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Opioid inhibition of adenylate cyclase in the striatum and vas deferens of the rat

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1 The activity of adenylate cyclase in striatal membrane-enriched fractions (25,000 *g*) was inhibited by morphine, β-endorphin, [D-Ala^2-D-Leu^5] enkephalin (DADLenk), fentanyl and bremazocine. Whereas guanosine triphosphate (GTP) appeared essential for the expression of this effect, sodium chloride seemed to enhance the degree of inhibition. Dopamine stimulation and sodium fluoride activation of the enzyme was also suppressed by morphine, β-endorphin and DADLenk.

2 β-Endorphin and DADLenk inhibited adenylate cyclase activity in vasa deferentia membrane-enriched fractions (25,000 *g*); both opioids required GTP and NaCl and were inhibited by a δ-opioid receptor antagonist and by naloxone. Morphine, bremazocine and tifluadom did not significantly alter the activity of the vas deferens enzyme.

3 Basal cyclic AMP values of striatal slices were not significantly altered by morphine, β-endorphin or DADLenk. However, dopamine-induced elevation of cyclic AMP was reduced by morphine and this effect of the opiate was suppressed by naloxone.

4 Only β-endorphin lowered the basal cyclic AMP values in the vas deferens.

5 The physiological relevance of adenylate cyclase coupling to opioid receptor subtypes is considered.

Introduction

A direct influence of opiates on intracellular levels of adenosine 3′:5′ cyclic monophosphate (cyclic AMP) was first demonstrated on a mouse neuroblastoma-glioma hybrid cell line (NG 108–15). The cyclic AMP forming enzyme, adenylate cyclase, was inhibited by opiates (Sharma *et al.*, 1975); this effect was stereospecific, naloxone reversible and subsequently shown to be dependent on sodium ions and guanosine triphosphate (GTP). As a result of these observations on cultured neuroblastoma cells, Klee *et al.* (1975) proposed that the analgesic effect of opiates was mediated through inhibition of adenylate cyclase. If neuroblastoma cells can be considered as models for morphine-sensitive cells in the brain, then the analgesic action of morphine should be accompanied by a fall in neuronal cell cyclic AMP and the analgesia reversed by an elevation in cyclic AMP (Ho *et al.*, 1973). Morphine withdrawal results in an increase in brain levels of cyclic AMP (Collier & Francis, 1975). In addition, opiates inhibit both prostaglandin (Collier & Roy, 1974a,b) and dopamine elevation (Wilkening *et al.*, 1976; Minneman, 1977) of basal cyclic AMP values in the brain. In contrast, prolonged exposure of hybrid neuroblastoma cells to morphine increases adenylate cyclase activity (Klee *et al.*, 1975).

The purpose of the present study was to delineate, characterize and compare the coupling of central (striatum) and peripheral (vas deferens) opioid receptors to adenylate cyclase. The functional relevance of such coupling to opioid receptor subtypes is discussed.

Methods

Animals

The animals used in this study (adult male rats of Porton strain, weighing between 200–400 g) were kept on a 12 h light and dark cycle at a constant temperature of 70°F, with free access to water and food. The rats were killed by cervical dislocation and appropriate tissue removed.

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Membrane preparations

Receptor-coupled and catalytic activation of adenylate cyclase was examined in rat striatal and vas deferens membranes.

(a) The brain was removed and placed on filter paper soaked in homogenizing buffer (mM: sucrose 250, $MgCl_2$ 5, KCl 25, EGTA 2, theophylline 8, Tris-HCl 50; pH 7.4). The striatum (caudate-putamen complex) was dissected free of surrounding cortical tissue on a petri-dish packed with ice. The striatal tissue was suspended in the homogenizing buffer (1:3, w/v) and disrupted by five strokes of a glass hand homogenizer (Jencons: 5 ml capacity; clearance 0.15–0.25). A 25,000 $g \times 30$ min membrane-enriched preparation was obtained by centrifugation after the initial removal of cell debris by spinning at 1000 $g \times 10$ min (MSE-Coolspin, 4°C).

(b) The vasa deferentia of adult male rats were removed and placed in a petri-dish containing ice-cold homogenizing buffer. The muscle was cleaned of connective tissue, gently squeezed to remove seminal fluid and subsequently cut into strips. The strips of vasa deferentia were suspended in homogenizing buffer (1:3, w/v) and homogenized by Ultra-turrax (Janke and Kunkel, Shaft 8 N) for 20 s. The homogenate was strained through a nylon mesh. After removal of 800 $g \times 10$ min pellet, a membrane-enriched fraction was separated at 25,000 $g \times 30$ min (MSE 50, 4°C).

Tissue slices

The turnover of cyclic AMP was determined in striatal and vasa deferentia slices.

