IN VITRO LIPOLYTIC ACTIVITY OF PORCINE β-ENDORPHIN NOT MEDIATED BY AN OPIATE RECEPTOR

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1. Introduction

The morphine-like peptide β-endorphin (β-EP) is identical with the C-terminal amino acid sequence 61–91 of β-lipotropin (β-LPH), binds to opiate receptors and inhibits the PGE_1-stimulated increase of adenosine 3′5-cyclic monophosphate in neuroblastoma × glioma hybrid cells [1–4].

In man β-EP was demonstrated in cerebrospinal fluid [5] as well as in plasma, where it increased together with ACTH after methyrapone administration [6]. β-EP was also found in patients with endocrine disorders associated with increased ACTH and β-LPH production [7]. Plasma β-EP was 4–5 ng/ml in normal [8] and 7–10 ng/ml in adrenalectomized rats [9].

The release of growth hormone [10,11] and prolactin [11,12] is stimulated by intraventricular injection of β-EP in the rat. Intravenous injection of an enkephalin analogue in man raised serum growth hormone, prolactin and glycerol while lactate, alanine and glucose were decreased [13].

Here we communicate that porcine β-EP has lipolytic activity in nearly physiologic concentrations in rabbit adipocytes which is accompanied by an activation of adenylate cyclase in fat cell ghosts. Because these effects are not inhibited by naloxone and cannot be imitated by opiate agonists, we conclude that this effect of β-endorphin is not mediated by an opiate receptor.

2. Materials and methods

From lyophilized pig pituitary glands, fraction D′ was prepared as in [14], β-LPH as in [15,16] and β-EP as in [17]. β-LPH and β-EP were characterized by amino acid analysis (Liquimat 3, Fa. Kontron, Munich), N- and C-terminal amino acid determinations, SDS–polyacrylamide gel electrophoresis. The opiate activity of β-EP in the guinea pig ileum, rat and mouse vas deferens systems (tested by Dr R. Schulz) and the inhibitory effect on PGE_1-stimulated cAMP level in neuroblastoma × glioma hybrid cells (tested by Dr M. Brandt) were equal to synthetic β-EP.

Lipolysis in isolated rabbit fat cells was measured as described with slight modifications [18]. Fat cells were isolated from perirenal adipose tissue of 12 h starved animals and all samples ($7–9 \times 10^4$ cells/ml) were tested in triplicate for free glycerol content. Fat cell ghosts were prepared by the method in [19] and adenylate cyclase activity was determined according to [20]. c[^{32}P]AMP was purified by column chromatography with Dowex (AG 50- ×4, 200–400 mesh, H^+) and neutral aluminium oxide [20].

Naloxone, levorphanol, etorphine, morphine, dextrorphan, and Leu-enkephalin were gifts from Dr Hamprecht; bovine albumin fraction V was obtained from Fa. Roth, Karlsruhe (defatted by the method in [21]), c[^{3}H]AMP and [α-^{32}P]ATP from Fa. Amersham-Buchler, Braunschweig, enzymes and co-substrates from Boehringer Mannheim, collagenase type CLs II from Fa. Worthington, NJ. α-Endorphin was purchased from Fa. Serva, Heidelberg.

3. Results

Porcine β-EP significantly stimulated glycerol release

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in isolated rabbit fat cells from 8 of 12 animals in minimal concentrations of 1 nM. At 10 nM β-EP was lipolytic in all animals. No lipolytic activity could be demonstrated for levorphanol, etorphine, morphine, dextrorphan, Leu-enkephalin at 10 μM and α-endorphin at 100 nM in two animals. The lipolytic activity could not be inhibited by 10 μM naloxone in the adipocytes of 8 animals (fig.1).

In accordance with the lipolytic activity in isolated adipocytes β-EP significantly activated adenylate cyclase in fat cell ghosts in the range from 10 nM to 10 μM (fig.2). In 5 different experiments the ED_{50} was in the range from 0.07–0.94 μM. The activation of adenylate cyclase by β-EP was not significantly inhibited by 10 μM naloxone (fig.2). No activation of adenylate cyclase could be demonstrated by the opiate agonists listed above at 10 μM.

