ELSEVIER

European Journal of Pharmacology 307 (1996) 157–162

ejp

Adenosine kinase inhibitors augment release of adenosine from spinal cord slices

Krystyna Golembiowska [1], Thomas D. White, Jana Sawynok *

Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

Received 18 January 1996; revised 21 March 1996; accepted 26 March 1996

Abstract

Inhibitors of adenosine kinase, but not adenosine deaminase, produce antinociception when administered spinally. In this study, we evaluated the relative contribution of adenosine kinase and adenosine deaminase to the regulation of adenosine release into the extracellular space within the spinal cord by determining the effects of the adenosine kinase inhibitors 5′-amino-5′-deoxyadenosine and 5-iodotubercidin, and the adenosine deaminase inhibitor 2′-deoxycoformycin on adenosine release from spinal cord slices in an in vitro perfusion system. Both 5′-amino-5′-deoxyadenosine (5–50 μM) and 5-iodotubercidin (5–50 μM), but not 2′-deoxycoformycin (50 μM), augmented adenosine release. 5-Iodotubercidin was slightly more potent and effective than 5′-amino-5′-deoxyadenosine in augmenting release except at the highest concentration, where it was considerably more effective. Combinations of 2′-deoxycoformycin (50 μM) and minimally active concentrations of 5′-amino-5′-deoxyadenosine and 5-iodotubercidin (5 μM each) produced a synergistic enhancement of release. These results support a predominant involvement of adenosine kinase in regulating extracellular adenosine levels in the spinal cord, but adenosine deaminase also can play a significant role.

Keywords: Adenosine; Spinal cord; Adenosine kinase; Adenosine deaminase

1. Introduction

The spinal application of adenosine analogs produces antinociception in thermal threshold (reviewed Sawynok, 1991), inflammatory (Malmberg and Yaksh, 1993; Poon and Sawynok, 1995) and neuropathic pain tests (Sosnowski and Yaksh, 1989; Minami et al., 1992). In view of this wide spectrum of activity, there is considerable interest in the possibility of developing adenosine-like compounds as therapeutic agents for the relief of pain (reviewed Sawynok, 1996). The spinal actions of adenosine analogs however may be limited by a modest separation between doses that produce antinociception and motor effects (DeLander and Hopkins, 1987; Karlsten et al., 1990). An additional approach may be the use of inhibitors of adenosine metabolism which potentially might recruit adenosine released endogenously (either as adenosine per se or as nucleotides which are metabolized to adenosine) either in an ongoing manner or due to neuronal stimulation. A basal release of both adenosine and adenosine triphosphate has been demonstrated from spinal cord preparations in vitro and in vivo (Sweeney et al., 1989; Sawynok et al., 1993), and a further release may occur due to sensory afferent stimulation (Fyffe and Perl, 1984; Salter et al., 1993). If such release can occur selectively within the dorsal but not the ventral spinal cord, fewer motor effects may occur following the indirect regulation of such release.

The intrathecal application of an adenosine kinase (but not an adenosine deaminase) inhibitor has been shown to produce antinociception in a thermal threshold (Keil and DeLander, 1992) and in an inflammatory pain test (Poon and Sawynok, 1995). This was not accompanied by motor effects, suggesting that the indirect approach may well be a useful way of recruiting adenosine systems in the spinal cord. These behavioural observations suggest adenosine kinase is significantly involved in regulating the levels of adenosine in the spinal cord. These data are consistent with observations that inhibitors of adenosine kinase, but less so adenosine deaminase, are anticonvulsant following central administration (Murray et al., 1993; Zhang et al., 1993). Neurochemical experiments in various brain regions also

* Corresponding author.

[1] Present address: Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Krakow, Poland.

0014-2999/96/$15.00 Published by Elsevier Science B.V.
PII S0014-2999(96)00248-8

have provided data in support of a predominant role of adenosine kinase in regulating endogenous adenosine levels in neural tissue (Sciotti and Van Wylen, 1993; Pak et al., 1994; White, 1996).

