INHIBITION BY BETA-ENDORPHIN OF DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN RAT STRIATUM

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SUMMARY. Beta-LPH 61-91 (beta-endorphin) inhibited the basal and dopamine-stimulated adenylate cyclase activity in a homogenate of rat striatum. Naloxone prevented this effect.

Endogenous peptides with opiate activity (endorphins) have been isolated and identified in animal brains and pituitaries (1-4). In addition to binding to brain opiate receptors and inhibiting of electrically stimulated contractions of mouse vas deferens or guinea pig ileum, beta-LPH 61-91 (beta-endorphin) has some actions on the central nervous system and can produce analgesia and catatonia (5-8). In our previous report (6), we demonstrated the involvement of a dopaminergic mechanism in the cataleptic effect of beta-LPH 61-91. Recent studies (9,10) have suggested the involvement of cyclic nucleotides in the cellular action of endorphins. Dopamine, a putative neurotransmitter, was shown to increase intracellar cyclic AMP levels in rat striatum (11). An inhibitory effect of morphine on dopamine-stimulated adenylate cyclase activity has been shown (12,13). In the present study, we observed the effect of beta-LPH 61-91 on the dopamine-stimulated adenylate cyclase (EC 4.6.1.1.) activity in rat striatum.

MATERIALS AND METHODS

Male Sprague Dawley rats weighing 200-250 g were used. The striatum were dissected by the method of Glowinski and Iversen

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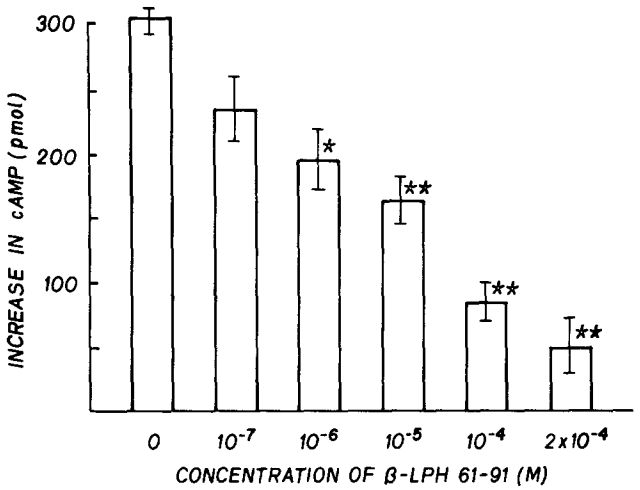

Figure 1. Effect of beta-LPH 61-91 on the dopamine-stimulated adenylate cyclase activity in a homogenate of rat striatum. The reaction mixtures contained 200 uM dopamine. In the absence of dopamine and beta-LPH 61-91, 370 ± 2 pmol of cyclic AMP was formed. The increase in cyclic AMP above this basal level is plotted. The data represent the mean ± SE from triplicate determinations.
* $P < 0.05$, ** $P < 0.01$

(14). The striatum were homogenized in 13 volumes of 2mM tris-maleate buffer (pH 7.4)-2mM EGTA.

Adenylate cyclase activity was determined by a modification of the method of Solomon et al. (15). Each tube contained 80mM tris-maleate (pH 7.4), 5mM $MgCl_2$, 20mM creatine phosphate, 10 u creatine phosphokinase, 1mM [alpha-^{32}P]-ATP (1-2 x 10^6 cpm), 1mM cAMP, 10mM theophylline and homogenate containing 200 ug of protein in a final volume of 100 ul. Incubation was for 5 min at 30°C. Cyclic AMP formed was purified using a combination of Dowex 50 and aluminum oxide chromatography. Data are presented as pmoles of cyclic AMP formed per mg protein per 5 min.

Beta-LPH 61-91 was isolated and purified as described previously (4). For statistical analysis of the results, Newman-Keuls multiple range test (16) was used.

RESULTS

Beta-LPH 61-91 inhibited dopamine-stimulated adenylate cyclase activity in a homogenate of rat striatum (Fig. 1). 10^{-6}, 10^{-5}, 10^{-4} and 2 x 10^{-4}M beta-LPH 61-91 caused progressively increased inhibition of cAMP production. Fifty % inhibition was achieved at a beta-LPH 61-91 concentration of just over 10^{-5}M.

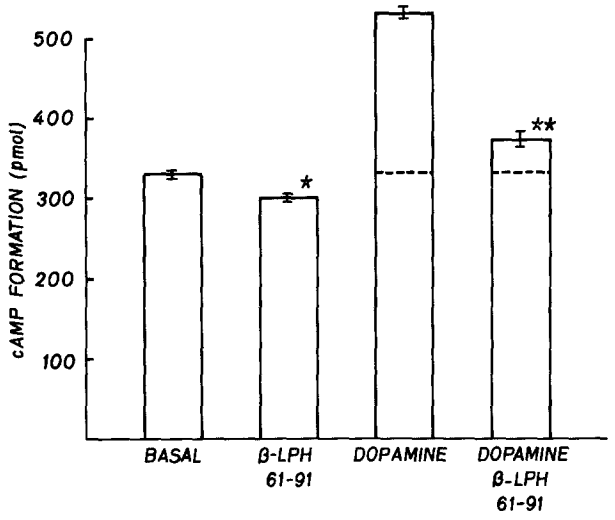

Figure 2. Effect of beta-LPH 61-91 on the basal and dopamine-stimulated adenylate cyclase activity in a homogenate of rat striatum. 200 uM beta-LPH 61-91 and 100 uM dopamine were used. Data represent the means ± SE from triplicate determinations.
* $P < 0.01$: basal vs beta-LPH 61-91
** $P < 0.01$: dopamine vs dopamine + beta-LPH 61-91.

