Beta-Endorphin in various phases of menstrual cycle

THESIS

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BY

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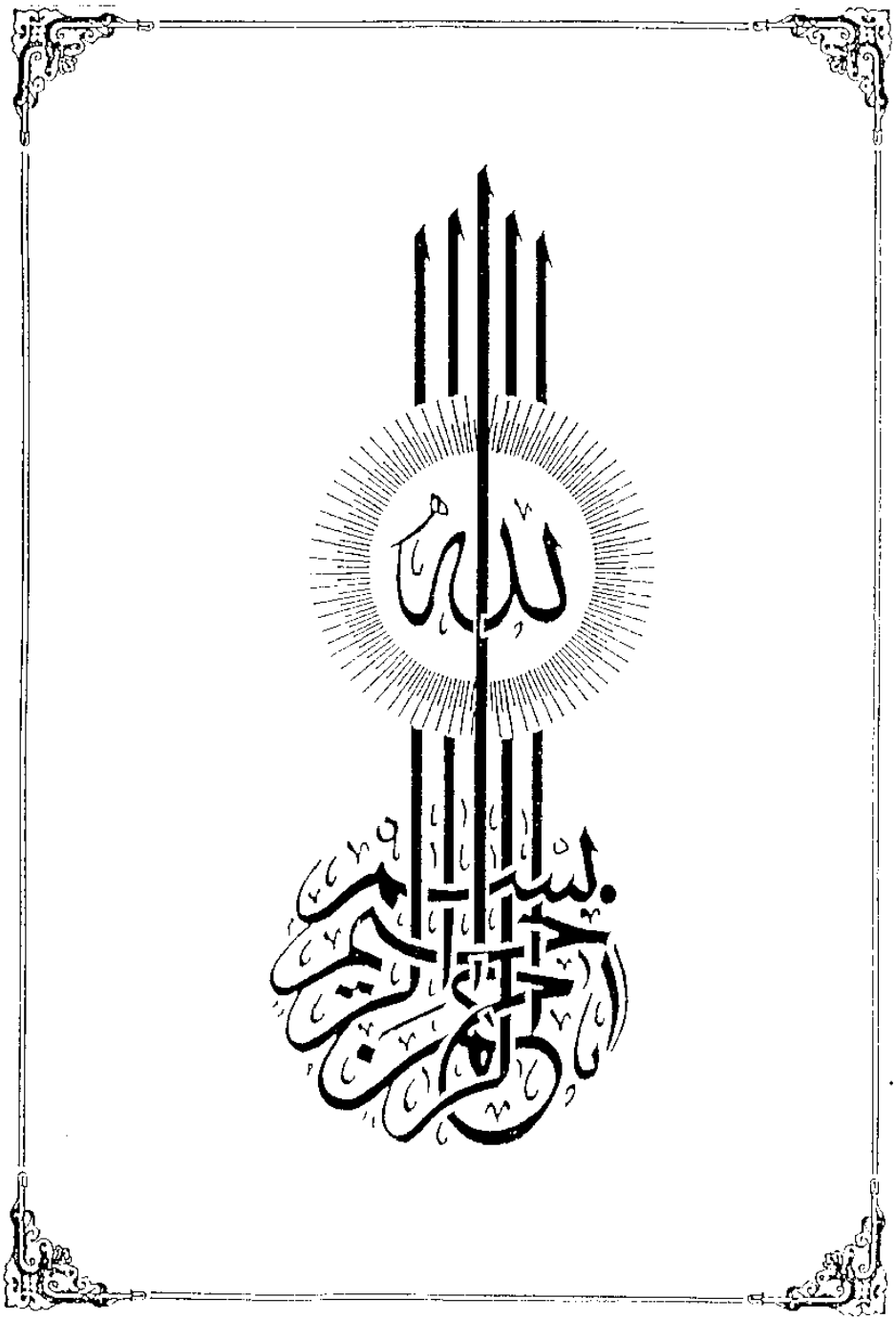

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Follicular Fluid Beta-Endorphin:

A Possible role in Follicle and Oocyte Maturation and Fertilization.

Introduction

Historical Review of Opioid Peptides

The ancient Sumerians, aware ..of the mood-elevating properties of the sticky juices that oozed from the flower seed capsule (later named opium), referred to the poppy as the "joy plant". It was prescribed by Greek and Roman physicians in 300 B.C. for various conditions. Early in the nineteenth century, Setürner isolated the morphine alkaloid from opium and named it after Morpheus, the Greek god of dreams. (*Bell and Malick, 1982*).

