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Review

# Adenosine receptor activation and nociception

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**Abstract**

Adenosine and ATP exert multiple influences on pain transmission at peripheral and spinal sites. At peripheral nerve terminals in rodents, adenosine $A_1$ receptor activation produces antinociception by decreasing, while adenosine $A_2$ receptor activation produces pronociceptive or pain enhancing properties by increasing, cyclic AMP levels in the sensory nerve terminal. Adenosine $A_3$ receptor activation produces pain behaviours due to the release of histamine and 5-hydroxytryptamine from mast cells and subsequent actions on the sensory nerve terminal. In humans, the peripheral administration of adenosine produces pain responses resembling that generated under ischemic conditions and the local release of adenosine may contribute to ischemic pain. In the spinal cord, adenosine $A_1$ receptor activation produces antinociceptive properties in acute nociceptive, inflammatory and neuropathic pain tests. This is seen at doses lower than those which produce motor effects. Antinociception results from the inhibition of intrinsic neurons by an increase in $K^+$ conductance and presynaptic inhibition of sensory nerve terminals to inhibit the release of substance P and perhaps glutamate. There are observations suggesting some involvement of spinal adenosine $A_2$ receptors in pain processing, but no data on any adenosine $A_3$ receptor involvement. Endogenous adenosine systems contribute to antinociceptive properties of caffeine, opioids, noradrenaline, 5-hydroxytryptamine, tricyclic antidepressants and transcutaneous electrical nerve stimulation. Purinergic systems exhibit a significant potential for development as therapeutic agents. An understanding of the contribution of adenosine to pain processing is important for understanding how caffeine produces adjuvant analgesic properties in some situations, but might interfere with the optimal benefit to be derived from others. © 1998 Elsevier Science B.V.

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## 1. Introduction

While the ability of adenosine and adenosine 5′-triphosphate (ATP) to alter nociceptive transmission by actions at peripheral and central sites has been recognized for some time (Keele and Armstrong, 1964; Collier et al., 1966; Vapaatalo et al., 1975; Yarbrough and McGuffin-Clineschmidt, 1981), the last decade has seen the development of a particular interest in the role of purines in nociception. A number of reasons account for this focus of attention. (a) Adenosine analogs produce antinociceptive properties in a wide range of test systems, including those for neuropathic pain where pain signalling mechanisms have been altered, and there is an interest in the potential development of adenosine-based pharmaceuticals. (b) Release of adenosine in the spinal cord contributes to the spinal efficacy of opioids, an unusual effect as most actions of opioids are considered inhibitory. (c) Caffeine, an adenosine receptor antagonist, produces adjuvant analgesic properties in combination with non-steroidal anti-inflammatory drugs and acetaminophen; an understanding of the role of adenosine in nociceptive processing is required to understand how caffeine produces such actions. (d) The $P_2$ purinoceptor subtypes involved in nociceptive activation have recently been identified, cloned and shown to have a unique distribution in sensory neurons; this may permit the development of $P_2$ receptor targets for conditions where ATP contributes to the etiology of pain. It is important to appreciate that effects of purines on nociception can be complex, with effects depending on particular receptor subtypes activated and on the localization of the receptor. The following review will summarize the profile of activity of adenosine agents following administration by different routes, consider cellular mechanisms implicated in such actions, and discuss the contribution of interactions

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with endogenous adenosine systems in the pharmacological actions of other agents. The actions of ATP on nociceptive processing have recently been reviewed elsewhere (Burnstock and Wood, 1996; Sawynok, 1997).

