Opiates and Endogenous Opioid Peptides
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DUAL REGULATION OF ADENYLATE CYCLASE BY ENDOGENOUS OPIATE PEPTIDES

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Morphine and related narcotics affect adenylate cyclase in two ways, both mediated by the opiate receptor. The first process is the inhibition of adenylate cyclase activity (1-3). The second phenomenon is a delayed increase in the specific activity of the enzyme, which compensates for the inhibitory action of the narcotics, but requires hours of exposure to the drugs before being expressed (4,5). This dual regulation of adenylate cyclase has been proposed to account for opiate tolerance and dependence, as well as for their acute effects (4).

The discovery and characterization of endogenous opiate peptides (6) prompted us to ask whether these substances also inhibit adenylate cyclase and evoke a delayed increase in enzyme activity. Our studies have shown that methionine enkephalin (Tyr-Gly-Gly-Phe-Met), and other endogenous opiate peptides, are potent, receptor mediated, inhibitors of adenylate cyclase activity in homogenates of neuroblastoma x glioma NG108-15 hybrid cells. Furthermore, upon prolonged incubation of NG108-15 cells with Met-enkephalin, the specific activity of adenylate cyclase is increased. Thus, Met-enkephalin causes both the immediate inhibition and the delayed increase in adenylate cyclase activity characteristic of dual regulation.

The high potency of Met-enkephalin as an inhibitor of basal and PGE_1 stimulated adenylate cyclase in homogenates of NG108-15 cells is shown in Fig. 1. The concentrations of Met-enkephalin required to inhibit both basal and PGE_1 stimulated adenylate cyclase half-

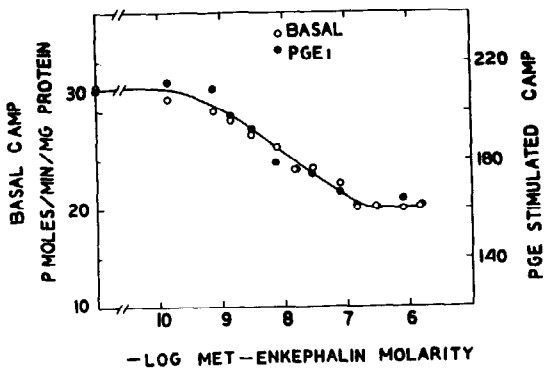

Fig. 1. Inhibition of basal — o — , and PGE_1 (10 μM) stimulated — • — , adenylate cyclase activity of homogenates (97 μg protein/tube of neuroblastoma x glioma NG108-15 cells by Met-enkephalin. Assays were performed as described by Sharma et al (2) except that incubations were for three minutes. Materials used in this work are as described elsewhere (8).

maximally, along with similar data for Leu-enkephalin, morphine, and etorphine (2), are shown in Table 1. Clearly, the enkephalins are of very high potency in this assay, as they also are in the mouse vas deferens (6). Indeed, in the adenylate cyclase assay, Met-enkephalin is comparable to etorphine in activity.

Inhibition of adenylate cyclase by Met-enkephalin is receptor mediated since it is reversed by naloxone, as shown in Fig. 2. As expected for a competitive interaction at the opiate receptor, the concentration of naloxone required to reverse the inhibitory action of Met-enkephalin increases with increasing peptide concentration. The dissociation constant for naloxone, K_e, calculated from these data by the dose-ratio method (7), is 30 nM, in good agreement with the value of 20 nM calculated from similar experiments with morphine (8), instead of enkephalin, or obtained by direct measurement of naloxone binding affinity for the opiate receptor (2).

TABLE 1

ACTIVITY OF PEPTIDE AND OTHER NARCOTICS AS INHIBITORS OF ADENYLATE CYCLASE IN NG108-15 HOMOGENATES(a)

Compound	K_i (b) (nM)	
	Basal	PGE_1 (c)
Tyr-Gly-Gly-Phe-Met (met-enkephalin)	12	20
Tyr-Gly-Gly-Phe-Leu (leu-enkephalin)	40	100
Morphine	1500	1500
Etorphine	10	-

(a) Assays performed as described previously (2).
(b) Concentration of inhibitor required for 50% of maximal effect.
(c) 10 μM in assays.

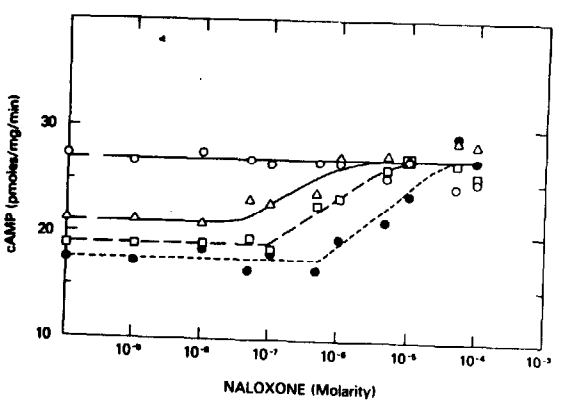

Fig. 2. The relation between naloxone concentration and the reversal of adenylate cyclase inhibition in homogenates (90 μg protein/tube) of NG108-15 cells by Met-enkephalin at the following concentrations: none, —o—; 10 nM —△—; 100 nM —□—; 1000 nM — • —.