(a) The method for striatal slices was based on that described by Quik *et al.* (1979). The striatum was placed in ice-cold Krebs-buffer A (mM: NaCl 124, $CaCl_2$ 0.8, $MgCl_2$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.2, glucose 10, KCl 5; pH 7.4; 95% O_2–5% CO_2). After recording the wet weight of the tissue, slices were cut on a McIlwain tissue chopper (0.26 × 0.26 mm, cross-chopped), suspended in 15 ml fresh Krebs-buffer A and pre-incubated for 60–90 min in a shaking water bath (Grant Instruments, Cambridge) (120 strokes min^{-1}) at 37°C. During the pre-incubation period the Krebs buffer was changed twice and finally suspended to give a concentration of 100 mg tissue ml^{-1}. A portion (100 µl) of the tissue slice preparation was added to each incubation tube, containing 100 µl Krebs-buffer with isobutyl-methyl xanthine (1 mM; IBMX) and 100 µl of drug or control solution. The tubes were incubated for varying periods of time at 37°C. At the end of the incubation, the tubes were heated at 110°C (Grant Instruments, Cambridge) for 5 min and stored at −20°C overnight. The next day the tubes were thawed and the denatured protein removed by centrifugation at 1750 $g \times 10$ min (MSE-Coolspin) and the supernatant was assayed for cyclic AMP.

(b) Cyclic AMP measurements in vasa deferentia slices followed that described by Albano *et al.* (1976). Slices of vasa deferentia were pooled into ice-cold Krebs-buffer B (mM: NaCl 121, KCl 5, $CaCl_2$ 2.6, $NaHCO_3$ 25.2, glucose 5.6, Na pyruvate 3.7, Na glutamate 3.7, Na fumarate 2.7; pH 7.4; 95% O_2 – 5% CO_2). The slices were pre-incubated at 37°C in a shaking water bath (120 strokes min^{-1}) transferred to a single flask containing 15 ml fresh Krebs-buffer B. After 40 min the slices were removed, blotted and weighed on a torsion balance. Slices of about 30–60 mg were transferred to tubes containing 100 µl Krebs-buffer B (and 0.1 µM IBMX), 0.1% bovine serum albumin (BSA) and 100 µl of drug or control solution, and incubated for varying periods of time. The reaction was terminated by freeze clamping the tissue in liquid nitrogen. Each sample was heated to 110°C for 10 min in 0.5 ml theophylline (6 mM). The denatured tissue was homogenized with an ultra-turrax for 45 s, acid-alcohol (0.5 ml) added and the tubes stored overnight at −20°C. Following centrifugation (1750 $g \times 10$ min: MSE-Coolspin), the pellet was washed once in acid-alcohol (0.5 ml). The supernatants were combined and taken to dryness at 55°C. The dried residue was dissolved in assay buffer for measurement of cyclic AMP.

Measurement of adenylate cyclase, cyclic AMP and protein

Adenylate cyclase activity of washed membrane fractions was determined by measuring the amount of cyclic AMP formed using unlabelled ATP as substrate (Albano *et al.*, 1973). The cyclic AMP concentration in membrane and tissue slice incubates was determined in the following manner: The standard assay contained in 75 µl of final volume, 50 µl ATP buffer (mM: 2 ATP, 3 $MgCl_2$, 10 NaCl, 10 KCl, 2 EGTA; pH 7.4) and 25 µl assay buffer (mM: 60 Tris base, 50 HCl, 8 theophylline: pH 7.4) or drug solution in assay buffer. The assay was performed in soda glass tubes. The enzyme reaction was started by the addition of the membrane suspension which was continuously stirred. The reaction mixture was incubated in a shaking water bath (120 strokes min^{-1}) at 30°C for the desired period. Incubations were terminated by placing the tubes in a Grant heating block at 110°C for 3 min and frozen at −20°C overnight. The next day the samples were thawed and resuspended in 1 ml assay buffer and centrifuged at 1600 $g \times 10$ min (MSE-Coolspin); 50 µl samples were taken for the measurement of cyclic AMP by the binding protein saturation assay of Brown *et al.* (1971).

Protein was determined by the Lowry method using serum albumen as the standard (Lowry *et al.*, 1951).

Statistics

All values are the mean ± s.e.mean of at least three experiments with each incubate in triplicate and assayed in triplicate ($n = 9$). In assessing significant differences between groups, data were analysed by a two-tailed Student's t test or analysis of variance.

Materials

All chemicals used in this study were, unless otherwise stated, purchased from British Drug Houses or Sigma and were of analytical grade. Morphine base was obtained from MacFarlane Smith, and all tritiated radiochemicals from Amersham International. Naloxone was a gift from Endo Laboratories, bremazocine and tifluadom from Sandoz and ICI 154129 (N,N-Bisallyl-Try-Gly-Gly-)CH_2S)-Phe-Leu-OH) from I.C.I.

