C- PEPTIDE RESPONSE TO IVI OF GLUCOSE IN EGYPTIAN DIABETICS

A THESIS

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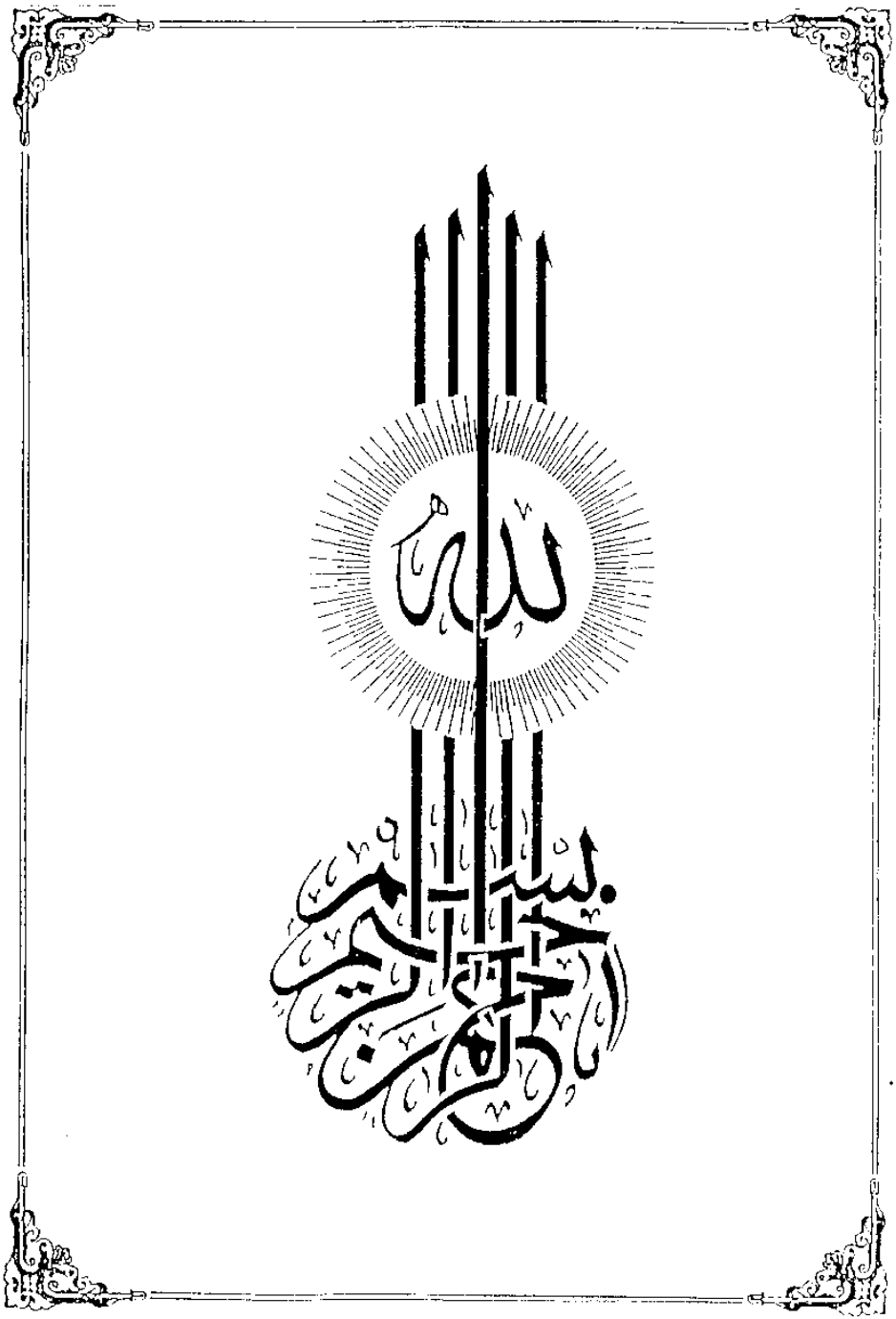

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The Aim of Work

Several studies have dealt with the potential role of endogenous insulin secretion on the metabolic control in diabetic patients.

