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RECEPTOR-RELATED INTERACTIONS OF OPIATES WITH PGE-INDUCED ADNEYLATE CYCLASE IN BRAIN

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Summary

The inhibition by opiates of the PGE_2-induced formation of cAMP in slices from rat brain striatum was investigated. A maximal, 3.5-fold increase over the basal level of cAMP was obtained with an EC50 for PGE_2 of 3 μM. Opiate agonists of both μ and κ type were inhibitory. The IC50 values for morphine, levorphanol and ethylketocyclazocine (EKC) were 110 nM, 80 nM and 25 nM, respectively. These values were similar to the potencies of the compounds in displacing stereospecifically bound ^{3}H-etorphine in rat brain membranes. As evidenced by the inactivity of dextrorphan, the inhibition of PGE_2-dependent cAMP formation was stereospecific. Also ineffective were the opiate antagonists naloxone, naltrexone and MR 2266. These compounds did, however, reverse the inhibition by agonists, displaying thereby selectivity toward the putative μ and κ opiates. Thus, the inhibition by morphine was antagonized to a greater degree by naloxone than by MR 2266, and the action of EKC was blocked more effectively by MR 2266 relative to naloxone.

According to an attractive hypothesis, opiates exert some of their effects by modifying the intracellular concentration of cAMP. Original observations that opiates inhibited PGE-stimulated adenylate cyclase in brain homogenates (1) were difficult to replicate (2,3). However, in transformed cell lines from brain, an opiate sensitive basal and PGE-dependent adenylate cyclase were described (4), and the long-term maintenance of cellular cAMP levels by opiates was implicated in the phenomena of tolerance, dependence and withdrawal (5). The inhibition of basal adenylate cyclase in homogenates from normal brain was recently investigated (6). In slices from normal rat brain, opiates were shown to inhibit the PGE_2-dependent increase of cAMP with partial reversal by naloxone (7). We have previously reported the inhibition of PGE_2-induced cAMP formation in slices from rat brain striatum, and the absence of that effect in primary cultures of astrocytes lacking opiates receptor (8). We now describe additional characteristics of the interaction of opiate with PGE_2-dependent adenylate cyclase in brain slices. Particular emphasis in this work was given to assess mediation of the phenomenon by opiate receptor, considering thereby its heterogeneity (9).

Materials and Methods

The drugs used in this study were made available by the Drug Abuse Basic Research Program, The University of Michigan. MR 2266: (-)-α-5,9-diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan. PGE_2 was obtained from the Upjohn Co., Kalamazoo, MI., while ^{3}H-cAMP and the radioassay kit for cAMP were

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purchased from the Amersham Corp., Arlington Heights, IL. Dowex resin and 3-isobutyl-2-methylxanthine (IBMX) were obtained from Sigma Chem. Corp., St. Louis, MO. All other chemicals were of reagent grade. The buffer medium used throughout this work had the following composition (mM): NaCl, 125.0; KCl, 1.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 10; IBMX (phosphodiesterase inhibitor), 1.0. The pH of the buffer was adjusted to 7.4 by gassing with 95% O_2 - 5% CO_2. A stock solution of 2.1 mg/100 µl PGE_2 was prepared in 95% ethanol. Further dilutions of this stock were made with the buffer medium.