Results

Modulation of brain striatal adenylate cyclase activity

Basal activation of adenylate cyclase in striatal membranes (33 ± 2.0 pmol cyclic AMP $min^{-1} mg^{-1}$ protein) was 2.3 times greater than for the enzyme in whole brain or cerebellum (14 ± 1.0 pmol cyclic AMP $min^{-1} mg^{-1}$ protein). NaCl (1 mM–1 M) and GTP (10^{-9} M–10^{-5} M) produced a dose-related increase in the basal activation of adenylate cyclase of both membranes (Figure 1). Catalytic site integrity was assessed with NaF, which produced maximal stimulation at 13.75 mM of 306 ± 12.0 pmol cyclic AMP $min^{-1} mg^{-1}$ $protein^{-1}$ ($P < 0.001$). Neither NaCl nor GTP influenced the NaF effect on the enzyme.

A neurotransmitter (dopamine) and an autocoid (prostaglandin E_1) were selected to examine receptor-linked enhancement of adenylate cyclase activity, particularly since both are considered to exert an important modulatory influence on the cellular actions of opiates. Both prostaglandin E_1 (PGE_1, 10^{-9} M–10^{-5} M; 10^{-6} M: 153 ± 6.0 pmol cyclic AMP $min^{-1} mg^{-1}$ protein; $P < 0.001$) and dopamine (10^{-7} M–10^{-3} M; 6×10^{-5} M: 212 ± 9.0 pmol cyclic AMP $min^{-1} mg^{-1}$ protein; $P < 0.001$) increased adenylate cyclase activity of striatal membranes (25,000 g) in a time- and dose-dependent manner. GTP (10 μM) but not NaCl was necessary to elicit the dopamine response. The dopamine dose-dependent (10^{-6} M–10^{-3} M) stimulation of adenylate cyclase was completely inhibited by (+)-butaclamol (10^{-8} M) but not (−)-butaclamol (10^{-8} M).

Adenylate cyclase activity associated with striatal membranes was inhibited by morphine but only on the addition of an appropriate concentration of GTP.

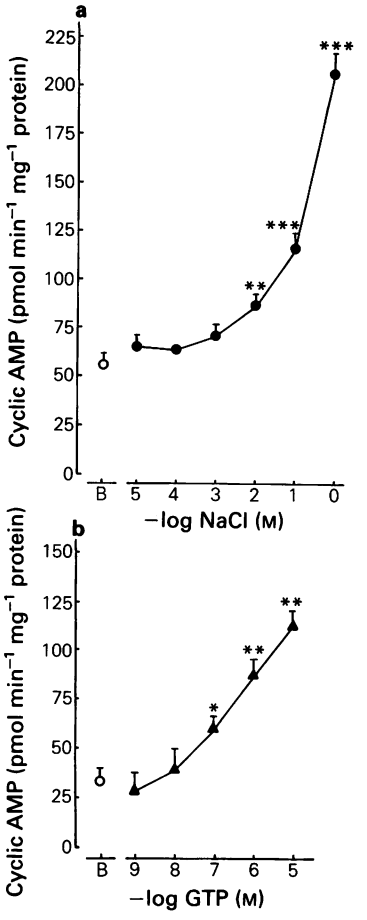


Figure 1 Dose-dependent stimulation of basal adenylate cyclase activity on 25,000 $g \times 10$ min membrane fraction by (a) NaCl (●) and (b) GTP (▲). B = basal (control, ○). Bar = one s.e.mean. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Morphine (10^{-4} M) reduced enzyme activity by 22 ± 3.9 pmol cyclic AMP $min^{-1} mg^{-1}$ protein ($P < 0.05$) at 1 μM GTP, and 35.9 ± 3.5 pmol cyclic AMP $min^{-1} mg^{-1}$ protein ($P < 0.05$) at 10 μM GTP. With NaCl, morphine (10^{-4} M) inhibited adenylate cyclase by 18.7 ± 5.5 pmol cyclic AMP $min^{-1} mg^{-1}$ protein ($P < 0.05$) at 10^{-3} M NaCl and by 43.7 ± 6.2 pmol cyclic AMP $min^{-1} mg^{-1}$ protein ($P < 0.01$) at 1 M NaCl. A maximal inhibition of 27% was achieved at a morphine concentration of 10^{-5} M in the presence of 10 μM GTP and after an incubation time of 30 min (Figure 2). This inhibition was antagonized by naloxone (10^{-7} M–10^{-5} M) which displayed no intrinsic effect on basal activation. A number of opioid receptor agonists were also tested. Fentanyl (μ-recep-

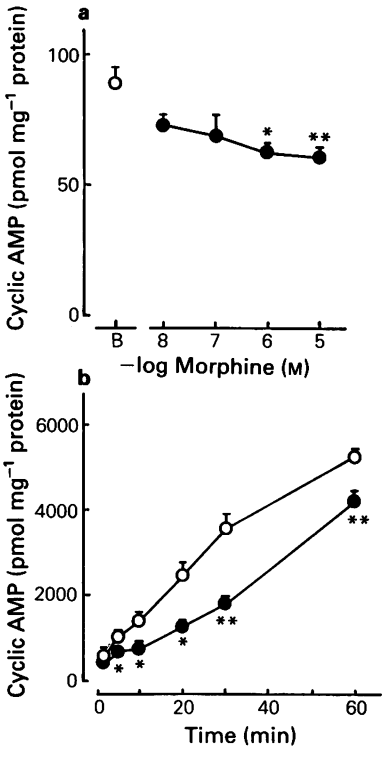


Figure 2 (a) Dose- and (b) time-related inhibition of adenylate cyclase in 25,000 g × 30 min striatal membrane fraction by morphine in the presence of NaCl (10 mM) and GTP (10 μM). Control (○) and morphine, 10^{-4} M (●). B = basal (control, ○). Bar = one s.e.mean. $*P<0.05$; $**P<0.01$.

