J. Pharm. Pharmacol. 1994, 46: 801–804
Received September 24, 1993
Accepted March 14, 1994

The Adenosine Agonist NECA Inhibits Intestinal Secretion and Peristalsis

IAN M. COUPAR AND DEBRA L. HANCOCK

Unit of Addictive Drug Research, School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia

Abstract—This study aimed to determine whether the antidiarrhoeal effect of the mixed A_1/A_2 adenosine agonist NECA (5′-*N*-ethylcarboxamido adenosine) is due to inhibition of intestinal fluid transport or to contractility. Intestinal secretion was stimulated in anaesthetized rats by intra-arterial infusions of PGE_2 ($4\,\mu g\,min^{-1}$) or vasoactive intestinal peptide ($0{\cdot}8\,\mu g\,min^{-1}$). NECA reversed PGE_2-induced secretion in the jejunum (ED50 $16\,\mu g\,kg^{-1}$) and ileum (ED50 $21\,\mu g\,kg^{-1}$, i.v.) and inhibited VIP-induced secretion in the jejunum (ED50 $21{\cdot}5\,\mu g\,kg^{-1}$). NECA inhibited twitch responses (0·1 Hz, 1 ms, IC50 11·2 nM) but not tetanic contractions at 10 Hz of the transmurally stimulated guinea-pig ileum. Likewise, NECA ($10\,\mu M$) did not inhibit frequency-related contractions over the range of 2·5 to 40 Hz of rat jejunum or ileum. However, NECA was shown to be a potent inhibitor (30 nM) of the peristaltic reflex in the rat ileum. The results indicate that adenosine receptors are involved in modulating peristalsis as well as the secretory activity of the mucosa in the rat small intestine.

Analgesia was one of the first documented effects of the adenosine agonists. In 1975 Valpaatalo et al showed that phenylisopropyladenosine (R-PIA) displayed potent antinociceptive activity in mice using the hot plate test. Several other adenosine agonists have subsequently been shown to have this property in both the hot plate and abdominal constriction tests (Herrick-Davis et al 1989; Contreras et al 1990). Low doses of R-PIA enhance morphine-induced analgesia, tolerance and dependence (Ahlijanian & Takemori 1985) and chloroadenosine has also been shown to induce tolerance to morphine in mice (Contreras et al 1990). The current evidence suggests that adenosine mediates the analgesic action of morphine in the corpus striatum (Perkins & Stone 1980) and spinal cord (Sweeney et al 1987).

Opiates exert potent actions on the intestine, where again, there is evidence for an interaction with adenosine. Early experiments using the guinea-pig isolated ileum identified that adenosine and chloroadenosine, like morphine, had the ability to suppress the release of acetylcholine. In addition, normorphine was shown to inhibit contraction induced by withdrawing adenosine (Collier & Tucker 1983). The assumption has also been made that adenosine is able to suppress the withdrawal contracture of morphine-dependent tissues (Collier 1984). More recently we have shown that the adenosine agonists PIA and 5′-*N*-ethylcarboxamido adenosine (NECA) are both potent inhibitors of naloxone-precipitated opiate withdrawal behaviour, as well as opiate-withdrawal diarrhoea in rats (Dionyssopoulos et al 1992).

The diarrhoea which is characteristic of the morphine-withdrawal syndrome is thought to be a consequence of both increased intestinal transit (Brown et al 1988) and decreased ability of the intestinal mucosa to absorb fluid (Chang et al 1984). Indirect evidence points to the release of acetylcholine, 5-HT and prostaglandins as mediators in this withdrawal reaction, since antagonists to these substances inhibit naloxone-precipitated opiate-withdrawal diarrhoea (Collier et al 1972; Francis et al 1975). Both 5-HT and PGE_2 are intestinal secretagogues and both are released into the lumen of the rat colon as a result of naloxone-precipitated withdrawal. As expected, this effect is associated with a reversal of fluid transport to net secretion (Beubler et al 1984).

The specific aim of this study was to determine whether the antidiarrhoeal effect of adenosine agonists is due to an action on neuromuscular contractility or on fluid transport. The adenosine receptor agonist NECA was selected since it acts equipotently on the two major adenosine receptors so far identified, the A_1 and A_2 receptors (Bruns et al 1986).

Correspondence: I. M. Coupar, Unit of Addictive Drug Research, School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia.