In the present study, we have evaluated the relative role of adenosine kinase and adenosine deaminase inhibitors in regulating adenosine release from spinal cord slices in an in vitro superfusion system. The adenosine kinase inhibitors used were 5′-amino-5′-deoxyadenosine (Miller et al., 1979) and 5-iodotubercidin (Davies et al., 1984, 1986), while the adenosine deaminase inhibitor was 2′-deoxycoformycin (Padua et al., 1990). Results indicate that inhibition of adenosine kinase plays a predominant role in regulating adenosine release in the spinal cord, yet adenosine deaminase also plays a significant role, since coadministration of inhibitors of both systems produces a synergistic enhancement of adenosine release.

2. Materials and methods

2.1. Tissue preparation

Adult male Sprague-Dawley rats (300–325 g; Charles River Canada, Quebec, Canada) were killed by decapitation and their spinal cords removed by hydraulic extrusion and placed into ice-cold Krebs-Henseleit bicarbonate buffer containing (mM) 111 NaCl, 26.2 $NaHCO_3$, 4.7 KCl, 1.2 NaH_2PO_4, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 11 glucose, gassed with 95% O_2/5% CO_2 to maintain a pH of 7.4. After the dura was removed, the thoracic-lumbar sections were cut into lengths of approximately 5 mm and longitudinal slices 450 μM thick were prepared with a McIlwain tissue chopper.

2.2. Superfusion of slices

Slices (8–10) were placed on a nylon mesh screen supported by a cylindrical platform in two tissue baths adjusted to an internal volume of 0.5 ml each. Slices were superfused from top to bottom with oxygenated Krebs-Henseleit medium at 37°C and a flow rate of 0.75 ml/min. Slices were equilibrated for 35 min prior to collection of 10 serial 2.5 min fractions. Three initial fractions were collected to determine the basal adenosine release, then the superfusing buffer was switched for 10 min to buffer containing the adenosine kinase or deaminase inhibitor, then the superfusing buffer was switched back to Krebs-Henseleit buffer for the final three fractions. Following collection of all fractions, slices were removed from the tissue baths, blotted and weighed.

2.3. Determination of adenosine

Samples of superfusate were deproteinated with $Ba(OH)_2$ and $ZnSO_4$ and then reacted with chloroacetaldehyde to form 1-N^6-ethenoadenosine, which was assayed by high-performance liquid chromatography with fluorescence detection as described previously (Craig and White, 1993). Adenosine standards were prepared in appropriate drug containing Krebs-Henseleit medium and processed identically to superfusate samples. The sample adenosine content was quantitated by a comparison of peak heights. Adenosine release for each fraction is expressed as pmol/g/min. Release over basal was obtained by subtracting the rate of release for the sample immediately preceding drug exposure. Total release is a summation of release at each interval during drug exposure.

2.4. Drugs

Adenosine and 5′-amino-5′-deoxyadenosine were purchased from Sigma Chemical (St. Louis, MO, USA), and 5-iodotubercidin from Research Biochemicals Int. (Natick, MA, USA). 2′-Deoxycoformycin (Pentostatin) was a gift from Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI, USA). 5-Iodotubercidin was dissolved in dimethylsulfoxide and diluted to the final volume with Krebs-Henseleit medium (final concentration 0.5%).

2.5. Statistical analysis

The effects of the drugs on the rates of adenosine release were analysed for differences by the unpaired Student's *t*-test.

3. Results

Superfusion of spinal cord slices with Krebs-Henseleit medium results in a detectable basal release of adenosine that exhibits some decline over the time course examined (Fig. 1). Superfusion of the slices with 5–50 μM 5′-amino-5′-deoxyadenosine and 5-iodotubercidin produced a concentration-dependent enhancement of adenosine release compared to pre-drug basal values (Fig. 1). Peak effects occurred within 5 min of drug addition and increases were sustained during drug application. 5-Iodotubercidin was slightly more potent and effective than 5′-amino-deoxyadenosine at concentrations up to 25 μM, but at the highest concentration (50 μM), was markedly more effective (Fig. 2). Perfusion of the spinal cord with 50 μM 2′-deoxycoformycin produced only a slight increase in adenosine release (Figs. 2 and 3). Coadministration of 50 μM 2′-deoxycoformycin with modestly active concentrations of 5′-amino-5′-deoxyadenosine and 5-iodotubercidin (5 μM each) produced a synergistic enhancement of release (Figs. 3 and 4). The extent of the enhancement presented in the figure insets is underestimated because the complete enhancement of release was not delineated during the time course used for these experiments.