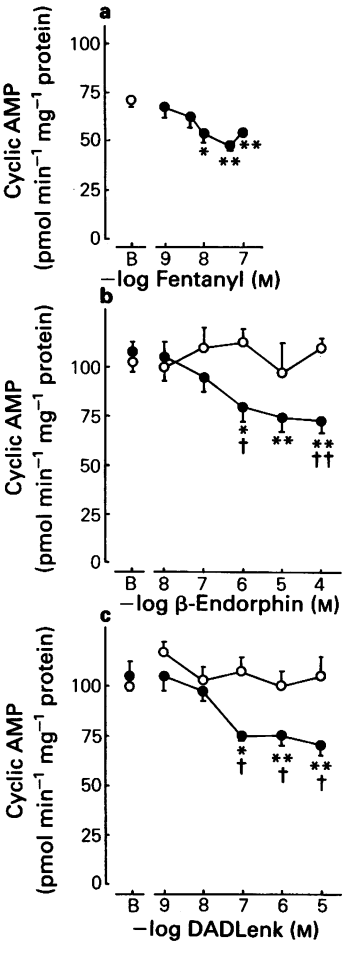


Figure 3 Dose-related inhibition of adenylate cyclase in 25,000 g × 30 min striatal membrane fraction in the presence of NaCl (10 mM) and GTP (10 μM) by (a) fentanyl (●), (b) β-endorphin (●) and antagonism by naloxone, 10^{-5} M (○), (c) DADL-enkephalin (●) and antagonism by naloxone, 10^{-5} M (○). B = basal (control, ○). Bar = one s.e.mean. Significance was tested for dose-dependency ($*P<0.05$; $**P<0.01$); and between control and opiate/opioid inhibition ($\dagger P<0.05$; $\dagger\dagger P<0.01$).

tor selective agonist, 5×10^{-7} M), bremazocine (κ-receptor selective agonist, 10^{-5} M), β-endorphin (10^{-5} M) and [D-Ala^2-D-Leu^5]enkephalin (DADLenk) (δ-receptor selective, 10^{-5} M) inhibited the striatal adenylate cyclase, achieving maximum inhibition of between 25 and 30% at the concentrations indicated (Figure 3). As with morphine, inhibition by the opioids was dependent on GTP (>1 μM) and was antagonized by naloxone (10^{-5} M). NaCl influenced this inhibition of the enzyme by β-endorphin and DADLenk but only at the higher concentrations (>10^{-2} M NaCl). It is conceivable that regulation of the enzyme by neurotransmitters may be influenced by opiates. Since dopamine is believed to play such a role we examined the interaction of opioids on the dopamine stimulated adenylate cyclase. At a concentration of 10^{-5} M morphine, β-endorphin and DADLenk inhibited the dopamine (10^{-7} M–10^{-4} M) and NaF (12.5 mM) enhancement of the enzyme by 30%. This inhibition by the opioids was completely reversed by naloxone (10^{-5} M), but was unaffected by (+)-butaclamol (10^{-8} M).