Materials and Methods

Fluid transport

Hooded Wistar rats of either sex, 230–300 g, were anaesthetized with pentobarbitone sodium ($60\,mg\,kg^{-1}$) subcutaneously. A cannula was inserted into the left jugular vein for administration of NECA and another into the left common carotid artery for constant infusion of vasoactive intestinal peptide ($0{\cdot}8\,\mu g\,min^{-1}$), PGE_2 ($4\,\mu g\,min^{-1}$) or saline as control, at a rate of $40\,\mu L\,min^{-1}$.

A 20 to 30 cm length of jejunum, starting distal from the ligament of Trietz, was perfused continuously with 8 mL of an iso-osmotic solution containing NaCl 148, KCl 5 and dextrose 5·5 mM. Phenol red, 0.05 mM, was also present in the solution to act as a non-absorbable water marker. The solution was contained in a reservoir maintained at 37°C and recirculated through the lumen by a gas-lift column of moistened 5% CO_2 in O_2. The pressure in the loop was 10 cm of H_2O and the flow rate was approximately $60\,mL\,min^{-1}$. A 20- to 30-cm loop of ileum was also perfused in experiments where PGE_2 was the secretagogue. Intra-arterial infusion started 5 min after injection of NECA and luminal perfusion commenced 5 min later and lasted for 20 min.

The recovered samples were diluted with buffer and peak absorbencies were measured at 560 nm as well as 520 and 600 nm to correct for non-specific interferences, as described

by Miller & Schedl (1972). Results are expressed as the net amount of water absorbed (+) or secreted (−) in μL (g wet weight tissue)$^{-1}$ during the 20-min perfusion.

Rat and guinea-pig isolated intestine

Segments of rat proximal jejunum (5 cm proximal to the ligament of Trietz) and ileum or guinea-pig ileum (5 cm proximal to the caecum), were removed from animals and mounted in 25-mL jacketed organ baths maintained at 37°C. The preparations were allowed to equilibrate under a resting load of 1 g for 45 min in Krebs–Henseleit containing (mM): NaCl 118, KCl 4·7, $NaHCO_3$ 25, KH_2PO_4 1·2, $CaCl_2$ 2·5, $MgSO_4$ 1·2, D-(+)-glucose 11. Changes in the length of the longitudinal smooth muscle strips were recorded using isotonic transducers (Ugo Basil) and displayed on a multichannel Grass model 79D polygraph.

The segments of rat jejunum and ileum were stimulated transmurally at pulse frequencies of 2·5, 5, 10, 20 and 40 Hz in trains lasting 8 s. The trains of pulses were delivered 3 min apart. Segments of guinea-pig ileum were also stimulated transmurally at 10 Hz for 8 s, and also at the conventionally used frequency of 0·1 Hz. Trains or single pulses were delivered through parallel platinum wire electrodes at 1 ms duration and supramaximal voltage from Grass S44 square wave stimulators.

The effect of NECA was also measured on the peristaltic reflex of the rat ileum in a separate series of experiments. Segments of ileum (6–8·5 cm) were tied at the distal end to a J tube. The proximal end was tied off and the reflex elicited by applying an intraluminal pressure of 7–9 cm of H_2O from a 5-mL reservoir containing physiological solution. A float in the reservoir was connected to an isotonic transducer to monitor volume changes related to circular muscle contraction and a second isotonic transducer was used to measure the associated changes in length of the longitudinal muscle. Segments were inflated for two periods of 15 min separated by a test period of 10 min.

Statistical analysis

The rates of water transport are given as means ± s.e.m. Student's unpaired *t*-test was used to compare single treatment means with their respective control and Dunnett's *t*-test to compare sets of means with their common control. IC50 and ED50 values were calculated using linear regression analysis with the 95% confidence interval of the estimates. The criterion for statistical significance was set at $P < 0{\cdot}05$.

Drugs

The drugs used were NECA (5′-*N*-ethylcarboxamido adenosine; RBI, Natick, USA), pentobarbitone sodium (Nembutal, Boehringer Ingelheim, Artarmon, Australia), prostaglandin E_2 (PGE_2, Sigma-Aldrich, Castle Hill, Australia), vasoactive intestinal peptide (VIP; Auspep, Melbourne, Australia).

