ESTIMATION OF BETA - ENDORPHIN

IN

PREGNANCY INDUCED HYPERTENSION

Submitted in Partial Fulfilment

Of M.Sc. Degree

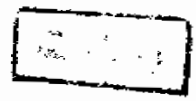
(Obst. and Gynec.)

BY

Dr. Mohamed Rabeih Mohamed EL- Ghorori

(M.B.B.CH.)

Supervised By :



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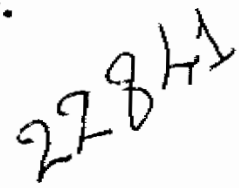

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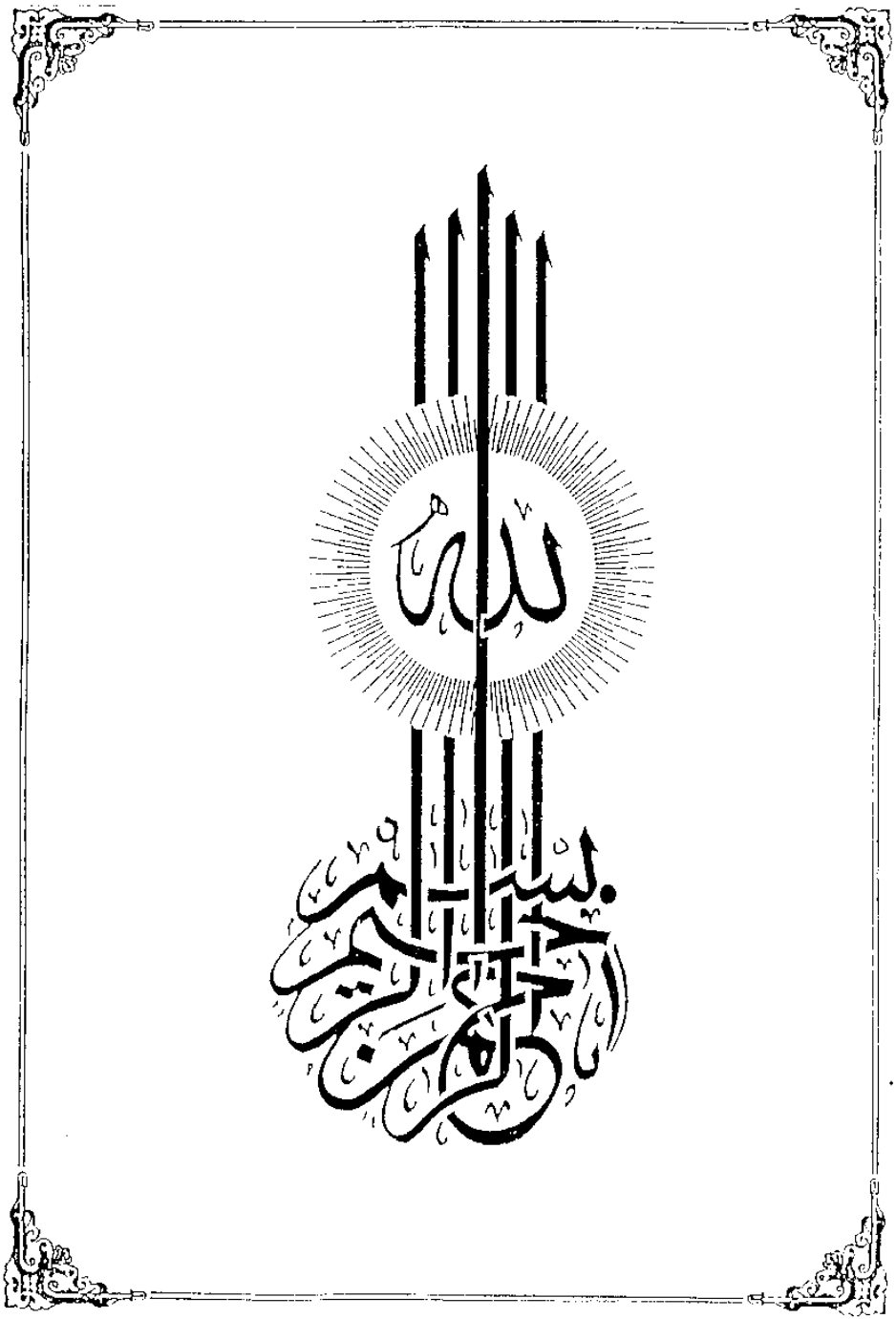

TO MY PARENTS

ACKNOWLEDGMENTS

I would like to express my cordial appreciation and deepest gratitude to Dr. FARIED ABDEL AZIZ ELTEHEWI Assist. Prof. of obst. & Gynec., Faculty of medicine, Ain Shams University & Prof. Dr. MOHAMED TALAAT ABDEL AZIZ, Head of Biochemistry Dept., Faculty of Medicine Cairo University, for their valuable advice, supervision, and continuous encouragement, through the whole work.

I am particularly indebted to Dr. MOHAMED YEHIA, lecturer of obst. & Gynec., Faculty of Medicine, Ain Shams University, for his valuable honest assistance, fruitful suggestions & follow up of the work, without his kind help & advice, this work would not have been fulfilled.

