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Tolerance and dependence evoked by an endogenous opiate peptide

(adenylate cyclase/methionine-enkephalin/narcotic dependence/neuroblastoma × glioma hybrid cells)

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ABSTRACT **Incubation of neuroblastoma × glioma hybrid cells for 12–97 hr with methionine-enkephalin results in an increase in adenylate cyclase activity [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] that is mediated by the opiate receptor. The results show that cells become tolerant to, and dependent upon, enkephalin.**

The members of a recently discovered family of peptides, which includes methionine-enkephalin (Tyr-Gly-Gly-Phe-Met) and leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu), behave as opiates in pharmacologic tests (1–7). The enkephalins‡, like morphine and other narcotics, are potential inhibitors of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] in cells that possess opiate receptors (8–11). As previously reported, exposure of neuroblastoma × glioma hybrid cells to morphine for 12–48 hr also results in a compensatory increase in adenylate cyclase activity (11, 12). Cells then have normal 3′:5′-cAMP levels and appear tolerant to morphine because the increase in adenylate cyclase activity is approximately equal to the inhibition. The cells also are dependent upon morphine to maintain normal cAMP levels because withdrawal of morphine, or displacement of the narcotic from the opiate receptor by the antagonist naloxone, reverses the inhibition and results in the synthesis of abnormally high levels of cAMP. Thus, the dual regulation of adenylate cyclase by narcotics accounts for the phenomena of narcotic dependence and tolerance.

We report here that incubation of NG108-15 hybrid cells for 12–97 hr with methionine-enkephalin results in an increase in adenylate cyclase activity that is mediated by the opiate receptor. The results show that cells become tolerant to, and dependent upon, enkephalin and suggest that tolerance and dependence are normal responses which regulate transynaptic communication coupled to adenylate cyclase activity.

MATERIALS AND METHODS

The source of each chemical and the medium and growth conditions for neuroblastoma × glioma hybrid NG108-15 cells were described previously (11), except that the medium contained 3% fetal bovine serum.

Adenylate cyclase assays were performed as described (10) with the following modifications: washed cell suspensions were stored at −191°; immediately before use frozen cell suspensions were thawed and homogenized in a motor-driven Potter-Elvejhem homogenizer at 1400 rpm for 40 sec.

RESULTS AND DISCUSSION

As shown in Fig. 1, exposure of cells to enkaphalin for 12–97 hr results in an increase in the specific activity of adenylate cyclase. The basal activity of the enzyme is increased in a parallel manner in the presence of either 10 μM enkephalin or 1 μM etorphine. Neither enkephalin nor etorphine affects the growth rate of the cells (data not shown). Adenylate cyclase activity stimulated by prostaglandin E_1 also is increased by incubation of cells in the presence of enkephalin or etorphine. The basal specific activity of adenylate cyclase continues to rise for 97 hr in the presence of enkaphalin or etorphine, whereas prostaglandin-E_1-stimulated activity ceases to increase after 12–25 hr. The relative increase in specific activity of basal adenylate cyclase is greater than that observed in the presence of prostaglandin E_1. Thus, the effects of methionine-enkephalin closely resemble those found with morphine (11). The increase in adenylate cyclase activity shows that NG108-15 cells exposed to methionine-enkephalin for 12 hr acquire tolerance to this compound as defined by the model of Sharma *et al.* (11).

In view of the short duration of action of enkephalin in many systems (13, 15), which may be due to rapid degradation, the experiments described in Fig. 1 were performed with 10 μM enkephalin, 100 times the concentration required to achieve maximum inhibition of adenylate cyclase‡, and the medium was changed twice daily. This regimen maintained saturating concentrations of enkephalin throughout the course of the experiment. As shown in Fig. 2, incubation of confluent cultures of NG108-15 with 10 μM methionine-enkephalin resulted in the rapid disappearance of methionine-enkephalin (50% loss in 90 min). The enkephalin concentration remaining after 12 hr of incubation was still 10 times the amount needed for maximum inhibition of adenylate cyclase.

Naloxone (50 μM) prevents the inhibition of adenylate cyclase by 1 μM enkephalin‡. This concentration of naloxone also prevents the increase in adenylate cyclase activity produced by exposure of cells to 1 μM methionine-enkephalin for 24 hr as shown in Table 1. Naloxone is a pure narcotic antagonist which binds to the opiate receptor but does not affect adenylate cyclase activity. Blockade by naloxone shows that the enkephalin-dependent increase in adenylate cyclase activity is mediated by the opiate receptor.

In cells which become tolerant to a narcotic, basal cAMP levels are normal or approach the normal level in the presence of the narcotic (11). The level of cAMP would be lowered by increasing the concentration of an opiate peptide, or elevated by decreasing the opiate concentration. The elevation in cAMP levels in tolerant cells due to a reduction in opiate peptide concentration would resemble the effect of an activator of adenylate cyclase because cAMP levels would rise above the basal value. Thus, the opiate peptides that are inhibitors of adenylate cyclase can also mimic the effects of activators of this enzyme.

Several other peptides with opiate activity have been isolated (3, 5–7). Those whose structures are known contain the amino acid sequence of methionine-enkephalin at the amino terminus.

Abbreviations: cAMP, adenosine 3′:5′-cyclic monophosphate; PGE_1, prostaglandin E_1.

‡ W. A. Klee and M. Nirenberg, submitted for publication.