Modulation of vas deferens adenylate cyclase

Experiments were designed to ascertain the subcellular

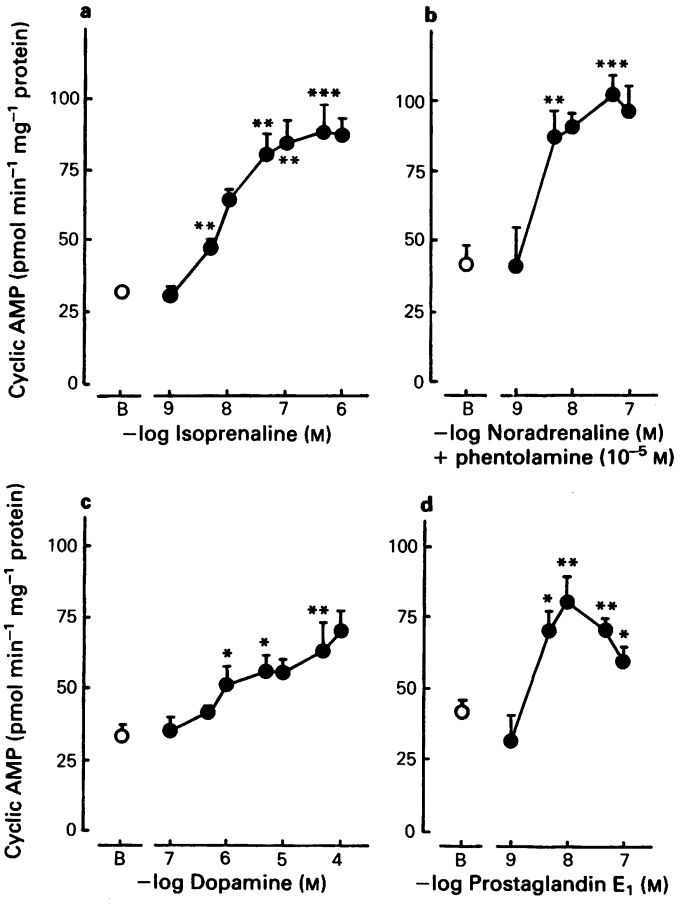


Figure 4 Dose-related stimulation of adenylate cyclase in the 25,000 g × 30 min vasa deferentia membrane fraction, in the presence of GTP (10 μM) and NaCl (10 mM) by (a) isoprenaline (●), (b) noradrenaline plus phentolamine, 10^{-5} M (●), (c) dopamine (●), (d) prostaglandin E_1 (●). B = basal (control, ○). Bar = one s.e.mean. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

distribution of adenylate cyclase in vasa deferentia fractions in order to achieve the optimum localization of the enzyme. The highest basal, receptor-linked (β-adrenoceptor, isoprenaline), and catalytic site (NaF), activation was attained with the membrane-enriched fraction. Basal activity (25 ± 10.9 pmol cyclic AMP min^{-1} mg^{-1} protein) was enhanced by GTP (>1 μM; 10 μM = 100 ± 6.3 pmol cyclic AMP min^{-1} mg^{-1} protein; $P<0.01$) and NaCl (>1 mM; 100 mM = 112 ± 7.8 pmol cyclic AMP min^{-1} mg^{-1} protein; $P<0.001$). NaF produced maximal activation of 450% at 12.5 mM (132.5 ± 10.0 pmol cyclic AMP min^{-1} mg^{-1} protein; $P<0.001$). Isoprenaline (peak, 300% at 5×10^{-7} M), noradrenaline (peak, 250% at 5×10^{-8} M) and prostaglandin E_1 (peak, 100% at 10^{-8} M) stimulated the vas deferens adenylate cyclase in a dose-related manner (Figure 4).

Although opioid receptors are widely distributed, little information exists with regard to the coupling of selective peripheral opioid receptor subtypes to adenylate cyclase. Unlike the striatum, morphine (μ-receptor selective, 10^{-4} M), bremazocine and tifluadom (κ-receptor selective, 10^{-5} M) produced no significant change in the activity of the vas deferens enzyme even in the presence of GTP (>1 μM) and NaCl (>10^{-3} M). On the other hand, β-endorphin (peak reduction of 25% at 10^{-5} M) and DADLenk (peak reduction of 22% at 10^{-5} M) inhibited adenylate cyclase in the

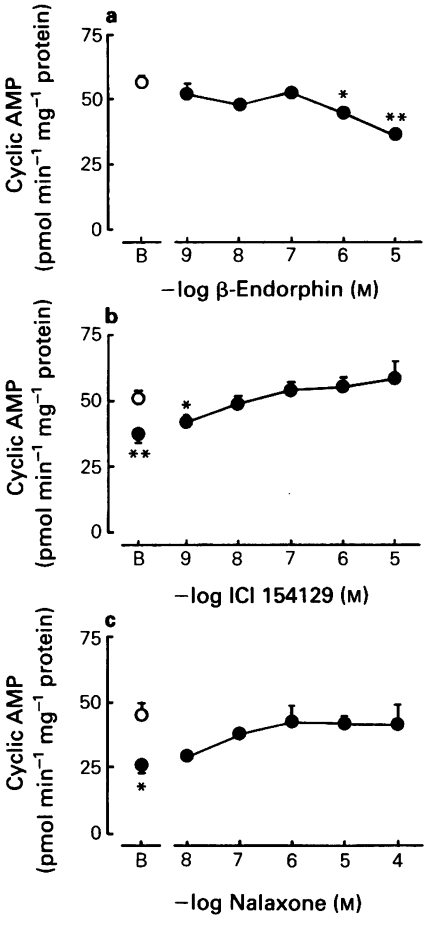


Figure 5 Inhibition of adenylate cyclase in 25,000 g × 30 min vasa deferentia membrane fraction in the presence of NaCl (10 mM) and GTP (10 μM) by (a) β-endorphin (●), (b) antagonism of β-endorphin (10^{-5} M) by ICI 154129 (●) and (c) antagonism of β-endorphin (10^{-5} M) by naloxone (●). B = basal (control, ○). Bar = one s.e.mean. $*P<0.05$; $**P<0.01$.