I am especially grateful to Dr. SAMYA MOUSTAFA Assist. Prof. of Biochemistry, Faculty of Medicine, Cairo University, who carried on the practical part of this work

I wish to acknowledge a deep indebtedness for her dedicated collaboration.

CONTENTS

INTRODUCTION

An extra dimension was added by the discovery that analgesia evoked by electrical stimulation of the brain (Akil et al., 1972) & in (1974) two independant laboratories identified a peptide in brain extracts that mimicked opiate activity both in its ability to inhibit electrically induced contraction in the guinea pig ileum (Kosterlitz & Hughes (1975) and to bind to opiate receptors (Terenius and Wahlstrom, 1975).

This discovery stimulated a tremendous effort toward further identification & characterization of endogenous opiates .

Then, Hughes et al., (1975) sequenced the amino acides of two pentapeptides, Met-enkephalin & Leu-enkephalin, which differ only in terminal amino acid, and have many pharmacological effects of opiates & produce analgesia (Loh et al.,1976 & Li & Chang 1976).

Within a short time,Goldstein (1975) discovered the presence of a pituitary factor with opiate receptor activity & properties significantly different from enkephalins Teschermacher et al.,(1975) was discovered that amino acid sequence of Met-enkephalin is identical to a peptide

sequence within β-lipotropin, a 91-amino acid peptide that was first found in the pituitary (Li & Chung, 1976).

Further work identified a number of other B-lipotropin fragment with varying degree of opioid activity , named β-endorphin (Bradbury et al., 1976; Li & Chung., 1976) , α-, γ- & δ- endorphins (Lin et al.,1976), then (Nakanishi et al., 1979) reported that β-endorphin , β-lipotropin, adrenocorticotrophic hormone & melanocyte-stimulating hormone are derived from a common multifunctional prohormone named pro-opio melanocortin , then, it took a further five years to identify totally the additional pituitary factor dynorphin (Goldstein et al., 1981).

The term "opioid peptides" has usually been applied to naturally occurring peptides with opiate-like activity, and the name endorphin from (end) signifying endogenous and orphin from a common suffix in the names of opiates.

However, this discovery open a new era for further investigations to discover properties & functions of these opioid peptides & to explain several observations & phenomena in human being.

CHEMISTRY & SYNTHESIS

β-endorphin is an opioid peptide formed of 31-amino-acid residues(Bradburg et al., 1976 ; Loh et al., 1976) with the five amino acid chain that constitutes the Met-enkephalin is present within its sequence (Li & Chung , 1976).

It was found to originate from a multifunctional prohormone by post translational proteolytic cleavage, and this prohormone is a common precursor of adreno-corticotrophic hormone (ACTH) , alpha- & beta - melanocyte stimulating hormone (α - & β - MSH), beta - endorphin & beta-lipotropin (B-LPH) (Mains et al., 1977; Nakanishi et al., 1979).

Thus adreno corticotrophic hormone (ACTH) & β-lipo-tropin share a high molecular weight (31,000 dolton) common precursor molecule called pro-opiomelanocortin (Mains et al., 1977) & the NH_2 terminal fragment comprises the NH_2 - terminal region, ACTH the middle region, and β-lipotropin the COOH terminal region of this common precursor.

β-lipotropin is a 91-amino acids molecule (Li & Chung, 1976) & has no opioid activity (Kosterlitz & Hughes, 1975).

with the sequence of β-endorphin at the extreme COOH-terminal end of β-lipotropin. (Loh et al., 1976) ,while alpha, beta - & gamma - melanocyte stimulating hormone (α -, β- & γ -MSH) are contained within the sequences of ACTH, B-lipotropin, and the NH_2-terminal fragment , respectively (Nakanishi et al., 1979) and all the major peptide products of pro-opiome lanocortin are flanked by pairs of basic amino acid residues, with potential for proteolytic cleavage.

β-lipotropin is broken down in a series of steps to β-melanocyte-stimulating hormone (β-MSH), enkephalin, and α -, γ & β-endorphin (Krierger et al., 1980).

The principal β-endorphin related peptides in anterior pituitary , however, are lipotropin & β-endorphin$_{1-31}$; lipotropin is the major peptide but β-endorphin is produced with a high specificity in its opioid active form (Smyth et al., 1982).

Pro-opiomelanocortin, an inactive pre-prohormone, , is synthesized with ribosomal participation & the endoplasmic reticulum probably involved under the direction of mRNA on the membrane-bound polyribosomes, then the cleavage of this sequence from the pre-prohormone on its entery into the Golgi apparatus yields the prohormone

(Hughes et al., 1980), then sequential cleavage of the prohormone yields one or more biologically active peptides that are available for secretion; the order of cleavage & its specificity, not all paired basic residues are cleaved, appears to be determined by a number of factors, including the conformation & the quaternary structure of the prohormone & the presence or absence of glycosylated asparagine residue, phosphorylated serine residue & possible O-sulphated tyrosine residue. (Docherty & Steiner, 1982).