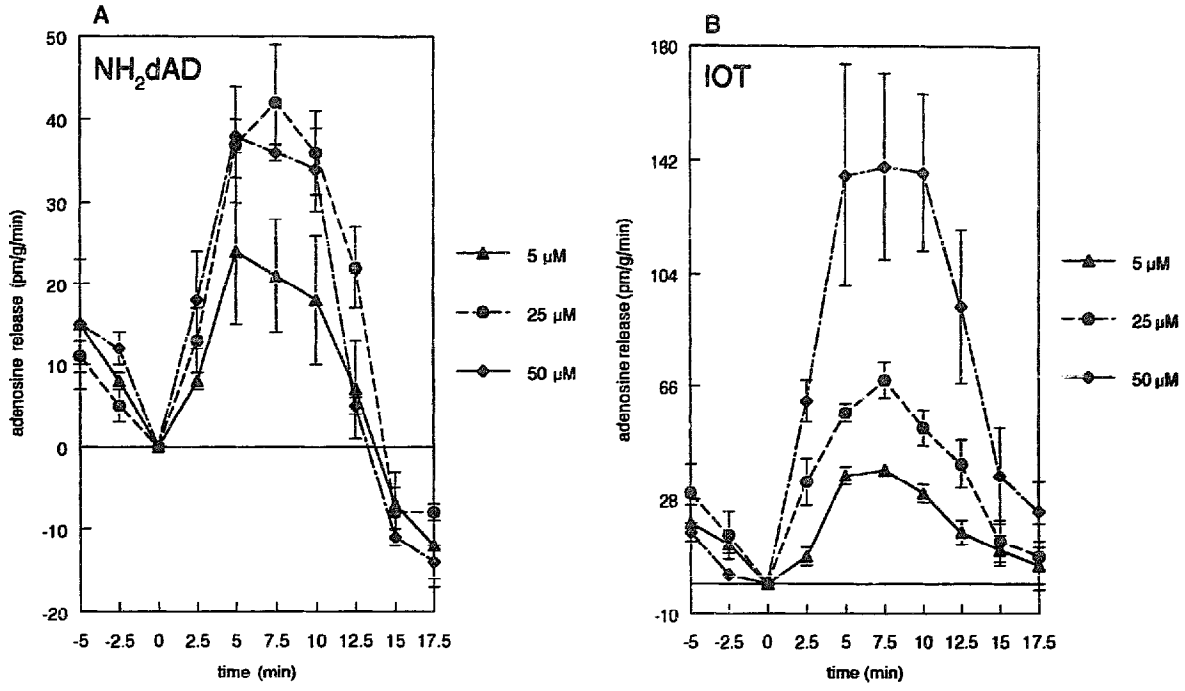

Fig. 1. Effect of inhibition of adenosine kinase with (A) 5'-amino-5-deoxyadenosine (NH_2dAD), and (B) 5-iodotubercidin (IOT) on levels of extracellular adenosine in rat spinal cord slices. Figure depicts time course of increase over basal adenosine release. Drugs were perfused from time 0 to 10 min. Data are expressed as the mean ± S.E.M. ($n = 4–7$).

