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Note

Release of insulin analogues in man, stimulated with glucose

In vitro and in vivo studies

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The circulating insulin immunoreactive material was characterized by disc gel electrophoresis and it was found that the so-called proinsulin fraction was heterogenous [1—3]. Also, Haën et al. [4] reported that the heterogeneity of proinsulin-size immunoreactive insulin (IRI) in circulation was more marked than that of insulin-size material. Moreover, Kimmel and Pollock [5] and Elliott et al. [6] suggested the presence of an abnormal insulin in circulation.

The presence of two groups of insulin was reported in human serum after extraction and gel filtration in an earlier report [7]. The present studies were undertaken to elucidate the presence of the insulin analogues in an incubation medium and incubated human pancreas, and to clarify the levels of these insulin analogues in human sera, obtained from normal adults, well controlled diabetic and poorly controlled diabetic patients.

MATERIALS AND METHODS

Subjects

A piece of human pancreatic tissue was obtained from a patient with localized esophageal cancer (53 years old, male) and from one with gastric cancer (69 years old, male) as the surgical specimen.

Four healthy adults (age 34—70 years old, fasting blood sugar level: 89—96 mg/dl), four well controlled diabetics (age 47—71 years old, fasting blood sugar level: 118—149 mg/dl) and two poorly controlled diabetics (age 54 and 50 years old, fasting blood sugar levels: 203 and 228 mg/dl respectively) were studied. There were no significant differences in obesity among these three groups.

Incubation

About 50 mg (wet weight) of the pancreatic tissue was incubated for 10 min at 37°C (95% O_2–5% CO_2) in Krebs Henselite buffer solution containing 3.0 mg/ml of glucose. Afterwards, the pancreatic tissue was incubated in the medium containing 0.6 mg/ml of glucose for an additional 30 min. After the incubation, the pancreas was incubated for another 4 h at 37°C (95% O_2–5% CO_2) in the medium containing 3.0 mg/ml of glucose.

Tests

Oral glucose tolerance tests (O-GTT) (50 g) were given to all normals and diabetics. The serum samples taken at fasting and 60 min after O-GTT were frozen at −20°C until used.

Extraction and gel filtration

After the incubation, the medium was withdrawn and frozen until used, and the pancreatic tissue was homogenized by Polytron PT 10-35 Kinetmatica for 1 min at setting 7 in the siliconized glass tube with an acid—ethanol mixture (350 ml 99.5% ethanol + 7 ml concentrated hydrochloric acid + 153 ml distilled water). Afterwards, extraction and gel filtration of the samples were the same as in the previously reported method [7].

Assay for IRI and calculations

The assay procedure and the calculation were performed as in the earlier report [7]. The results were expressed as mean ± S.D.

RESULTS AND DISCUSSION

In the incubation study of human pancreas, two peaks of insulin were detected both in the medium and in the pancreatic tissue in the case of 4 h incubation as shown in Fig. 1, but in the case of the first 10-min incubation, the trace of Peak I of insulin was recognized, besides Peak II of insulin as shown in Fig. 2. When the pancreas was directly stimulated with 3.0 mg/ml glucose for 10 min, Peak II of insulin was markedly released into the medium, compared with Peak I of insulin. But in the 4-h incubation, the ratio of Peak I to Peak II of insulin in the medium was higher than that in the pancreatic tissue. From these data, Peak II of insulin is released from the human pancreas by the rapid change of glucose stimulation. On the contrary, maintained in the same condition for 4 h, the pancreatic tissue releases not only Peak II but Peak I of insulin. The proportion of Peak I of insulin in the medium, especially, is increased under the stable concentration of 3.0 mg/ml glucose for 4 h.

The serum samples of fasting and 60 min after O-GTT were gel filtrated after extraction, and the insulin contents of each fraction were assayed in normals, well controlled and poorly controlled diabetic patients. Two groups of insulin were apparently shown in all serum samples as reported elsewhere [7]. But the fraction of proinsulin-like component was not revealed.