In an effort to synthesize a non-addictive analgesic, the structural requirements for the analgesic action of morphine were thoroughly studied. It was recognized that only the levorotatory isomer has an analgesic action. Moreover, parts of the morphine molecule, when changed, may lead to changes in the pharmacological potency; i.e., the replacement of the methyl group on the tertiary nitrogen with an allyl or cyclopropyl methyl group results in a potent specific antagonist to morphine's pharmacological action. Some antagonists retain some of their agonist properties (nalorphine and cyclozoceine), while others are pure antagonists (*Simon 1982*).

The recognition of the remarkable stereospecificity and structural constraints placed on the analgesic actions of the opiates led to the **receptor hypothesis**; i.e., there should be specific sites for opiates to bind to in order to trigger a physical or chemical step that results in the response observed. (*Simon 1982*).

The idea that the brain may possess its own analgesic system (the "body's own morphine") has been suggested even prior to the biochemical demonstration of opiate receptors, and was supported by the discovery that analgesia could be evoked by electrical stimulation of the brain (*Mayer and Hayes 1975*).

It was only in 1973 that three groups of investigators (*Simon et. al. 1973, Pert and Synder 1973, and Terenius 1973*) reported the presence of high-affinity

stereospecific opiate receptors in mamallian brain homogenate The presence of these brain receptors initiated the search for a natural ligand.

Later, Akil and co-workers, 1976, were able to demonstrate that the specific opiate antagonist naloxone partially reverses the analgesia produced by electrical stimulation of brain areas such as periaqueductal gray, suggesting that electrically stimulated analgesia was mediated in part by endorphins. Moreover, tolerance was shown to develop to this phenomenon (*Mayer and Hayes 1975*).

Reports of endogenous opiate activity in aqueous extracts of animal brain first came simultaneously from two laboratories; Terenius and Wahlstrom (1974) in Uppsala, Sweden, and John Hughes (1975) in Hans Kosterlitz's, Aberdeen, Scotland. Opiate activity was assessed by the ability of brain extract to compete for opiate binding sites (*Terenius and Wahlstrom 1974*), or its ability to inhibit neurally evoked contractions of the mouse vas deferens which was antagonized by the narcotic antagonist naloxone 4*Hughes 1975*). At the same time, Teschemacher et. al. 1975 reported the presence of opioid activity in extracts of bovine pituitary glands.

Late in 1975, Hughes et. al. identified two pentapeptides in extracts of bovine brain which differed in their terminal amino acid residue. They determined their amino acid sequences and named them methionine (met)- and leucine (leo)-enkephalin respectively. They also noted that met-enkephalin (met-ENK) is sequence (61-65) of the amino acid peptide named beta-lipotropin (ß-LPH). ß-LPH is a 91-residue polypeptide first isolated by C. H. Li (1964) from the pituitary gland. Its name was derived from its rather weak lipid-mobilizing activity, yet its real function is still unknown.

Later, a group of investigators independently isolated and characterized a potent opiate activity from porcine pituitaries (*Bradbury et. al. 1976*), camel pituitaries (Li and Chung 1976), and human pituitaries (*Chretien et. al. 1976, Li et. al. 1976*). The peptide was obtained in milligram quantities, contained 31 amino acid residues, and was provisionally named the **C fragment of lipotropin**, as it was

found to represent AA residue (61-91) of ß-LPH.

Moreover, Bradburg et. al. 1975 reported another opioid peptide found in the porcine pituitary which contained 27 amino acid residues and was provisionally named the **'C fragment of lipotropin**, comprised of residues (61-87) of the B-LPH structure.

After the isolation of the enkephalins and the C and 'C fragments of lipotropin, several other peptides with consequences corresponding to portions of the C terminal region of ß-LPH were reported to occur in the pituitary and possess a degree of opiate activity. Ling et. al. 1976 isolated two extracts from sheep pituitary which represent amino acid fragments (61-76) and (61-77) of ß-LPH; Gráf et. al. 1976 further identified residues (61-79) of ß-LPH in porcine pituitary extracts. These longer peptides were later named after Simon as **alpha** (61-76), **beta** (61-91), **gamma** (61-77) and **delta** (61-79) **ENDORPHIN** (*Simon 1982*).

