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INCUBATION STUDIES OF PANCREAS FOR EVALUATION OF INSULIN ANALOGUES IN MAN, USING GEL CHROMATOGRAPHIC PROCEDURES

*Keiji KAKITA, *Masaharu HORINO, *Atsuko TENKU, *Seikoh NISHIDA, *Shigeichi MATSUMURA, *Michihiro MATSUKI, **Sachiko KAKITA, **Soichi NISHISHITA and ***Kaiso SANO

*Division of Endocrinology, Department of Medicine, **Department of Radiology, ***Department of Surgery, Kawasaki Medical School, Kurashiki 701-01, Japan

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Abstract

Human pancreas was taken from three patients, one was a patient with esophageal cancer, two with gastric cancer as surgical specimen. The incubation medium and incubated pancreas were gel chromato graphed on the Bio-Gel P-30 column after extraction with acid-ethanol. Each of fraction was assayed for immunoreactive insulin, C-peptide immunoreactivity and glucagon immunoreactivity.

Two peaks of insulin were detected in the incubation medium and the incubated pancreas at the position of 6000 molecular weight region. The pancreatic tissue abounded in intermediate molecular size of insulin derivatives, but the secreted insulin was eluated only at the position of 6000 molecular weight region. Therefore the secretion mechanism of insulin might be selective.

These findings suggest that the two groups of insulin could be directly secreted from human pancreas with the selective mechanism.

INTRODUCTION

The presence of two kinds of immunoreactive insulin (IRI) was reported in human sera after extraction and gel filtration in earlier reports of Kakita *et al*[1,2]. Two kinds of insulin were also detected both in the incubation medium and in the incubated pancreas[3]. Therefore, these two insulin analogues are directly secreted from human pancreas. Moreover, the early eluting insulin on the Bio-Gel column is not secreted with the stimulation of glucose and controls the fasting blood sugar level. The late one is released with the same stimulation and regulates the elevated blood sugar level[3].

The incubation study was undertaken to elucidate the presence of insulin

垣田敬治，堀野正治，天工厚子，西田聖幸，松村茂一，松木道裕，垣田さち子，西下創一，佐野開三

analogues in incubation medium and incubated pancreatic tissue, and the secretion mechanism of these analogues.

MATERIALS AND METHODS

Pieces of human pancreatic tissue were obtained from a patient with esophageal cancer (53 years old, male) and two patients with gastric cancer (69 years old, male ; 73 years old, female). All three patients were normoglycemic and had no family history of diabetes mellitus.

Pieces of the pancreatic tissue (wet weight, approximately 50 mg) were incubated for 10 or 60 minutes at 37°C (95 % O_2/5 % CO_2) in Krebs Henseleit buffer solution containing 3.0 mg/ml of glucose, and for additional 30 minutes in the medium containing 0.6 mg/ml of glucose.

After these incubation, the medium was extracted by the reported method[1,2]. The extraction from the pancreas was performed according to the reported method[3]. The Procedure of gel filtration was done with the reported method[1,2,3]. The column was calibrated with ^{125}I-labelled C-peptide (Daiichi Radioisotope Lab., Japan) and ^{125}I-labelled glucagon (Dainabot Radioisotope Lab., Japan), besides porcine ^{125}I-labelled proinsulin and porcine ^{125}I-labelled insulin. Assay for IRI and C-peptide immunoreactivity (CPR) was performed according to the reported methods[4,5]. Glucagon immunoreactivity (GLI) was assayed with the kit of Dainabot Radioisotope Lab.