In addition to the effect on the dopamine-stimulated enzyme, beta-LPH 61-91 showed a slight but significant inhibition of basal adenylate cyclase activity (Fig. 2). 2×10^{-4}M beta-LPH 61-91 reduced basal activity to about 90% of the control value.

The inhibitory effect of beta-LPH 61-91 on the dopamine-stimulated adenylate cyclase activity was prevented by naloxone (Fig. 3). 2×10^{-4}M naloxone completely prevented the inhibition of adenylate cyclase by 10^{-6} and 10^{-5}M of beta-LPH 61-91 and partially prevented the inhibition by 10^{-4}M beta-LPH 61-91 ($P < 0.01$)

DISCUSSION

Our results suggest a direct antagonistic action of beta-LPH 61-91 on dopaminergic neurones. The idea of involvement of opiate receptors in cyclic AMP regulation is also supported by our results.

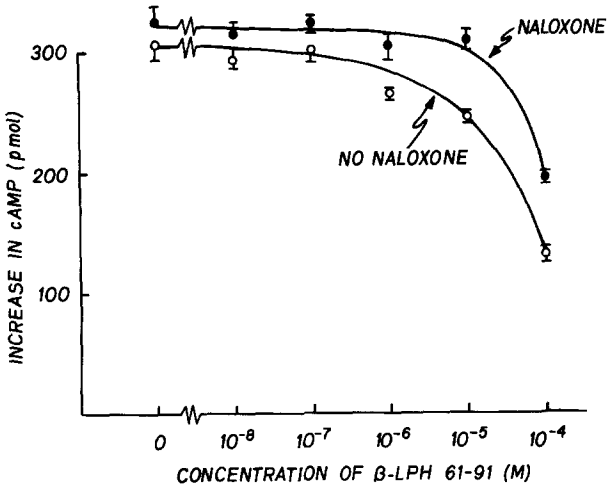

Figure 3. Effect of beta-LPH 61-91, alone or in combination with 100 uM naloxone, on the dopamine-stimulated adenylate cyclase activity in a homogenate of rat striatum. Reaction mixtures contained 100 uM dopamine. In the absence of dopamine, beta-LPH 61-91 and naloxone, 379 ± 2 pmol of cyclic AMP was formed. The increase in cyclic AMP above this basal level is plotted. Data represent the means ± SE from triplicate determinations.

In a previous report (6), we demonstrated the antagonistic effects of L-DOPA on beta-LPH 61-91-induced catalepsy in rat. Apomorphine, a specific blocker of the dopamine receptor, has also been shown to abolish the cataleptic effect of beta-LPH 61-91 in rats (17).

The present results are consistent with the earlier observations. The ED50 of beta-LPH 61-91 for catalepsy was determined as 5 nmol per rat when given intraventricularly. The concentration in cerebrospinal fluid following this dose would be about 5 uM, if it were evenly distributed in 1 ml of cerebrospinal fluid. The present experiment shows that concentrations of beta-LPH 61-91 over 1 uM significantly changed the dopamine-sensitive adenylate cyclase activity in vitro.

Morphine-like drugs were already reported to inhibit the stimulation by E_1 and E_2 prostaglandins of cyclic AMP formation by rat brain homogenate (24). Collier and Roy (24), however, did not observe any change of basal production of cyclic AMP by morphine. In our present work we report a slight but significant decrease in basal production of cyclic AMP by beta-LPH 61-91. This difference could be probably explained by the differences in methods used and by the use of rat striatum instead of whole brain homogenate (24).

The inhibition by beta-LPH 61-91 of the basal activity in rat striatum is less than that obtained by Klee and Nierenberg (10), who showed a marked inhibition (by methionine-enkephalin) of the basal adenylate cyclase activity in neuroblastoma x glioma hybrid cells. This difference may be due to the lower density of opiate receptors in the striatum than in the hybrid cells. Recently Loh et al. (18) also reported the inhibitory effect of beta-LPH 61-91 on dopamine release in rat striatum. Extrapyramidal symptoms such as akinesia and muscular rigidity are known to involve a dopaminergic mechanism. Parkinson's syndrome with akinesia and rigidity as the main symptoms involves the hypofunction of dopaminergic neurones in the nigro-striatal pathway (19). Endorphins therefore, may play a pathophysiological role in Parkinson's syndrome and related disorders.

The involvement of a dopaminergic mechanism in schizophrenia has been extensively discussed (20,21). Inhibitory effects of antipsychotic drugs on the dopamine-sensitive adenylate cyclase have been shown in rat striatum (22). Hyperactivity of a dopaminergic mechanism in the brain has therefore been suggested to be involved in the pathogenesis of schizophrenia. Our results indicate the possibility of decreased activity of endorphins in the brain of some schizophrenic patients. Gunne

et al. (23), however, reported a benefical effect of naloxone on the auditory hallucination in schizophrenia. This result seems to contradict our speculation. More extensive studies will be required to elucidate the real pathophysiological role of endorphins.

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