TYPES OF β-ENDORPHIN:

There are 3 forms of β-endorphin (Massey & Smyth, 1980)

$\beta\text{-endorphin}_{1-31}$

$\beta\text{-endorphin}_{1-27}$

$\beta\text{-endorphin}_{1-26}$

In human pituitary the principal form of β-endorphin is the biologically potent $\beta\text{-endorphin}_{1-31}$ & it is accompanied by relatively small quantities of $\beta\text{-endorphin}_{1-27}$ (Smyth & Zakarian,1982a).

$\beta\text{-endorphin}_{1-26}$ & its acetylated form not exist in human & generally the acetylated form of β-endorphin might have a non-opioid activities (Smyth et al., 1982).

STORAGE:-

Fractionation studies indicate that immuno-reactivity of opioid peptides is highly concentrated in synaptosomes (Simantov, 1976), and the electron-microscopical studies indicate that the opioid peptides are largely concentrated in large-granular (≲ 70-100 nm) type vesicles, (Höfkelt et al., 1982), later, the opioids are transported down to the nerve terminals where they are released in a neurotransmitter fashion (Cuello et al., 1982).

DISTRIBUTION:-

β-endorphin, the most potent known naturally occurring analgesic agent (Loh et al., 1976), was first isolated & identified as one of a series of prohormone fragments present in porcine pituitary (Bradbury et al., 1976) & also found to be present in the pituitary of different species including man (Dragon et al., 1977) & has been shown to be present also in the brain of man (Swann & Li, 1980) but the highest concentrations of β-endorphin appear to be in the hypothalamus (Bloom et al., 1978) & the pituitary (Guillemin et al., 1977).

According to Bloom et al., (1978), the cell bodies where biosynthesis takes place are concentrated

in the arcuate nucleus & median eminence close to the ventromedial border of the third ventricle & immunoreactive fibre system originating from these neurones are abundant in mid-line periventricular regions of the hypothalamus & Bloom et al.,(1978) also demonstrated that these loose fibres appears to continue dorsally & to terminate in a number of brain-stem structures especially peri-aqueductal grey matter, the nucleus of tractus solitarius & the locus coeruleus & in ventral portions of the brain-stem suggesting the existence of these fibres in dorsal & ventral bundles of hypothalamic projections , and also found immunoreactive fibres in parts of olfactory cortex & hippocampus.

In the pituitary, β-endorphin , β-lipotropin (β-LPH) & adrenocorticotrophic hormone (ACTH) occur in same cells of the anterior lobe (Watson et al., 1978)& this is consistent with the view that they are derived from the same precursor molecule, proopiomelanocortin (Nakanishi,et al., 1979), so there are some cells in the pituitary that produce both ACTH & β-endorphin (Deftos & Catherwood, 1980) & in this case it would seem likely that the two biologically active peptides are generated together for a coordinated function in the periphery, however, β-endorphin has no more than slight analgesic properties when

administered intravenously (Feldberg & Smyth, 1977),it appears that this peptide poorly penetrates the blood-brain barrier & the pituitary peptide is therefore unlikely to fulfil a direct role in brain function.

Feurle et al., (1980) reported that high proportion of the β-endorphin related peptides were present in pancreas & also in pyloric antrum (Tanaki et al., 1982).

β-endorphin is present as an independent peptide in the circulation of the man (Nakao et al., 1977) & β-endorphin together with ACTH, β-LPH, ϒ-LPH appear to circulate in ratios that suggest their origin from the common precursor proopiomelanocortin , synthesized in the pituitary but fully processed before secretion (McLoughlin et al., 1980), the major peaks of β-LPH & β-endorphin were detected in the human circulation without significant peaks of higher-molecular weight precursor material (Ratter et al., 1980).

In man , circulating β-endorphin has the same pattern of circadian secretion as has been demonstrated for ACTH & β-LPH ; (Shanks et al., 1981) ; like these hormones, it is subject to the same corticosteroid negative-feed back secretory inhibition & its release is stimulated in response to stress ;(Smith et al.,1981)

& its release is suggested to be under the control of corticotrophin-releasing factor (CRF) as (Grossman et al., 1982a) demonstrated equimolar secretion of ACTH & β-LPH under the effect of CRF.

β-endorphin & β-LPH are found also in cerebrospinal fluid (CSF) (Jeffcoate et al., 1978) & (Terenius & Wahlstrom 1975), however, more β-endorphin appears to be present in CSF than in plasma & the presence of significant levels in CSF in pan-hypopituitary subjects with undetectable plasma levels would suggest that the two compartments for β-endorphin, the brain-SCF & pituitary-plasma, are separate & that the brain itself is an important source of CSF β-endorphin .

B-endorphin has been detected in human placenta (Fraioli & Genazzani, 1980) & in placental tissue fluid (Kimball & Chang 1981) & particularly upon finding of Liotta & associates (1982) who reported that cultured human placental cell produce β-endorphin.

Human placenta contains & synthesizes ACTH (Genazzani ,et al.1975 Odagiri et al.1979) & the family of related peptides , β-endorphin & β-LPH (Odagiri et al., 1979), as with pituitary cells, a common precursor for these peptides (proopiomelanocortin) has also been found