## 2. Peripheral regulation of pain

### 2.1. Adenosine $A_1$, $A_2$ and $A_3$ receptors

Multiple chemical mediators contribute to the transduction of pain at peripheral sensory nerve terminals. These include amines (histamine, 5-hydroxytryptamine), kinins (bradykinin), prostanoids (prostaglandins, leukotrienes, hydroxyacids), cytokines (interleukins, tumor necrosis factor), cations ($H^+$, $K^+$), reactive oxygen species, as well as adenosine and ATP (reviewed Levine and Taiwo, 1994; Dray, 1995). The peripheral actions of adenosine in rodents have been characterized primarily using behavioural approaches. These actions depend very much upon the adenosine receptor subtype activated. Thus, the exogenous administration of adenosine $A_1$ agonists locally to the hindpaw of the rat produces antinociception in a pressure hyperalgesia model (Taiwo and Levine, 1990) and in the low concentration formalin model (Karlsten et al., 1992). In contrast, local administration of adenosine $A_2$ receptor agonists enhances pain responses in both models (Taiwo and Levine, 1990; Karlsten et al., 1992), an action most likely due to adenosine $A_{2a}$ receptor activation as it is elicited by CGS21680 (Doak and Sawynok, 1995). Local application of an adenosine $A_3$ receptor agonist produces an intrinsic nociceptive response resembling that produced by formalin, as well as augmenting the response to a low concentration of formalin (Sawynok et al., 1997). Fig. 1 summarizes the influence of multiple adenosine receptors on pain transmission at sensory nerve terminals.

The action of adenosine $A_1$ receptor agonists appears to be directly on the sensory nerve terminal itself and results from inhibition of adenylate cyclase and a decreased production of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Taiwo and Levine, 1991; Khasar et al., 1995). While not visualized directly on sensory terminals, adenosine $A_1$ receptors are present on the cell body of dorsal root ganglion cells (MacDonald et al., 1986) and on the central terminals of primary afferent neurons (Santicioli et al., 1993), such that transport of these receptors to the peripheral aspect of this nerve is likely. The adenosine $A_1$ receptor has been proposed to exist as a part of a $\mu$ opioid and $\alpha_2$-adrenergic multireceptor complex on the basis of a demonstrated cross antagonism, cross tolerance and cross withdrawal between these systems (Aley and Levine, 1997). Actions due to adenosine $A_2$ receptor activation have been proposed to result from stimulation of adenylate cyclase resulting in an increase in cyclic AMP levels in the sensory nerve terminal (Taiwo and Levine, 1991; Khasar et al., 1995). The pronociceptive actions of adenosine $A_3$ receptor activation are mediated by an action on mast cells to release histamine and 5-hydroxytryptamine (5-HT) (Sawynok et al., 1997), an action likely mediated by increased inositol 1,4,5-triphosphate production and enhanced intracellular $Ca^{2+}$ (Ramkumar et al., 1993). These agents interact with histamine $H_1$ and 5-$HT_2$ receptors located on the sensory nerve terminal itself (Ninkovic et al., 1982; Pierce et al., 1996), with subsequent transduction mechanisms including changes in inositol phosphates and $Ca^{2+}$ (Hill, 1990; Hoyer et al., 1994).

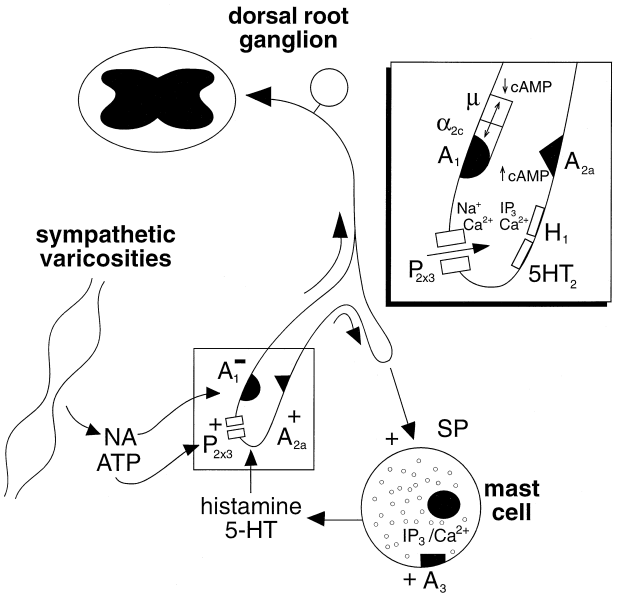


Fig. 1. Schematic rendition of the role of adenosine $A_1$, $A_2$ and $A_3$ receptors and $P_{2x3}$ purinoceptors in peripheral pain signalling in rodents. Mediators which may interact with these receptor systems are included. Inset depicts second messenger systems activated by adenosine receptors on sensory nerve terminals.