Results

Fluid transport

The rates of fluid absorption were 270 ± 30 μL g^{-1} in 20 min (n = 8) from the jejunum and 278 ± 45 μL g^{-1} in 20 min (n = 7) from the ileum. Infusion of PGE_2 at 4 μg min^{-1} induced a net secretion of −56 ± 23 μL g^{-1} in 20 min (n = 10) into the lumen of the jejunum and −17 ± 43 μL g^{-1} in 20 min (n = 8) into the ileum. NECA did not affect the rates of absorption in the jejunum or ileum of control animals but produced dose-related reversals of the PGE_2-induced effects in these two regions of the small intestine. The ED50 values of NECA in reversing the secretory effect of PGE_2 were 16·3 μg kg^{-1} (95% CI = 2·9, n = 23) in the jejunum and 20·1 μg kg^{-1} (95% CI = 2·7, n = 27) in the ileum (Fig. 1).

NECA also produced a dose-related reversal of VIP-induced secretion in the jejunum (ileum not studied), although a high dose of 100 μg kg^{-1} did not fully restore fluid absorption. The secretory response to VIP was −327 ± 22 μL g^{-1} in 20 min and this was converted to net absorption at a rate of 114 ± 47 μL g^{-1} in 20 min (n = 4) by 100 μg kg^{-1} NECA. The response to this dose of NECA in VIP-infused animals was significantly different from the absorption rate of 239 ± 24 μL g^{-1} in 20 min as occurred in the control group of rats (saline i.a., $P = 0{\cdot}03$, n = 6). The ED50 of NECA was 21 μg kg^{-1} (95% CI = 1·4, n = 13).

Isolated intestine

Transmural stimulation. NECA caused large inhibitions (80 ± 1·8% at 40 nM) in twitch responses of the guinea-pig ileum with an IC50 value of 11·2 ± 0·9 nM. This concentration of NECA did not alter the response of the tissues to stimulation at 10 Hz (n = 4, Fig. 2). Likewise, NECA at 10 μM did not inhibit the frequency-dependent contractions of rat jejunum (n = 3, Fig. 3) or ileum.

Peristaltic reflex. The rat ileum displayed rhythmical contractions of the longitudinal muscle associated with volume expulsions in each of the 15-min stimulation periods. The frequency of these parameters was in the order of 1 min^{-1} (n = 4). Low concentrations of NECA

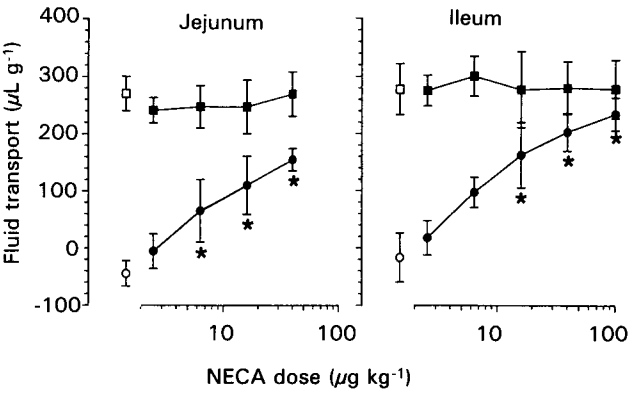

FIG. 1. Inhibition by NECA of PGE_2-induced secretion in the jejunum and ileum. The lower point (open circle) on the left of each graph is the value of fluid transport in response to intra-arterial infusion of PGE_2 at 4 μg min^{-1}. The filled circles show the dose-related inhibition of PGE_2-induced secretion by NECA. The top point (open square) on the left of each graph shows the resting value of absorption in animals infused intra-arterially with saline as control. The filled squares indicate the levels of absorption in groups treated with NECA. Bars indicate s.e.m. and asterisks indicate that the means are significantly different from the control (saline, i.v.) groups infused with PGE_2 (Dunnett's *t*-test, $P < 0{\cdot}05$).

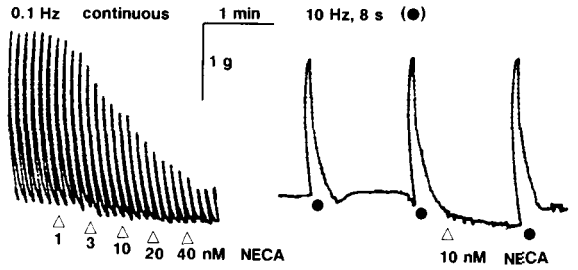

FIG. 2. Representative trace showing inhibition of twitch responses (0·1 Hz, 1 ms) of the guinea-pig ileum by cumulative addition of NECA but lack of effect of the ED50 concentration of an 8-s train of pulses delivered at 10 Hz, 1 ms duration.

