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Morphine Enhances the Release of ^{3}H-Purines from Rat Brain Cerebral Cortical Prisms

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WU, P. H., J. W. PHILLIS AND H. YUEN. *Morphine enhances the release of ^{3}H-purines from rat brain cerebral cortical prisms*. PHARMAC. BIOCHEM. BEHAV. 17(4) 749–755, 1982.—*In vitro* experiments have shown that ^{3}H-purines can be released from ^{3}H-adenosine preloaded rat brain cortical prisms by a KCl-evoked depolarization. The KCl-evoked release of ^{3}H-purines is dependent on the concentration of KCl present in the superfusate. At concentrations of 10^{-7}–10^{-5}M morphine did not influence the basal release of ^{3}H-purines from the prisms, although it enhanced the KCl-evoked release of ^{3}H-purines. The enhancement of KCl-evoked ^{3}H-purine release by morphine was concentration-dependent and was antagonized by naloxone, suggesting the involvement of opiate receptors. Uptake studies with rat brain cerebral cortical synaptosomes show that morphine is a very weak inhibitor of adenosine uptake. Comparisons with dipyridamole, a potent inhibitor of adenosine uptake, suggest that this low level of inhibition of the uptake did not contribute significantly to the release of ^{3}H-purine by morphine seen in our experiments. It is therefore suggested that morphine enhances KCl-evoked ^{3}H-purine release by an interaction with opiate receptors and that the resultant increase in extracellular purine (adenosine) levels may account for some of the actions of morphine.

Morphine Adenosine Cerebral cortex Release Dipyridamole

A NUMBER of observations suggest that a purinergic (adenosine) receptor is involved in some of the central actions of morphine. Methylxanthines (caffeine, theophylline), which antagonize the central effects of adenosine [24], inhibit the analgesic actions of morphine and the endogenous opioids [5,14]; and enhance the effects of nociceptive stimulation [23]. Methylxanthines also antagonize the depressant actions of morphine on acetylcholine release from the intact cerebral cortex [15,25]. Phillis *et al.* [25] have shown that morphine enhances the release of adenosine and its metabolites from the rat cerebral cortex. Adenosine depresses acetylcholine release from the cerebral cortex [15]. This morphine-elicited increase in extracellular adenosine levels could therefore be responsible for the reduction in acetylcholine release. Naloxone, an opiate receptor antagonist, inhibits the facilitatory and depressant effects of morphine on purine and acetylcholine release respectively, indicating that both effects are a result of the activation of a specific opiate receptor. The antagonism of morphine-induced depression of acetylcholine release by methylxanthines apparently involves a "purine" receptor rather than a morphine receptor as these agents, unlike naloxone, do not affect the morphine-elicited release of labelled purines [18]. It was therefore proposed that morphine inhibition of cortical acetylcholine release is mediated by a purinergic step, the "purine link" hypothesis [17,25].

In this report, we demonstrate that morphine enhances purine release *in vitro* and that this enhancement can be blocked by naloxone. Although inhibition of the reuptake of adenosine may make a small contribution to the observed increase in purine efflux, our findings indicate that the major factor is an increased release of adenosine from neural tissues.

METHOD

Male Wistar rats (weighing 200–350 g) were used in these experiments. The rats were sacrificed and their brains removed. Each brain was placed on an ice-cold stage. The cerebral cortices were dissected. One slice of approximately 2 mm thickness was removed from each cortex. The 2 mm thick slices were then sliced with a McIlwain tissue chopper to prepare prisms with dimensions of 0.1 × 0.1 × 2 mm. The prisms were dispersed by gentle vortexing in 5 ml of warmed Krebs improved Ringer I solution (37°C). The Krebs improved Ringer I solution consisted of NaCl (0.0947 M); KCl (0.0047 M); $CaCl_2$ (0.0025 M); KH_2PO_4 (0.00118 M), $MgSO_4$ (0.00118 M); $NaHCO_3$ (0.025 M); pyruvate (0.0049 M);

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glutamate (0.0049 M); fumarate (0.0053 M) and glucose (0.0115 M) [7]. The solution was kept at 37°C and gassed with 95% O_2 and 5% CO_2 mixture for a period of one hour before use. The solution had a pH of 7.4.