In addition to the direct administration of adenosine agonists, the local administration of an adenosine kinase inhibitor can also modify behaviours produced by an inflammatory stimulus. Thus, 5′-amino-5′-deoxyadenosine ($NH_2$dAD) produces antinociception in the low concentration formalin test and this is augmented by the simultaneous administration of deoxycoformycin, an adenosine deaminase inhibitor (Sawynok et al., 1998). This local antinociception is mediated by activation of adenosine $A_1$ receptors (Sawynok et al., 1998). Local levels of adenosine are enhanced by the inflammatory process itself and can be further enhanced by inhibition of adenosine kinase (Cronstein et al., 1995). Adenosine inhibits a number of aspects of inflammation including phagocytosis, generation of toxic oxygen metabolites and cellular adhesion and has been proposed to be an anti-inflammatory autacoid (Cronstein, 1994, 1995). Systemic administration of an adenosine kinase inhibitor produces local anti-inflammatory effects which are mediated by accumulation of adenosine and

activation of adenosine $A_2$ receptors (Cronstein et al., 1995; Rosengren et al., 1995). Inhibitors of adenosine metabolism thus produce both antinociceptive and anti-inflammatory properties which are mediated by different adenosine receptor populations (via adenosine $A_1$ and $A_2$ receptors, respectively) and are due to independent actions. This broad spectrum of activity makes this class of agents an attractive one for development. However, it must be appreciated that with both adenosine $A_2$ and $A_3$ receptor activation producing peripheral pronociceptive actions, there may well be circumstances in which antinociceptive actions mediated by adenosine $A_1$ receptors are compromised by endogenously released adenosine activating these receptors as well.

### 2.2. Algogenic actions of adenosine in humans

The application of adenosine to the human blister base produces an algogenic action (Bleehen and Keele, 1977). Similarly, the i.v. administration of adenosine by bolus injection or 2 min infusion can provoke a pain response that resembles that produced by ischemic cardiac pain (Sylvén et al., 1986, 1988a; Crea et al., 1990, 1992). The provocation of pain is not restricted to cardiac sites as arm pain is elicited by administration into the brachial artery (Sylvén et al., 1988b) and pain is reported in other regions as well following i.v. administration (Watt et al., 1987). Adenosine released endogenously by ischemia may contribute to ischemic pain as aminophylline can reduce ischemic cardiac and muscle pain (Crea et al., 1989; Jonzon et al., 1989). Cardiac pain appears to be due to a direct activation of cardiac afferents as it is not seen in subjects who have undergone heart transplantation (Bertolet et al., 1993).

The pronociceptive action of adenosine in humans has been attributed to adenosine $A_1$ receptor activation (Pappagallo et al., 1993; Gaspardone et al., 1995), which is in direct contrast to rodent studies where adenosine $A_2$ receptor activation has uniformly been implicated in pain enhancing properties of adenosine. It is not clear whether this reflects a species difference. Thus, in dogs, both adenosine $A_1$ and $A_2$ receptors have been implicated in activation of cardiac afferent nerves (Dibner-Dunlap et al., 1993; Huang et al., 1995). While adenosine $A_1$ receptor mediated events are primarily considered to be inhibitory (Fredholm et al., 1994), excitatory actions on mediator systems also have been described (Akbar et al., 1994). Pain initiating effects of adenosine in humans are augmented by substance P (Gaspardone et al., 1994) and nicotine (Sylvén et al., 1990). The sites of action of these agents in producing this enhanced response have not been characterized. The clear characterization of adenosine receptors mediating the pronociceptive action of adenosine in humans is an important issue to resolve for the purposes of drug development.

## 3. Spinal regulation of pain

### 3.1. Antinociceptive properties of adenosine in animals

The first studies to describe antinociceptive activity of adenosine analogs were screening studies in which drugs were administered systemically and a number of behavioural endpoints were assessed (Vapaatalo et al., 1975; Crawley et al., 1981) or when a role of cyclic AMP in the actions of morphine was being addressed (Gourley and Beckner, 1973; Ho et al., 1973). Interest in intrinsic antinociceptive properties of adenosine analogs became much more prominent when the activity of systemically administered adenosine analogs was attributed to actions at a spinal site (Post, 1984; Holmgren et al., 1986). Since that time, most studies have used a direct spinal route of administration to examine the antinociceptive properties of adenosine, its analogs and inhibitors of metabolism. Such studies demonstrate that both directly and indirectly acting adenosine agents produce antinociception in a variety of test systems including acute nociceptive pain tests, inflammatory pain tests and neuropathic pain tests (Table 1).