(median concentration 30 nM, n = 6) prevented the peristaltic reflex which could be re-established in the second period of stimulation after the wash and zero pressure rest period (Fig. 4).

Discussion

This study shows that the mixed A_1/A_2-adenosine agonist NECA is a potent inhibitor of both intestinal fluid secretion and peristalsis. As such, the results add to those of our previous study which showed NECA inhibits naloxone-precipitated withdrawal diarrhoea in morphine-dependent rats (Dionyssopoulos et al 1992).

The diarrhoea of morphine withdrawal is caused partly by the release of PGE_2 which reverses normal fluid movement across the mucosa to net secretion (Beubler et al 1984). This study has shown that this secretory effect of PGE_2 is inhibited by low doses of NECA in both jejunum and ileum. In addition, it was confirmed that the antisecretory

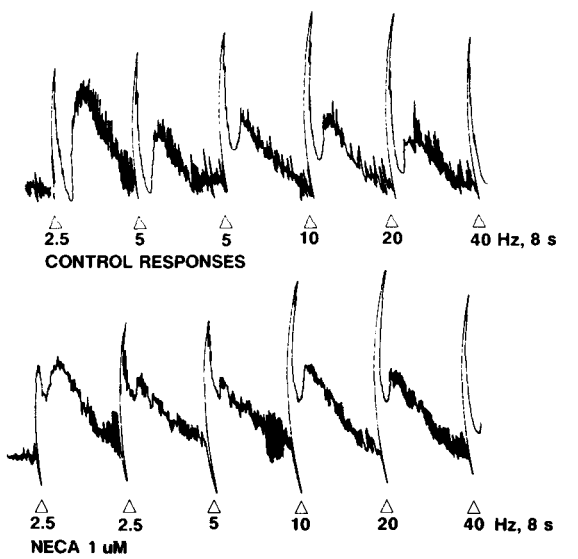

FIG. 3. Representative trace showing responses of the rat isolated jejunum to transmural stimulation at different frequencies. Pulses were delivered in 8-s trains of 1 ms duration (△) which induced a frequency-dependent fast cholinergic contraction during the period of stimulation. This was followed by partial recovery and then a slow atropine-resistant after-contraction. A 10-min incubation with NECA at 10 μM did not alter the contractile responses but unmasked or potentiated an initial fast relaxation during stimulation.

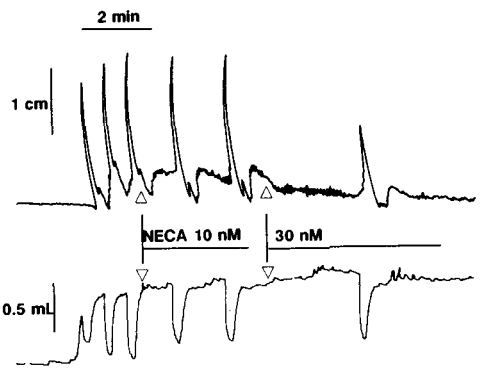

FIG. 4. Inhibition by NECA of the peristaltic reflex in a segment of rat ileum. The upper trace shows the contraction of the longitudinal muscle and the lower trace the associated volume expulsion as a result of circular muscle contraction in response to raising the intramural pressure to 9 cm H_2O. NECA at 10 nM slowed the reflex while 30 nM virtually abolished it. Peristalsis was restored 10 min later after a zero pressure rest period and replacing the bathing solution with NECA-free solution.

effect of NECA is not restricted to PGE_2 since it also inhibited the secretion induced by VIP. While this secretagogue does not appear to be released during morphine withdrawal, it is known to be a potent neurotransmitter involved in the regulation of intestinal fluid transport. Like PGE_2, its effect is mediated by cyclic AMP (Brown & Miller 1991). NECA itself did not influence the basal rate of fluid absorption from either jejunum or ileum, showing that it exerts true antisecretory activity rather than a proabsorptive effect opposing secretion.

The present results would not have been predicted from those obtained in studies of adenosine agonist action on the intestinal mucosa using electrophysiological techniques. Results from experiments measuring short circuit current from sheets of rabbit ileal and colonic mucosa have indicated that both NECA and adenosine induced Cl^- secretion (Grasl & Turnheim 1984; Dobbins et al 1984). This emphasizes the importance of functional studies and the need to be aware of possible species differences in responses to drugs. This latter point is illustrated further by the present results obtained from the smooth muscle preparations.