Preloading of Rat Cerebral Prisms

The rat cerebral cortical prisms (0.1 × 0.1 × 2 mm) were incubated with 1 μM ^{3}H-adenosine (specific activity 1~10 μCi/nmole) for a period of 1 min. Following rapid washing in 2 × 5 ml fresh incubation solution, the brain prisms were collected and incubated in 20 ml of Krebs improved Ringer I solution for 1 to 60 min. At regular intervals, 500 μl of incubation medium containing brain prisms were withdrawn. After removal of the incubation medium, the prisms were homogenized and protein denatured by heating at 100°C for 2 min. The denatured brain homogenate was centrifuged at 10,000 × g to separate the protein. The supernatant was lyophilized and the residue dissolved in 50 μl of distilled H_2O. This tissue extract was then subjected to silica gel thin layer chromatography (TLC) using the solvent system n-butanol/ethylacetate/methanol/NH_4OH (7:4:3:4 v/v) as described by Shimuzu *et al.* [34]. The radioactivity associated with adenosine, cyclic AMP, hypoxanthine, inosine and adenine nucleotides on the chromatogram were determined by scintillation spectroscopy. It was found that purine metabolites reached stable levels in the prisms only after 30 min of incubation. A 60 minute incubation time was therefore chosen for loading of the tissue with ^{3}H-adenosine in the remaining experiments.

Release of Purines

The equipment (perfusion chambers) used in this release study were similar to those described by Raiteri *et al.* [29]. Brain prisms which had been preloaded with ^{3}H-adenosine for 60 min were transferred to the bottom of the chamber onto a 24 mm Whatman filter paper. The prisms were washed rapidly with 20 ml of incubation solution saturated with 95% O_2 and 5% CO_2 at 37°C. After washing, the brain prisms were superfused with 20 ml of fresh incubation solution gassed with a 95% O_2 and 5% CO_2 mixutre at 37°C. The perfusion chamber contents were maintained at 37°C throughout the study. Routinely, a volume of 20 ml of incubation solution containing 80 mM KCl was added to the perfusion chamber containing the washed rat brain prisms. Incubation fluid was perfused past the prisms and the filter at a flow rate of 1.0 ml/min using a Pharmacia P-3 peristaltic pump directly into scintillation counting vials. Morphine was added to the incubation medium after collection of the 8 or 10th fraction. Eight ml of Aquassure high performance (LSC) cocktail (NEN, New England Nuclear) was added to the superfusate fractions and the sample was counted in a Nuclear Chicago ISOCAP-300-spectrophotometer. The counting efficiency as determined by the sample-channel ratio method was approximately 36%. At the end of perfusion, the filter bearing the brain prisms was placed in a scintillation vial. One ml of 12% perchloric acid was added to extract the radioactivity from the tissues. After addition of 8 ml Aquassure (NEN) solution, samples were counted as indicated above.

Identification of Purine Metabolites in the Superfusates

Rat brain cortical prisms were incubated with 1 μM ^{3}H-adenosine (100 μCi/nmole) for a period of 60 min. Following incubation and washing, the brain prisms were superfused with incubation solution containing 80 mM KCl. After ten minutes of superfusion, morphine solution was added to the remaining perfusion solution in the perfusion chamber to a final concentration of 5 × 10^{-5}M. Each one minute fraction was collected in a small test tube. The superfusate was lyophilized and the residue was dissolved in 50 μl of distilled H_2O. Fifty μl of the dissolved residue was transferred to the origin of a silica gel thin layer chromatogram and was developed in the solvent system: n-butanol/ethyl acetate/methanol/NH_4OH (7:4:3:4, v/v) as described by Shimizu *et al.* [34]. The spots corresponding to adenosine, cyclic AMP, hypoxanthine, inosine and adenine nucleotides were isolated and the radioactivity associated with these metabolites was determined using the same procedures described in the previous section.

Inhibition of Adenosine Uptake into Rat Brain Cortical Synaptosomes

Rat brain cortical synaptosomes were prepared according to the method described in our previous publications [1,37]. Morphine or dipyridamole was added to the incubation medium to final concentrations of 10^{-9}–10^{-4} M. Synaptosomes were preincubated in the presence or absence of these agents for 2 min. The uptake of ^{3}H-adenosine was initiated by addition of ^{3}H-adenosine (specific activity 1 μCi/nmole) to the incubation solution at a final concentration of 1 μM. Uptake by the synaptosomes was allowed for 30 sec. The reaction was then terminated by the addition of washing solution (composition, see Bender *et al.* [1]) and filtration in a GF/C glass fibre filter. The radioactivity associated with the synaptosomes was then determined by scintillation spectroscopy. The dose-response curves of adenosine uptake were obtained by semilogarithmic plots. The IC_{50} values were obtained from the curves.