The inhibitory action of Met-enkephalin on NG108-15 adenylate cyclase is short lived, typically only 15 minutes or so (Fig. 3). Note that morphine continues to inhibit the activity of the enzyme for at least 90 minutes. The short duration of action of Met-

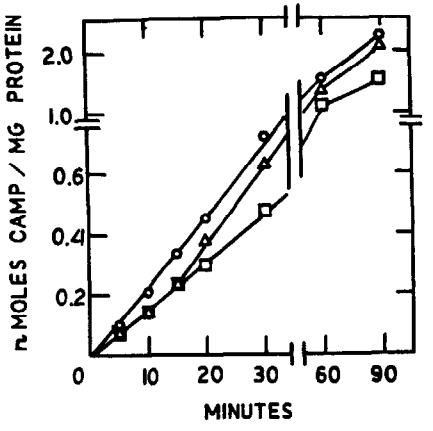

Fig. 3. Adenylate cyclase activity of a homogenate (111 μg protein/tube) of NG108-15 cells, as a function of time. Additions to the standard assay mixture were as follows: none —o—; Met-enkephalin, 0.16 nM —△—; morphine, 20 μM —□—.

enkephalin is due to its destruction during the incubation rather than to desensitization since the enzyme is inhibited by a freshly added portion of methionine enkephalin after 25 minutes of pre-incubation with the peptide (Table 2). The relative lability of Met-enkephalin activity compared with that of morphine is in agreement with results of analgesic assays of these materials in intact animals (9-11), and with tests performed with the guinea pig ileum preparation (6).

Incubation of cultures of NG108-15 cells for 12 or more hours with methionine enkephalin results in a marked increase in the specific activity of adenylate cyclase as shown in Table 3. In view of the lability of Met-enkephalin, the experiments shown were performed with a 100 fold excess of peptide over that required to saturate the receptors, and the medium was changed twice daily. Control experiments showed that approximately 90% of the enkephalin activity in such cultures is destroyed in 12 hours of incubation, and so the

TABLE 2

ADENYLATE CYCLASE ACTIVITY OF HOMOGENATES OF NG108-15 CELLS PREINCUBATED WITH AND WITHOUT MET-ENKEPHALIN

Additions to assay	Adenylate cyclase activity pmoles cAMP/min/mg protein	
	Preincubation	
	Control	Enkephalin
Water	16.4	16.5
Naloxone (10 μM)	16.4	17.2
Met-enkephalin (0.16 μM)	11.5	11.5

Homogenates of NG108-15 cells (111 μg protein/tube) were pre-incubated at 37° in the standard adenylate cyclase assay mixture, omitting only radioactive ATP, for 25 min, with and without 0.16 μM Met-enkephalin. Control experiments showed that adenylate cyclase was inhibited by enkephalin for less than 20 minutes. After 25 min, 1 μCi of [α^{32}P] ATP was added to each tube along with other additions as shown in column 1 of the table. Incubations at 37° were continued for an additional 5 min and [^{32}P] cAMP formed during this interval was determined in triplicate.

amount of enkephalin present is in excess throughout the experiment (12). Note that the extent of the increase in adenylate cyclase activity elicited by enkephalin is similar to that found with etorphine; the time dependence is also similar.

The experiments described here demonstrate that an endogenous opiate peptide acts as a dual regulator of adenylate cyclase exactly as morphine and other conventional narcotics. Thus, these neurohormones, or transmitters, can be expected to inhibit adenylate cyclase activity of neurons with opiate receptors and thereby suppress the effects of other neurohormones or transmitters which activate adenylate cyclase. As a result of the delayed increase in adenylate cyclase activity caused by the endogenous opiates, some neurons may be rendered supersensitive to the effects of agents which stimulate the enzyme. Shifts in the levels of endogenous opiate peptides may increase or decrease the flow of information

TABLE 3

ADENYLATE CYCLASE ACTIVITY IN HOMOGENATES OF NG108-15 CELLS CULTURED FOR THE TIMES SHOWN WITH MET-ENKEPHALIN OR ETORPHINE

Hours of treatment	Adenylate cyclase activity pmoles cAMP/min/mg protein		
	Control	Met-enkephalin	Etorphine
0	7	-	-
12	9	17	19
25	11	19	22
48	15	23	31
97	17	38	43

Logarithmically growing cultures of NG108-15 cells were maintained with additions to the culture media as follows: Met-enkephalin, 10 μM, and etorphine, 1 μM. Media were changed twice daily, and cells were harvested, homogenized, and assayed for basal adenylate cyclase activity in the presence of 100 μM naloxone. PGE_1 stimulated adenylate cyclase activities also were increased, although to a smaller extent.(12).

through specific neural circuits, and also regulate the responses of adenylate cyclase to other kinds of receptor mediated reactions. Cellular tolerance and dependence induced by the endogenous opiate peptides may play a normal role in switching certain neural pathways on or off.

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