Reynolds et al., (1977) studied C-peptide concentration in stable and unstable diabetic patients with adult-onset insulin-requiring diabetes and found higher c-peptide levels in the former group. Metabolic stability was determined from the absolute level and variability in diurnal and urinary glucose estimations. Grajwer et al., (1977) studied 35 patients with juvenile onset diabetes. All patients whose immunoreactive c-peptide concentrations were higher than 2.0 ng/ml (0.67 pmol/ml) were in the group of patients considered to be in good control, while patients whose c-peptide levels were less than 2.0 ng ml were equally distributed between the poorly controlled and well controlled groups.

Shima et al. (1977) measured the response of c-peptide to a glucose load in 46 insulin-treated patients. They assessed the degree of instability of diabetes by standard deviation of 10 measurements of fasting plasma glucose for each patients.

Yue et al. (1978) divided diabetic patients into metabolically stable and labile group on the basis of their physicians clinical impression. C-peptide response to glucose was present in 58 of the stable diabetic patients but in none of the labile patient. On the other hand, Ikeda et al. (1975) could not find a correlation between c-peptide response to glucose and either the state of control or the dose of insulin in young insulin dependent diabetic patients.

The aim of this work is to assess whether is a difference between diabetic patients treated with oral hypoglycemic agents and those treated by insulin, in addition to estimate beta cell function in diabetic patients by measurement of C-peptide in their serum after intravenous glucose load.

Syntheses of C-peptides and human proinsulin

Human proinsulin is a single chain polypeptide of 86 amino acid residues, in which the N-terminus of the A chain of human insulin is linked with the C-terminus of the B chain via connecting segment comprising 35 amino acid residues, following the elucidation of its structure (Ko et al., 1971 and Oyer et al., 1971), Synthesis of the human proinsulin connecting peptide derivative and the c-peptide, which lacks two basic dipeptide at both of its termini, have been accomplished, and the ensuing synthetic polypeptides were proved to be as immunologically active as natural human C-peptide (Yanaithani et al., 1974, Naithani et al., 1973, Naithani et al., 1975, Geiger et al., 1973 and Noboru et al., 1978) have reported synthesis of human C-peptide and its analogues by differnt approaches.

In contrast of insulin, the fully reduced form of proinsulin recovers a high proportion of its native immunologic reactivity by air oxidation under mildly alkaline conditions (Bromer et al., 1970, Noboru et al., 1978 and Steiner et al., 1968) . Evidence is now defined structures serve as excellent substrates (Noboru et al., 1978).

Species variations in the primary structure of the proinsulin connecting segment give rise to unique immunologic determinants (Noboru et al., 1978).
In the case of porcine proinsulin (Chance et al., 1968), they found that the smallest peptide that was as immunologically active as porcine proinsulin or the connecting peptide on an equimolar basis in an assay system using a purified guinea pig antiserum to porcine proinsulin (Yanaihara et al., 1976) was an undecapeptide possessing the proinsulin sequence. Fragments retated to porcine proinsulin connecting peptide were synthesized. Naithani et al., (1973) also reported that the major antigenic determinant was located within the sequence 41-54 in the same assay system that was used, thus agreeing with original conclusion (Chance, 1972 and Yanaihara et al., 1972).

Availability of the synthetic human connecting peptide and its analogues led to develop a radioimmunoassay system specific for the c-peptide (Kaneko et al., 1974 and Noboru et al., 1978) which has proved to be as useful for the available indicating that synthesis of the linear polypeptide comprising 86 amino acid residues, based on the proposed primary struture of human proinsulin, followed by air oxidation, leads to the formation of the proinsulin molecule with its characteristic conformation.

Human proinsulin C-segment and its analogues

In the synthesis of the human connecting segment, the C-terminal tetra decapeptide, which corresponds to positions (HP) 52-65 in human proinsulin, was prepared by coupling the azide derived from Z-Ser-Leu-Glu-Pro-Leu-Ala-Leu-Glu-(OBU)-Gly-N HNH - Boc (positions HP 52-6p) with H-Ser-Leu Gln-e-Formly-Lys-Arg (H+) - OH (positions HP 61-65) followed by hydrogenolysis.