While initial behavioural studies using adenosine analogs and non-selective antagonists attributed both adenosine $A_1$ and $A_2$ receptors to spinal antinociception, more recent studies using more selective agonists and antagonists have implicated primarily adenosine $A_1$ receptors in spinal antinociception (Karlsten et al., 1991; Reeve and Dickenson, 1995; Lee and Yaksh, 1996). The dorsal spinal cord contains both adenosine $A_1$ and $A_2$ receptors (Goodman and Snyder, 1982; Geiger et al., 1984; Choca et al., 1987). For both receptor subtypes, dorsal spinal cord levels exceed ventral spinal cord levels, and adenosine $A_1$ receptors are selectively concentrated in the substantia gelatinosa (Goodman and Snyder, 1982; Geiger et al., 1984; Choca et al., 1988). Adenosine receptors are localized primarily on neurons post-synaptic to primary afferents and descending projections within the dorsal horn (Geiger et al., 1984; Choca et al., 1988), but some receptors are present on central terminals of primary afferent neurons (see below).

A number of cellular mechanisms have been implicated in spinal antinociception. This includes a postsynaptic hyperpolarization of transmission neurons by interactions with ATP-sensitive-$K^+$ channels to increase conductance (Salter et al., 1993; Li and Perl, 1994). This action most likely accounts for inhibition of the postsynaptic actions of substance P and excitatory amino acids (Doi et al., 1987; DeLander and Wahl, 1991). The latter action may be of particular relevance to the inhibition of 'wind up' that has been observed in the spinal cord following application of an adenosine $A_1$ analog (Reeve and Dickenson, 1995). There is also evidence for a presynaptic action of adenosine on afferent terminals, as adenosine analogs inhibit the release of substance P and calcitonin gene-related peptide when spinal cord slices are stimulated electrically (Santi-

Table 1
Spinal efficacy of adenosine, adenosine analogs and inhibitors of adenosine metabolism (via adenosine kinase) in rodent tests for antinociception. Drugs were administered either via chronically implanted intrathecal cannulas, or by acute percutaneous lumbar puncture

| Test | Agents | Refs. |
|---|---|---|
| Nociceptive tests | | |
| Tail flick, tail immersion and/or hot plate | NECA | Post, 1984 |
| | L-PIA, NECA | DeLander and Hopkins, 1986 |
| | L-PIA | Holmgren et al., 1986 |
| | L-PIA, CHA, NECA | Sawynok et al., 1986 |
| | L-PIA, CHA, NECA | Sosnowski et al., 1989 |
| | L-PIA, NECA | Aran and Proudfit, 1990a |
| | L-PIA, NECA | Karlsten et al., 1990 |
| | adenosine, L-PIA, CHA | Karlsten et al., 1991 |
| | $NH_2$dAD | Keil and DeLander, 1992 |
| I.t. capsaicin | NECA | Hunskaar et al., 1986 |
| I.t. substance P | ATP, ADP, AMP | Doi et al., 1987 |
| I.t. substance P, NMDA, kainic acid | NECA, L-PIA, CADO | DeLander and Wahl, 1988 |
| | NECA | DeLander and Wahl, 1991 |
| | $NH_2$dAD | Keil and DeLander, 1996 |
| Acetic acid writhing | L-PIA, NECA | Sosnowski et al., 1989 |
| Inflammatory tests | | |
| Formalin (phase 2) | L-PIA | Malmberg and Yaksh, 1993 |
| | CHA, NECA, CGS21680, $NH_2$dAD | Poon and Sawynok, 1995 |
| | CPA | Reeve and Dickenson, 1995 |
| Carrageenan thermal hyperalgesia | CHA, CGS21680, $NH_2$dAD | Poon and Sawynok, 1997 |
| Neuropathic tests | | |
| I.t. strychnine | L-PIA | Sosnowski and Yaksh, 1989 |
| | L-PIA, NECA | Sosnowski et al., 1989 |
| I.t. prostaglandin $E_2$ | L-PIA | Minami et al., 1992 |
| Chronic constriction injury | NECA | Yamamoto and Yaksh, 1991 |
| | L-PIA | Sjölund et al., 1996 |
| | L-PIA | Cui et al., 1997 |
| Dorsal root ligation | L-PIA | Lee and Yaksh, 1996 |