RESULTS

Metabolism of Adenosine in Rat Brain Cortical Prisms

Rat brain cortical prisms were preloaded with 1 μM adenosine (10 μCi/nmole) for 1 min. After rapid washing, the preloaded prisms were incubated in fresh incubation medium for various periods of time. The metabolites of adenosine were then analyzed. Figure 1 shows that adenosine contributes approximately 70% of the total radioactivity associated with the radioactively labelled brain prisms during the first 10 minutes of incubation. Nucleotides represent approximately 15–20% of total radioactivity. Hypoxanthine and inosine accounted for less than 10% of total radioactivity. When the incubation time was prolonged to 20 min, the adenosine level dropped to approximately 54%, while nucleotide levels increased to 33%. Hypoxanthine and inosine levels remained in the 10% range. After 30 minutes of incubation, adenosine, its nucleotides and hypoxanthine each accounted for about 30% of total radioactivity, while inosine levels remained unchanged. These levels remained stable with longer incubation periods. Similar results were obtained by incubating brain cortical prisms with the continuing presence of ^{3}H-adenosine for 1–60 min. In order to obtain a constant level of adenosine in the brain prisms, the time period for preloading of ^{3}H-adenosine was chosen at 60 min.

Release of ^{3}H-Purines from Rat Brain Cortical Prisms

Rat brain cortical prisms were pre-loaded with ^{3}H-

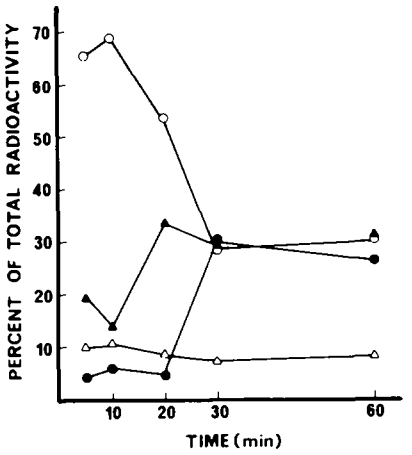

FIG. 1. Distribution of ^{3}H-purines in rat brain cortical prisms. Rat brain cortical prisms were pre-loaded with 10 μCi/nmole of ^{3}H-adenosine at concentrations of 1 μM for 1 min at 37°C in Krebs improved Ringer I solution. Following pre-loading and washing, the brain cortical prisms were transferred to a fresh incubation solution. A portion of the prisms was withdrawn at 5, 10, 20, 30 and 60 min intervals of the incubation period. Radioactively labelled purine metabolites were analyzed. The distribution of adenosine (—○—), hypoxanthine (—▲—), inosine (—△—) and nucleotides (—●—) is shown as the percent of total radioactivity analyzed in the rat cortical prisms.

adenosine (1 μM, specific activity 10 μCi/nmole). The prisms were washed and then used for the release studies. When KCl was added to the perfusion medium to a final concentration of 50 mM or 80 mM at the 8th fraction, there was an enhancement of ^{3}H-purine efflux. Table 1 shows that KCl enhances the release of ^{3}H-purine from rat brain cortical prisms. The magnitude of the increase in ^{3}H-purine release was dependent on the KCl concentration present in the perfusion medium ($0.025 > p$). Whilst morphine (10^{-7} ~ 10^{-5} M) did not affect significantly ($p > 0.1$, Student "t" test) the basal release of ^{3}H-purine *in vitro* (Purine released in the absence of excess KCl), it enhanced the KCl (80 mM) evoked release of ^{3}H-purine ($0.025 > p$). The enhancement of KCl-evoked ^{3}H-purine release by morphine was dose dependent in that increases in the morphine concentration caused a more pronounced increase in the KCl-evoked ^{3}H-purine release (see Table 2 and Fig. 2). Figure 2 demonstrates typical KCl-evoked ^{3}H-purine efflux curves in the absence and the presence of 1×10^{-5} M and 5×10^{-5} M morphine. The effect of morphine on KCl-evoked ^{3}H-purine release is gradual with the maximum increase occurring approximately 3 minutes after the addition of morphine to the incubation medium. At a lower concentration of morphine (1×10^{-5} M), the enhancement of ^{3}H-purine release subsided after it had reached the maximal effect, however it had not returned to the original level of KCl-evoked release of ^{3}H-purine 7 minutes after the addition of drug. The morphine-evoked enhancement of release of ^{3}H-purine did not show any significant sign of return to pre-morphine levels 7 minutes after the addition of a higher concentration (5×10^{-5} M) morphine (see Fig. 2). A dose-response curve (Fig. 3) shows that the enhancement of KCl-evoked ^{3}H-purine release by morphine was significant at morphine concentrations between 10^{-7}–10^{-5} M, with a much more pronounced effect at morphine concentrations higher than 10^{-5} M. Naloxone (10^{-7} M) did not significantly change the KCl-evoked release of ^{3}H-purine from rat brain cortical prisms (see Table 2). However, naloxone (10^{-7} M) significantly reduced the effect of morphine (10^{-5} M) on KCl-evoked ^{3}H-purine release ($0.001 > p$). When the morphine concentration was reduced to 10^{-6} M and the naloxone concentration was increased to 10^{-5} M, a complete blockade of the morphine effect on the KCl-