4. Discussion

Within the cell, adenosine levels are regulated by phosphorylation by adenosine kinase and deamination by adenosine deaminase (reviewed Meghji, 1991). Adenosine kinase has a higher affinity for adenosine than does adenosine deaminase, and it is likely that adenosine is phosphorylated at low concentrations and deaminated at high concentrations. As some differences may occur within specific cell types (Meghji, 1991), it is important to determine which mechanism(s) is (are) responsible for regulation of intracellular and extracellular adenosine at specific sites. In the present study, it appears that adenosine kinase is more important than adenosine deaminase for regulating extracellular adenosine levels in the spinal cord, as adenosine kinase inhibitors are both more potent and more effective than an adenosine deaminase inhibitor in enhancing adenosine levels collected in the superfusate. The spinal cord is thus similar to the striatum, hippocampus and cortex where similar observations have been made (Sciotti and Van Wylen, 1993; Pak et al., 1994; White, 1996). While there might be some concern that the relative inactivity of 2'-deoxycoformycin might be due to delayed cellular uptake (Newby, 1981), marked effects for 2'-deoxycoformycin are seen in combination with adenosine kinase inhibitors (see below) and in the in situ spinal cord (Golembiowska et al., 1995) using this time course of application, which suggests this may not be a major factor in determining its lesser activity.

The demonstration of a greater effect on adenosine release from spinal cord slices by adenosine kinase inhibitors than by an adenosine deaminase inhibitor is also consistent with observations in behavioural experiments following the spinal administration of these agents. Thus,

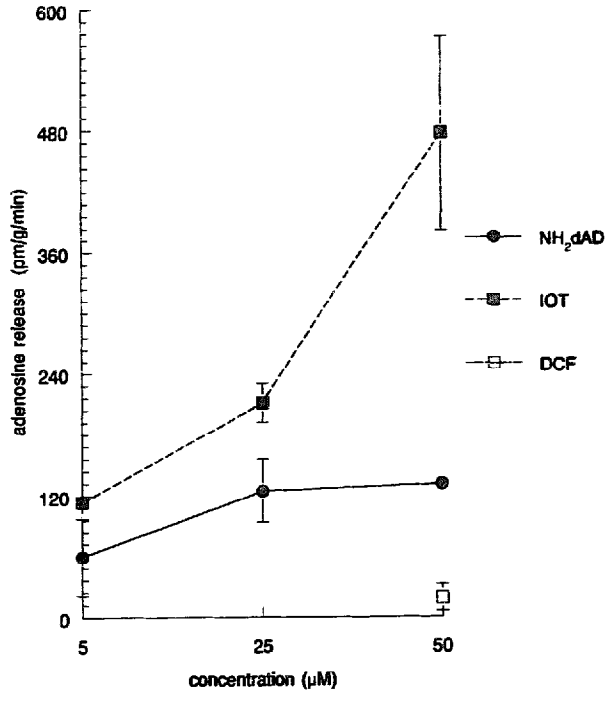

Fig. 2. Dose-response relationship for the effect induced by the adenosine kinase inhibitors 5'-amino-5'-deoxyadenosine (NH_2dAD) and 5-iodotubercidin (IOT) and the adenosine deaminase inhibitor 2'-deoxycoformycin (DCF) on adenosine release from rat spinal cord slices. Data are expressed as a mean ± S.E.M. for total increase over basal adenosine release during exposure to drug ($n = 4–7$ except for DCF where $n = 3$).