The octadecapeptide, H-Gly-Gly-Pro-Gly-Ata-Gly-Ser-Leu-Glau-Pro-Leu-Ala-Leu-Glu-Glu-Gly-Ser-Leu-Gln-OH (HP 46-63), which was produced by coupling the azide derived from Z-Gly-Pro-Gly-Ala-Gly-NHNH-Boc (positions HP 46-5l) with fragment HP 52-63 followed by hydrogenolysis, was purified by gel filtration on Bio-Gel-P-6 to give a product shown to be homogenous in two different systems (1-butanolacetic acid-Hzo 4. 1 . 5 and 1 butanol-pyridinacetic acid H_2O= 30 . 20. 6. 24) mp 210-213; (α) $^{D}_{27}$ -102 . 4^{o}.

In addition to the C-peptide, tyrosylated (64-formyllysine) -HP 7l-65 was prepared by coupling Z-try (Z) Arg (H+)- Arg (H+)- Glu-Ala-Glu-Asp-Leu-Glan azide with (64-formyllysine) -HP 39-65 followed by hydrogenolysis (Yanaihara et al., 1974).

The crude material was purified by gel filtration on sephadex G-50 to give homogenous-product (α) D 24-97. 9^{o} (c 0. 21, 10 percent acetic acid), amino acid composition of acid hydrolysate Asp 0-97 Seri-60 Glu-13 pro 1.94 Gly 7.15 Alaz 3.08 val 2.05 Leu 5.96 Tyro 0.92 Tyro 0.92 Lyso 0.75 Arg 2.85.

Radioiodination of the tyrosylated analogue gave 125-tyrosylated (64-formyllysine)-HP 31-65, which can be used as tracer in radiommunoassay for human c-peptide introduction of the terminal basic residues increased solubility of the c-peptide in aqueous solution significantly.

Immunologic evaluation of human proinsulin c-peptide fragments and analogues:

Attempts have been made do determine the structural features of the connecting peptide that determine its immunoreactivity with various proinsulin or C-peptide antisera. For this purpose, synthetic peptide fragments of measurement of C-peptide in human blood (Horwitz et al., 1976 and Kaneko et al., 1975) as the assay system using natural human C-peptide (Block et al., 1972, Melani et al., 1970 and Rubenstein et al., 1973).

In order to determine the structural features responsible for the antigen-antibody interaction in the immunoassay system for human c-peptide, five related synthetic peptides HP 44-50, HP 45-56, HP 31-40 , (64 formyllysine) HP - 65, and (46-formyllysine) -HP 39-65 were used. HP 44-56 and HP 45-56 were constructed by condensation of Z-Leu-Gly-Gly-Pro-Gly-azide or Z-Gly-Gly-Gly-Pro-Gly-Ala-Gly-azide with H-ser-Leu-Glu-Pro-Leu-OH followed by hydrogenolysis, for the synthesis of HP 31-40, H-val-Gly-OH was used as the starting material, and the peptide chain was elongated by consecutive acylation with Z-Glu-Asp-Leu-Glu-azide and Z-Arg (No.2) - Arg (H+)- Glu-Ala-zide-Hydrogenolysis was employed for Z and No. 2 dprotection. HP 44-56 and HP 45-56 were purified by CM-Sep-hadex C-25 (Free form) with the use of increasing concentration of acetic acid for column elution-(64-formyllysine) HP 46-65 and (64-formyllysine)-HP 39-65 are intermediates previously used for the synthesis of human connecting peptide, and they were purified by gel filtration on Bio-Gel-P-6.

Each of these five synthetic peptides behaved as a hemogenous substance on two different solvent systems when detected by the chlorine tolidine and ninhydrin reagents. Their acid hydrolysates contained the constituent amino acids in theortic ratios. The chemical and physical properties

of the synthetic human connecting peptide fragments are as follows:

HP 44-56: (α)D 25-78.0° (C 1.00, 10 per cent acetic acid) amino acid composition of acid hydrolysate sero, 89 Gluo 91 Pro_2 2.00 Gly 5.21 Ala 0.99 Leu 3.00 HP 45-56. (α)D 25-100. 5° 9 (C 1.00, 10 per cent acetic acid) amino acid composition of acid hydrolysate Sero 0. 22 Cho 0. 90 Pro 1. 99 Gly 5.34 Ala 0. 98 Leu, 1.97 HP 31-40 (α)D 24-62 4° (CO.52, 10 percent aceitic acid), amino acid composition of acid hydrolysate Arg 1.94 Asp. 1.02 Glu 3. 01 Gly 0.97 Ala 1.00 Val 1.07 Leu 0.99.