In addition to intestinal fluid secretion, morphine withdrawal diarrhoea is also due to increased intestinal transit (Brown et al 1988). In this regard the present results show clearly that NECA is able to block motility, since low concentrations blocked the peristaltic reflex in the rat isolated ileum. The neuronal site of this action is unclear at present but is partly revealed from the results of the experiments using transmural stimulation.

In the guinea-pig ileum, adenosine and chloroadenosine inhibit the release of acetylcholine evoked at conventional low frequencies of transmural stimulation (Gintzler & Musacchio 1975; Sawynock & Jhamandas 1976). NECA however, does not appear to have been screened by others for activity at 0·1 Hz, although it has been shown to inhibit contractions to repetitive stimulation at the considerably higher frequency of 3 Hz for 5 s every min. Under these conditions the IC50 of NECA was shown to be approximately 33 nM (Gustafsson et al 1985). This is higher than the value of 11·2 nM obtained in this study which was obtained

at 0·1 Hz. Taken with our observation that NECA did not affect the response to stimulation at 10 Hz, the findings show that NECA inhibits cholinergic contractions of the guinea-pig ileum only at low frequency. This frequency-dependent block of acetylcholine release in the guinea-pig ileum also occurs with morphine.

Unlike the ileum of the guinea-pig, segments of rat small intestine do not respond to single pulses of transmural stimulation; the threshold for contractions occurs at around 2·5 Hz and peaks at 10 to 20 Hz (Coupar & De Luca 1994). The fast contractions induced at 10 Hz have been characterized pharmacologically on the basis that they are blocked by atropine. In contrast to the findings in the guinea-pig ileum, NECA did not affect cholinergic contractions of the rat jejunum or ileum at any of the frequencies investigated, even though the concentration of NECA used in these experiments was nearly 1000 times the IC50 value derived from the guinea-pig ileum. This discounts an inhibitory action of NECA on cholinergic neuroeffector transmission in the regions studied. A separate study has established that NECA relaxes the rat duodenum (EC50 400 nM) by activating smooth muscle A_2 receptors (Nicholls et al 1992), but this seems to be the only region of the gut which is sensitive to NECA. The present results showing that NECA is a potent antagonist of the peristaltic reflex but not of transmural stimulation indicate that the adenosine receptors that mediate inhibition of peristalsis are located proximal to the final cholinergic neurons in the reflex arc.

We have not characterized the adenosine receptors which mediate the antimotility or antisecretory effects of NECA, nor have we identified the tissue localization of the receptors that control these effects. Indeed, the issue as to whether adenosine receptors are present in the rat intestine has not been resolved, since only two studies have been undertaken and both give ambiguous findings. Bruns et al (1986) showed that although NECA showed specific and high affinities for A_1 and A_2 binding sites in the rat brain, it did not bind to homogenates of whole small intestine. However, Reymann & Gniess (1988) provided evidence that adenosine receptors are present in rat intestinal mucosa where agonists reduce cAMP levels which would be expected to lead to a reduction in fluid secretion (Brown & Miller 1991). The A_1 agonist R-PIA was moderately potent in this regard which would corroborate the present results if it were not for their finding that the approximate IC50 of NECA was in the order of 100 μM.

Overall, the results of this study support the hypothesis that NECA exerts its antidiarrhoeal action against morphine withdrawal, as previously described, by blocking intestinal fluid secretion as well as by preventing motility.

References

Ahlijanian, M. K., Takemori, A. E. (1985) Effects of (−)-N^6-(R-phenylisopropyl)-adenosine (PIA) and caffeine on nociception and morphine-induced analgesia, tolerance and dependence. Eur. J. Pharmacol. 112: 171–179

Beubler, E., Bukhave, K., Rask-Madsen, J. (1984) Colonic secretion mediated by prostaglandin E_2 and 5-hydroxytryptamine may contribute to diarrhoea due to morphine withdrawal in the rat. Gastroenterology 87: 1042–1048

Brown, D.R., Miller, R. (1991) Neurohormonal control of fluid and electrolyte transport in intestinal mucosa. In: Schultz, S. G., Field, M., Frizzell, R. A. (eds) Handbook of Physiology – The Gastrointestinal System, Vol. iv, pp 527–589

Brown, N.J., Coupar, I.M., Rumsey, R. D. E. (1988) The effect of acute and chronic administration of morphine and morphine withdrawal on intestinal transit time in the rat. J. Pharm. Pharmacol. 40: 844–848