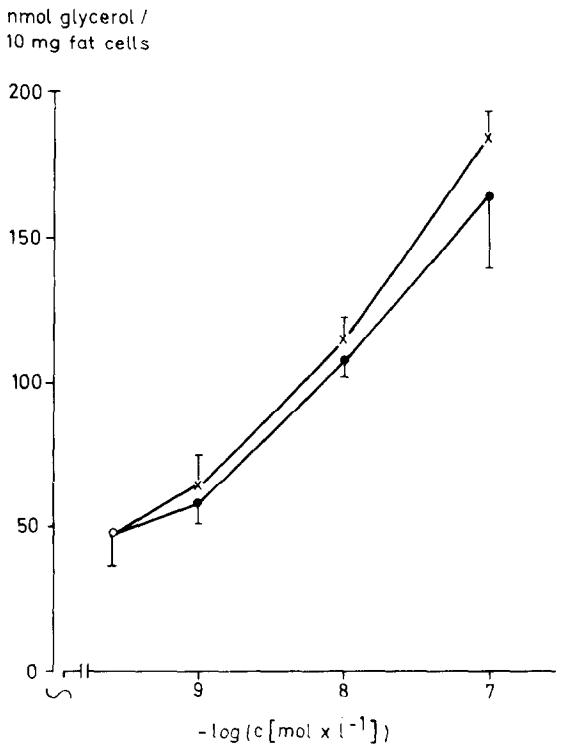

Fig.1. Lipolysis in isolated rabbit fat cells by β-EP (X——X) and β-EP + naloxone (10 μM) (•——•). Basal lypolysis (○). Mean values of triplicate determination ± SE of a typical experiment.

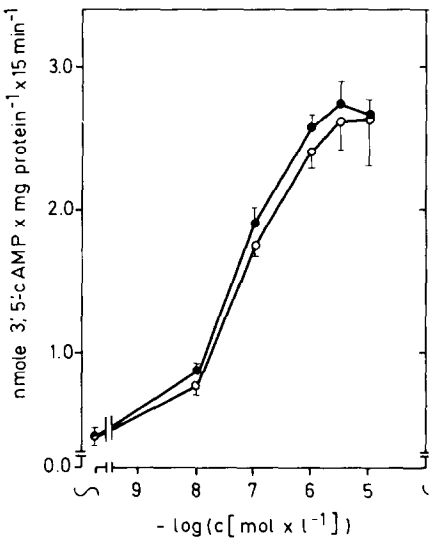

Fig.2. Activation of adenylate cyclase in rabbit fat cell ghosts by porcine β-endorphin (•——•). Mean values of triplicate determination ± SE of a representative experiment is shown (n = 5). Values of β-endorphin + naloxone (10 μM) (○——○) are compared (n = 2).

4. Discussion

The homogenous porcine β-EP preparations tested had the same or higher opiate activity in comparison to synthetic preparations in organ and cultured cell systems. Two new aspects of biological β-EP activity could be demonstrated in concentrations comparable to plasma levels in rodents [8,9,22]: the stimulation of adenylate cyclase and of lipolysis in adipocytes which could not be inhibited by naloxone and could not be initiated by opiate agonists.

The activation of adenylate cyclase in fat cell ghosts by β-EP is in contrast to the data from neural tissue where adenylate cyclase is inhibited by opioids [23]. Though the actual cAMP concentration remains to be determined it is suggested that the intracellular activation of lipolysis by β-EP is mediated by the cAMP pathway. This might correspond to the 2-fold increase of the intracellular cAMP concentration in the mouse prostate gland by morphine [24].

The β-EP preparations tested had a lipolytic activity comparable to synthetic β-EP [25] but were more potent than another porcine β-EP in rabbit fat pads

[26]. Lipolysis of β-EP obviously is not mediated by an opiate receptor because there is no inhibition by naloxone and no stimulation by several opiate agonists. So far an opiate receptor in adipose tissue has not been described in literature. Although there is good evidence for different types of opiate receptors [27,28], the lipolytic activity of β-EP in isolated adipocytes is more likely to be mediated by another kind of receptor. Further characterization of this peptide receptor is needed. Work is in progress to determine the lipolytically active sequence of β-EP. This is obviously located in the C-terminal part of the molecule, because neither Leu-enkephalin nor α-endorphin stimulated lipolysis.

From our data we conclude that the lipolytic and adenylate cyclase stimulating effect of β-EP is not mediated by an opiate receptor, which was shown to be responsible for other peripheral effects of β-EP.

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