TABLE 1

RELEASE OF ^{3}H-PURINE FROM RAT BRAIN CORTICAL PRISM BY POTASSIUM DEPOLARIZATION

Experimental Conditions	Release of ^{3}H-purine (arbitrary unit)
Control	10.25 ± 0.51 (22)
50 mM KCl	11.15 ± 0.77 (11)*
80 mM KCl	12.02 ± 0.81 (5)*

Rat brain cortical prisms (100 μ × 100 μ × 2 mm) were pre-loaded with ^{3}H-adenosine (1 μM; 10 μCi/n mole) for a period of 60 min. Following rapid washing to remove unbound radioactivities, the prisms were used for the efflux study. The rate of superfusion was 1.0 ml per min at 37°C using a Pharmacia pump. The fraction was collected and radioactivities were measured in an ISOCAP 300 spectrofluorometer. The efflux curves were plotted on a graph paper with 1 × 1 cm division. The area under the efflux curve between fractions 8 and 18 was then integrated. Results are expressed as area under time × release curve in arbitrary units.

*Different from control, one way analysis of variance $p < 0.025$.

TABLE 2

EFFECT OF MORPHINE AND NALOXONE ON THE POTASSIUM CHLORIDE EVOKED RELEASE OF ^{3}H-PURINES FROM RAT BRAIN CORTICAL PRISM

Experimental Condition	^{3}H-purine release (arbitrary unit)
80 mM K^+	12.02 ± 0.81 (5)
80 mM K^+ plus 10^{-6} M morphine	15.50 ± 0.50 (5)[a,c]
80 mM K^+ plus 10^{-5} M morphine	21.90 ± 0.52 (4)[a,d]
80 mM K^+ plus 10^{-7} M naloxone	11.53 ± 0.91 (3)[b]
80 mM K^+ plus 10^{-6} M morphine and 10^{-5} M naloxone	12.82 ± 0.54 (4)[c]
80 mM K^+ plus 10^{-5} M morphine and 10^{-7} M naloxone	17.05 ± 0.49 (4)[d]

Rat brain cortical prisms (100 μ × 100 μ × 2 mm) were pre-loaded with ^{3}H-adenosine (1 μM, 10 μCi/n mole) for 60 min. Following rapid washing to remove unbound radioactivities, the prisms were used for the efflux study by superfusing with buffer solution containing various compounds as indicated above. Results are mean ± S.E. of the number of experiments in parentheses. Data are presented as in Table 1.

[a]Significantly different from 80 mM K^+-evoked release of ^{3}H-purine, one way analysis of variance $p < 0.025$.

[b]10^{-7} M naloxone did not affect K^+-evoked ^{3}H-purine release, Student t-test, not significant.

[c]Naloxone blocked 10^{-6} M morphine enhanced K^+-evoked ^{3}H-purine release, Student t-test, $0.001 < p < 0.01$.

[d]Naloxone antagonized 10^{-5} M morphine enhanced K^+-evoked ^{3}H-purine release, Student t-test, $p < 0.001$.

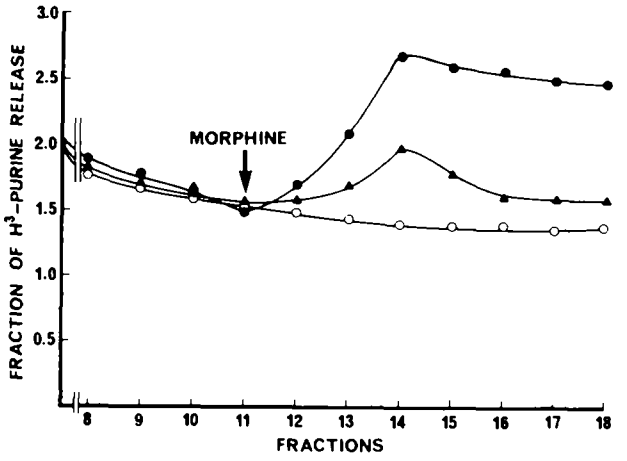

FIG. 2. Effect of morphine on the KCl-evoked ^{3}H-purine release from rat brain cortical prisms. After pre-loading with ^{3}H-adenosine, rat brain cortical prisms were transferred to a perfusion chamber and were superfused with Krebs improved Ringer I solution containing 80 mM KCl (—○—); Morphine (1×10^{-5}M) (—▲—) or morphine (5×10^{-5}M (—●—) which was added to the superfusion solution after collection of the 10th fraction of perfusate.