Bruns, R. F., Lu, G. H., Pugsley, T. A. (1986) Characterization of the A_2 adenosine receptor labelled by [^{3}H]NECA in rat striatal membranes. Mol. Pharmacol. 29: 331–346

Chang, E. B., Brown, D. R., Field, M., Miller, R. J. (1984) An antiabsorptive basis for precipitated withdrawal diarrhoea in morphine-dependent rats. J. Pharmacol. Exp. Ther. 228: 364–369

Collier, H. O. J. (1984) Cellular aspects of opiate tolerance and dependence. In: Hughes, J., Collier, H. O. J., Rance, H.J., Tyres, M. B. (eds) Opioids, Past, Present and Future. Taylor and Francis, London and Philladelphia, pp 109–125

Collier, H. O. J., Tucker, J. F. (1983) Novel form of drug dependence on adenosine in guinea-pig ileum. Nature 302: 618–621

Collier, H. O. J., Francis, D. L., Schneider, C. (1972) Modification of morphine withdrawal by drugs interacting with humoral mechanisms: some contradictions and their interpretation. Nature 237: 220–223

Contreras, E., Germany, A., Villar, M. (1990) Effects of some adenosine analogues on morphine-induced analgesia and tolerance. Gen. Pharmacol. 21: 763–767

Coupar, I. M., De Luca, A. (1994) Opiate and opiate antidiarrhoeal drug action on rat isolated intestine. J. Auton. Pharmacol. 14: 69–78

Dobbins, J. W., Laurenson, J. P., Forrest, J. N. (1984) Adenosine and adenosine analogues stimulate adenosine cyclic 3′,5′-monophosphate-dependent chloride secretion in the mammalian ileum. J. Clin. Invest. 74: 929–935

Dionyssopoulos, A., Hope, W., Coupar, I. M. (1992) Effect of adenosine analogue on the expression of opiate withdrawal in rats. Pharmacol. Biochem. Behav. 42: 201–206

Francis, D. L., Roy, A. C., Collier, H. O. J. (1975) Morphine abstinence and quasi abstinence effects after phosphodiesterase inhibitors and naloxone. Life Sci. 16: 1901–1906

Gintzler, A. R., Musacchio, J. S. (1975) Interactions of morphine, adenosine triphosphate and phosphodiesterase inhibitors on the field-stimulated guinea-pig ileum. J. Pharmacol. Exp. Ther. 194: 575–582

Grasl, M., Turnheim, K. (1984) Stimulation of electrolyte secretion in rabbit colon by adenosine. J. Physiol. 346: 93–110

Gustafsson, L. E., Wiklund, N. P., Lundin, J., Hedqvist, P. (1985) Characterization of pre- and post-junctional adenosine receptors in guinea-pig ileum. Acta Physiol. Scand. 123: 195–203

Herrick-Davis, K., Chippari, S., Luttinger, D., Ward, S. J. (1989) Evaluation of adenosine agonists as potential analgesics. Eur. J. Pharmacol. 162: 365–369

Miller, D. L., Schedl, H. P. (1972) Non-absorbable indicators: a comparison of phenol red and inulin ^{14}C and effects of perfusion techniques. Gastroenterology 62: 48–55

Nicholls, J., Houranni, S. M. O., Kitchen, I. (1992) Characterization of P_1-purinoceptors on rat duodenum and urinary bladder. Br. J. Pharmacol. 105: 639–642

Perkins, M. N., Stone, T. W. (1980) Blockade of striatal neurone responses to morphine by aminophylline: evidence for adenosine mediation of opiate action. Br. J. Pharmacol. 69: 131–137

Reymann, A., Gniess, A. (1988) Evidence for adenosine A_1 receptor action in rat jejunal mucosa. Eur. J. Pharmacol. 149: 155–158

Sawynock, J., Jhamandas, K. H. (1976) Inhibition of acetyl choline release from cholinergic nerves by adenosine, adenosine nucleotides and morphine: antagonism by theophylline. J. Pharmacol. Exp. Ther. 197: 379–390

Sweeney, M. I., White, T. D., Jhamandas, K. H., Sawynok, J. (1987) Morphine releases endogenous adenosine from the spinal cord in vivo. Eur. J. Pharmacol. 141: 169–170

Valpaatalo, H., Onken, D., Neuvonen, P. J., Westermann, E. (1975) Stereospecificity in some central and circulatory effects of phenylisopropyl-adenosine (PIA). Arzneim. Forsch. 25: 407–409