NOTE

Qualitative Abnormality of Insulin Secretion in a Case with Insulinoma

YUTAKA HARANO, MOTOO KOHAMA, NOBORU ARAKI,
ARITOSHI SAKAMOTO*, MITSURU HOSHI,
MOTOAKI SHICHIRI, KENJI SHIMA**, JUN OKAMURA***
AND YUKIKO SHIGETA

First Department of Medicine, Osaka University Medical School,
Fukushima-ku, Osaka 530,
*Kansai Rosai Hospital, Hyogo 663,
**Central Laboratory for Clinical Investigation, Osaka University
Medical School, Fukushima-ku, Osaka 530
and
***Second Department of Surgery, Osaka University
Medical School, Fukushima-ku, Osaka 530

Synopsis

We have presented here a case of atypical insulinoma. Despite the recurrent
episodes of hypoglycemic symptoms, the plasma level of insulin has never been
excessive at fasting or by regular provocative tests. Detailed examination had demon-
strated qualitative rather than quantitative abnormality of insulin secretion. Hypo-
suppressibility of insulin secretion by hypoglycemia, borderline diabetic curve of glucose
tolerance test, blunted response of insulin to glucagon and leucine were the principal
characteristics of these abnormalities. After removal of adenoma, insulin response to
glucose, glucagon and leucine was improved. Only secretin provoked a high level
of insulin and this abnormal elevation was no longer seen after the removal of
adenoma. A removed insulinoma contained 25 U of immunoreactive insulin per
gram tissue, but was negative for aldehyde-fuchsin staining. On electronmicroscopy,
only atypical β-cell granules were seen.

Even if the Whipple’s triad (Whipple, 1952), consisting of a history of hypogly-
cemic episode, a fasting blood sugar level of 50 mg/100 ml or less during attacks and
immediate recovery upon administration of glucose are fulfilled, failure of demonstrat-
ing a high level of circulating insulin either at fasting or by various insulin-provocative
tests makes the diagnosis of insulinoma difficult to establish. An attempt to demon-
strate the high level of insulin in portal vein (Samols and Marks, 1963) is not an
easy procedure. Determination of an elevated fasting plasma level of proinsulin may
be helpful in establishing the diagnosis of

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Case Report

A 50-year-old working woman was admitted to the hospital because of frequent hypoglycemic symptoms with two episodes of syncope before lunch. Determination of circadian changes of blood sugar revealed fasting blood sugar ranging from 35 to 75 mg/100ml with plasma immuno-reactive insulin (IRI) from 10 to 25 μU/ml. Liver function tests and pancreatic exocrine function test (pancreozymin-secretin) were normal. There was no family history of diabetes. Physical examinations were unremarkable. One of the repeated selective celiac angiography demonstrated vascular staining in the head of the pancreas suggesting insulinoma. On laparotomy, 1 cm × 1 cm-sized adenoma was found at the junction of the pancreatic head and the body, and was enucleated (Fig. 1).

Materials and Methods

Immuno-reactive insulin (IRI) was measured by double antibody method (Hales and Randle, 1963). Biological activity of insulin contained in the tumor was determined by blood sugar-lowering effect in rabbits, using an aliquot of acid alcohol-extracted fraction (Lacy, 1941). Immuno-reactive glucagon (IRG) was measured by the method of Shima and Foa (Shima and Foa, 1968). Blood sugar was measured by Hoffman’s method (Hoffman, 1937).

Laboratory Findings

Preoperative data are shown in comparison with postoperative results. Oral glucose tolerance test (50g) raised plasma IRI only by 16.3 μU/ml before operation and by 50.5 μU/ml postoperatively (Fig. 2). Results of the blood sugar response curve were interpreted as borderline diabetes1) in both of them. Intravenous administration of 1 mg glucagon provoked no substantial amounts of plasma insulin while blood sugar was elevated by 36 mg/100ml within 10 min. After excision of the tumor, normal response of

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Fig. 1. Gross appearance of the adenoma removed at surgery.

Fig. 2. Pre-and post-operative results of the oral glucose tolerance tests (50g).
insulin secretion was observed (Fig. 3). By the tolbutamide tolerance test, blood sugar was 22 mg/100 ml at 30 min and hypoglycemia below 35 mg/100 ml was sustained for two hours (Fig. 4). An exaggerated IRI response was seen only when secretin was infused intravenously (1 Harper U/kg body wt.) as shown in Fig. 5. Blood sugar dropped from 45 mg/100 ml to 39 mg/100 ml 15 min after secretin. After enucleation, this abnormal increase of plasma IRI was no longer seen. The plasma IRI response to oral leucine was nil, but the normal response was seen postoperatively. There was a moderate increase of plasma IRI (26 μU/ml at 20 min) following the arginine infusion. After the operation, the response was diminished to 16.3 μU/ml.

Fig. 3. Effects of i.v. injection of 1 mg glucagon on blood sugar and IRI before and after the removal of adenoma.

Fig. 4. Pre-and post-operative results of the tolbutamide tolerance tests (1 g i.v.).

Fig. 5. IRI response to i.v. secretin injection. One Harper U per kg body wt. of the same lot no. of secretin (Boots Co., Ltd.) was injected before and after the removal of adenoma.