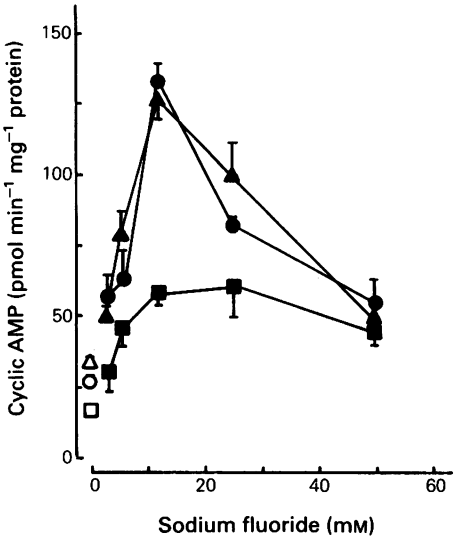


Figure 6 Inhibition of sodium fluoride-activated adenylate cyclase of vasa deferentia 25,000 g × 30 min membrane fraction by β-endorphin (10^{-5} M) and its antagonism by ICI 154129 (10^{-7} M). NaF (●), NaF plus β-endorphin (10^{-5} M) (■), NaF plus β-endorphin (10^{-5} M) plus ICI 154129 (10^{-7} M) (▲). Open symbols = basal values. Bar = one s.e.mean.

presence of GTP (10 μM) and NaCl (10 mM). This inhibition was antagonized by a δ-receptor antagonist (ICI 154129) and by naloxone (Figure 5). Of the two opioids, β-endorphin was more effective in reducing the NaF catalytic site stimulated enzyme by 55% (Figure 6). The degree of inhibition of adenylate cyclase through N_i protein could be modulated by the simultaneous activation of more than one subtype (Figure 7). We therefore examined the question whether the combined application of agonists would reveal the presence of multiple subtypes. The simultaneous incubation of β-endorphin (10^{-5} M) and DADLenk (δ-receptor selective, 10^{-5} M) did not produce an inhibition greater than that previously observed in the presence of either opioid. Furthermore, no unmasking of μ-receptor subtype (morphine 10^{-4} M plus β-endorphin 10^{-5} M) occurred. These results seem to suggest the presence of δ opioid receptor subtype on rat vas deferens and would be in agreement with the structure activity data of Corbett *et al.* (1984) and Sheehan & Hayes (1985) that indicate δ opioid subtypes on the hamster vas deferens.

Turnover of cyclic AMP in striatal tissue slices

Brain striatal slices were pre-incubated for 60 to 90 min in order to stabilize basal turnover of cyclic nucleotides (Kakiuchi & Rall, 1964). Whereas dopamine (10^{-7} M–10^{-4} M) caused a dose- and time-dependent increase, neither morphine (10^{-6} M) (Figure 8), β-endorphin (10^{-9} M–10^{-5} M) nor DADLenk (10^{-9} M) significantly altered cyclic AMP in striata slices incubated for 10, 20 or 30 min. In contrast, morphine (10^{-4} M) significantly decreased the elevation of cyclic AMP produced by dopamine (10^{-6} M–10^{-3} M; 10^{-5} M reduced by 21%) in striatal slices prepared from control animals. This inhibition was completely reversed by naloxone (10^{-5} M).

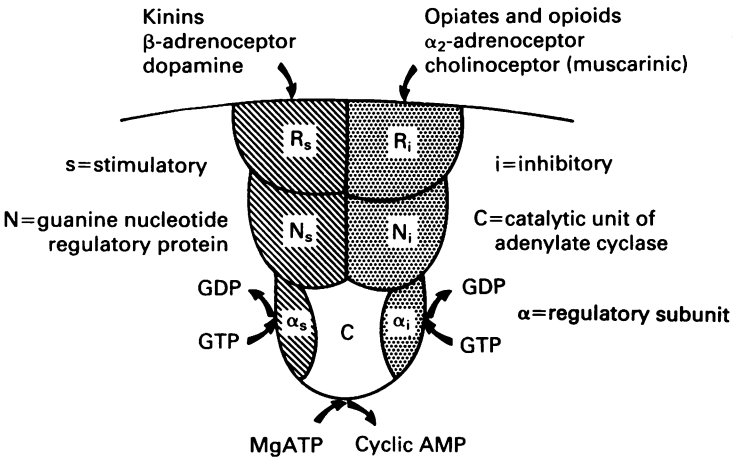
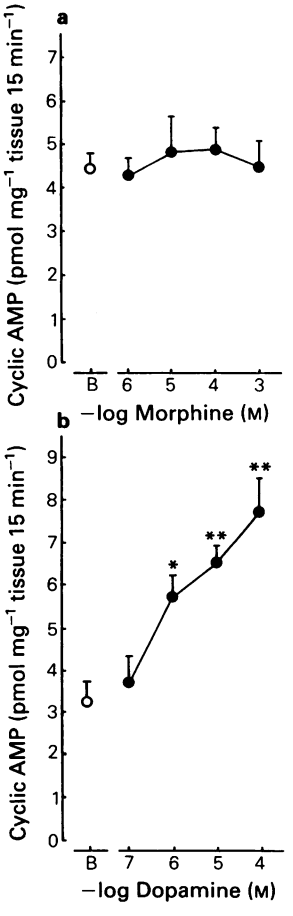


Figure 7 Receptor coupling to adenylate cyclase.