TABLE 3

IDENTIFICATION OF PURINE METABOLITES IN THE SUPERFUSATE OF ^{3}H-ADENOSINE PRE-LOADED RAT BRAIN CORTICAL PRISM

Metabolites	Per Cent of Total Radioactivity Analyzed: 80 mM K^+-evoked release	Per Cent of Total Radioactivity Analyzed: 5×10^{-5} M morphine enhanced 80 mM K^+-evoked release
Adenosine	39.12 ± 3.67	33.35 ± 2.92
Cyclic AMP	13.27 ± 0.77	15.28 ± 1.61
Hypoxanthine	21.47 ± 3.54	23.77 ± 1.40
Inosine	9.42 ± 0.60	11.87 ± 0.54
Nucleotides	16.72 ± 0.88	15.73 ± 1.01

Rat brain cortical prisms were pre-loaded with 10 μCi (1 μM) ^{3}H-adenosine for 60 min at 37°C. The prisms were transferred to the perfusion chamber and were superfused with buffer solution containing 80 mM KCl or 80 mM KCl and 5×10^{-5} M morphine. Each one minute fraction of the superfusate was collected and the metabolites were analyzed according to the procedures described in Method section (see text). Results are the mean ± S.E. of six fractions at different time intervals (2, 6, 12, 13, 14, 15 min).

evoked ^{3}H-purine release was observed (Table 2). Naloxone (10^{-5} M) did not, by itself, significantly change KCl-evoked ^{3}H-purine release.

^{3}H-Purine Metabolites Released by KCl-Evoked Depolarization and Morphine

The superfusate from the rat brain prisms preloaded with ^{3}H-adenosine was collected at 1 min intervals. Fractions 2, 6, 12, 13, 14, 15 of superfusate were lyophilized, redissolved in 50 μl of distilled water, and the metabolites were separated by silica gel TLC. Morphine was added to the superfusate at the beginning of the experiment. As the levels of each metabolite (present as a percent of total radioactivity analyzed) were very similar in all factions of the superfusate, the results have been pooled and are presented in Table 3. Adenosine was the major metabolite released into the superfusate in the presence of 80 mM KCl. Hypoxanthine contributed approximately 21% of total radioactivity. Other metabolites, nucleotides (16%), cyclic AMP (13%) and inosine (9%) were also identified in the superfusate. Morphine (5×10^{-5} M) enhanced KCl-evoked release of ^{3}H-purines, however, the distribution of radioactivity among the metabolites was similar to that of KCl-evoked release. In morphine (5×10^{-5} M) treated samples, adenosine represented 33% of radioactivity analyzed in the superfusate. Hypoxanthine accounted for 24%, and the other metabolites; nucleotides (16%), cyclic AMP (15%), and inosine (12%) made up the rest of total radioactivity analyzed in the superfusate of ^{3}H-adenosine preloaded brain prisms.

Dipyridamole Enhanced KCl-Evoked ^{3}H-Purine Release

Dipyridamole (final concentrations ranging from 10^{-7} to 10^{-5} M) was used to investigate the effect of inhibition of adenosine uptake on KCl-evoked ^{3}H-purine release from rat brain prisms preloaded with ^{3}H-adenosine. Dipyridamole (10^{-7} M) enhanced the KCl-evoked release of ^{3}H-purine from rat brain prisms by 9.9±3.2%. At a dipyridamole concentration of 10^{-6} M, there was a 32.9 ± 5.9% increase in the ^{3}H-purine release evoked by 80 mM KCl. Dipyridamole (10^{-5} M) caused an even more pronounced enhancement (41.0 ± 3.5%) of 80 mM KCl-evoked ^{3}H-purine release. As dipyridamole is poorly soluble at concentrations higher than 10^{-5} M, the effect of higher concentrations of dypyridamole on ^{3}H-purine release was not observed.