Abbreviations: ADP, adenosine 5′-triphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; CADO, 2-chloroadenosine; CGS21680, 2-[ *p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; CHA, $N^6$-cyclohexyl adenosine; CPA, $N^6$-cyclopentyl adenosine; $NH_2$dAD, 5′-amino-5′-deoxyadenosine; L-PIA, L-phenylisopropyl adenosine; NECA, $N^6$-ethylcarboxamido adenosine; NMDA, *N*-methyl-D-aspartate; i.t., intrathecal.

cioli et al., 1993). Adenosine analogs inhibit release of substance P but not calcitonin gene-related peptide release in vivo (Sjölund et al., 1997). While not directly demonstrated for the spinal cord preparation, it is often assumed that adenosine also can inhibit the release of excitatory amino acids, as demonstrated in other parts of the central nervous system (Corradetti et al., 1984); this action results from inhibition of $Ca^{2+}$ entry into nerve terminals (Burke and Nadler, 1988). Interactions with pertussis toxin sensitive G-proteins and a decrease in adenylate cyclase have also been implicated in spinal antinociception by an adenosine $A_1$ analog (Sawynok and Reid, 1988).

The above intrinsic profile of adenosine analogs suggests that this class of agents may be useful for therapeutic development as analgesic agents. One limitation to this approach may be side effects such as motor weakness or autonomic changes. Many studies observing antinociception have noted motor effects resulting from the spinal administration of adenosine analogs, but only a few quantified motor responses and derived a potency comparison. For L-PIA, one of the most extensively characterized agents, there is an order of magnitude difference in doses required to produce antinociceptive and motor effects in rats (Karlsten et al., 1990; Lee and Yaksh, 1996), but less difference is noted in mice (DeLander and Hopkins, 1987). The delay in onset of motor effects seen with higher doses of L-phenylisopropyl adenosine (L-PIA) compared to those seen with $N^6$-ethylcarboxamido adenosine (NECA), probably reflects pharmacokinetic differences which allow for a greater degree of penetration of spinal cord tissue by NECA (Fastbom et al., 1990). Antinociception has been attributed to the activation of adenosine $A_1$ receptors, with motor effects reflecting actions at adenosine $A_2$ receptors (Lee and Yaksh, 1996). However, a recent study which characterized adenosine actions on C-fibre-evoked responses (slow ventral root potential) and a monosynaptic reflex (non-nociceptive transmission related to motor function) observed that adenosine $A_1$ agonists inhibited both responses, but nociceptive responses were inhibited at lower doses (5–8 fold) (Nakamura et al., 1997). The possibility of autonomic changes following spinal administration of adenosine analogs was observed directly in one

study, but no significant changes in heart rate and blood pressure were observed (Sosnowski et al., 1989).

While more recent studies continue to emphasize the involvement of adenosine $A_1$ receptors in spinal antinociception (see above), there are a number of observations not necessarily accounted for by this homogeneity of action. Thus, there is the recent suggestion that spinal adenosine $A_2$ receptors may mediate the actions of supraspinally administered morphine, while both adenosine $A_1$ and $A_2$ receptors mediate the actions of supraspinally administered $\beta$-endorphin (Suh et al., 1997). Earlier studies had demonstrated that NECA, a mixed adenosine $A_1$ and $A_2$ receptor agonist, but not the more selective adenosine $A_1$ receptor agonist L-PIA, exhibited synergy with adrenoceptor agonists (Aran and Proudfit, 1990a,b) and that the actions of $N^6$-cyclohexyl-adenosine (CHA) and NECA could be differentially modulated (Sawynok and Reid, 1988). The selective adenosine $A_{2a}$ receptor agonist 2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680) does exhibit efficacy in threshold (Suh et al., 1997), inflammatory (Poon and Sawynok, 1997) and neuropathic pain tests (Lee and Yaksh, 1996), but this usually occurs in higher doses and could thus still reflect adenosine $A_1$ receptor activation. Adenosine $A_2$ receptors clearly are present in the dorsal spinal cord in comparable numbers with adenosine $A_1$ receptors (Choca et al., 1987), such that their potential involvement in spinal adenosine pharmacology should continue to be considered. The use of more selective adenosine $A_2$ receptor antagonists which have recently been developed (Ongini and Frendhom, 1996), should help to clarify their involvement in pain regulation.

