Br. J. Pharmac. (1984), **83**, 883–888

Purine involvement in morphine antinociception

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1 The effects of a series of adenosine derivatives on morphine antinoceptive effect were investigated in rats by the 'tail-flick' method.

2 2-Chloroadenosine (CADO) and L-N^6-phenylisopropyladenosine (L-PIA), given intraperitoneally, caused decreased morphine antinociception.

3 Intracerebroventricular injections of CADO, L-PIA and 5'-N-ethylcarboxamide adenosine (NECA), but not of 2'-deoxyadenosine, antagonized morphine antinociception.

4 The effects of both central and peripheral injections of CADO and L-PIA on morphine antinociception were partially reversed by caffeine.

5 Intracerebroventricular injection of dibutyryl-cyclic 3',5' adenosine monophosphate (db cyclic AMP) had no effect on morphine antinociception.

6 These data indicate that adenosine plays a role in morphine-induced antinociception. The results are discussed in terms of postulated effects of adenosine derivatives on adenylate cyclase.

Introduction

There is evidence that adenosine may be involved in such effects of morphine as sedation, motor changes, respiratory depression (Stone, 1982) and analgesia (Gourley & Beckner, 1973).

Adenosine is a metabolite of adenosine 5'-triphosphate (ATP), adenosine 5'-phosphate (AMP) and cyclic adenosine 3'-5'-monophosphate (cyclic AMP) (Stone, 1981) and in many systems adenosine and cyclic AMP have similar activities. One of the most important actions of adenosine is to control intracellular levels of cyclic AMP through external membrane located adenosine receptors (Ri, Ra) (Londos *et al.*, 1980) and an intracellular site associated with adenylate cyclase (P-site) (Londos & Wolff, 1977). Both types of R receptors are blocked by methylxanthines, while the P-site is not (Phillis & Wu, 1981).

It has been suggested that opiate agonists exert their action by decreasing the intracellular levels of cyclic AMP, since opiates have been shown to reduce basal or pharmacologically stimulated levels of cyclic AMP in homogenates prepared from whole brain or from specific brain regions (Collier & Roy, 1974; Wilkening *et al.*, 1976; Tsang *et al.*, 1978). However, stimulation of adenylate cyclase by opiates (Puri *et al.*, 1975; Tang & Cotzias, 1978) and no effect on this enzymatic activity (Katz & Catravas, 1977) have also been reported. Ho *et al.* (1973) found that prior administration of cyclic AMP to mice or rats antagonized morphine analgesia. The specificity of this effect of cyclic AMP was questioned by Gourley & Beckner (1973) who found that the reversal of morphine analgesia is not specific for cyclic AMP, because it also occurred after treatment with cyclic AMP catabolic products, such as adenosine and adenine. In contrast to the findings of Ho *et al.* (1973), another group has found that microinjections of dibutyryl cyclic nucleotides into brain stem sites resulted in analgesia (Levy *et al.*, 1981). In addition, it has been reported that adenosine-related compounds have antinociceptive effects in mice and rats (Vapaatalo *et al.*, 1975; Snyder *et al.*, 1981; Yarbrough & McGuffin-Clineschmidt, 1981; Holmgren *et al.*, 1983).

Hence, because of these discordant findings, the present study was undertaken to determine the ef-

[1] Correspondence

fects of purinergic drugs, alone or in combination with morphine, on nociception in the rat, using the tail-flick method. The effects of purine analogues on morphine analgesia were assessed after both central and peripheral administration of the analogues, with and without caffeine, which is known to be an adenosine receptor antagonist in many systems (Phillis & Wu, 1981).

Furthermore, to see if the purine effects on morphine analgesia might be mediated through changes in intracellular cyclic AMP, we tested the effects of intracerebroventricular (i.c.v.) injections of dibutyryl cyclic AMP (db cyclic AMP) alone or in combination with morphine on morphine antinociception.

As modification of body temperature in animals treated with purine analogues has been described (Vapaatalo *et al.*, 1975; Yarbrough & McGuffin-Clineschmidt, 1981; Wager-Srdar *et al.*, 1983), and since adenosine is known to be a potent vasodilator in various vascular beds (Fox & Kelly, 1978) the tail temperatures were measured, because the temperature of the tail is an important factor in the tail-flick response (Minfeng & Jisheng, 1979).

