Hypersecretion of Proinsulin in Thyrotoxicosis

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Summary. The plasma insulin, C-peptide and proinsulin concentrations were investigated in thyrotoxic patients and in normal controls after an overnight fast, during a 36 h fasting period, an intravenous glucose tolerance test and an oral glucose tolerance test. The main finding was a significantly raised concentration of proinsulin in plasma of patients with thyrotoxicosis. After an overnight fast the plasma proinsulin was 0.048 ± 0.005 pmol/ml in 15 thyrotoxic patients compared with 0.023 ± 0.012 pmol/ml in 15 euthyroid controls. A twofold rise of plasma proinsulin concentration was also found in thyrotoxic patients during a prolonged fast, and during intravenous and oral glucose tolerance tests. The immunoreactivity of proinsulin in the insulin radioimmunoassay gave rise to slightly elevated concentrations of immunoreactive insulin in thyrotoxic patients in all the conditions investigated. When insulin values were corrected for proinsulin crossreactivity, they were similar in euthyroid controls and thyrotoxic patients. The concentration of plasma C-peptide was not significantly altered in thyrotoxic patients during intravenous and oral glucose tolerance tests.

Key words: Thyrotoxicosis, insulin secretion, proinsulin, C-peptide, insulin immunoreactivity.

Introduction

The effect of thyrotoxicosis on insulin secretion in man has not been completely clarified. In thyrotoxic patients insulin determined radioimmunochemically may be either normal or increased both in the fasting state and after a glucose challenge [1–4], while glucose tolerance is impaired [1–3]. On the other hand, it has been shown experimentally that insulin secretion is decreased in hyperthyroid animals [5–7], and

the pancreatic insulin content from such animals is decreased [8]. All studies to date have used radioimmunoassays for the measurement of insulin. Such assays measure not only insulin but also proinsulin, and the contribution of the latter moiety is variable. In order to see whether proinsulin contributes significantly to the immunoreactive insulin concentrations in thyrotoxic patients, we have measured proinsulin, insulin, and C-peptide in a group of thyrotoxic patients following short and prolonged fasting and during glucose tolerance tests.

Subjects and Methods

Subjects

The clinical details of the patients and controls are given in Table 1. The study included three different patient groups with thyrotoxicosis which underwent either A) an overnight fast, B) a 36 h fast or C) oral and IV glucose tolerance tests. No subject appeared in more than one group. None of the subjects were taking drugs apart from sedatives of the benzodiazepine group, and none suffered from any other major disease. Before the study informed consent was obtained from all patients.

Each group had a separate group of euthyroid controls, which for groups B and C were selected to match the patient group with respect to age and sex. Group A were consecutive patients appearing in the outpatient clinic, and the control group consisted of outpatients with minor illnesses, mostly varicose veins.

Protocols

Plasma insulin, C-peptide, and proinsulin concentrations were determined in all patients and controls in the three study groups (Table 1). The prolonged fasting period was preceded by a light meal (1600 kJ). Blood samples were obtained from an indwelling needle in the antecubital vein every 6 h. During the fasting period smoking was prohibited. An oral glucose tolerance test was performed by giving 1.75 g glucose/kg body weight and blood samples were taken eight times during the following 180 min. An IV glucose tolerance test was performed by giving 0.5 g glucose/kg body weight in a 50% (w/v) solution IV over a 3-min period. Five plasma samples were collected over the following 60 min from an indwelling needle.

Table 1. Data for thyrotoxic patients and control subjects

		No of patients	Age (years)	Deviation from ideal body weight (%)	♀/♂	L-thyroxine (µg/100 ml) (4.3–10.0)	Triiodothyronine (ng/100 ml) (106–182)
Group A	Overnight fast (controls)	5	39±6	-9.7±13	4/1	6.7-0.9	147-5
	Overnight fast (thyrotoxic)	6	62±4	-9.9±7.9	6/0	12.1±2.0	309±48
Group B	Prolonged fast (controls)	4	36±6	-7±10	3/1	6.7±1.8	114±20
	Prolonged fast (thyrotoxic)	4	35±5	-9±11	3/1	19.4±5.4	412±292
Group C	Glucose tolerance tests (controls)	6	43±18	-3.8±12	6/0	6.2±1.2	121±17
	Glucose tolerance tests (thyrotoxic)	5	46±16	-1.2±11	5/0	14.8±4.0	432±199