### *3.2. Analgesic actions of adenosine in humans*

There is a single case report of the spinal administration of L-PIA producing an alleviation of neuropathic pain in a human subject (Karlsten and Gordh, 1995). In all other human studies in which analgesic properties have been reported, adenosine itself (50–80 $\mu$g/kg per min) was infused intravenously for 45–60 min. (Note that the lowest i.v. infusion dose producing pain is 0.347 $\mu$mol/kg per min or 92.7 $\mu$g/kg per min (Lagerqvist et al., 1992); analgesia thus occurs in doses lower than those which produce pain). This paradigm produces analgesic properties in experimental pain in healthy volunteers (Ekblom et al., 1995; Segerdahl et al., 1994, 1995b), reduces the requirement for analgesics in post operative pain (Segerdahl et al., 1995a, 1996) and produces pain relieving properties in neuropathic pain cases (Belfrage et al., 1995; Sollevi et al., 1995). The latter finding is of considerable interest in view of the observation that plasma and cerebrospinal fluid levels of adenosine are reduced by half in subjects with neuropathic pain (Guieu et al., 1996). While analgesic properties of adenosine in the above studies have generally been attributed to actions within the spinal cord on the basis of spinal actions of adenosine analogs when administered systemically, it is not clear to what extent adenosine itself gains access to the perispinal space. Thus (a) adenosine has a half life of 10 s in blood (Möser et al., 1989), (b) there is a metabolic barrier to adenosine entering the central nervous system consisting of adenosine kinase within the endothelial cells in addition to the cell membranes of the endothelial cells (Pardridge et al., 1994) and (c) even when adenosine is administered directly to the perispinal space, its activity is weak unless degradative enzymes are inhibited (Keil and DeLander, 1994). These issues raise the question of whether adenosine might act peripherally to activate adenosine $A_1$ receptors (Section 2.1) to produce analgesic actions following i.v. administration in human studies.

## 4. Involvement of adenosine in antinociception by other agents

### *4.1. Caffeine*

Caffeine has been combined with aspirin, acetaminophen and other non-steroidal anti-inflammatory agents for some time, but it is only relatively recently that its efficacy in this regard has been convincingly established in humans (Laska et al., 1984; Forbes et al., 1991; Sawynok and Yaksh, 1993). More recent animal based studies have continually demonstrated its adjuvant activity in combination with acetaminophen, aspirin and ketolorac in a newly developed functional impairment model (Granados-Soto et al., 1993; Castañeda-Hernández et al., 1994; López-Muñoz et al., 1996) and revealed consistent intrinsic antinociceptive properties in the formalin test (Sawynok et al., 1995). Most pharmacological actions of caffeine are currently understood to result from adenosine receptor blockade (Fredholm, 1995). If this is the case, the potential block of pronociceptive actions of adenosine at peripheral nerve terminals (particularly if this adenosine were to interact with other inflammatory mediators such as prostaglandins) is an appealing hypothesis to account for its actions. However, the local administration of caffeine has not demonstrated any antinociceptive properties for caffeine, perhaps because of its mixed profile of activity against both adenosine $A_1$ and $A_2$ receptors (Doak and Sawynok, 1995). Intrinsic antinociceptive actions of caffeine have been proposed to result from actions of caffeine at supraspinal sites because manipulation of central monamine pathways can inhibit such actions (Sawynok and Reid, 1996). This effect appears paradoxical as the supraspinal administration of adenosine agonists also can produce antinociception (Herrick-Davis et al., 1989), but caffeine antinociception may involve inhibition of presynaptic adenosine receptors on cholinergic nerve terminals (Ghelardini et al., 1997), while adenosine analogs act postsynaptically at other sites to produce antinociception.

While usually assumed to be the case, it is not entirely clear whether the mechanism that underlies intrinsic antinociception is the same as that for adjuvant analgesia.