Methods

Male Sprague-Dawley Charles River rats (130–150 g) were used. Animals were housed at a constant temperature (23–24°C). The tail-flick assay of D'Amour & Smith (1941) was used to assess the antinociceptive effect. The reaction time to heat was measured in tenths of a second. The cut-off time was 8 s. To prevent tissue damage, only one tail-flick response was induced per time point. The tail temperature was measured at room temperature (24°C) with a digital precision thermometer. The groups were compared by the Dunnett test (Dunnett, 1964) which is applicable to comparisons of more than 2 groups at a time.

Under barbiturate anaesthesia (Nembutal 35 mg kg^{-1} i.p.), permanent polyethylene cannulae (PE10) were implanted into both lateral ventricles by the method of Altaffer *et al.* (1970). Cannula position was verified by the outflow of clear cerebrospinal fluid. The tail-flick tests were carried out 7 days after implantation.

Drugs and vehicle (0.9% w/v NaCl solution) were administered simultaneously by intracerebroventricular (i.c.v.; in 5–10 µl) or intraperitoneal (i.p.) or subcutaneous (s.c.) injections (route of administration designated in the text). Each dose of each drug was tested in at least 6 rats. Adenosine, 2-chloroadenosine (CADO), 2'-deoxyadenosine, N^6, O^2-dibutyryl adenosine 3':5'-cyclic monophosfate (db cyclic AMP) and caffeine were obtained from the Sigma Chemical Co., St. Louis; L-N^6-phenylisopropyladenosine (L-PIA) was obtained from Boehringer Mannheim and 5'-N-ethylcarboxamide adenosine (NECA) was a gift from Dr Cattabeni.

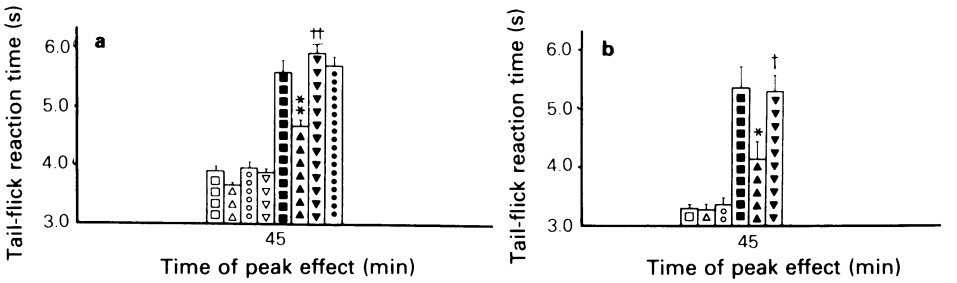

Figure 1 (a) Antagonism by caffeine (○) of the inhibitory effect of 2-chloroadenosine (CADO; △) on the antinociceptive effect of morphine (■) in rats (6–8 per group). Morphine (1.25 mg kg^{-1} s.c.), CADO (5 µmol kg^{-1} i.p.) and caffeine (15 mg kg^{-1} i.p.) were injected simultaneously. Mean values are shown, vertical lines indicate s.e.mean. NaCl/NaCl/morphine group (■) compared to CADO/NaCl/morphine group (▲): $^{**}P<0.01$. CADO/NaCl/morphine group (▲) compared to CADO/caffeine/morphine (▼): $‡P<0.01$. (□) Effect of NaCl; (▽) CADO + caffeine + NaCl.

(b) Antagonism by caffeine (○) of the inhibitory effect of L-phenylisopropyladenosine (L-PIA; △) on the antinociceptive effect of morphine (■) in rats (6 per group). Morphine (1.25 mg kg^{-1} s.c.), L-PIA (0.26 µmol kg^{-1} i.p.) and caffeine (15 mg kg^{-1} i.p.) were injected simultaneously. Mean values are shown, vertical lines indicate s.e.mean. NaCl/NaCl/morphine group (■) compared to L-PIA/NaCl/morphine group (▲): $^{*}P<0.05$. L-PIA/NaCl/morphine group (▲) compared to L-PIA/caffeine/morphine group (▼): $†P<0.05$. (□) NaCl group.

