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INCORPORATION OF TRITIATED LEUCINE INTO INSULIN ANALOGUES IN VITRO

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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. Excised pancreas was incubated in Krebs Henseleit bicarbonate buffer with 0.6 or 3.0 mg/ml glucose and L-[4,5-^{3}H(N)]-leucine. The incubation medium and incubated pancreas were gel chromatographed on the Bio-Gel column after extraction with acid-ethanol. The radioactivity of each fraction was determined by a liquid scintillation counter after addition of Bray's scintillator liquid. 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. Each peak of insulin was not corresponded to that of ^{3}H-leucine radioactivity in all experiments. Also the peak of tritiated leucine was not corresponded to that of C-peptide and glucagon.

These findings suggest that there are two kinds of insulin both in the incubation medium and in the incubated pancreas, but the estimation of incorporated radioactivity of ^{3}H-leucine has to be verified for evaluation of insulin biosynthesis itself in this way of experiment.

INTRODUCTION

The presence of two kinds of immunoreactive insulin (IRI) was reported both in human serum and in human pancreas[1,2,3]. The release of insulin was also discussed in earlier report[3].

It is well known that the labeled amino acids are incorporated into proinsulin and insulin in incubated pancreatic slices[4,5] or pancreatic islets[6,7].

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The incubation study was undertaken to elucidate the presence of insulin analogues in incubation medium and incubated pancreatic tissue, and the concordance of the peak of insulin with that of incorporated ^{3}H-leucine radioactivity was reassessed, using gel filtration after acid-ethanol extraction of the incubation medium and the incubated pancreas.

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 (approximately 50 mg wet weight) 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 the incubation, the pancreas from all three patients was incubated at 37°C in the medium containing 3.0 mg/ml of glucose and ^{3}H-leucine (L-[4,5-^{3}H(N)]-, 100 μCi, 5.0 Ci/mmol, New England Nuclear) for another 4 and 8 hours, respectively. Moreover, after the 8 hours incubation, the 30 minutes incubation of the piece of pancreas from a male patient with gastric cancer was followed by the 60 minutes incubation in the medium of 3.0 mg/ml glucose.

After these incubation, the medium and the incubated pancreas were extracted and gel chromatographed by the reported methods[1,2]. The column was calibrated with ^{125}I-labeled C-peptide and ^{125}I-labeled glucagon, besides porcine ^{125}I-labeled proinsulin and porcine ^{125}I-labeled insulin.

Assay for IRI and C-peptide immunoreactivity (CPR) was performed according to the reported methods[8,9]. Each of the fraction was counted for ^{3}H radioactivity in the liquid scintillation counter (Packard, Tri-carb. Model 3385), using Bray's scintillator liquid[10].

RESULTS

After the 10 minutes and following 30 minutes incubation, two peaks of IRI were detected both in the incubation medium and in the incubated pancreas (Peak I of IRI was reassured with the increased sample volume.) incubated for 4 hours, but those peaks were not corresponded to that of the incorporated ^{3}H-leucine. CPR was also detected as aggregated form (Fig. 1, 2). After the 8 hours incubation, the piece of pancreas was incubated for an hour in the 3.0 mg/ml glucose medium after the preceding 30 minutes incubation. The results of those study were shown in fig. 3. In the medium (Fig. 3, A, A′), the small shoulder of the incorporated ^{3}H-leucine profile was faintly corresponded