Results expressed as mean ± SEM (Normal range for thyroid hormones given in parentheses)

Fig. 1. Mean changes in blood glucose, plasma IRI, proinsulin and C-peptide concentrations in four euthyroid control subjects (●) and four thyrotoxic patients (○) during a 36 h fasting period following a light meal. Significant differences were found at 6 and 12 h for proinsulin ($p < 0.05$)

Chemical Methods

Plasma glucose was determined by the glucose oxidase method (Boehringer Mannheim, Germany). Serum thyroxine and triiodothyronine concentrations were determined radioimmunochemically [9, 10]. Plasma immunoreactivity of insulin (IRI) was determined as described previously [11]. Plasma C-peptide was determined after removal of proinsulin by Sepharose-bound insulin antibodies (S-AIS) by a radioimmunoassay using antiserum M 1181, synthetic ^{125}I -Tyr-C-peptide and synthetic C-peptide [12]. Plasma proinsulin was determined after binding to S-AIS, using human proinsulin (0.005–0.5 pmol/ml; donation from Professor A. Rubenstein, Chicago) as a standard and synthetic ^{125}I -Tyr-C-peptide as tracer [13]. The antiserum was either anti-crude proinsulin serum M 1181 or anti-synthetic C-peptide serum M 1219.

The sensitivity expressed as SD within assay was for IRI 0.0025, for proinsulin 0.001, and for C-peptide 0.004 pmol/ml. The detection limit was for IRI 0.0062, for proinsulin 0.002, and for C-peptide 0.01 pmol/ml.

The insulin concentration was calculated as the IRI concentration with subtraction for proinsulin crossreactivity, which in our assay amounts to 67% of the proinsulin concentration [14].

Values are given as mean ± SEM. Differences in the mean values of individual points and differences in the areas under the concentration curves during the tolerance tests were evaluated by Student's unpaired t-test.

Results

Plasma Hormone Concentrations After an Overnight Fast

The thyrotoxic patients in group A had a significantly elevated plasma proinsulin concentration (0.049 ± 0.005 pmol/ml) ($n = 6$) compared with euthyroid

controls (0.027 ± 0.010 pmol/ml ($n = 5$, $p < 0.05$). The concentrations of insulin and C-peptide did not differ in the patients and controls.

Plasma concentrations of the peptides measured after 12 h fast in all 15 controls and in the 15 thyrotoxic patients were also analysed. The plasma IRI concentration was 0.042 ± 0.017 pmol/ml in euthyroid controls and significantly higher in thyrotoxic patients (0.064 ± 0.028 pmol/l, $p < 0.02$). In contrast the plasma C-peptide concentration was identical in the two groups (0.57 ± 0.06 pmol/ml in the controls and 0.57 ± 0.01 pmol/ml in the thyrotoxic patients). Again the most important difference was in the plasma proinsulin concentration, which was 0.023 ± 0.012 pmol/ml in the control subjects and 0.048 ± 0.006 pmol/ml in the thyrotoxic patients ($p < 0.001$).

If, however, the IRI concentration is corrected for crossreacting proinsulin in the assay, the insulin concentration in the normal controls was 0.026 ± 0.011 pmol/ml, which is similar to 0.033 ± 0.010 pmol/ml found in the thyrotoxic patients. Moreover it can be concluded that on a molar basis insulin and proinsulin are circulating in equal concentrations in fasting euthyroid subjects (0.026 and 0.027 pmol/ml, respectively), whereas in thyrotoxic patients the proinsulin concentration is 50% higher than insulin concentration after an overnight fast (0.049 versus 0.033 pmol/ml, respectively).

Plasma Hormone Concentrations During a Prolonged Fast

The concentrations of glucose, insulin, C-peptide and proinsulin during a 36-h fasting period are shown in Fig. 1. Concentrations of IRI, proinsulin, and C-peptide achieved a basal state 6 h after a light breakfast in the four normal controls, but not in the thyrotoxic patients. The concentrations of plasma insulin and C-peptide were not significantly different in the two groups. The general level of the proinsulin concentration was two times higher in the thyrotoxic group than in the controls, with significantly different mean values at 6 and 12 h after the light meal.