Results

Effects of intraperitoneal injections of CADO and L-PIA on the antinociceptive effects of morphine and their antagonism by caffeine

Intraperitoneal injections of CADO (5 μmol kg^{-1}) and L-PIA (0.26 μmol kg^{-1}) were able to reduce significantly the antinociceptive effects elicited by the subcutaneous injection of morphine (1.25 mg kg^{-1}) (Figure 1a and b). The animals injected with CADO and L-PIA exhibited sedation. The intraperitoneal injection of caffeine (15 mg kg^{-1}) significantly antagonized the inhibitory effects of CADO and L-PIA on the antinociceptive effect of morphine. CADO, L-PIA and caffeine alone had no significant effects on the threshold of pain, as compared with that of controls injected with 0.9% NaCl solution.

Effects of i.c.v.-injected purine analogues on the antinociceptive effect of morphine

CADO at a dose of 1 nmol per ventricle and L-PIA and NECA at a dose of 0.1 nmol per ventricle, injected simultaneously with morphine, (1.25 mg kg^{-1} s.c.) induced a significant decrease in the antinociceptive effect of morphine, with no effect on basal responses (Figure 2).

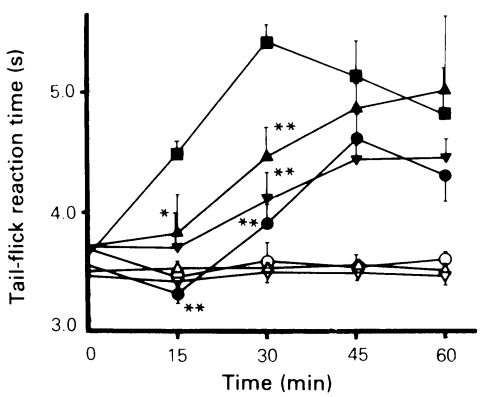

Figure 2 Antinociceptive effect of morphine (■) in rats (6 per group) treated with 2-chloroadenosine (CADO; △), L-phenylisopropyladenosine (L-PIA; ▽) and 5'-N-ethylcarboxamide (NECA; ○). Morphine (1.25 mg kg^{-1}) was administered s.c. simultaneously with CADO (1 nmol per ventricle), L-PIA (0.1 nmol per ventricle) and NECA (0.1 nmol per ventricle). Mean values are shown, vertical lines indicate s.e.mean. NaCl/morphine group (■) compared to CADO/morphine (▲), L-PIA/morphine (▼) and NECA/morphine (●) groups: $*P<0.05$; $**P<0.01$.

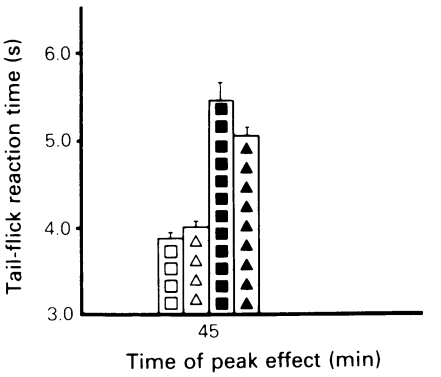

Figure 3 Antinociceptive effect of morphine (■) in rats (6–7 per group) treated with 2'-deoxyadenosine (▲). Morphine (1.25 mg kg^{-1}) was administered s.c. simultaneously with 2'-deoxyadenosine (100 nmol per ventricle). (□) Effect of NaCl alone; (△) effect of 2'-deoxyadenosine + NaCl.

As shown in Figure 3, 2'-deoxyadenosine (100 nmol per ventricle) had no effect on tail-flick reaction time when administered simultaneously with morphine (1.25 mg kg^{-1} s.c.).

Effects of caffeine on i.c.v.-administered CADO and L-PIA-induced inhibition of the antinociceptive effect of morphine

Treatment of rats with caffeine (15 mg kg^{-1} i.p.) significantly antagonized the inhibitory effects of CADO (4 nmol per ventricle) and L-PIA (0.13 nmol per ventricle) on the antinociceptive effect of morphine (1.25 mg kg^{-1} s.c.). CADO, L-PIA and caffeine alone had no significant effects on basal responses (Figure 4a and b).

Effects of db cyclic AMP on the antinociceptive effect of morphine

db cyclic AMP injected simultaneously with morphine (1.25 mg kg^{-1} s.c.) at a dose of 7.5 μg per ventricle did not modify the tail-flick responses elicited after morphine. db cyclic AMP alone had no effect on the threshold of pain; each animal served as his own control (Figure 5). The animals injected with this dose of db cyclic AMP exhibited a modest hyperreactivity.