Sharing similar peptide sequences (except leucine-enkephalin (Leu-ENK), these series of peptides (endorphins and enkephalins) were frequently described as a "**family of endogenous opiates**", and were thought to share a common origin. Later, lipotropin was found to represent an intermediate form of a 31.000 dalton prohormone which includes the sequences of both lipotropin and corticotropin, which was named **propiomelanocortin** (POMC) (*Nakanishi et. al. 1979*).

However, recent evidence suggests that, despite the coincidence of sequence with the N-terminus of lipotropin C fragments, the enkephalins do not originate biosynthetically from that peptide. The five resiudes of leu-ENK have also been located at the N-terminus of two recently discovered opioid peptides, **neo-endorphin** (*Kangawa et. al. 1979*) and **dynorphin** (*Goldstein et. al. 1979*).

Biochemistry and Physiology of Opioid Peptides

Three sets of precursor proteins for opioid peptides have been identified and are composed of a similar number of amino acids (from 257 to 265). Each have different distributions and functions in the body. Cleavage by trypsin-like enzymes appears to be a major mechanism by which these precursors are processed (*Douglass et. al. 1984*).

Opioid peptide production is regulated at the level of gene transcription and at the post-translational level, where the proteolytic processing of the various precursors generates bioactive peptides. This regulatory mechanism is tissue-specific. Some factors that modulate gene transcription in one tissue may be inactive in other tissues. (*Facchinetti et. al. 1987* a).

Moreover, acetylation of these peptides may greatly modify their biological activity; for instance, acetylated Beta-endorphin (β-EP) posesses much less affinity for its opiate receptor than it does in its unacetylated form, and therefore has a weaker biological activity. The reverse is true for alpha melanocyte stimulating hormone (alpha-MSH), where the acetylated form is more active (*Zakarian and Smyth 1979*). This again appears to be tissue-specific, as Sertoli cells are equally responsive to alpha-MSH and des-acetyl alpha-MSH, unlike the central nervous system (*O'Donahue et. al. 1982*) and melanocyte (*McCormack et. al. 1982*), which show a differential response to these peptides.

The three distinct precursors of the endogenous opioid peptides (Figure 1) that have been isolated from various endocrine and nervous tissues are:

I. Proopiomelanocortin (POMC)

II. Proenkephalin A

III. Proenkephalin B.

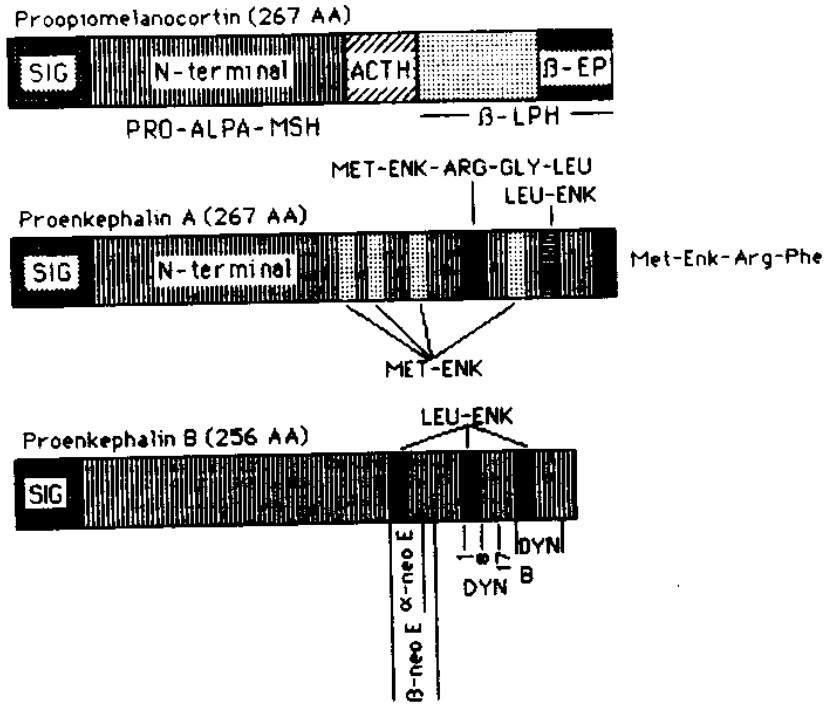

Figure 1 Schematic representation of the three opioid precursor molecules as deduced from the sequencing of their respective mRNAs *(Fasshinetti et al. 1983a)*

