRG neurons. This result illustrates that the inhibition of adenosine A_{2A}Rs alleviate bladder hyperalgesia in cyclophosphamide induced cystitis by inhibiting sensitization of TRPV1 [66].

The regulatory role of A_{2B}Rs in inflammatory pain has also been studied. In a formalin experiment, intrathecal injection of adenosine A_{2B}R antagonist could reverse the antinociceptive effect of adenosine, alluding the antinociceptive role for spinal A_{2B}Rs [15]. In another formalin experiment, Bilkei-Gorzo et al. found that the A_{2B}R antagonist PSB-1115 has a dose-dependent effect on the alleviation of inflammatory pain, which reached the maximum effect at a low dose of 3 mg/kg, while being nociceptive over a broad dose range [67]. These results suggest that A_{2B}Rs may have a pronociceptive effect.

Overall, the abovementioned data showed that A_1Rs and A_{2A}Rs are mainly involved in inflammatory pain in the CNS. A_1Rs alleviate inflammatory pain by inhibiting excitatory amino acids through the Gαi/PKAα3 and Gβγ/α1^{ins} pathways. A_{2A}Rs have both pronociceptive and antinociceptive effects, the pronociceptive effect of A_{2A}Rs may be related to TRPV1. A_{2B}Rs may play a promoting role in inflammatory pain; however, there are few studies on A_{2B}Rs and A_3Rs, so the mechanisms need more research to prove.

Adenosine signaling and postoperative and visceral pain

A_1Rs also seem to be involved in postoperative pain. Intrathecal injection of the A_1R agonist R-PIA can reduce postoperative mechanical hyperalgesia and improve the pain threshold in incisions. Zahn et al. assessed the different K$^+$ channels for A_1R-mediated antinociception in the surgical incision of rats. The results illustrated that the analgesic effect of R-PIA was alleviated by the intrathecal administration of a K_{ATP} channel blocker, while Ca^{2+}-activated K$^+$ channels or Kv1.3 or Kv1.6 channel blockers failed to decrease R-PIA-induced hypoalgesia. This result implies that the opening of the K_{ATP} channel is conducive for the analgesic effects of A_1R agonist [68]. Another report suggests that intrathecal adenosine may be an effective treatment for neuropathic pain but not for postoperative pain. Yamaoka et al. compared the

effects of intrathecal injection of adenosine on three types of pain signals: two neuropathic pains (spinal cord compression and chronic constriction of the sciatic nerve) and one postoperative pain (plantar incision). Following the intrathecal injection of Cl-adenosine 24 h after the surgery, the pain levels of the three models were significantly improved. Intrathecal administration of adenosine at 72 h after the surgery suppressed hyperalgesia in a neuropathic pain model, but it had no effect on the postoperative pain model. When compared with the other two models, the expressions of A_1R messenger RNA (mRNA) and protein were significantly decreased in the plantar incision. This observation signifies that the decrease in A_1Rs may be the main reason for the ineffective analgesia of adenosine [69]. Moreover, A_{2A}Rs are involved in the regulation of postoperative pain perception in the median preoptic nucleus (MnPO). When compared with ad libitum sleep, complete sleep deprivation (the hypothalamus pituitary adrenal axis was not activated) 6 h before the surgical incision significantly enhanced the postoperative mechanical hypersensitivity of the affected paw and prolonged the recovery time. Microinjection of the adenosine A_{2A}R antagonist ZM241385 into the MnPO not only effectively blocked the surgical pain in the rats but also eliminated the thermal hyperalgesia caused by sleep deprivation in a group of non-surgical rats [70].

The potential role of A_1Rs in postoperative pain was also investigated. Intracisternal injection of CPA increased the threshold of abdominal withdrawal reflex induced by colon dilation in rats. In addition, the administration of DPCPX, an A_1R antagonist, completely blocked the brain orexin-induced antinociceptive action against colonic distension [71]. Subsequently, Okumura et al. confirmed the antinociceptive effects of CPA in visceral pain. In their study, intracisternal injection of 5-HT$_{1A}$ or 5-HT$_{2A}$ receptor antagonist was given, and subcutaneous injection of dopamine D_1 receptor antagonist, cannabinoid 1 (CB$_1$) receptor antagonist, or naloxone blocked the CPA-induced visceral antinociception. However, intracisternal injection of 5-HT$_{1B}$ and subcutaneous injections of dopamine D_2 receptor and CB$_2$ receptor antagonists failed to block the CPA-induced antinociception. It could therefore be inferred that the visceral antinociception of A_1Rs is caused by the activation of 5-HT$_{2A}$, 5-HT$_{1A}$, and dopamine D_1 or CB$_1$ receptors [72].

As far as current studies are concerned, in CNS, the regulation of postoperative and visceral pain is mainly mediated by A_1Rs. A_1Rs relieve postoperative pain through the K_{ATP} pathway, and A_1Rs relieve visceral pain through 5-HT$_{2A}$, 5-HT$_{1A}$, and dopamine D_1 or CB$_1$ receptors. A_{2A}Rs have also been implicated in regulating postoperative pain; however, relatively few studies have been conducted on A_{2A}Rs, A_{2B}Rs, and A_3Rs, and the related mechanisms need to be further explored (Table 1).