RESULTS

In the elution profile of human pancreas, IRI and CPR were detected as larger molecule than monomer of each hormone (Fig. 1, upper panel), but GLI was eluated at the position of ^{125}I-labelled glucagon (Fig. 1, lower panel). In the direct incubation of pancreas for 60 minutes in the medium containing 3.0 mg/ml of glucose after the excision of pancreas, two peaks of insulin which were corresponded to the reported two peaks from human serum were detected both in the medium (Peak I of IRI was reassured with the increased sample size.) and in the pancreas, though intermediate size of insulin was recognized in the pancreas. The peaks of CPR in the pancreas were not corresponded to those in the medium which were corresponded to the peak of ^{125}I-labelled C-peptide. There were no peak corresponded to that of porcine ^{125}I-labelled proinsulin (Fig. 2). In the direct incubation in the medium of 3.0 mg/ml glucose for 10 minutes and in that of 0.6 mg/ml glucose for the following 30 minutes, two peaks of IRI were also revealed. The most peaks of CPR were detected as aggregated form (Fig. 3).

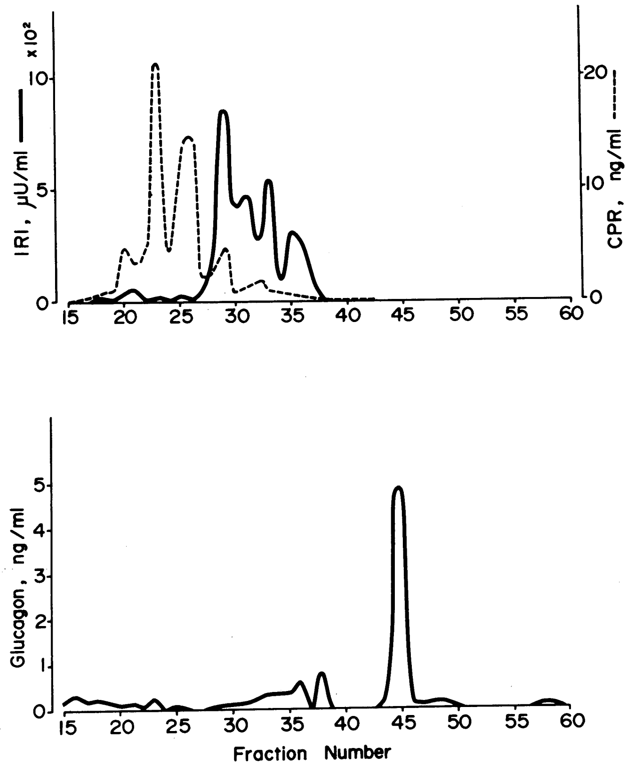

Fig. 1. Elution profiles of IRI, CPR and GLI extracted directly from human pancreas after the excision (I. M., 69 y. o., M.) on the Bio-Gel P-30 column.

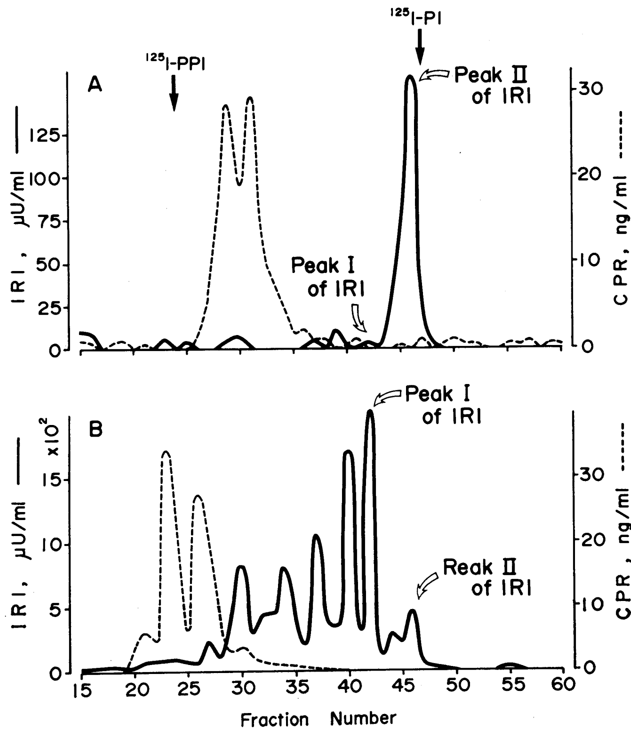

Fig. 2. Elution profiles of IRI and CPR extracted from incubation medium and incubated human pancreas (I. M.,) on the Bio-Gel P-30 column. Krebs Henseleit bicarbonate buffer (pH 7.4, 95% O_2/5% CO_2) with 3.0 mg/ml glucose for 60 minutes at 37°C. Upper panel: Elution profiles of IRI and CPR from incubation medium. Lower panel : Elution profiles of IRI and CPR from incubated pancreas. PPI represents porcine proinsulin. PI represents porcine insulin.