Effects of purine analogues and db cyclic AMP on tail temperature

As shown in Table 1, the doses of CADO (4 nmol per ventricle) and L-PIA (0.13 nmol per ventricle) used

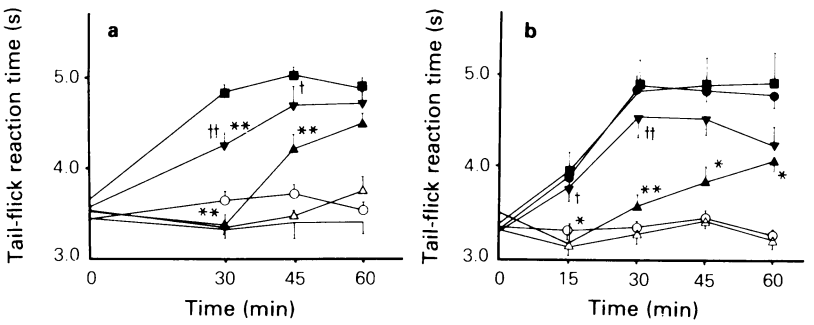

Figure 4 (a) Antagonism by caffeine (○) of the inhibitory effect of 2-chloroadenosine (CADO; △) on the antinociceptive effect of morphine (■) in rats (6–8 per group). Morphine (1.25 mg kg^{-1}) was injected s.c. simultaneously with CADO (4 nmol per ventricle) and caffeine (15 mg kg^{-1} i.p.). Mean values are shown, vertical lines indicate s.e.mean. NaCl/morphine/NaCl group (■) compared to CADO/morphine/NaCl group (▲): $^{**}P<0.01$. NaCl/morphine/NaCl groups (■) compared to CADO/morphine/caffeine groups (▼): $^{**}P<0.01$. CADO/morphine/NaCl group (▲) compared to CADO/morphine/caffeine group (▼): †$P<0.05$; ‡$P<0.01$. (—) NaCl alone.

(b) Antagonism by caffeine (○) of the inhibitory effect of L-phenylisopropyladenosine (L-PIA; △) on the antinociceptive effect of morphine (■) in rats (6–8 per group). Morphine (1.25 mg kg^{-1}) was injected s.c. simultaneously with L-PIA (0.13 nmol per ventricle) and caffeine (15 mg kg^{-1} i.p.). Mean values are shown: vertical lines indicate s.e.mean. NaCl/morphine/NaCl group (■) compared to L-PIA/morphine/NaCl group (▲): $^{*}P<0.05$; $^{**}P<0.01$. L-PIA/morphine/NaCl group (▲) compared to L-PIA/morphine/caffeine group (▼): †$P<0.05$; ‡$P<0.01$. (●) Effect of morphine + caffeine + NaCl.

in the experiments for assessing the specific effects of these compounds on morphine antinociception did not change the tail temperature during a 60 min period, nor did the dose of db cyclic AMP (7.5 μg per ventricle). The tail temperature was measured in the tail segment we selected for the tail-flick latency study.

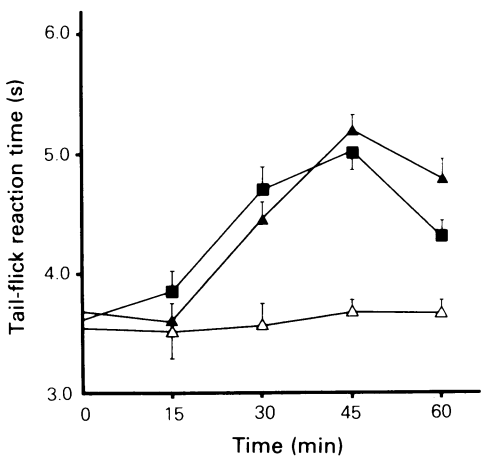

Figure 5 Antinociceptive effect of morphine (■) in rats (7 per group) treated with dibutyryl cyclic AMP (▲). Morphine (1.25 mg kg^{-1} s.c.) was administered simultaneously with dibutyryl cyclic AMP (7.5 μg per ventricle). (△) Dibutyryl cyclic AMP + NaCl.