Inhibition of Adenosine Uptake into Rat Brain Cortical Synaptosomes by Morphine and Dipyridamole

Rat brain cortical synaptosomes were prepared and the uptake of adenosine was carried out according to standard procedures [1]. Morphine (10^{-7}–10^{-4} M) or dipyridamole (5×10^{-9}–5×10^{-5} M) was included in the incubation solution which also contained 50 μl (0.4–0.5 mg protein) of rat brain cortical synaptosomal preparation. The mixture was preincubated for 2 min. The uptake reaction (30 sec) was then initiated by the addition of ^{3}H-adenosine to a final concentration of 1 μM. Figure 4 shows that dipyridamole was a potent inhibitor of adenosine uptake with an IC_{50} value of 4.5×10^{-7} M. Morphine was a weaker adenosine uptake inhibitor. Its dose-response curve on a semilogarithmic plot was linear from concentrations of 1×10^{-7} to 1×10^{-4} M. The inhibition of adenosine uptake was approximately 23% at a morphine concentration of 1×10^{-4} M.

DISCUSSION

Adenosine is a potent depressant of neurons in the central nervous system [24]. In the *in vitro* preparations, adenosine markedly reduces the amplitude of monosynaptic responses [9, 20, 22, 32]. These observations have formed the basis for the suggestion that adenosine may play a neurotransmitter or neuromodulator role in CNS. Successful demonstrations of

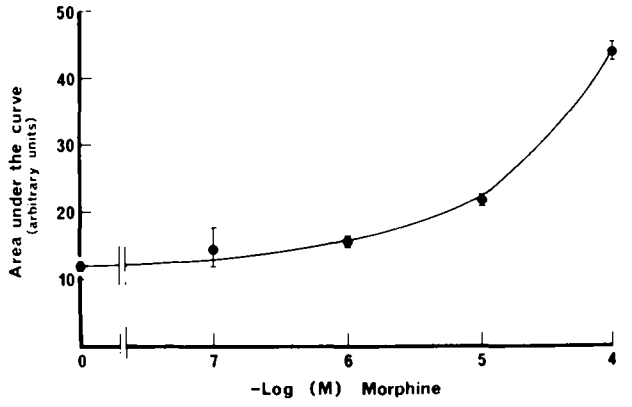

FIG. 3. Dose-response curve for morphine enhancement of KCl-evoked release of ^{3}H-purines. Morphine at concentrations of 1×10^{-7}, 1×10^{-6}, 1×10^{-5} and 1×10^{-4} were added to the superfusion solution containing 80 mM KCl after collection of the 8th fraction. The ^{3}H-purine release curve was obtained and the areas under the curve between fractions 8 and 18 were integrated. The area under the curve was then presented as arbitrary units. The enhancement of ^{3}H-purine release was shown by the increase in the area under the release curve. Results are mean±S.E. of 5 to 8 experiments.

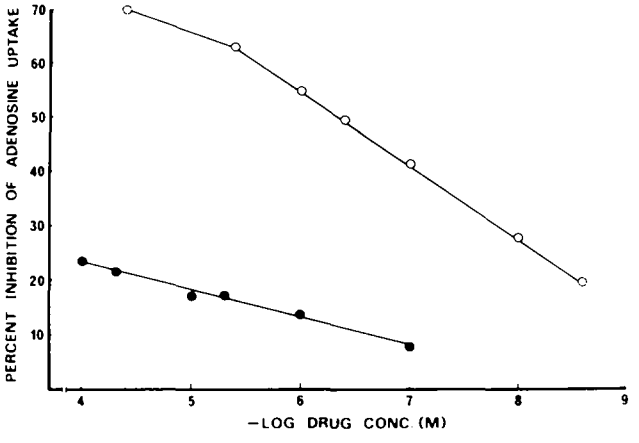

FIG. 4. Inhibition of adenosine uptake into rat brain cortical synaptosomes by morphine and dipyridamole. Morphine (—●—) in concentrations ranging from 1×10^{-7}M to 1×10^{-4}M and dipyridamole (—○—) in concentrations ranging from 5×10^{-9}M to 5×10^{-5}M were added to rat brain synaptosomal preparations for a 2 min pre-incubation period. Following the addition of ^{3}H-adenosine to initiate the uptake process, inhibition of adenosine uptake was estimated as the percent inhibition as compared to the control level of adenosine uptake. Results are mean of 4 experiments in triplicate.

adenosine release in a calcium-dependent manner from various *in vivo* and *in vitro* preparations [6, 16, 19, 27, 33, 35] have further strengthened this concept that adenosine may have an important function in central neurotransmission (for detail see review [26]). In the current paper, we address specifically the effect of morphine on the release of adenosine and its metabolites from an *in vitro* preparation of rat brain cortex.