Total insulin quantity of each group was calculated as shown in Table I. In normal adults, the calculated fasting values of Peak I of insulin and Peak II of insulin were 0.05 ± 0.01 and 0.01 ± 0.01 p*M*/ml, respectively. The level of Peak

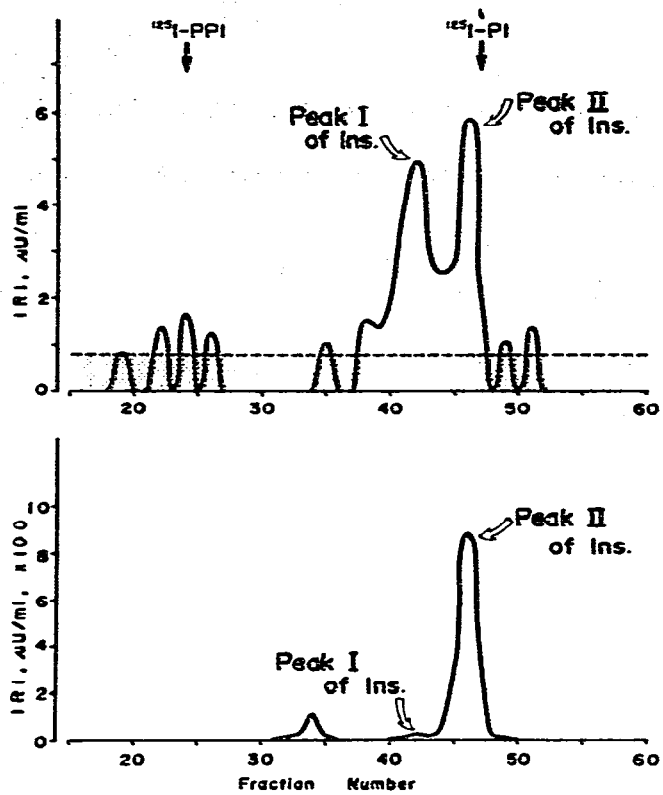

Fig. 1. Elution profiles of extracted human insulin on the Bio-Gel column with 4.05 ml fraction size obtained from incubation medium containing 3.0 mg/ml glucose for 4 h at 37°C (95% O_2—5% CO_2) (above) and from the pancreatic tissue incubated in the same medium under the same conditions (below). Pancreatic tissue was obtained from a patient with localized esophageal cancer (53 years old, male). PPI = porcine proinsulin; PI = porcine insulin. Dotted area shows detection level of IRI.

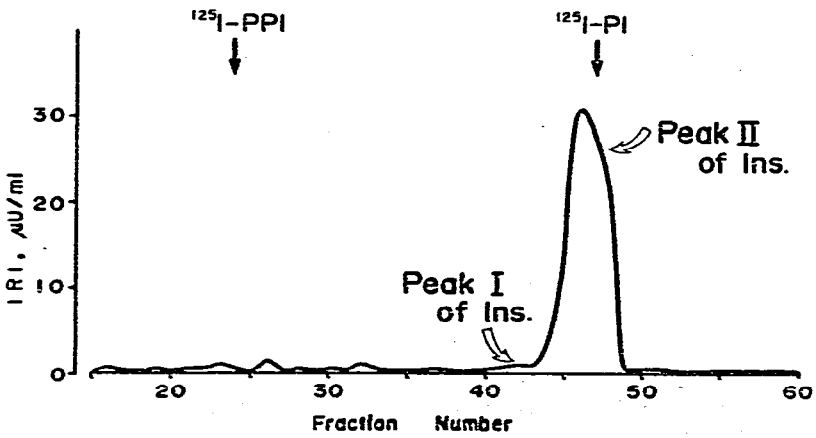

Fig. 2. Elution profile of extracted human insulin on the Bio-Gel column with 4.05 ml fraction size obtained from incubation medium containing 3.0 mg/ml glucose for 10 min at 37°C (95% O_2—5% CO_2) with pancreatic tissue obtained from a patient with localized esophageal cancer (53 years old, male). PPI = porcine proinsulin; PI = porcine insulin. Detection level is 0.1 μU/ml for IRI.

TABLE I

LEVELS OF INSULIN OBTAINED AT FASTING AND 60 MIN AFTER GLUCOSE LOADING

F, fasting; n.s., not significant. These values were corrected with the recovery rate of extraction (0.833) and gel filtration (0.647).