Turnover of cyclic AMP in vasa deferentia slices

Pre-incubation experiments indicated that cyclic AMP levels show an initial rise up to 10 min but stabilize after 40 to 60 min. The tissue was therefore pre-incubated for 60 min. Isoprenaline caused a dose- (10^{-8} M–10^{-4} M) and time- (peak 30 min, 10^{-5} M) dependent increase in cyclic AMP; basal value of 3.4 ± 0.9 pmol cyclic AMP mg^{-1} tissue 15 min^{-1} was increased to 8.7 ± 0.45 pmol cyclic AMP mg^{-1} tissue 15 min^{-1} ($P<0.01$). A similar rise was obtained by dopamine (10^{-7} M–10^{-3} M; maximum elevation of 160% at 10^{-3} M). Noradrenaline appeared to exercise a dual regulation of cyclic AMP levels. Noradrenaline alone had no effect on cyclic AMP level. However, when the tissue was preincubated with phentolamine (10^{-5} M), noradrenaline increased basal levels in a dose- (10^{-8} M–10^{-4} M) and time- (peak, 20–30 min) related manner; this effect was significantly reduced when the vas was preincubated with propranolol (Figure 9), but when yohimbine was also added the reduction was blocked. Therefore noradrenaline appears to modulate cyclic AMP levels in the vas deferens by the simultaneous activation of adenylate cyclase coupled to stimulatory β- and inhibitory α_2- (yohimbine sensitive) adrenoceptors. The inhibitory control shown by activation of α_2-adrenoceptors was similar to the dose-dependent decrease in cyclic AMP produced by acetylcholine (10^{-8} M–10^{-4} M; at 10^{-4} M reduced by 32%).

Figure 8 Dose-related effect of (a) morphine (●) and (b) dopamine (●) on cyclic AMP levels in striatal slices. B = basal (control, ○). Incubation time 15 min. Bar = one s.e.mean. $*P<0.05$; $P<0.01$.

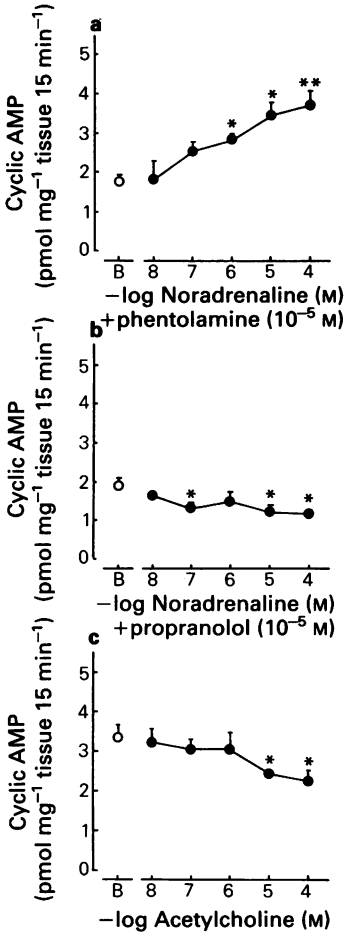


Figure 9 Dose-related effect on cyclic AMP levels in the vasa deferentia slices in the presence of (a) noradrenaline after prior incubation with phentolamine (10^{-5} M) (●), (b) noradrenaline after prior incubation with propranolol (10^{-5} M) (●) and (c) acetylcholine (●). B = basal (control, O). Bar = one s.e.mean. $*P<0.05$; $**P<0.01$.

As observed in the striatum, both morphine and DADLenk were without effect on basal cyclic AMP levels in vasa deferentia slices. In contrast, β-endorphin evoked a dose- (10^{-9} M–10^{-6} M, max 28% at 10^{-7} M) and time- (peak 30%, 30 min, 10^{-7} M) related fall in cyclic AMP levels (Figure 10).

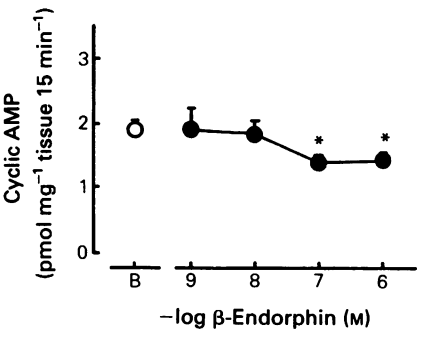


Figure 10 Dose-related decrease in cyclic AMP levels in vasa deferentia slices by β-ebdorphin (●). B = basal (control, O). Bar = one s.e.mean. $*P<0.05$; $**P<0.01$.