Discussion

Recently, binding sites for radiolabelled metabolically stable adenosine analogues, such as NECA, L-PIA and CADO, have been characterized in mammalian brain (Schwabe, 1981). These compounds have low affinity for the uptake systems and for adenosine deaminase (ADA) (Stone, 1981), and thus remain in the extracellular environment to act as 'long-lasting' adenosine. Furthermore, these adenosine analogues have the advantage that they react with the R-site receptors and only weakly, if at all, with the 'P-site' (Daly *et al.*, 1981). In our experimental conditions, both CADO and L-PIA, when injected intraperitoneally at relatively high doses, decrease morphine antinociception. The CADO and L-PIA reductions of morphine analgesia seem to be specific, since caffeine treatment reversed the effects of these purines on morphine antinociception. CADO, L-PIA and caffeine, at the doses used, did not *per se* alter the tail-flick responses. Caffeine was chosen as the methylxanthine to use because it enters the brain more rapidly than theophylline after parenteral administration (Sattin, 1971) and is even less potent than theophylline as a phosphodiesterase inhibitor (Fredholm, 1980).

Table 1 Effect of i.c.v.-injected 2-chloroadenosine, L-N^6-phenylisopropyladenosine and dibutyryl cyclic AMP on tail temperature

Treatment (dose per ventricle)	*Tail temperature* ($\bar{x} \pm$s.e.)				
	0	*15 min*	*30 min*	*45 min*	*60 min*
NaCl	26.7±0.5	26.4±0.5	26.9±0.8	26.2±0.5	26.3±0.6
CADO (4 nmol)	27.0±0.4	28.5±0.8	26.1±0.4	25.9±0.4	26.1±0.3
L-PIA (0.13 nmol)	27.6±0.9	25.9±0.6	26.1±1.0	25.8±0.4	25.1±0.8
db cyclic AMP (7.5 μg)	26.3±0.3	26.3±0.7	25.8±0.8	26.2±0.3	25.9±0.4

As we do not know whether the effects of peripherally administered purines are exerted directly on the central nervous system or occurs as a consequence of actions elsewhere in the body, the effects of i.c.v. injections of the purine analogues on morphine antinociception were assessed. L-PIA and NECA were the most potent of these compounds, eliciting equivalent decreases in morphine action when tested at equimolar doses. Adenosine, at a dose of 100 μg per ventricle, caused a brief decrease in morphine antinociception (data not shown). This is consistent with the poor activity of adenosine when administered *in vivo*, as it is rapidly metabolized in the organism by adenosine deaminase (ADA) (Davies *et al.*, 1980). CADO was less potent than L-PIA and NECA, but more potent than adenosine. These central effects of CADO and L-PIA also appear to be specific.

In contrasr, 2′-deoxyadenosine, a known agonist at the so-called P-site (Londos & Wolff, 1977), was ineffective in decreasing morphine analgesia. Because 2′-deoxyadenosine is deaminated by ADA (Fox & Kelly, 1978), it was injected at high doses.

Furthermore, the data presented here support the idea that blockade of the purine effects on morphine antinociception involves the R-site adenosine receptors. The site of purines action seems to be central, since they act in much smaller quantities when given centrally than after peripheral administration.

Our data on the inhibition of morphine analgesia by purines are in accordance with the findings of Gourley & Beckner (1973). However, several other investigators have obtained results seemingly in conflict with ours. Antinociceptive effects after both intracisternal injection of L-PIA, CADO and NECA (Yarbrough *et al.*, 1981) and peripheral injection of L-PIA (Vapaatalo *et al.*, 1975; Snyder *et al.*, 1981; Holmgren *et al.*, 1983) have been described. The reason(s) for the disparity is not at present clear, but one possibility is that the increase in the hot plate reaction time that follows purine administration might be secondary to a decrease in body temperature. In this context, we measured the tail temperatures after central injection of L-PIA and CADO and found no change after the doses we used.