### 4.2. Opioids

A link between adenosine systems and opioids was initially encountered when the role of cyclic AMP in the action of morphine was being investigated. In this context, cyclic AMP and methylxanthines (as phosphodiesterase inhibitors) were shown to modify antinociception by morphine (Gourley and Beckner, 1973; Ho et al., 1973). Subsequently, a number of studies have shown that systemic administration of adenosine and adenosine analogs enhance antinociception by morphine (Ahlijanian and Takemori, 1985; Contreras et al., 1990; Malec and Michalska, 1990). While methylxanthines inhibit antinociception by morphine when administered systemically (Jurna, 1984; Ahlijanian and Takemori, 1985; Malec and Michalska, 1988) (an action presumably reflecting a spinal release of adenosine by morphine, see below), some studies have demonstrated an enhancement of the action of morphine by caffeine (Misra et al., 1985; Person et al., 1985). Some of this diversity of outcome may be accounted for by different species being used (rats, mice) (Malec and Michalska, 1988), different doses of caffeine whereby intrinsic actions of caffeine become manifest at higher doses (Sawynok et al., 1995) and pharmacokinetic factors (Misra et al., 1985).

In examining effects of adenosine on tolerance to and dependence upon morphine, somewhat variable results are reported. Thus, some adenosine analogs do not affect, while others decrease, tolerance and dependence (Contreras et al., 1990; Germany et al., 1990; Michalska and Malec, 1993). Conversely, subanalgesic doses of L-PIA were reported to potentiate morphine induction of tolerance and dependence (Ahlijanian and Takemori, 1985). Interactions between chronically administered opioids and adenosine analogs may reflect changes in central adenosine receptor and adenosine transporter expression. Thus, chronic exposure to morphine has been demonstrated to upregulate adenosine $A_1$ receptors in cortex (Kaplan et al., 1994) and adenosine transport binding sites in striatum and hypothalamus (Kaplan and Leite-Morris, 1997), and to downregulate adenosine $A_{2a}$ receptors in striatum (De Montis et al., 1992) and adenosine $A_1$ receptors in the spinal cord (Tao and Liu, 1992; Tao et al., 1995). The outcome of interaction studies may thus depend on the contribution of these different receptor and transport systems to parameters being considered in a particular paradigm.

Within the spinal cord, a component of the antinociceptive action of morphine is due to the local release of morphine. This was originally proposed on the basis of the ability of methylxanthines to inhibit spinal antinociception by morphine (Jurna, 1984; DeLander and Hopkins, 1986; Sweeney et al., 1987). Subsequently, morphine was shown to release adenosine from in vitro and in vivo spinal cord preparations (Sweeney et al., 1987, 1989); such release occurs from capsaicin sensitive afferents (Sweeney et al., 1989), is due to activation of protein kinase C (Cahill et al., 1995; Smart and Lambert, 1996) and occurs via a bidirectional carrier system (Sweeney et al., 1993). Release is due to the activation of $\mu$- but not $\delta$- or $\kappa$-opioid activation (DeLander and Keil, 1994; Cahill et al., 1995). When coadministered spinally with opioids, both adenosine analogs and an adenosine kinase inhibitor produce an additive interaction in combination with $\mu$-opioids, but a synergistic interaction in combination with $\delta$- and $\kappa$-opioid receptors (DeLander and Keil, 1994). In an electrophysiological preparation of the spinal cord, an adenosine $A_1$ receptor agonist augments the actions of morphine under some conditions, but additional interactions also occur (Reeve and Dickenson, 1995).

Adenosine release by opioids also appears to contribute to hypotensive actions of morphine (Calignano et al., 1992; White et al., 1995) and muscle rigidity induced by fentanyl (Lui, 1997). It is of interest to note that morphine has been demonstrated to release adenosine from parts of the central nervous system other than the spinal cord (Fredholm and Vernet, 1978; Stone, 1981; Wu et al., 1982), and this release could contribute to other actions of morphine. Interactions of opioids with endogenous systems appear to be multifaceted and contribute to a number of aspects of opioid pharmacology.