(64 Formyllysine) - HP 46-65 (a) D 29-92-7° (C 0. 74,10 percent acetic acid), amino acid composition of acid hydrolysate Ser 1.76 Glu 3.09 Pro 1.90 Gly 5.90 Ala 2-14 Leu 3. 92 Ly 1.02 Arg 1.01.

(64-Formyllysine)-HP 39-65 (α)D 29-87. 6° (c 0.25, 10 percent acetic acid), Ser 1. 78 Glu 5.0 Pro 1. 95 Gly 7.08 Ala 1.92 Val 2.07 Leu 4.97 Luy 0.78 Arg 0. 97.

Immunoussay of HP 44-56, HP 45-56, HP 31-40, (64-formyllysine) -HP 46-65, and (64-formyllysine)-HP 39-65 was performed according to a modification of the double-actibody

method of Morgan and Lazarow (Morgan et al., 1963). The antiserum used in the present study had been raised in a rabbit against synthetic (64-formyllysine 1:10,000 Synthetic connecting peptide was used as the standard, and tyrosylated synthetic human connecting peptide was radioiodinated by the method of (hunter and Greenwood, 1962) quoted by (Noboru et al., 1978) which was used as tracer.

Human pancreatic proinsulin also competed with the tracer for binding sites in the antiserum. On the other hand HP 44-56, HP 45-46, HP 46-63, and (64-formyllysine) HP 46-65 with amino acid sequences corresponding to the central or carboxylterminal region of human connecting peptide reacted poorly or not at all HP 31-40, the decapeptide of amino terminus of the connecting peptide did not displace the tracer. (64-Formyllysine) HP 39-65 and HP 39-63 having the additional sequence Val-Gly-Gln-Val Glu-Leu-Gly at the amino terminus of HP 46-65 showed cross reaction, although the reactivity was low.

It can therefore be concluded that in human connecting peptide, the region between positions 39-45,-Val-Gly-Cln-Val-Glu-Leu-Gly-, contains important immunologic determinants for the antiserum studied.

Total Synthesis of human proinsulin:

Synthesis of linearly protected hexaocta contapeptide:

In a previous communication Yanaihara et al., (1976) outlined the synthesis of the partially protected hexaoctaconta-peptide possessing the entire sequence of human proinsulin. This polypeptide was shown to significantly displace ^{125}I human C-peptide in the amino acid composition of an acid hydrolysate of this polypeptide agreed, within experimental error, with theory. The synthetic strategy employed for the synthesis of the linear, partially protected hexaoctacontapeptide was similar to that successfully used in the synthesis of the c-peptide. The construction of this protected polypeptide was planned to be undertaken exclusively by the azide-fragment condensation in solulion starting from the terminal undecapetide (HP 75 - 86).

On the role of the proinsulin C-peptide

In the biosynthesis of insulin the c-peptide fullfills an important biologic role by facilitating the formation of the correct secondary and tertiary structure of the hormone (Steiner, 1968). Once this function has been accomplished at the level of the rough endoplasmic reticulum, the newly formed proinsulin is transferred to the Golgi region of the beta cell, where its proteolytic cleavage to insulin and c-peptide is initiated (Steiner et al., 1970, and Kemmler et al., 1973).

The sequestration of this process within the Golgi lamellae and immature secretory granules, results in the retention of the c-peptide along with the insulin in the granules, and both peptides are then released together into the circulation during exocytosis (Melani et al., 1970 and Rubenstein et al., 1969). Thus, in most species the beta-cell secretory products consist mainly of insulin and c-peptide (95%) along with small amounts of proinsulin and related intermediate cleavage forms (5%) (Rubenstein et al., and Sando et al., 1972).

In some species the c-peptide undergoes additional cleavages at internal positions. Such cleavage was first