Case	*n*	Maximum BS level after 50-g O-GTT (mg/dl)	Time (min)	Peaks (p*M*/ml)	
				Peak I	Peak II
Normals	4	139.7 ± 25.9*	F	0.05 ± 0.01	0.01 ± 0.01
				n.s.	$p < 0.05$
		$p < 0.02$	60	0.07 ± 0.02	0.05 ± 0.03
Well controlled diabetics	4	279.0 ± 40.3	F	0.04 ± 0.01	0.01 ± 0.00
				$p < 0.01$	$p < 0.01$
		$p < 0.02$	60	0.10 ± 0.01**	0.08 ± 0.02**
Poorly controlled diabetics	2	453.0 ± 7.0*	F	0.04 ± 0.01	0.00 ± 0.00
				n.s.	n.s.
			60	0.05 ± 0.01**	0.02 ± 0.01**

*Significant difference ($p < 0.001$).
**Significant difference ($p < 0.05$).

I of insulin at the fasting state was observed to be higher than that of Peak II of insulin. At 60 min after 50 g O-GTT, the calculated values of Peak I of insulin and Peak II of insulin were 0.07 ± 0.02 and 0.05 ± 0.03 p*M*/ml, respectively. Peak II of insulin was elevated after glucose load ($p < 0.05$), but Peak I of insulin was not changed.

In well controlled diabetics, the levels of Peak I and Peak II of insulin were 0.04 ± 0.01 and 0.01 ± 0.00 p*M*/ml, respectively, at the fasting state. The level of Peak I of insulin obtained was also higher than that of Peak II of insulin at the fasting state. At 60 min after O-GTT, the calculated values of Peak I and Peak II of insulin were 0.10 ± 0.01 and 0.08 ± 0.02 p*M*/ml, respectively. In this group, both Peak I and Peak II of insulin were elevated in the serum after the stimulation of glucose ($p < 0.01$). Well controlled diabetics showed better response to glucose loading than normals in both insulin peaks, especially in Peak I of insulin. It was first noted by Yalow and Berson [8] that the total insulin secretory response in diabetics with glucose loading was significantly greater than normals and this has been subsequently confirmed by many laboratories [9—13]. The increase of total insulin in diabetics should be responsible in part for increments of Peak I of insulin, though Peak I of insulin was not increased by the stimulation of glucose in normals.

In poorly controlled diabetics, the values of Peak I and Peak II of insulin were 0.04 ± 0.01 and 0.00 ± 0.00 p*M*/ml, respectively at the fasting state. The level of Peak I of insulin was observed to be higher at the fasting state than that of Peak II of insulin. The fasting serum level of Peak I of insulin was 0.04—0.05 p*M*/ml amongst the three groups. At 60 min after glucose loading, the

total amounts of Peak I and Peak II of insulin were 0.05 ± 0.01 and 0.02 ± 0.01 p*M*/ml, respectively. In poorly controlled diabetics, increments in Peak I and Peak II of insulin were not observed. There were significant differences in the calculated values of Peak I and Peak II of insulin at 60 min after glucose loading between well controlled and poorly controlled diabetics ($p < 0.05$). It seems that the Peak II reservoir to glucose stimulation is restricted in poorly controlled diabetic patients.

The fraction of proinsulin-like material was not detected in the experiment of incubation and the study of human serum. These facts are quite compatible with the level of proinsulin in human plasma, reported by Turner and Heding [14].

From the above mentioned results, the following conclusions are suggested. (1) There are two groups of insulin not only in circulating human sera but in incubation medium and incubated human pancreas. (2) In normals, Peak II of insulin is released by glucose loading, but Peak I of insulin is not. (3) In a well controlled diabetic patient, both Peak I and Peak II of insulin are secreted by the same stimulation. Therefore, the secretion mechanism of Peak I of insulin could be different from that of a normal. (4) In poorly controlled diabetics, neither Peak I nor Peak II of insulin are released by the same loading. In this case, the impairment could be mainly in the secretion mechanism of Peak II of insulin. (5) The insulin secretion from human B-cell could firstly be hyperresponsive to the loading of glucose in the early stage of diabetes mellitus; according to the progress of the disease, the function of B-cell might be impaired gradually.

Further studies of Peak I and Peak II of insulin could lead to a better understanding of the mechanisms regulating the synthesis and secretion of insulins and its patho-physiological roles in man.

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