Discussion

Opioid receptor-linked adenylate cyclase in neuronal membranes

No conclusive evidence was available concerning opioid modulation of adenylate cyclase until the conditions required to demonstrate inhibition of the enzyme were delineated in experiments on neuroblastoma-glioma hybrid cells. The striatal tissue contains the highest opioid receptor density in the brain and, consequently, we chose this region for our study. Opioids inhibited adenylate cyclase activity in the 25,000 *g* but not in the 1600 *g* striatal particulate fractions (Bhoola & Pay, 1982), even under similar experimental conditions in which receptor (dopamine, PGE_2) and catalytic (NaF) activation of the enzyme could be readily achieved. All of the opiates and opioids tested on the striatal membrane-enriched fractions inhibited adenylate cyclase to a maximum of 20%–30% and this effect was antagonized by naloxone. The order of potency was fentanyl > DADLenk = β-endorphin > morphine > bremazocine. Thereby indicating a high functional relationship of adenylate cyclase with μ- and δ-opioid receptors and a low efficacy with κ subtypes. Opioid receptor coupling to adenylate cyclase could be demonstrated in the presence of GTP and NaCl. Our results support the previous experiments of Law *et al.* (1981) and Cooper *et al.* (1982) who have shown a similar requirement for both GTP and NaCl in striatal homogenates.

Opiates may regulate the release of dopamine since lesions of substantia nigra, which interrupt dopamine input into the striatum, lead to a loss of opioid receptors in the striatum. Morphine, DADLenk and β-endorphin inhibited the dopamine-stimulated adenylate cyclase in the striatal membrane-enriched fraction. The inhibitory action of opiates was not antagonized by (+)-butaclamol, suggesting a direct effect mediated through the N_i protein and not through the dopamine receptor as proposed by Govani *et al.* (1975). Although morphine did not significantly alter basal cyclic AMP levels in striatal slices, elevation by dopamine was decreased by morphine. This finding supports the report of Minneman (1977) indicating

that dopamine, but not isoprenaline, adenosine or prostaglandin-induced elevation of cyclic AMP, is inhibited by morphine in the striatum.

Opiate receptor-linked adenylate cyclase in vasa deferentia membranes

The present study appears to be the first detailed account of the receptor coupling of nucleotide cyclase and turnover of cyclic nucleotide in the vas deferens. Our results indicate that adenylate cyclase is coupled through N_s to β-adrenoceptors, prostaglandin E_1 and dopamine, and through N_i to α_2-adrenoceptors, opioid and muscarinic receptors (see Figure 7). Another aim of this study was to delineate the opiate subtype coupled to adenylate cyclase in vas deferens membranes. β-Endorphin is known to be released by the pituitary gland into the circulation (Guillemin, 1977), and although there is little information as to where it may exert its action, any peripheral organ possessing opioid receptors could respond physiologically. The vas deferens of the rat is sensitive to β-endorphin (Lemaire *et al.*, 1978), and although enkephalin itself is less potent, its analogue DADLenk is almost as active as β-endorphin. Our study confirms the insensitivity of the rat vas deferens to morphine (Hughes *et al.*, 1975). Adenylate cyclase in the membrane enriched fraction of the vas deferens was inhibited by β-endorphin and DADLenk in the presence of GTP and Na^+; whereas morphine, bremazocine and tifluadom were without effect. This inhibition of adenylate cyclase was reversed by naloxone and by a selective δ-receptor antagonist (ICI 154129). Our results are in agreement, therefore, with the physiological experiments of Lemaire *et al.* (1978) who, using field stimulation, reported sensitivity of the vas to β-endorphin and DADLenk but not to morphine. These observations led us to suggest that the predominant opioid subtype coupled to adenylate cyclase in the rat vas deferens was the δ-receptor (Bhoola & Pay, 1983). Clearly the opioid receptor subtypes coupled to adenylate cyclase in neuronal tissue (striatum) may differ from the subtypes coupled to peripheral membranes (vas deferens).

Functional importance of opiate inhibition of adenylate cyclase

Ever since the demonstration of opioid receptors and the occurrence of endogenous opioids, experiments have been concerned mainly with the selective opioid receptor binding ligands and less so with molecular events responsible for physiological events initiated by opioid receptor occupancy. The importance of opioid inhibition of adenylate cyclase is as yet unknown. Opioids are known to have many different effects *in vivo* and it is possible that some of these are mediated through activation of adenylate or guanylate cyclase. An essential aspect for the expression of inhibition of adenylate cyclase is the requirement for GTP and Na ions. In addition to a primary action, opioid inhibition of basal and receptor stimulated enzyme suggests a modulatory role. A number of peptides are known to co-exist with classical neurotransmitters and may be co-released to modulate synaptic function. In addition, opioids are believed to play a regulatory role in the release of known neurotransmitters. For instance, opioids inhibit substance P release from the trigeminal nucleus (Jessel & Iversen, 1977). Should such an effect be associated with both reduced neuronal formation of cyclic AMP and initiation of analgesia, then the opioid occupancy of specific receptor subtypes coupled to adenylate cyclase could influence nociception.

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