### 4.3. Biogenic amines

Noradrenaline releases a nucleotide which is converted to adenosine from an in vitro spinal cord preparation (Sweeney et al., 1987), raising the possibility that adenosine release might contribute to spinal adrenergic analgesia. Several studies report that methylxanthines do not inhibit antinociception by spinally administered noradrenaline or clonidine (DeLander and Hopkins, 1987; Sweeney et al., 1987), but one laboratory has reported such effects (Yang et al., 1994, 1995). The basis of this difference is not immediately clear. Significant interactions between spinal adrenergic and adenosine systems can occur in other ways. Thus, the coadministration of noradrenaline with adenosine analogs produces synergistic interactions (Aran and Proudfit, 1990a,b), while the efficacy of adenosine analogs in nociceptive tests is dependent on an intact noradrenergic system (Sawynok et al., 1991).

Methylxanthines inhibit spinal antinociception by 5-HT (DeLander and Hopkins, 1987) and certain 5-$HT_1$ receptor selective ligands, but not 5-$HT_2$ or 5-$HT_3$ selective ligands (Sawynok and Reid, 1996). 5-HT has been demonstrated directly to release adenosine from capsaicin-sensitive afferents in the dorsal spinal cord (Sweeney et al., 1990). When opioids are administered supraspinally, antinociception can be blocked by the spinal administration of methylxanthines

indicating a spinal release of adenosine contributes to such actions (DeLander and Hopkins, 1986; DeLander and Wahl, 1989); this action is secondary to the spinal release of 5-HT by opioids (Sawynok et al., 1991; Sweeney et al., 1991).

### 4.4. Tricyclic antidepressants

A number of years ago, tricyclic antidepressants were demonstrated to inhibit the uptake of adenosine into neuronal preparations (Phillis and Wu, 1982). Tricyclic antidepressants were subsequently shown to enhance electrophysiological actions of adenosine but not adenosine analogs not subject to uptake (Stone and Taylor, 1979; Phillis, 1984). Tricyclic antidepressants are used in the treatment of neuropathic pain (Portenoy, 1993). While interactions with amines, opioids, substance P and excitatory amino acids have been considered, their mechanism of action in this regard is far from clear (Eschalier et al., 1994). Methylxanthines have been shown to inhibit antinociception by a number of tricyclic antidepressants in the writhing test (Sierralta et al., 1995). Our own results have shown that caffeine can block the antihyperalgesic action of systemically and spinally administered amitriptyline in the rat dorsal root ligation model, a test for neuropathic pain (Esser and Sawynok, in preparation). Adenosine thus appears to contribute significantly to the mechanism by which tricyclic antidepressants produce antinociception.

### 4.5. Transcutaneous electrical nerve stimulation

Caffeine can block analgesia produced by transcutaneous electrical nerve stimulation in humans at doses relevant to normal intake levels (Marchand et al., 1995). This was hypothesized to be due to activation of large diameter afferent fibres releasing ATP which is subsequently converted to adenosine and then activates adenosine $A_1$ receptors. Electrophysiological studies have provided considerable evidence for vibration-induced analgesia being mediated by the same mechanism (Salter and Henry, 1987; Salter et al., 1993).

## 5. Conclusions

Purines can exert complex effects on pain transmission, with prominent actions at both peripheral and spinal sites in preclinical models. The nature of the modulation of pain signalling depends very much upon the receptor subtype activated. In the periphery, adenosine $A_1$ receptor activation produces pain suppression, while adenosine $A_2$ and $A_3$ receptor activation produces pain enhancement. Within the spinal cord, adenosine $A_1$ receptor activation produces antinociception; there are some observations suggesting an additional involvement of adenosine $A_2$ receptors in such actions, but no data on adenosine $A_3$ involvement. On the basis of these effects, adenosine $A_1$ receptors show a significant potential for therapeutic development. At both peripheral and spinal sites, the manipulation of endogenous adenosine levels by inhibition of adenosine kinase can produce antinociception by activating adenosine $A_1$ receptor mechanisms. These actions, combined with the peripheral anti-inflammatory effects mediated by adenosine $A_2$ receptor activation, may make these agents particularly useful in inflammatory pain where there is an enhanced production of adenosine. There is also a particular interest in the efficacy of adenosine in neuropathic pain, as adenosine and its analogs appear to have unique properties in case studies and limited trials. Attention to the multiple effects of adenosine on the regulation of pain may well yield novel therapies for pain control. In parallel with the development of adenosine-based pharmaceuticals in this context must be the recognition of the role of endogenous adenosine in other therapeutic modalities and the potential need to control dietary caffeine intake in order to achieve optimal benefit from these regimens.

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