ENDORPHINS EXERT OPIATE-LIKE ACTION ON NEUROBLASTOMA × GLIOMA HYBRID CELLS

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1. Introduction

Leucine and methionine–enkephalin [1] and α-, β- and γ-endorphin [2–6] are peptides with opiate-like activity that have been isolated from brain. With the exception of leucine–enkephalin they appear to be fragments of β-lipotropic hormone (β-LPH). Like morphine they bind to opiate-receptors [3,5,7,8], inhibit the electrically stimulated contraction of guinea-pig ileum [1,3,9] and cause analgesia [6,11] and tolerance [12–14]. In addition, in rats γ-endorphin was demonstrated to enhance irritability [15] and β-endorphin to cause long-lasting catatonia [15, 16]. All these effects of the opioid peptides can be prevented by the opiate-antagonist naloxone [1–3, 9–11,15,16]. This confirms the notion that the opioid peptides act by binding to opiate receptors.

Opiates [17,18], the enkephalins [19,20] and some of their analogues [21,22] have also been demonstrated to act on the opiate-receptors of neuroblastoma × × glioma hybrid cells in culture. These cells exhibit many properties characteristic of neurons [23–25]. In the presence of prostaglandin E_1 (PGE_1) the concentration of adenosine 3′5′-cyclic monophosphate (cyclic AMP) is increased in the hybrid cells [26]. The rise in the level of cyclic AMP is much more strongly suppressed by the two equipotent enkephalins than by opiates [19]. Here we communicate that on the hybrid cells α-, β- and γ-endorphins act qualitatively similar to methionine-enkephalin, although they are less potent (potency: methionine–enkephalin > α- and β-endorphin > γ-endorphin). A preliminary report of some of the data has appeared [27].

2. Materials and methods

Methionine–enkephalin (β-LPH-[61–65]) and leucine–enkephalin were synthesized by Moroder and Wünsch [19,28], α-endorphin (β-LPH-[61–76]) [2], β-endorphin (β-LPH-[61–91]) [3] and γ-endorphin (β-LPH-[61–77]) [3] were generous gifts from Drs R. Guillemin and N. Ling. Naloxone · HCl and PGE_1 were gifts from Hoffman-LaRoche, Grenzach, Germany, and Dr J. Pike, Upjohn Co., Kalamazoo, Michigan, respectively. 2.4×10^5 viable (exclusion of nigrosin) neuroblastoma × glioma hybrid cells 108CC15 were seeded onto plastic dishes (85 mm in diameter, containing 20 ml of growth medium) and cultured as described [29]. Four days later the medium was removed, the cells were washed once with 5 ml of incubation medium (37°C) and, unless stated otherwise, were incubated (37°C, 10 min) with 5 ml of this medium containing PGE_1 (0.3 μM) and the peptides. Thereafter, the intracellular concentration of cyclic AMP was determined [29].

3. Results

Like methionine-enkephalin, also the three endorphins inhibit the action of PGE_1 (fig.1). The endorphins are, however, less potent than the enkephalins. The concentrations at which the effect of PGE_1 is inhibited by 50% (IC_{50}) increases from 1 nM for methionine-enkephalin to 5, 10 and 40 nM for β-, α-, and γ-endorphin, respectively. From one experiment to another these values may vary within the range of approximately half an order of magnitude.

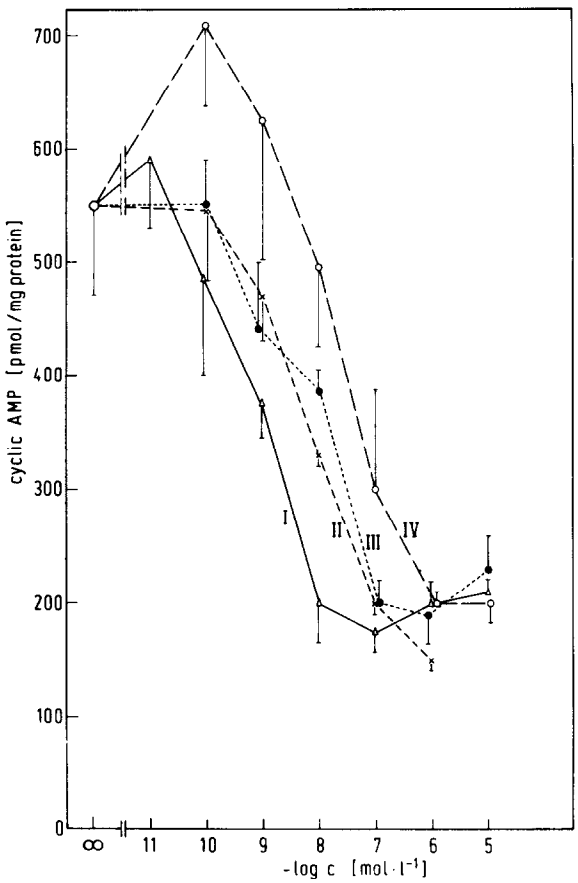

Fig.1. Methionine–enkephalin (curve I), β-endorphin (curve II), α-endorphin (curve III) and γ-endorphin (curve IV) inhibit the increase in the level of cyclic AMP caused by PGE_1. Mean values ± s.d. from 3 parallel incubations. 1.6×10^6 viable hybrid cells, viability 91%, passage number 13.