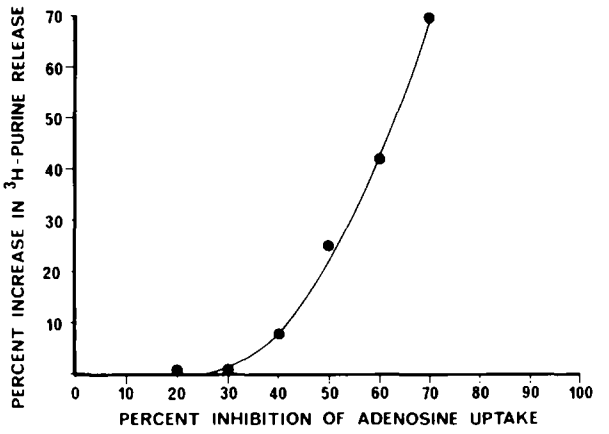

FIG. 5. The effect of uptake inhibition on ^{3}H-purine release from rat brain cortical prisms. Various dipyridamole concentrations (1×10^{-6} to 1×10^{-5}M) were tested for their ability to enhance ^{3}H-purine release in the presence of 80 mM KCl. The data obtained from the release studies were correlated with those obtained from uptake inhibition studies and are plotted as the correlation between the inhibition of adenosine uptake (percent inhibition of adenosine uptake) and the enhancement of ^{3}H-purine release (percent increase in ^{3}H-purine release).

Morphine has been shown to inhibit the release of acetylcholine in central and peripheral tissues [8, 15, 31, 36]. In the guinea-pig myeneteric plexus-longitudinal muscle preparation, morphine exerted a hyperpolarizing effect on the neuron from which acetylcholine was released, thus reducing their rate of discharge and therefore decreasing the rate of release of acetylcholine from their terminals [36]. Morphine also hyperpolarized neuronal elements which were not spontaneously active [13,21], raising the threshold of some of these units above the applied depolarization. Such units failed to release acetylcholine as a result of stimulation in the presence of morphine [36]. These observations indicate that morphine can modulate the stimulation-evoked release of acetylcholine by the CNS or peripheral tissues. As methylxanthines antagonize the morphine-evoked inhibition of field stimulated contractions of the guinea pig longitudinal muscle [31] and its inhibition of acetylcholine release from cerebral cortical preparations [15,25], it was postulated that morphine's action on acetylcholine release is mediated by a purinergic mechanism [18].

Fredholm and Vernet [10] were able to demonstrate that morphine can enhance the veratridine-evoked release of purines from rat cortical slices. Phillis *et al.* [25] have confirmed that the release of labelled purine from intact rat cortex is enhanced by morphine. The increase in purine release was associated with a corresponding decrease in acetylcholine release. Naloxone readily antagonized the morphine-enhanced release of purines, while theophylline and caffeine did not block the morphine-enhanced release of purine, although they antagonized the reduction of acetylcholine release associated with the enhanced levels of purine [18]. This evidence suggested that a "purinergic link" was involved in morphine's action in the central nervous system. In order to substantiate this hypothesis, we have investigated the morphine enhancement of purine release in a carefully controlled system.

^{3}H-adenosine taken up by the cortical prisms is continuously metabolized to form various metabolites. The major metabolites are adenosine, hypoxanthine and adenine nucleotides. When short incubation intervals were used, adenosine (70%) and nucleotides (20%) accounted for most of the radioactivity associated with the brain prisms. However, as the incubation period was prolonged, the adenosine level dropped sharply, whilst nucleotide and hypoxanthine levels increased to reach a state of equilibrium at which adenosine, nucleotides and hypoxanthine represented 28%, 29% and 30% respectively of the total radioactivity analyzed. Inosine levels in the brain prisms did not change significantly over the different incubation periods. This seems to suggest that adenosine taken up by the brain tissue is metabolized, distributed and stored in different compartments until eventually a steady-state condition is achieved. The rate of release of ^{3}H-purine from such brain prisms is dependent on the degree of depolarization, in that the magnitude of the KCl-evoked release of ^{3}H-purines is a reflection of the concentration of potassium chloride present in the superfusate. Although morphine (10^{-7}~10^{-5} M) had no effect on the resting release of ^{3}H-purines, it strongly enhances the KCl-evoked release of ^{3}H-purines. The enhancement of KCl-evoked ^{3}H-purine release was dose-dependent, however, as indicated by Fig. 3, the slope of the dose-response curve was not linear over the range of morphine concentrations tested. This could suggest that there is more than one mechanism involved in morphine's action on ^{3}H-purine release. It is interesting to note that the Krebs improved Ringer I solution we used in these experiments contains 5 mM L-glutamate. There are reports showing that excitatory amino acids including L-glutamate elicited a release of ^{3}H-purine [16,28]. Jhamandas and Dumbrille [16] also reported that the L-glutamate-evoked ^{3}H-purine release was inhibited by morphine (10^{-5} M). However, there are indications that the purine release evoked by L-glutamate is different from that elicited by potassium ion [16]. We found that morphine (10^{-7}~10^{-5} M) did not alter the basal release of ^{3}H-purine in our preparation and morphine enhanced the K^+-evoked release of ^{3}H-purine, again suggesting that there are probably different mechanisms which mediate the release of ^{3}H-purine. Such a view is consistent with that suggested by Jhamandas and Dumbrille [16].