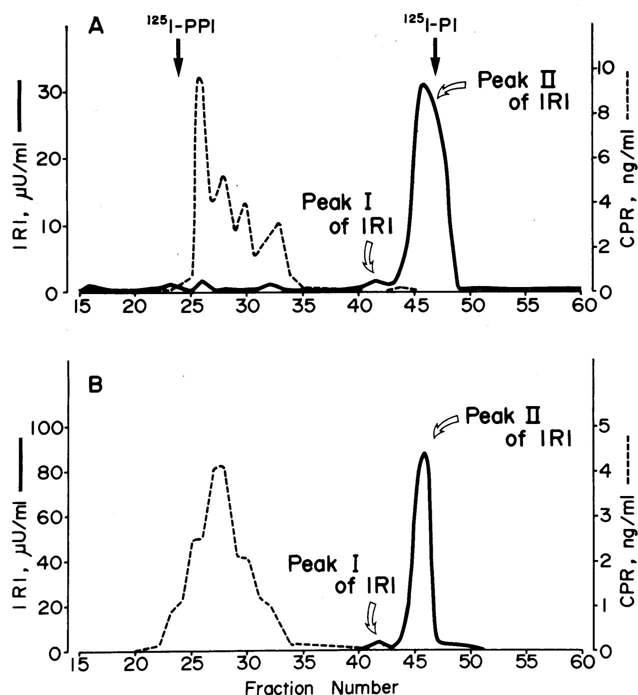

Fig. 3. Elution profiles of IRI and CPR extracted from incubation medium (N. M., 53 y. o., M.) on the Bio Gel P-30 column. Krebs Henseleit bicarbonate buffer (pH 7.4, 95% O_2/5% CO_2) with 3.0 mg/ml glucose for 10 minutes and with 0.6 mg/ml glucose for following 30 minutes at 37°C. Upper panel : Elution profiles of IRI and CPR from incubation medium containing 3.0 mg/ml of glucose for 10 minutes. Lower panel : Elution profiles of IRI and CPR from incubation medium with 0.6 mg/ml glucose for following 30 minutes. PPI represents porcine proinsulin. PI represents porcine insulin.

DISCUSSION

The presence of two kinds of insulin was first reported in human sera after extraction and gel filtration[1,2]. Two kinds of insulin were also detected both in the incubation medium and in the incubated human pancreas[3]. Moreover, the secretion of these insulin analogues was evaluated[3]. The incubation study was undertaken to elucidate the presence of insulin analogues in incubation medium and incubated pancreatic tissue, and the secretion mechanism of these analogues.

In the human pancreatic tissue, insulin and C-peptide were detected as larger molecule than monomer of each hormone, but glucagon was eluted at

the position of ^{125}I-labelled glucagon. In the direct incubation for 60 minutes after excision, the two peaks of insulin which was corresponded to the reported peak in human sera[1,2] were selectively secreted into the medium from the human pancreas, though the pancreas abounded in intermediate molecules of insulin. CPR in the pancreas was not the same as that in the medium. In other all incubation study for 10, 30 and 60 minutes, two peaks of insulin were also recognized both in the incubation medium and in the incubated pancreas. The C-peptide peaks was eluated at the larger molecular region than that of monomer, the facts might be due to the properties of aggregation.

From above mentioned results, following conclusions are obtained : The two groups of insulin are detected in the human pancreas. These insulins could be directly released from human pancreas with the selective mechanism. C-peptide molecules are aggregated in the incubation medium and the incubated pancreas. C-peptide might be secreted from human pancreas with the selective mechanism.

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