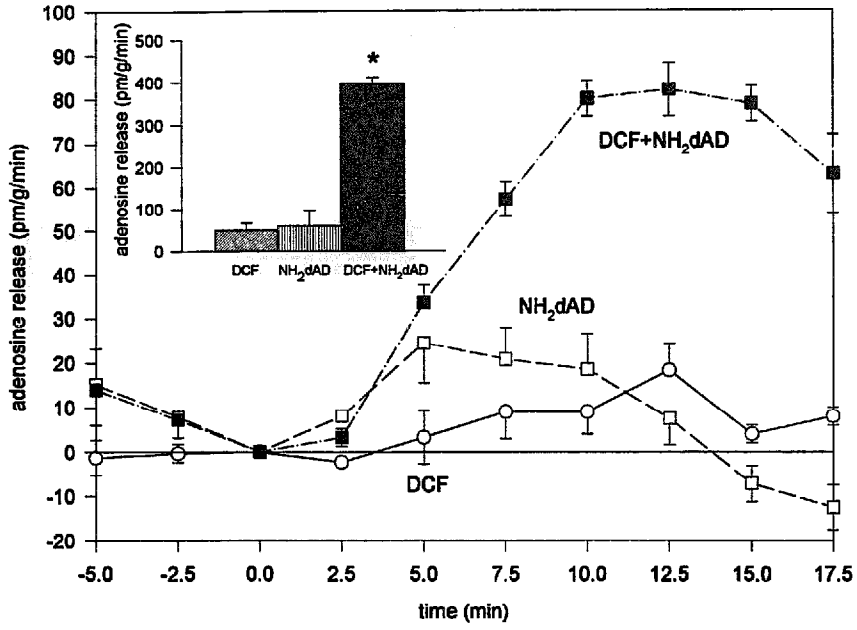

Fig. 3. Effect of 2′-deoxycoformycin (DCF) 50 μM and 5′-amino-5′-deoxyadenosine (NH_2dAD) 5 μM administered in combination on adenosine release from rat spinal cord slices. Drugs were perfused from time 0 to 10 min. Values are mean ± S.E.M. for $n = 4$. Inset indicates total adenosine release during drug perfusion. * $P < 0.01$ compared to DCF or NH_2dAD alone.

following intrathecal application, adenosine kinase but not deaminase inhibitors produce antinociception in the tail flick (Keil and DeLander, 1992) and formalin tests (Poon and Sawynok, 1995). Antinociception is inhibited by methylxanthines indicating it is due to activation of cell surface adenosine receptors, most likely by adenosine which accumulates as a result of inhibition of metabolism, rather than by the inhibitor interacting directly with the adenosine receptor (cf. Davies et al., 1984, 1986). Adenosine deaminase is localized in the superficial layers of the dorsal spinal cord in small diameter sensory afferents (Nagy and Dadonna, 1985; Geiger and Nagy, 1986), and is thus preferentially localized in good proximity to adenosine receptors believed to mediate spinal antinociception (Geiger et al., 1984; Choca et al., 1988). To our knowledge, there is no specific data on the distribution of adenosine kinase

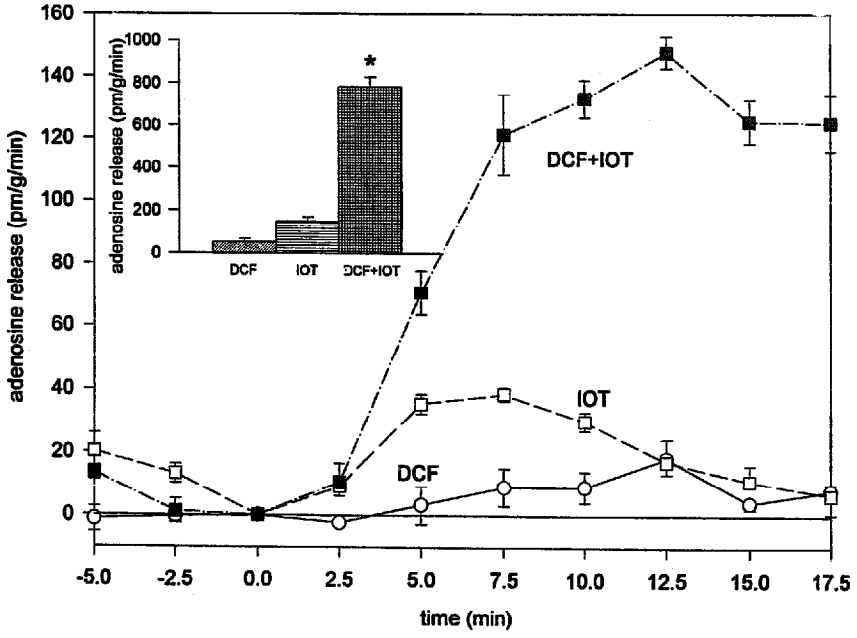

Fig. 4. Effect of 2′-deoxycoformycin (DCF) and 5-iodotubercidin (IOT) administered in combination on adenosine release from rat spinal cord slices. Drugs were perfused from time 0 to 10 min. Inset indicates total adenosine release during drug perfusion. Values are mean ± S.E.M. for $n = 4$. * $P < 0.01$ compared to DCF or IOT alone.