One of the most important actions of adenosine is to increase intracellular levels of cyclic AMP by reacting with external membrane-located adenosine receptors (Sattin & Rall, 1970; Shimizu & Daly, 1970; Schultz, 1975). As it is not altogether clear if the effects of adenosine derivatives on cyclic AMP generating systems parallel their physiological activity (Dunwiddie & Hoffer, 1982), we tested the possibility that cyclic AMP might be involved in the effects of purines on morphine antinociception. Therefore, db cyclic AMP, known to gain access to the intracellular space (Posternak *et al.*, 1962), was injected into the lateral ventricles of the rat simultaneously with morphine to reduce the possibility that db cyclic AMP would be deacylated to monobutyryl cyclic AMP and then to cyclic AMP, 5′-AMP and adenosine. To make sure that the analgesiometric tests were not affected by severe motor disturbance, we used doses of db cyclic AMP (15 μg) that have no substantial motor effects and did not modify the tail temperature. This dose of db cyclic AMP also had no effect on the tail flick latency time or morphine antinociception.

In view of the depressant effects of adenosine analogues on morphine antinociception and the lack of modification of the responses after db cyclic AMP administration it is difficult to argue that these effects of adenosine are mediated by elevation of intracellular cyclic AMP levels. Nevertheless, adenosine might increase intracellular cyclic AMP in a different compartment, and as a consequence, the physiological effect might be far different from that induced by an injection of db cyclic AMP.

In conclusion, even though the antagonistic effect of adenosine analogues on morphine antinociception seems to be specific, the way in which adenosine analogues act to block morphine analgesia remains to be determined.

References

ALTAFFER, F.B., DE BALBIAN WERSTER, F., HALL, S., LONG, C.J. & D'ENCARNACAO, P. (1970). A simple and inexpensive cannula technique for chemical stimulation of the brain. *Physiol. Behav.*, **5,** 119–121.

COLLIER, M.O.J. & ROY, A.C. (1974). Morphine-like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate. *Nature*, **248,** 24–27.

D'AMOUR, E.F. & SMITH, D.L. (1941). A method for determining loss of pain sensation. *J. Pharmac. exp. Ther.*, **72,** 74–79.

DALY, J.W., BRUNS, R.F. & SNYDER, S.H. (1981). Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. *Life Sci.*, **28,** 2083–2097.

DAVIES, L.P., TAYLOR, K.M., GREGSON, R.P. & QUINN, R.J. (1980). Stimulation of guinea-pig brain adenylate cyclase by adenosine analogues with potent pharmacological activity *in vivo*. *Life Sci.*, **26,** 1079–1088.

DUNNET, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics*, **20,** 482–491.

DUNWIDDIE, T.V. & HOFFER, B.J. (1982). The role of cyclic nucleotides in the nervous system. In *Cyclic Nucleotides II. Physiology and Pharmacology* ed. Kebabian, J.W. & Nathanson, J.A., pp. 389–463. Berlin, Heidelberg, N.Y.: Springer-Verlag.

FREDHOLM, B.B. (1980). Are methylxanthine effects due to antagonism of the endogenous adenosine? *Trends pharmac. Sci.*, **1,** 129–132.

FOX, I.H. & KELLY, W.N. (1978). The role of adenosine and 2'-deoxyadenosine in mammalian cells. *Ann. Rev. Biochem.*, **47,** 655–686.

GOURLEY, D.R.H. & BECKNER, S.K. (1973). Antagonism of morphine analgesia by adenine, adenosine, and adenine nucleotides. *Proc. Soc. exp. biol. Med.*, **144,** 744–778.

HO, I.K., LOH, H.H. & WAY, E.L. (1973). Cyclic adenosine monophosphate antagonism of morphine analgesia. *J. Pharmac. exp. Ther.*, **185,** 336–346.

HOLMGREN, M., HEDNAR, T., NORDBERG, G. & MELLSTRAND, T. (1983). Antinociceptive effects in the rat of an adenosine analogue, N^6-phenyl-isopropyl-adenosine. *J. Pharm. Pharmac.*, **35,** 679–680.

KATZ, J.B. & CATRAVAS, G.N. (1977). Absence of morphine antagonism by prostaglandin E_1 stimulated ^{3}H-3',5'-cyclic adenosine monophosphate accumulation in rat brain mince system. *Brain Res.*, **120,** 263–268.

LEVY, R.A., GOLDSTEIN, B.D. & ELYJIW, M.M. (1981). Analgesia following local injection of dibutyryl cyclic nucleotides at sites in the rat CNS. *Eur. J. Pharmac.*, **71,** 139–142.