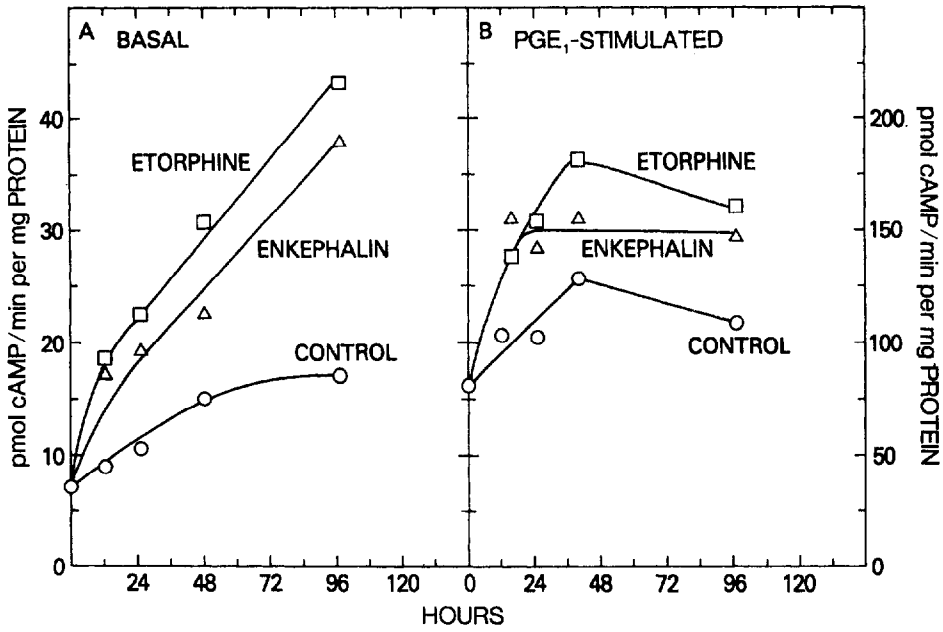

FIG. 1. Basal (A) and prostaglandin E_1 (PGE_1)-stimulated (B) adenylate cyclase activity of homogenates of NG108-15 cells cultured for the times shown in the presence of 10 μM methionine-enkephalin (△), 1 μM etorphine (□), or in the absence of these compounds (○). NG108-15 cells were grown in 60 mm petri dishes (20 cm^2 surface area) as described (11), except that 3% fetal bovine serum was used. The medium was changed twice daily. The cells in the early logarithmic growth phase (0.4 mg of protein per plate at zero hours and 2.4 mg of protein at 97 hr) were harvested at the times shown.

Each of these peptides, including methionine-enkephalin, corresponds to a portion of the carboxyl-terminal sequence of β-lipotropin (5–7). Some have a much longer duration of action than enkephalin, and a long-acting opiate peptide should be more effective in producing cellular tolerance and dependence. Using the method of Teschemacher *et al.* (3), we have prepared from crude adrenocorticotropic hormone a peptide with a molecular weight of 3000–4000 by gel filtration. This peptide, when added to cultures of NG108-15 hybrid cells for 4 days, elicits an increase in adenylate cyclase activity. The increase in activity is blocked by naloxone in the manner demonstrated with enkephalin and other opiates. The finding that at least two opiate peptides induce cellular tolerance and dependence suggests that these phenomena represent normal mechanisms which enable opiate peptides to regulate the efficiency of communication across certain synapses. Shifts in the levels of endogenous opiate peptides may increase or decrease the activity of specific neural circuits by regulating the responses of adenylate cyclase to different kinds of receptor-mediated activators of the enzyme.

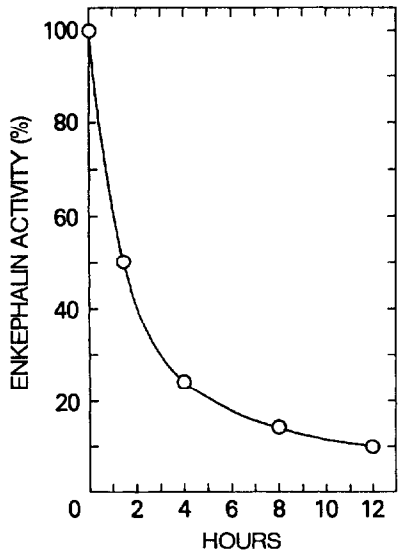

FIG. 2. Methionine-enkephalin activity remaining in filtrates of NG108-15 cultures exposed to 10 μM enkephalin for the times shown. Quadruplicate confluent cultures (60 mm petri dishes) were used for each time point. The media were removed and 2.5 ml portions were passed through Millipore filters (GS 0.22 μm pores, 25 mm diameter). After dilution with 9 volumes of water, 5 μl aliquots were assayed in standard 100 μl reaction mixtures for inhibition of NG108-15 adenylate cyclase activity in the presence and absence of 100 μM naloxone. Methionine-enkephalin concentration was proportional to naloxone-reversible inhibition of adenylate cyclase activity within the range of 2–50 nM.

Table 1. Blockade by naloxone of methionine-enkephalin-induced increase in adenylate cyclase activity

Additions	Adenylate cyclase activity, pmol cAMP/min per mg of protein	
	Basal	PGE_1-stimulated
Control	14.2	158
Met-enkephalin	20.2	203
Met-enkephalin + naloxone	16.3	149

NG108-15 cells were cultured for 24 hr where indicated with 1 μM enkephalin, 50 μM naloxone, or water. Each value represents the average specific activity of six homogenates, each assayed at three to four protein concentrations.

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