within the spinal cord, but it might be reasonable to assume a meaningful juxtaposition with adenosine deaminase. Adenosine kinase inhibitors also potentiate antinociception produced by opioids (Keil and DeLander, 1994) which probably occurs due to opioid-induced release of endogenous adenosine from the spinal cord (DeLander and Hopkins, 1987; Sweeney et al., 1989; DeLander et al., 1992; Cahill et al., 1995). Interestingly, when adenosine itself is administered into the intrathecal space, inhibition of adenosine deaminase produces a greater enhancement of antinociception than does inhibition of adenosine kinase (Keil and DeLander, 1994). This could reflect an involvement of an extracellular adenosine deaminase (see Meghji, 1991) in spinal cord metabolism of adenosine under certain conditions, or a more prominent role for adenosine deaminase when adenosine levels are high due to exogenous administration.

The present study also demonstrates a marked synergy between coadministration of adenosine kinase and adenosine deaminase inhibitors. Such synergy has previously been noted in behavioural studies where combinations of 5′-amino-5′-deoxyadenosine and 2′-deoxycoformycin produce an augmented anticonvulsant activity (Zhang et al., 1993). Although such synergy has also been noted in neurochemical studies in brain (Sciotti and Van Wylen, 1993; Pak et al., 1994), the extent of the increase seen in those studies was less than that observed in the present study. It may be that in the spinal cord, there is less differential in the relative affinities and capacities of adenosine for adenosine kinase and adenosine deaminase, and only a modest inhibition of adenosine kinase is required to recruit the involvement of adenosine deaminase. This might explain why in the intact spinal cord preparation, where test conditions are somewhat different from those in the present study, a prominent role for adenosine deaminase in regulating extracellular adenosine levels is apparent (Golembiowska et al., 1995).

The in vitro characterization of 5′-amino-5′-deoxyadenosine and 5-iodotubercidin indicates that 5′-amino-5′-deoxyadenosine is more potent than 5-iodotubercidin in inhibiting adenosine kinase (Ki 0.02 μM vs. 1–5 μM) (Miller et al., 1979; Davies et al., 1984) (although some of this difference may be due to different tissue sources of adenosine kinase). When applied directly into the prepiriform cortex, both agents exhibit a similar potency and efficacy at suppressing bicuculline-induced convulsions (Zhang et al., 1993). In the present study, 5′-amino-deoxyadenosine and 5-iodotubercidin exhibited a similar ability to enhance adenosine levels at concentrations up to 25 μM, but at 50 μM, 5-iodotubercidin was considerably more effective than 5′-amino-5′-deoxyadenosine. While 5-iodotubercidin also inhibits adenosine uptake (Davies et al., 1984; Davies et al., 1986), this is likely to be secondary to inhibition of adenosine kinase as 5-iodotubercidin does not affect rapid transport of adenosine into synaptosomes (Wu et al., 1984). As this effect occurs at low concentrations of 5-iodotubercidin, it may not be an adequate explanation for enhanced activity at the higher concentration. 5-Iodotubercidin inhibits a number of protein kinases (including protein kinase C) in addition to adenosine kinase at 50 μM (Massillon et al., 1994), and it is more likely that actions of this kind contribute to the enhanced activity at the highest concentration of 5-iodotubercidin.

In summary, inhibition of adenosine kinase produces a greater degree of enhancement of efflux of adenosine into the extracellular space from spinal cord slices than does inhibition of adenosine deaminase. This enhanced appearance of adenosine probably contributes to spinal antinociception following intrathecal administration of adenosine kinase inhibitors. Simultaneous inhibition of adenosine deaminase produces a significant further enhancement of adenosine release. The consequences of simultaneous administration of adenosine kinase and deaminase inhibitors in nociceptive paradigms have yet to be reported.