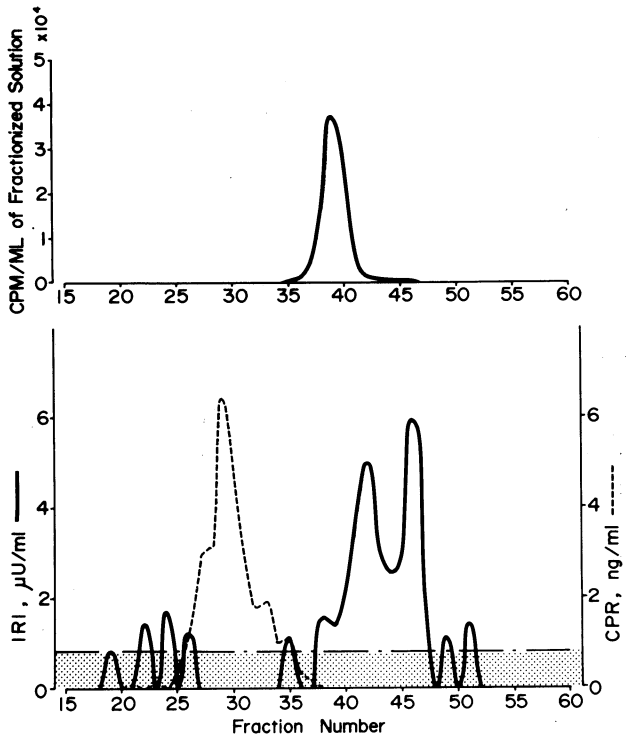

Fig. 1. Elution profiles of incorporated ^{3}H-leucine radioactivity, insulin and C-peptide extracted from incubation medium (N. M., 53 y. o., M.) on the Bio-Gel P-30 column. Krebs Henseleit bicarbonate buffer solution (pH 7.4, 95% O_2/5% CO_2) with 3.0 mg/ml glucose and ^{3}H-leucine for 4 hours at 37°C. Upper panel : Elution profile of incorporated ^{3}H-leucine radioactivity. Lower panel : Elution profiles of insulin and C-peptide. Dotted area : Detection level of IRI

to the peak of IRI, though the two peaks of insulin which was detected both in the incubation medium and in the pancreas incubated for 60 minutes were changed in shape. In the pancreas (Fig. 3, B, B'), the later peak of the incorporated ^{3}H-leucine was corresponded to the peak of IRI, besides the incorporation of ^{3}H-leucine into macromolecular substance. In the incubation study for 4 hours, the main peak of glucagon in the pancreas was corresponded to that in the medium containing 3.0 mg/ml glucose (Fig. 4). But in the 8 hours incubation, the peak of glucagon was shifted to the later eluting position.

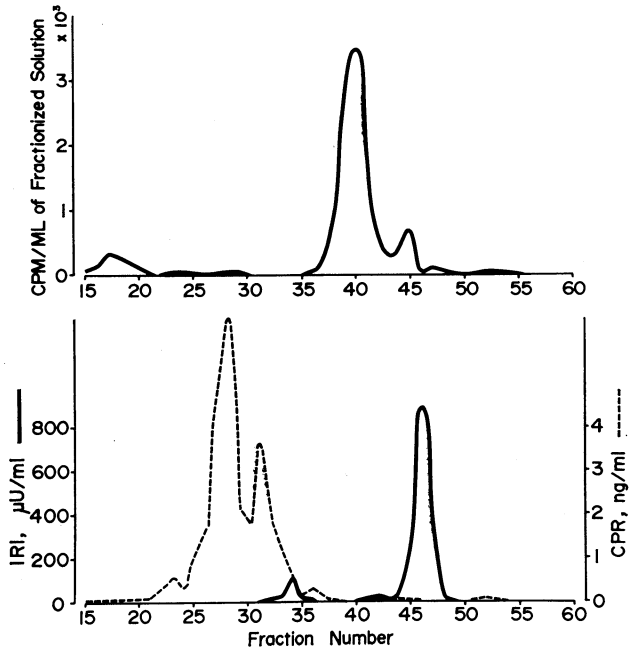

Fig. 2. Elution profiles of incorporated ^{3}H-leucine radioactivity, insulin and C-peptide extracted from incubated human pancreas (M. N., 53 y. o., M.) on the Bio-Gel P-30 column. Krebs Henseleit bicarbonate buffer solution (pH 7.4, 95% O_2/5% CO_2) with 3.0 mg/ml glucose and ^{3}H-leucine for 4 hours at 37°C. Upper panel : Elution profile of incorporated ^{3}H-leucine radioactivity. Lower panel : Elution profiles of insulin and C-peptide.