The effects of the endorphins can be blocked by the opiate antagonist naloxone. In table 1 this is exemplified for β-endorphin. As positive controls, similar data for leucine-enkephalin are included. Previous experiments had demonstrated that enkephalins are slowly inactivated, probably by proteolysis, when exposed to the hybrid cell culture [19,20, 30]. The possibility had to be considered that also the endorphins are degraded by proteolysis. This could imply that the endorphins are inactive peptides not affecting the formation of cyclic AMP in the hybrid cells. From the endorphins active peptides such as methionine–enkephalin could be generated during the 10 min incubation. From this hypothesis would follow that the endorphin would, at best, only slightly inhibit the effect of PGE_1 during the early phase of the incubation. The inhibition should increase with the time of the incubation. However, this is not the case. Like leucine–enkephaline, β-endorphin inhibits the action of PGE_1 already at the shortest time of incubation that was chosen (0.5 min; fig. 2).

4. Discussion

The experiments demonstrate that α-, β- and γ-endorphin act on neuroblastoma X glioma hybrid cells in a way comparable to that of the enkephalins and opiates. They inhibit the increase in the level of cyclic AMP evoked by PGE_1. Naloxone blocks the effects of the endorphins. This indicates that, in order to cause their effects, these peptides must bind to the opiate receptors on the hybrid cells. These results

Table 1
The opiate antagonist naloxone inhibits the action of β-endorphin. Mean values ± s.d. from 3 parallel incubations. 2.2×10^6 viable cells per plate, viability 92%, passage number 21

Addition (μM)	Cyclic AMP (pmol/mg protein)
none	19 ± 2
PGE_1 (0.3)	815 ± 40
PGE_1 + naloxone (10)	860 ± 45
PGE_1 + leucine–enkephalin (0.1)	200 ± 10
PGE_1 + leucine–enkephalin + naloxone	680 ± 60
PGE_1 + β-endorphin (0.01)	450 ± 45
PGE_1 + β-endorphin (0.1)	280 ± 90
PGE_1 + β-endorphin (0.01) + naloxone	880 ± 110
PGE_1 + β-endorphin (0.1) + naloxone	720 ± 90

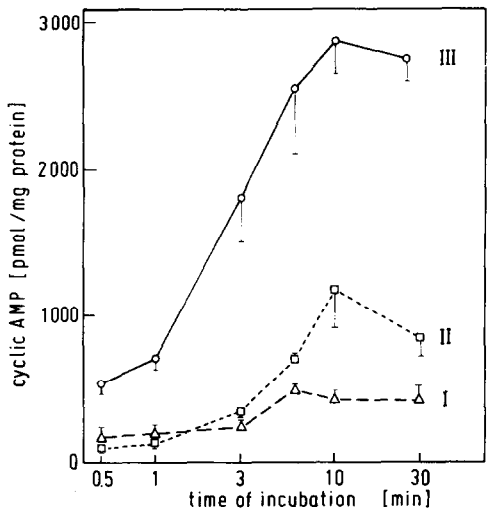

Fig.2. β-Endorphin (0.1 μM; curve I) and leucine–enkephalin (0.1 μM; curve II) inhibit the increase in the intracellular level of cyclic AMP evoked by PGE_1 (0.3 μM; curve III) in hybrid cells. In order to provide a better constancy of the pH value during the short-term incubations, the incubations were carried out in Dulbecco's Modified Eagle's Medium (pH 7.4), in which $NaHCO_3$ had been replaced by 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 19 mM NaCl. The alteration of the medium has no influence on the results. Note that for better display of the data at short times, the time values (abscissa) are plotted logarithmically. Mean values ± s.d. from 3 parallel incubations. 1.4×10^6 viable cells, viability 94%, passage number 15.

agree with the observations that the actions of the endorphins on small intestine [1] and brain [11,17, 18] are also antagonized by naloxone.

From the fact that β-endorphin inhibits already at short periods of incubation it is concluded that it acts directly on the cells without prior degradation to shorter peptides such as methionine–enkephalin. Probably this is also true for the other endorphins. In their influence on the hybrid cells the endorphins are approximately one order of magnitude less potent than the enkephalins. On the other hand it is comparable to that of two other fragments of β-LPH, β-LPH-[61–69] and β-LPH-[61–75] [21]. Thus, it appears that elongation at the carboxyl end of the peptide chain of enkephalin yields peptides of reduced potency. However, the potency does not decrease inversely to the length of the peptide chain. This is exemplified best by the fact that the potency of β-endorphin is higher than that of the shorter peptide γ-endorphin.

The neuroblastoma × glioma hybrid cells can be considered as a model system for neurons with which they have in common characteristic neuronal markers [24,25]. Thus, it is hypothesized that the mechanisms of action of opioid peptides observed with the hybrid cells will be found in neurons carrying opiate receptors.

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