Acknowledgements

This work was supported by the Medical Research Council of Canada. We thank Parke Davis Pharmaceuticals for the provision of 2′-deoxycoformycin (Pentostatin).

References

Cahill, C.M., T.D. White and J. Sawynok, 1995, Spinal opioid receptors and adenosine release: neurochemical and behavioural characterization of opioid subtypes, J. Pharmacol. Exp. Ther. 275, 84.

Choca, J.I., R.D. Green and H.K. Proudfit, 1988, Adenosine A_1 and A_2 receptors of the substantia gelatinosa are located predominantly on intrinsic neurons: an autoradiographic study, J. Pharmacol. Exp. Ther. 247, 757.

Craig, C.G. and T.D. White, 1993, *N*-Methyl-D-aspartate- and non-*N*-methyl-D-aspartate-evoked adenosine release from rat cortical slices: distinct purinergic sources and mechanisms of release, J. Neurochem. 60, 1073.

Davies, L.P., J. Baird-Lambert and J.F. Marwood, 1986, Studies on several pyrrolo[2,3-*d*]pyrimidine analogues of adenosine which lack significant agonist activity at A1 and A2 receptors but have potent pharmacological activity in vivo, Biochem. Pharmacol. 35, 3021.

Davies, L.P., D.D. Jamieson, J.A. Baird-Lambert and R. Kazlauskas, 1984, Halogenated pyrrolopyrimidine analogues of adenosine from marine organisms: pharmacological activities and potent inhibition of adenosine kinase, Biochem. Pharmacol. 33, 347.

DeLander, G.E. and C.J. Hopkins, 1987, Involvement of A_2 adenosine receptors in spinal mechanisms of antinociception, Eur. J. Pharmacol. 139, 215.

DeLander, G.E., H.I. Mosberg and F. Porreca, 1992, Involvement of adenosine in antinociception produced by spinal or supraspinal receptor-selective opioid agonists: dissociation from gastrointestinal effects in mice, J. Pharmacol. Exp. Ther. 263, 1097.

Fyffe, R.E.W. and E.R. Perl, 1984, Is ATP a central synaptic mediator for certain primary afferent fibres from mammalian skin?, Proc. Natl. Acad. Sci. USA 81, 6890.

Geiger, J.D. and J.I. Nagy, 1986, Distribution of adenosine deaminase activity in rat brain and spinal cord, J. Neurosci. 6, 2707.

Geiger, J.D., F.S. LaBella and J.I. Nagy, 1984, Characterization and localization of adenosine receptors in rat spinal cord, J. Neurosci. 4, 2303.

Golembiowska, K., T.D. White and J. Sawynok, 1995, Modulation of adenosine release from rat spinal cord by adenosine deaminase and adenosine kinase inhibitors, Brain Res. 699, 315.

Karlsten, R., T. Gordh, P. Hartvig and C. Post, 1990, Effects of intrathecal injection of the adenosine receptor agonists *R*-phenylisopropyladenosine and *N*-ethylcarboxamide-adenosine on nociception and motor function in the rat, Anesth. Analg. 71, 60.

Keil, G.J. and G.E. DeLander, 1992, Spinally-mediated antinociception is induced in mice by an adenosine kinase-, but not by an adenosine deaminase-, inhibitor, Life Sci. 51, 171.

Keil, G.L. and G.E. DeLander, 1994, Adenosine kinase and adenosine deaminase inhibition modulate spinal adenosine- and opioid agonist-induced antinociception in mice, Eur. J. Pharmacol. 271, 37.

Malmberg, A.B. and T.L. Yaksh, 1993, Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction, Anesthesiology 79, 270.

Massillon, D., W. Stalmans, G. Van de Werve and M. Bollen, 1994, Identification of the glycogenic compound 5-iodotubercidin as a general protein kinase inhibitor, Biochem. J. 299, 123.