LONDOS, C., COOPER, D.M.F. & WOLFF, J. (1980). Subclasses of external adenosine receptors. *Proc. natn. Acad. Sci. U.S.A.*, **77,** 2551–2554.

LONDOS, C. & WOLFF, J. (1977). Two distinct adenosine-sensitive sites on adenylate cyclase. *Proc. natn. Acad. Sci. U.S.A.*, **74,** 5482–5486.

MINFENG, R., JISHENG, H. (1979). Rat tail flick acupuncture analgesia model. *Chinese Medical J.*, **92,** 576–582.

PHILLIS, J.W. & WU, P.H. (1981). The role of adenosine and its nucleotides in central synaptic transmission. *Prog. Neurobiol.*, **16,** 187–239.

PURI, S.K., COCHIN, J. & VOLICER, L. (1975). Effect of morphine sulfate on adenylate cyclase and phosphodiesterase activities in rat corpus striatum. *Life Sci.*, **16,** 759–768.

POSTERNAK, T., SUTHERLAND, E.W. & HENION, W.F. (1962). Derivatives of cyclic 3',5'-adenosine monophosphate. *Biochim. biophys. Acta*, **65,** 558–560.

SATTIN, A. (1971). Increase in the content of adenosine 3',5'-monophosphate in mouse forebrain during seizures and prevention of the increase by methylxanthines. *J. Neurochem.*, **18,** 1087–1096.

SATTIN, A. & RALL, T.W. (1970). The effect of adenosine and adenine nucleotides on the cyclic adenosine 3',5'-monophosphate content of guinea pig cerebral cortex slices. *Molec. Pharmac.*, **6,** 13–23.

SCHULTZ, J. (1975). Cyclic adenosine 3',5'-monophosphate in guinea pig cerebral cortical slices: studies on the role of adenosine. *J. Neurochem.*, **24,** 1237–1242.

SCHWABE, U. (1981). Direct binding studies of adenosine receptor. *Trends pharmac. Sci.*, **2,** 299–303.

SHIMIZU, H. & DALY, J.W. (1970). Formation of adenosine 3',5'-monophosphate from adenosine in brain slice. *Biochim. biophys. Acta*, **222,** 465–473.

SNYDER, S.H., KATIMS, J.J., ANNAU, Z., BRUNS, R.F. & DALY, J.W. (1981). Adenosine receptors and behavioral action of methylxanthines. *Proc. natn. Acad. Sci. U.S.A.*, **78,** 3260–3264.

STONE, T.W. (1981). Physiological roles for adenosine and adenosine 5'-triphosphate in the nervous system. *Neuroscience*, **6,** 523–555.

STONE, T.W. (1982). Adenosine symposium. *Trends pharmac. Sci.*, **3,** 423–425.

TANG, L.C. & COTZIAS, G.C. (1978). Morphine sulfate stimulates the adenylate cyclase in mouse caudate nuclei. *Proc. natn. Acad. Sci. U.S.A.*, **75,** 1546–1548.

TSANG, D., TAN, A.T., HENRY, J.L. & LAL, S. (1978). Effect of opioid peptide on noradrenaline-stimulated cyclic AMP formation in homogenates of rat cortex and hypothalamus. *Brain Res.*, **152,** 521–527.

VAPAATALO, H., ONKEN, D., NEUVONEN, P.J. & WESTERMANN, E. (1975). Stereospecificity in some central and circulatory effects of phenylisopropyl-adenosine (PIA). *Arzneim-Forsch. (Drug Res.)*, **25,** 407–410.

WAGER-SRDAR, S.A., OKEN, M.M., MORLEY, J.E. & LEVINE, A.S. (1983). Thermoregulatory effects of purines and caffeine. *Life Sci.*, **33,** 2431–2438.

WILKENING, D., MISHRA, R.K. & MAKMAN, M.H. (1976). Effects of morphine on dopamine-stimulated adenylate cyclase and on cyclic GMP formation in primate brain amygdaloid nucleus. *Life Sci.*, **19,** 1129–1138.

YARBROUGH, G.G. & McGUFFIN-CLINESCHMIDT, J.C. (1981). *In vivo* behavioural assessment of central nervous system purinergic receptors. *Eur. J. Pharmac.*, **76,** 137–144.

(Received April 4, 1984. Revised July 2, 1984.)