Chesselet *et al.* [4] have suggested that modulation of the *in vivo* release of only certain neurotransmitters (acetylcholine, substance P and noradrenaline) by opiates is mediated by a μ-receptor because the opiate effect in their studies was antagonized by naloxone. However, the effect of morphine and μ-opiate agonists on dopamine release was not antagonized by naloxone, suggesting that the μ-receptors were not involved. These investigators suggested that the dopamine-releasing action of opioid agonists may be mediated via delta-opioid receptors. An involvement of more than one type of opiate receptor could account for the non-linear relationship of the dose-response curve we have obtained in morphine's enhancement of ^{3}H-purine release. Obviously, selective agonists of δ- and μ-receptors should be used for further studies of ^{3}H-purine release.

Adenosine, hypoxanthine and the adenine nucleotides were released into the superfusate in the same proportions as those present in the tissue. 5×10^{-5} M morphine did not change the distribution of the metabolites released into superfusate, suggesting that morphine promotes the KCl-evoked ^{3}H-purine release process. In our experiments, it is not possible to trace the exact cellular locations from which these ^{3}H-purines were released by morphine.

The possibility that the enhanced release of ^{3}H-purine by morphine is a result of morphine inhibition of adenosine uptake must be considered. Morphine is a weak inhibitor of adenosine uptake into rat brain synaptosomes (Fig. 4). A 17% inhibition of adenosine uptake was found at a morphine concentration of 1×10^{-5} M and it is possible that this effect could significantly influence the release of ^{3}H-purines. In order to investigate this possibility, dipyridamole, a known potent inhibitor of adenosine uptake, was tested for its effect on the KCl-evoked release of ^{3}H-purine in our preparations. Figure 5 shows that no increase in ^{3}H-purine release was observed until at least 30% of the re-uptake of adenosine was inhibited. However, the inhibition of uptake contributed very significantly to the release when the uptake inhibition reached 40% and higher. The highest morphine concentration used in these experiments was 1×10^{-4} M and at this concentration morphine would have caused only a 22% inhibition of the adenosine uptake mechanism (Fig. 4). According to the data presented in Fig. 5 it is unlikely that a reduction in uptake of this magnitude would have contributed significantly to the enhancement of ^{3}H-purine release by morphine observed in our experiments.

In conclusion, we have demonstrated that morphine enhances the KCl-evoked ^{3}H-purine release from rat brain prisms *in vitro*. The effect of morphine on purine release can be effectively antagonized by naloxone, and does not appear to be a result of the inhibition of adenosine uptake by morphine. These findings support the hypothesis that morphine can depress acetylcholine release from the cerebral cortex through a purinergic step, by enhancing extracellular levels of adenosine [15,19]. An adenosine-mediated reduction in calcium entry into nerve terminals could account for this reduction in transmitter release [26]. There are several indications that morphine administration also results in a reduced entry of calcium into brain tissues. Ross *et al.* [30] have demonstrated that, in analgesic doses, opiates can cause a loss of brain Ca^{2+} in mice or rats. This loss of Ca^{2+} was stereospecific, antagonized by naloxone and was confined to the synaptic fractions of brain [2,3,11]. Harris *et al.* [12] were able to antagonize opiate analgesia by using the Ca^{2+} ionophore A23187 to facilitate the entry of Ca^{2+} into nerve cells. These similarities between the actions of morphine and adenosine on calcium fluxes are consistent with our suggestion that morphine can act by enhancing extracellular adenosine levels.

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