Table 1 Mechanisms of ARs in the different pain models of CNS

Pain model	Adenosine receptors	Site	Analgesic mechanism	References
Neuropathic pain	A_1Rs	Corpus striatum	p-ERK1/2	[39]
		ACC, vlPAG	Neuronal activity	[34]
		Spinal cord	TNF-α, MPO	[35]
			C fiber	[37]
	$A_{2A}Rs$	L4–L6 lumbar Spinal cord	IL-10	[44]
		Spinal cord	TNF-α	[42]
			NMDA	[18]
	$A_{2B}Rs$	DRG	IL-6 / sIL-6R,TRPV1	[45]
	A_3Rs	Spinal cord	TNF-α, ICAM-1, MIP-2, MCP-1	[49]
		Spinal dorsal horn	p-p38, MAPK	[46]
		Spinal cord (L4–6)	TNF, IL-1β, IL-10, IL-4	[47, 48]
			TNF-α, IL-1β, IL-10, Glu	[50]
		DRG	N-type VDCC	[51]
		Spinal cord	5-HT, NE	[52]
		RVM	5-HT, NE	[52]
		Spinal cord	GABAergic, p-KCC2	[53]
		Brainstem	BDNF	[73]
Inflammatory pain	A_1Rs	Spinal dorsal horn	Gly	[57]
			NMDAR 1	[59]
		Spinal cord	Glu	[62]
	$A_{2A}Rs$	Lateral ventricle	NPS	[63]
		DRG	TRPV1	[66]
Postoperative pain	A_1Rs	Spinal cord	K_{ATP} channels	[68]
Visceral pain	A_1Rs	Cisterna	5-HT1A, 5-HT2A, D1 or CB1receptors	[72]

Abbreviations: ACC anterior cingulate cortex, *vlPAG* Ventrolateral periaqueductal gray, *DRG* Dorsal root ganglion, *RVM* Rostral ventromedial medulla, *p-ERK1/2* Phosphorylation, extracellular signal regulated protein kinase1/2, *TNF-α* Tumor necrosis factor α, *MPO* Myeloperoxidase, *IL-10* Interleukin 10, *NMDAR 1* N-meth D-aspartate receptor 1, *IL-6* Interleukin 6, *sIL-6R* Soluble IL-6 receptor, *TRPV1* Transient receptor potential V1, *ICAM-1* Intercellular adhesion molecule 1, *MIP-2* Macrophage inflammatory protein 2, *MCP-1* Monocyte chemoattractant protein 1, *p-p38* Phosphorylation of p38, *MAPK* Mitogen-activated protein kinases, *IL-1β* Interleukin 1β, *IL-4* Interleukin 4, *Glu* Glutamate, *N-type VDCC* N-type, voltage-dependent Ca^{2+} channels, *5-HT* Either serotonin, *NE* Norepinephrine, *p-KCC2* Phosphorylation-K⁺-Cl⁻ cotransporter 2, *BDNF* Brain-derived neurotrophic factor, *Gly* Glycinergic, *NPS* Neuropeptide S, *KATP* channel Adenosine triphosphate, sensitive K channels, *5-HT₁A* Either serotonin 1A, *5-HT₂A* Either serotonin 2A, *D₁* Dopamine1; *CB₁* Cannabinoid 1

Conclusion and perspectives

Purinergic signaling induces potent modulation in different pain models. Most studies have shown that activation of A_1Rs contributes to relieve a variety of pain, but recent studies have also demonstrated that some agonists are ineffective in relieving neuralgia in mice, which needs to be confirmed by further studies. The role of $A_{2A}Rs$ in pain regulation is related to different pain models. Activation of $A_{2A}Rs$ can relieve neuralgia, while $A_{2A}Rs$ have both pronociceptive and antinociceptive effects in inflammatory pain. $A_{2B}R$ regulation of pain in the CNS is relatively poorly studied, and specific mechanisms need further exploration. The activation of A_3Rs contributes to the relief of neuropathic pain, and its regulation of inflammatory pain has been relatively poorly reported in the CNS.

Even though great achievements have been made for the past decades in regulation of pain mediated by adenosine signaling, there still exist a lot of challenges for further investigations: (1) Compared with other receptors, $A_{2B}Rs$ have been less studied in the CNS. It is still not clear how $A_{2B}Rs$ regulate pain. And the specific pathways and downstream molecular mechanisms that regulate pain need to be further studied. (2) Adenosine degrades rapidly in vivo, making it difficult to detect adenosine signals in time. It might be most helpful to introduce new detection tools to elucidate the regulatory role of adenosine signaling in pathological conditions. In summary, adenosine signaling exerts a significant role at pain regulation in the CNS, and as research continues, adenosine signaling pathway may become effective target of drugs for the treatment of pain in the near future.

Declarations

Conflict of interest All authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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