The first part of the methodology described below represents an adaptation of the procedure reported by Havemann and Kuschinsky (7). Male Sprague Dawley rats weighing 120-180 g were decapitated, the brains quickly excised and transferred to 2-4°. The striata were dissected and cut into 400 µm slices using a McIlwain tissue chopper. The slices were suspended with 4 ml of buffer medium, and incubated for 15 min at 37° with 95% O_2 - 5% CO_2 bubbling through the medium. At the end of each 5 minute period, 3.5 ml of the medium were removed and replaced by an identical aliquot of fresh 37° buffer. After the third wash, 3.5 ml buffer containing PGE_2 (final concentration 35 µM), or containing PGE_2 and drug were added and the slices incubated for 5 min at 37° with gassing. The incubation was terminated by placing the samples in a boiling water bath for 5 min. In experiments with antagonists, the slices were first incubated in the presence of 35 µM PGE_2 and 10 µM antagonist for 10 min, then the agonist (final concentration 10 µM) was added in a small volume of buffer, and the incubation continued for 5 min before termination by boiling. The contents of the vial were then disrupted for 1 min, using a Polytron homogenizer (Model P-10, Brinkman Instruments, Inc.) at the power output 6.5. In an aliquot of the homogenate, protein was determined according to Lowry et al. (10). The remainder of the homogenate was centrifuged at 10,000 x g for 15 min, and the supernatant removed and saved. The pellet was washed by resuspension for 30 sec using a Bronson Sonifier with microtip at power output 6, then centrifuged as described above. The two supernatants were combined, and an aliquot of ^{3}H-cAMP (5000 cpm) was added to monitor recovery. After application to a Dowex 1x2 column (11), the fractions containing ^{3}H-cAMP were pooled and lyophilized. The samples were then reconstituted to the desired volume with 0.5 M Tris.HCl, pH 7.5 at 0°, and cAMP determined by a competitive binding assay (Amersham radioassay kit).

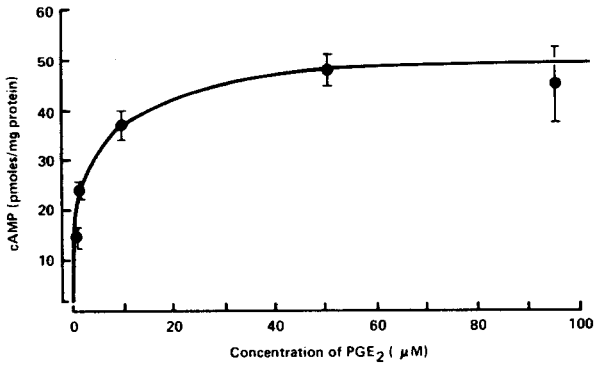

FIG. 1. PGE_2-induced formation of cAMP. Striatal slices were incubated as described under Materials and Methods for 5 min at 37° in the absence and presence of PGE_2 as indicated. Control samples, without PGE_2, contained appropriate concentrations of ethanol, present in solutions of the prostaglandin. cAMP was extracted and determined as described above. Each point is the mean of quadruplicate determinations.

Results and Discussion

PGE_2 increased the concentration of cAMP in striatal slices approximately 3.5-fold, from 15.1 ± 2.7 pmole cAMP/mg protein in the control sample to the maximally stimulated value of 52.6 ± 8.6 pmole cAMP/mg protein. Maximal stimulation was obtained after 5 min incubation at 37°, with an EC50 for PGE_2 of approximately 3 µM (Fig 1).

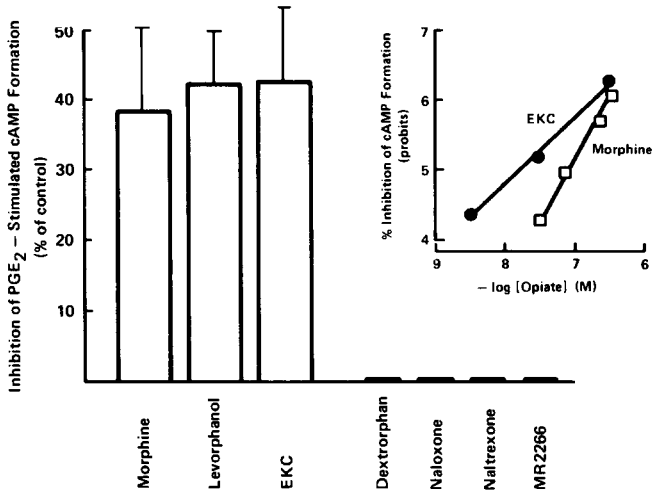

FIG. 2. Inhibition of PGE_2-induced cAMP formation. Striatal slices were incubated as described above with and without PGE_2 and 10 μM of the compounds as indicated. Measured cAMP was expressed as pmole/mg protein. The inset shows log-probit plots of the inhibition obtained at several drug concentrations. Percent inhibition was defined as

$$\frac{(cAMP_{PGE} - cAMP_{PGE+drug})}{(cAMP_{PGE} - cAMP_{basal})} \times 100.$$

Shown are mean values and standard deviations of 3-6 experiments.

