PRELIMINARY COMMUNICATIONS

The Biosynthesis of Insulin and 'Proinsulin' in Foetal Bovine Pancreas

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Summary. Incubation of foetal bovine pancreas slices resulted in the incorporation of ^3H -leucine into a protein fraction which appeared to have the properties of a 'proinsulin'. This protein fraction was larger in size than insulin, possessed the immunoreactivity of insulin and was converted by limited trypsin treatment to a peptide similar to insulin.

Biosynthèse d'insuline et de «proinsuline» par le pancréas d'embryon de veau

Résumé. Après incubation de tranches de pancréas d'embryon de veau, la leucine- H^3 est incorporée dans une fraction protéique qui semble avoir les propriétés d'une "proinsuline". Cette fraction protéique est de taille supérieure à l'insuline, possède l'immunoréactivité propre

à l'insuline, et après traitement limité par la trypsine elle est transformée en un peptide semblable à l'insuline.

Die Biosynthese des Insulins und 'Pro-Insulins' durch fötales Rinderpankreas

Zusammenfassung. Die Inkubierung von Dünnschnitten des fötalen Rinder-Pankreas in Gegenwart vom H^3 -Leucin ergab einen Einbau dieser Aminosäure in eine Eiweißfraktion, die die Eigenschaften eines 'Pro-Insulins' aufwies. Das Molekulargewicht dieser Eiweißfraktion war größer als dasjenige des Insulins; sie besaß die Immunreaktivität des Insulins und konnte durch teilweisen Abbau mit Trypsin in ein insulinähnliches Peptid umgewandelt werden.

Key-words: Insulin, proinsulin, biosynthesis.

Introduction

The possibility of a precursor protein being involved in insulin biosynthesis has been studied by various investigators [1, 4, 7]. This possibility has been explored also in this laboratory during the past three years using ligated rabbit pancreas and foetal calf pancreas. Recently, STEINER et al. [5, 6] reported on the synthesis of a 'proinsulin' in human islet adenoma tissue and in isolated islets of Langerhans from the rat. In this laboratory, a 'proinsulin' has been isolated from crystalline bovine insulin and its amino acid composition analysed and reported [9].

The experiments reported here were designed to study the biosynthesis of 'proinsulin' and insulin in foetal bovine pancreas slices. Results obtained showed that leucine was incorporated into a protein which had the immunoreactivity of insulin, but was larger than insulin, and similar to the 'proinsulin' obtained from crystalline bovine insulin preparations.

Material and Methods

Foetal bovine pancreas obtained from a local slaughter house was brought to the laboratory in ice-cold HANK'S buffer [3]. The tissue contained no detectable proteolytic enzyme activity. 1.5 g of slices were incubated with 500 μC of leucine-4,5- ^3H , 5C/mM (New England Nuclear Corps.) at 37°C under 95% O_2 -5% CO_2 in HANK'S buffer containing 3200 mg/l glucose and 20 mg/l of each of the 18 natural occurring amino acids. After incubation, the slices were rinsed, then homo-

genized in 10% TCA. The resultant precipitate was washed three times with 5% TCA, then extracted with acid alcohol [2] for 3 h at room temperature. After centrifugation for 20 min at 2000 g, the extract was evaporated to dryness *in vacuo* at 37°C. The residue was dissolved in 5 ml of 1 M acetic acid and centrifuged for 20 min at 2000 $\times$ g. The supernatant was partially purified by chromatography on a Sephadex G-50 (fine) column (1.5 cm $\times$ 86 cm) using 1 M acetic acid as eluant. Fractions of 1.5 ml were collected. Fractions eluted after the void volume were pooled, evaporated to dryness *in vacuo*, and re-chromatographed on a similar column, with bovine insulin added as marker. Aliquots of 0.5 ml were used for measuring radioactivity and UV absorption at 275 m μ . The procedure for immunoassay was that of YALOW and BERSON [8].

Results and Discussion

It may be seen (Fig. 1) that three major radioactive components (I, II and III) were present in the extract obtained after a 1 h incubation. (I) probably consisted of large proteins excluded by the gel and was not studied further. After partial purification, component II showed the immunoreactivity of insulin according to the method of YALOW and BERSON [8]. Purified material from (II) and 'proinsulin' obtained from crystalline insulin preparations [9] had similar elution properties on the Sephadex G-50 column. Limited tryptic hydrolysis converted the ^3H -labelled material from (II) to ^3H -labelled material which behaved like insulin on the Sephadex G-50 column in 1M acetic acid. Com-

ponent III coincided with the insulin marker and was immunologically identified as insulin. Analysis of the extract after the 5 h incubation (Fig. 1) showed that, although there was an increase of radioactivity in II and III, the radioactivity associated with III (Insulin) remained low.

[5, 6]. By contrast with their findings, however, these data show that leucine incorporation into the 'proinsulin' fraction remained considerably higher than that into insulin even after 5 h of incubation, suggesting a very slow conversion of 'proinsulin' to insulin in the incubated foetal material.

Fig. 1. Elution diagrams of the rechromatography of acid alcohol extracts of foetal bovine pancreas slices after incubation. The arrow indicates the insulin marker

Fig. 2. Immuno-electrophoresis of material from component (II)
(A) Guinea pig anti-porcine insulin antibody and ^{131}I -labelled porcine insulin
(B) Same as A, except purified material from component II was added. F — free insulin;
B — antibody-bound insulin

These experiments suggest that in foetal bovine pancreas slices, leucine-4,5- ^3H was incorporated into a protein larger than insulin, which had the immunological properties of insulin and was partially convertible to an insulin-like molecule. The larger protein is probably similar to the 'proinsulin' reported by STEINER et al.

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