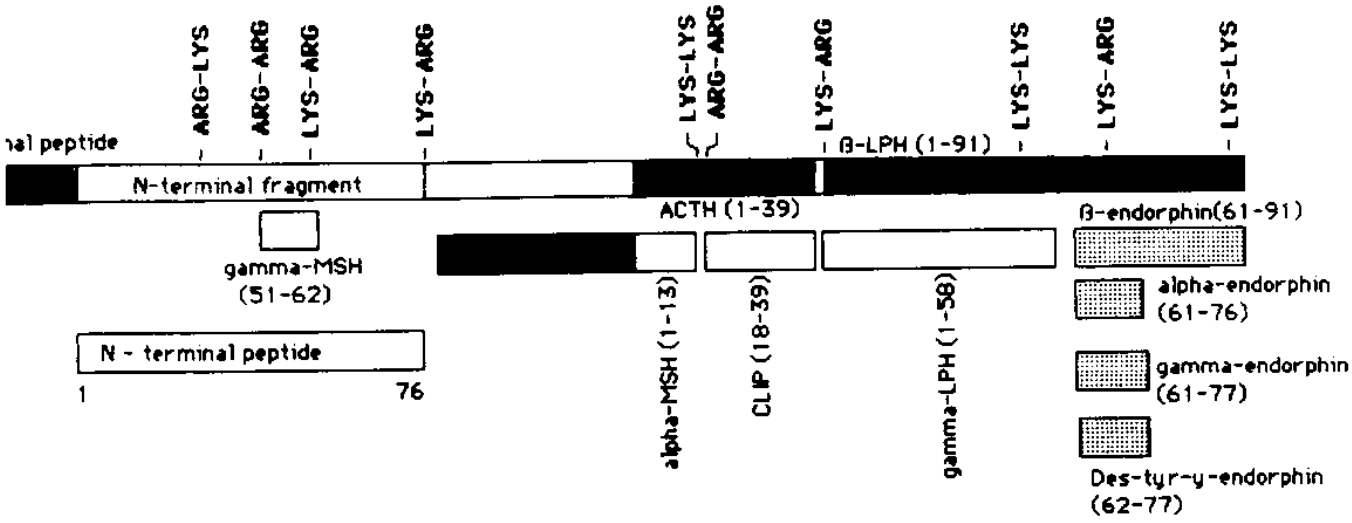

Figure 2. Schematic representation of the bovine pro-opiomelanocortin molecule, and the structures of the various component peptides derived from it. (Bardin et. al. 1987).

I- Proopiomelanocortin (POMC)

A glycoprotein with a carbohydrate molecule attached near the N-terminus of the molecule was the first precursor to be identified. Nakanishi et. al. 1979 purified the m-RNA coding for POMC from bovine pituitaries. It consists of 267 AA, of which residues 1 to 26 represent the signal peptide. ß-lipotropin (ß-LPH) (91AA) is located at the carboxyl end of this molecule and its terminal 31AA extension constitutes beta-endorphin (ß-EP). The amino acid terminal of ß-LPH is adjacent to the sequence constituting adrenocorticotropin hormone (ACTH) (39AA). In addition, the sequence of three different melanocyte stimulating hormones (MSH) are contained within the POMC molecule. These are alpha-MSH (13AA, contained in ACTH), ß-MSH (18AA, present in ß-LPH), and gamma-MSH (11AA, found in the aminoterminal half of POMC) (Figure 2).

The POMC polypeptide was originally described in the anterior and intermediate pituitary lobes in the rat, but not in the posterior lobe (*Zakarian and Smyth 1982*). While the hypothalamus and pituitary appear to be the main sites of POMC synthesis, there is inconclusive evidence that this 31K precursor is present and synthesized in other parts of the central nervous system and brain stem, and in a number of non-pituitary peripheral tissues which include the gastrointestinal tract, the lungs (*Eipper and Mains. 1980*), the male reproductive tract, including the testes and semen (*Tsong et. al. 1982*), and the female reproductive tract, including the ovaries (*Shaha et. al. 1984a*) and the placenta (*Krieger. 1982*)

Processing of POMC to release its peptide fractions appears to be tissue-specific. Moreover, it differs from fetal to adult life. During the embryotic life of a human pituitary, POMC is processed to shorter chain peptides such as alpha-MSH and ß-EP, rather than their larger precursors, ACTH and ß-LPH respectively (*Facchineti et. al. 1987b*). By the fifteenth week of pregnancy, POMC is processed in patterns similar to those seen in the adult pituitary. In the adult human pituitary, the processing of POMC in the anterior lobe produces ß-LPH and ACTH (1-39), with only a small amount of