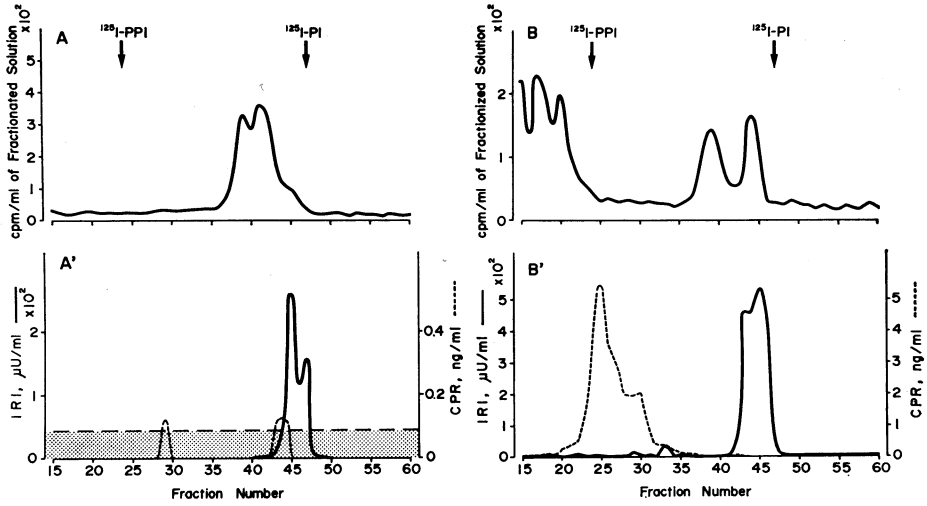

Fig. 3. Elution profiles of incorporated ^{3}H-leucine radioactivity, insulin and C-peptide extracted from incubation medium and incubated human pancreas (I. M., 69 y. o., M.) on the Bio-Gel P-30 column. Krebs Henseleit bicarbonate buffer solution (pH 7.4, 95% O_2/5% CO_2) with 3.0 mg/ml glucose and ^{3}H-leucine for 8 hours at 37°C. Details are shown in the text. A : Elution profile of incorporated ^{3}H-leucine radioactivity extracted from incubation medium. A' : Elution profiles of insulin and C-peptide extracted from incubation medium. B : Elution profile of incorporated ^{3}H-leucine radioactivity extracted from incubated human pancreas. B' : Elution profiles of insulin and C-peptide extracted from incubated human pancreas. Dotted area : Detection level of CPR.

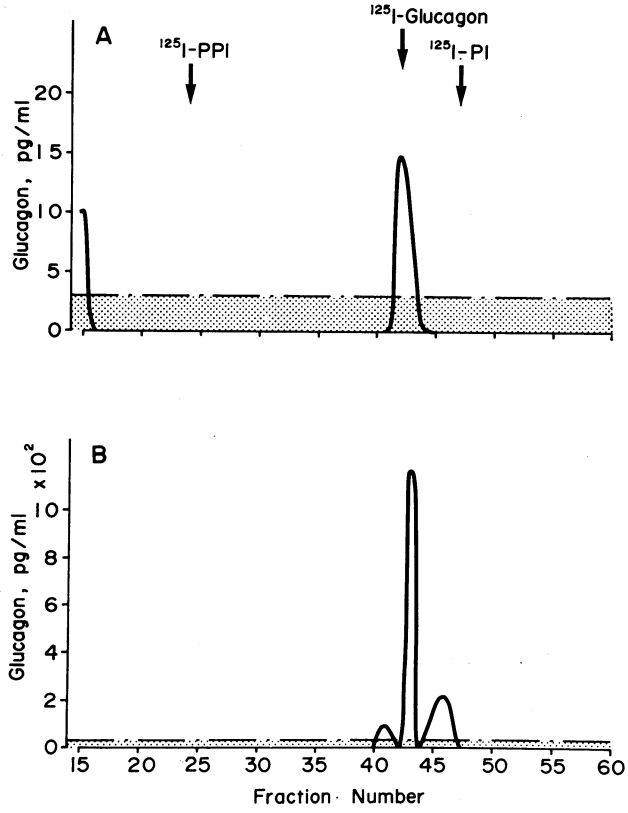

Fig. 4. Elution profiles of glucagon extracted from incubation medium and incubated human pancreas (H. M., 73 y. o., F.) on the Bio-Gel P-30 column. Krebs Henseleit bicarbonate buffer solution (pH 7.4, 95% O_2/5% CO_2) with 3.0 mg/ml glucose for 4 hours at 37°C. Upper panel : Elution profile of glucagon extracted from incubation medium. Lower panel : Elution profile of glucagon extracted from incubated human pancreas. Dotted area : Detection level of GLI. PPI : Porcine proinsulin. PI : Porcine insulin.

DISCUSSION

The presence of two kinds of insulin was first reported both in human sera and in human pancreas after extraction and gel filtration[1,2,3]. The secretion of these insulin analogues was evaluated in earlier report[3]. In this report, the presence of insulin analogues in incubation medium and incubated pancreas was elucidated, and the incorporation of ^{3}H-leucine into these analogues was also reassessed.

In fig. 1,2 the peaks of insulin were not corresponded to that of the incorporated ^{3}H-leucine both in the incubation medium and in the incubated pancreas, though the two peaks of insulin were detected in both. In fig. 3, the change of insulin peaks might be due to digestive action of pancreatic peptidase. The minor peak of the incorporated ^{3}H-leucine was corresponded to that of the insulin. But the incorporation into C-peptide was hardly recognized. A proinsulin molecule has 12 leucine molecules, namely insulin has 6 molecules[11]. From this facts, it is not understandable that no incorporation of ^{3}H-leucine into C-peptide, but the incorporation into insulin were recognized. Therefore, this concordance between the peak of insulin and that of the incorporated ^{3}H-leucine should not mean the result of the incorporation of the radioactive leucine into insulin molecule.

In fig. 4, the peak of glucagon was eluated at the region of ^{125}I-glucagon both in the incubation medium and in the incubated pancreas. But the longer the incubation time, the more the late eluting component of GLI was increased, because of the instability of this hormone, especially due to the susceptibility to pancreatic protease action.

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