Plasma Hormone Concentrations During an Oral Glucose Tolerance Test (Fig. 2)

After an oral glucose tolerance test there were no significant differences between the insulin (and C-peptide) values at individual points or between the areas under the insulin (or C-peptide) curve in the normal and the thyrotoxic subjects. However, the

Fig. 2. Mean changes in blood glucose, plasma IRI, proinsulin and C-peptide concentrations in six euthyroid control subjects (●) and five thyrotoxic patients (○) during an oral glucose tolerance test. Significant differences were found for proinsulin at 30, 45, 60 and 180 min ($p < 0.05$) and for glucose at 30, 45 and 60 min ($p < 0.05$).

subjects with thyrotoxicosis showed a marked difference in the blood glucose levels.

The pattern of secretion of proinsulin was very similar to that of C-peptide with an approximately linear increase in the plasma concentration up to 60 min after the glucose load. However, the proinsulin concentration in the thyrotoxic group was twofold greater than in the control group, and significant differences were attained at the individual points 30, 45, 60, and 180 min.

Plasma Hormone Concentrations During a IV Glucose Tolerance Test (Fig. 3)

As with the oral glucose tolerance test, these subjects showed no significant differences with regard to IRI and C-peptide concentrations between the groups

Fig. 3. Mean changes in the blood glucose, plasma IRI, proinsulin and C-peptide concentrations in six euthyroid control subjects (●) and five thyrotoxic patients (○) (the same subjects as in Fig. 2) during a glucose tolerance test. Significant differences were found for proinsulin at all sampling points ($p < 0.05$) except 60 min

during an IV glucose tolerance test. However, the plasma proinsulin concentration before and during a glucose tolerance test was increased by 100% in the thyrotoxic patients compared with the normal controls. Significant differences were attained at all individual sample points except 60 min.

In one patient an oral glucose tolerance test and an IV glucose tolerance test were performed before and after treatment of the thyrotoxicosis. Following therapy with propylthiouracil, the fasting proinsulin concentration decreased from 0.046 to 0.015 pmol/ml. During the oral glucose tolerance test, the peak value decreased from 0.500 (at 60 min) to 0.140 pmol/ml (at 150 min) following treatment. The peak values during the IV glucose tolerance test were 0.180 (at 5 min) and 0.060 pmol/ml (at 60 min) before and after treatment respectively.

Discussion

The main finding in this study is that thyrotoxicosis is accompanied by significant elevations in the plasma concentration of circulating proinsulin. This phenomenon in thyrotoxic patients was observed in the fasting state and also during both oral and IV glucose tolerance tests. Thus, in hyperthyroidism it could be argued that the pancreatic B cell has an increased capacity to secrete proinsulin compared with the euthyroid state, and proinsulin is circulating in high concentrations because of the relatively slow rate of proinsulin removal [15, 16]. In contrast to insulin independent diabetes, where the fasting proinsulin concentration is also increased [17], the concentration of proinsulin in thyrotoxicosis is frequently so high that it is in the range which is considered characteristic of patients with insulinomas [14, 18].

The phenomenon of hyperinsulinaemia may be far more common than generally appreciated since proinsulin crossreactivity with insulin has been usually ignored in studies of various conditions in which insulin responses to glucose or other stimuli have been determined.

The circulating "true insulin" concentration is the same in thyrotoxic patients and in controls. However, as judged from the C-peptide response to glucose, the capacity for insulin secretion is unchanged or somewhat decreased in thyrotoxicosis. The latter possibility is in line with experimental studies which show a decreased response of insulin secretion to glucose in hyperthyroidism [5–7, 19]. In such animals the pancreatic islet mass is also decreased [8].

A combination of decreased insulin secretion and normal circulating insulin concentrations may be explained by a decreased rate of degradation of insulin in hyperthyroidism, but there is no direct evidence for this in man [20, 21]. Proinsulin can displace native insulin from the insulin receptor [22, 23], but the affinity of proinsulin for the insulin receptor and its biological effect is only about 2% of that of insulin [22, 23]. Therefore, neither decreased insulin degradation nor glucose intolerance in thyrotoxic patients can be understood on the basis of receptor interactions between insulin and proinsulin.

As shown previously [11] glucose tolerance may be impaired in thyrotoxic patients despite a normal level of circulating insulin. During an oral glucose tolerance test and an IV glucose tolerance test the inability of the thyrotoxic liver to accumulate glycogen [24] may play a major role in glucose intolerance. The mechanism behind this phenomenon is unknown, but, again, it seems unlikely that it is due to interference of proinsulin with insulin, since in

other hyperproinsulinaemic states, such as insulin independent diabetes, the liver content of glycogen is increased after an overnight fast [25].

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