The PGE_2-induced increase in cAMP levels was inhibited by putative μ and κ opiate agonists. The maximum effect leveled off below complete inhibition (Fig 2). The inhibitory potency (Fig 2, inset) ranged from 110 nM for morphine, to 80 nM and 25 nM for levorphanol (not shown on the log-probit plot) and EKC. The magnitude and order of the IC50 values were similar to those of the affinities of the agonists in binding to opiate receptor. The IC50 for morphine (12,13), levorphanol (12), and EKC (13) in displacing stereospecifically bound ^{3}H-etorphine in rat brain membranes, in the presence of 150 mM NaCl, were 142 nM, 21 nM and 21 nM, respectively. Opiate antagonists of both μ and κ type did not inhibit the PGE_2-induced cAMP formation. Also ineffective was dextrorphan, the pharmacologically inactive isomer of levorphanol (Fig 2), thus providing evidence for stereospecificity of the phenomenon. The receptor-related nature of the inhibition by opiate agonists of cAMP formation was also underlined by the ability of opiate antagonists, including naloxone, to block the inhibition by agonists (Fig 3). Furthermore, in the course of their antagonism these compounds displayed selectivity toward putative agonists of the μ and κ type. Thus, naloxone but not MR 2266, a benzomorphan antagonist (14), was effective in reversing the inhibitory effect of morphine. On the other hand, inhibition of cAMP formation by EKC, a putative κ agonist (14,15) was antagonized to a significantly lesser degree by naloxone relative to MR 2266. A major obstacle

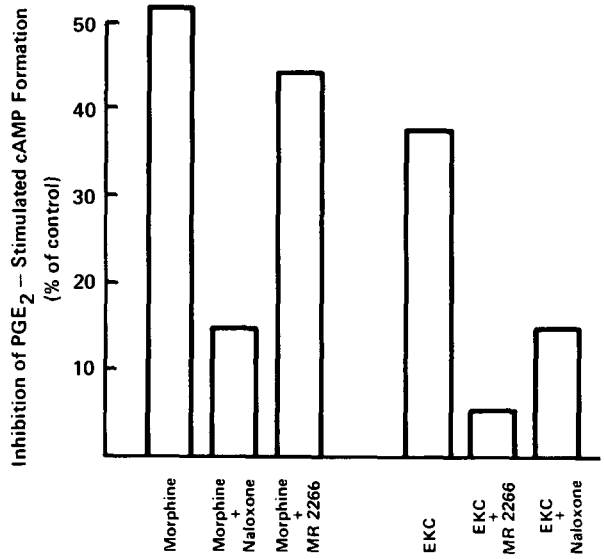

FIG. 3. Effect of antagonists on the inhibition of PGE_2-induced cAMP formation by opiate agonists. Striatal slices were incubated as described under Materials and Methods. The results shown were replicated twice. Within one experiment each point was the mean of 4 determinations.

in assessing opiate receptor heterogeneity is the equivocal specificity of the employed ligands, particularly as it relates to alkaloids of the κ type. In view of the systematic evidence for dynorphin as a specific endogenous ligand for the putative κ opiate receptor (16), it will be of interest to assess its effect on the functional relationship between receptor and putative effector, described here.

Additional support for the mediation by opiate receptor of the investigated phenomenon was provided by the results obtained with isolated glial cells (8). Primary cultures of astrocytes from normal rat brain displayed virtually no opiate receptor binding. In these cells cAMP formation was stimulated 20-fold by PGE_2, but opiates were ineffective in inhibiting this increase. Assuming that glial cells are deficient in opiate receptor, the observed plateau in the inhibitory action of opiate agonists in brain slices (Fig 1) becomes plausible. While the glial component of the tissue responds to PGE-stimulation of cAMP production, it is insensitive to opiate inhibition. In this respect our data disagree with the previously reported complete inhibition of the PGE-induced increase of cAMP in striatal slices (7).

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