Meghji, P., 1991, Adenosine production and metabolism, in: Adenosine in the Nervous System, ed. T. Stone (Academic Press, London) p. 25.

Miller, R.L., D.L. Adamczyk, W.H. Miller, G.W. Koszalka, J.L. Rideout, L.M. Beacham, E.Y. Chao, J.J. Haggerty, T.A. Krenitsky and G.B. Elion, 1979, Adenosine kinase from rabbit liver, II. Substrate and inhibitor specificity, J. Biol. Chem. 254, 2346.

Minami, T., R. Uda, S. Horiguchi, S. Ito, M. Hyodo and O. Hayashi, 1992, Allodynia evoked by intrathecal administration of prostaglandin $F_{2\alpha}$ to conscious mice, Pain 50, 223.

Murray, T.F., G. Zhang and P.H. Franklin, 1993, Manipulation of endogenous adenosine affects seizure susceptibility, Drug Dev. Res. 28, 410.

Nagy, J.I. and P.E. Dadonna, 1985, Anatomical and cytochemical relationships of adenosine deaminase-containing primary afferent neurons in the rat, Neuroscience 15, 799.

Newby, A.C., 1981, The interaction of inhibitors with adenosine metabolizing enzymes in intact isolated cells, Biochem. Pharmacol. 30, 2611.

Padua, R., J.D. Geiger, S. Dambock and J.I. Nagy, 1990, 2′-Deoxycoformycin inhibition of adenosine deaminase in rat brain: in vivo and in vitro analysis of specificity, potency, and enzyme recovery, J. Neurochem. 54, 1169.

Pak, M.A., H.L. Haas, U.K.M. Decking and J. Schrader, 1994, Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices, Neuropharmacology 33, 1049.

Poon, A. and J. Sawynok, 1995, Antinociception by adenosine analogs and an adenosine kinase inhibitor: dependence on formalin concentration, Eur. J. Pharmacol. 286, 177.

Salter, M.W., Y. De Koninck and J.L. Henry, 1993, Physiological roles for adenosine and ATP in synaptic transmission in the spinal dorsal horn, Prog. Neurobiol. 41, 125.

Sawynok, J., 1991, Adenosine and pain, in: Adenosine and Adenine Nucleotides as Regulators of Cellular Function, ed. J.W. Phillis (CRC, Boca Raton) p. 391.

Sawynok, J., 1996, Purines and nociception, in: Purinergic Approaches in Experimental Therapeutics, ed. K. Jacobson and M.F. Jarvis (Wiley and Sons).

Sawynok, J., J.W. Downie, A.R. Reid, C.M. Cahill and T.D. White, 1993, ATP release from dorsal spinal cord synaptosomes: characterization and neuronal origin, Brain Res. 610, 32.

Sciotti, V.M. and D.G.L. Van Wylen, 1993, Increases in interstitial adenosine and cerebral blood flow with inhibition of adenosine kinase and adenosine deaminase, J. Cereb. Blood Flow Metab. 13, 201.

Sosnowski, M. and T.L. Yaksh, 1989, Role of spinal adenosine receptors in modulating the hyperesthesia produced by spinal glycine receptor antagonism, Anesth. Analg. 69, 587.

Sweeney, M.I., T.D. White and J. Sawynok, 1989, Morphine, capsaicin and K^+ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord, J. Pharmacol. Exp. Ther. 248, 447.

White, T.D., 1996, Potentiation of excitatory amino acid-evoked adenosine release from rat cortical slices by inhibitors of adenosine kinase and adenosine deaminase and by AICAR (acadesine), Eur. J. Pharmacol.

Wu, P.H., R.A. Barraco and J.W. Phillis, 1984, Further studies on the inhibition of adenosine uptake into rat brain synaptosomes by adenosine derivatives and methylxanthines, Gen. Pharmacol. 15, 251.

Zhang, G., P.H. Franklin and T.F. Murray, 1993, Manipulation of endogenous adenosine in the rat prepiriform cortex modulates seizure susceptibility, J. Pharmacol. Exp. Ther. 264, 1415.