1) Using criteria for whole blood recommended by the Japan Diabetic Society, the GTT is abnormal when the 1-hour peak value is 160 mg/100 ml or higher, and the 2-hour level is 130 mg/100 ml or higher. If the peak level fell between 130 mg/100 ml and 160 mg/100 ml or 2-hour level between 100 mg/100 ml and 130 mg/100 ml, the test is considered borderline.
Plasma or urine levels of insulin-antagonistic hormones are shown in Table 1. Fasting plasma cortisol, growth hormone, glucagon and thyroxine were all within normal range in spite of hypoglycemia with blood sugar around 50 mg/100 ml. Urinary excretion of vanillylmandelic acid (VMA) was also not elevated.

Table 1. Serum or urine levels of insulin-antagonistic hormones at fasting or during the day

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Unit</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>ng/ml</td>
<td>1.0</td>
<td>(0.5–1.0)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>µg/100 ml</td>
<td>10.6</td>
<td>(5.0–18.0)</td>
</tr>
<tr>
<td>GH</td>
<td>ng/ml</td>
<td>5.6</td>
<td>(&lt;5.0)</td>
</tr>
<tr>
<td>TSH</td>
<td>ng/ml</td>
<td>1.3</td>
<td>(&lt;10.0)</td>
</tr>
<tr>
<td>PBI</td>
<td>µg/100 ml</td>
<td>4.6</td>
<td>(3.0–8.0)</td>
</tr>
<tr>
<td>VMA (urine)</td>
<td>mg/day</td>
<td>6.3</td>
<td>(2.5–9.5)</td>
</tr>
<tr>
<td>17-OHCS (urine)</td>
<td>mg/day</td>
<td>4.1</td>
<td>(3.0–11.0)</td>
</tr>
</tbody>
</table>

Values are the average of 2 to 4 determinations. Values in parentheses show normal range.

Hormonal Contents and Histology of the Enucleated Adenoma

Excised adenoma per gram contained 25 U of immunoreactive or 14.7 U of biologically active insulin. The insulin amount was at least 5 fold more than those contained in the pancreas obtained from normal control subjects (Table 2). Immuno-reactive glucagon was also contained to the similar extent to the normal pancreas. Histological study demonstrated this tumor to be a benign adenoma, which was not stained by aldehyde fuchsin. The adenoma contained considerable amount of connective tissue. By electron microscopy, typical cored granules of β-cells were not seen and instead atypical diffuse granules were seen (Fig. 6).

After the operation fasting blood sugar never fell below 80 mg/100 ml. The patient remained borderline diabetic at 16 months after the enucleation.

Table 2. Insulin and glucagon contents of the excised adenomas

<table>
<thead>
<tr>
<th>Hormonal Content</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoreactive insulin</td>
<td>U/g</td>
<td>25.0</td>
</tr>
<tr>
<td>Biological activity of insulin</td>
<td>U/g</td>
<td>14.7</td>
</tr>
<tr>
<td>Immunoactive glucagon</td>
<td>U/g</td>
<td>746</td>
</tr>
</tbody>
</table>

a) Hormonal contents were determined using an aliquot of acid-alcohol-extracted fraction of the adenoma. Values are expressed per g fresh tissue weight.
b) Normal value ranges from 1 to 3 U/g.
c) Normal range; 200-800 ng/g.

Table 3. Relative hyperinsulinism in relation to fasting blood sugar (FBS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preoperative</th>
<th>Postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/100 ml)</td>
<td>(16) 54±12.3*</td>
<td>(13) 104±13.9</td>
</tr>
<tr>
<td>IRI (U/ml/FBS)</td>
<td>(10) 0.3±0.01*</td>
<td>(6) 0.14±0.03</td>
</tr>
<tr>
<td>FF A (pEq/l/FBS)</td>
<td>50/72±0.7</td>
<td>200/70±3.0</td>
</tr>
<tr>
<td>3-hydroxybutyrate (pEq/l/FBS)</td>
<td>50/36±1.4</td>
<td>200/80±2.5</td>
</tr>
</tbody>
</table>

* By t-test v.s. postoperative results, P<0.001. No. in parentheses indicates no. of determinations.

Fig. 6. An electronmicrograph of the removed adenoma (×56,000). The adenoma cell contains electron dense spherical granules which mimic α-granules rather than typical β-granules. (Fixed in glutaraldehyde and in osmium tetroxide, and then doubly stained with uranium acetate and lead acetate).
Discussion

Despite the frequent episodes of hypoglycemia, an excessive response of plasma IRI was not quantitatively demonstrable by regular provocative agents. However, fasting plasma IRI was relatively high, considering the mean fasting blood sugar of 54 mg/100 ml (Table 3). A ratio of fasting IRI to FBS was higher than normal. After the operation this ratio decreased from 0.3 to 0.14. A low level of serum free fatty acids (FFA) and ketone bodies indicates the suppression of lipolysis by this level of fasting insulin. Insulin is known to suppress lipolysis in adipose tissue in small amounts (Fain and Rosenberg, 1972) and ketogenesis in the liver (Harano et al., 1972). In normal subjects, hypoglycemia induced by fasting or by exogenous insulin renders circulating endogenous insulin level extremely low (Turner et al., 1971; Turner et al., 1973), allowing the elevation of FFA and ketone bodies to replace glucose as an energy source. In the present case, shutting-off mechanism of insulin secretion seemed abnormal, presumably because of a disorder in the fine control mechanism of insulin secretion in adenoma cells.

Low or no response of insulin release to glucose, glucagon and leucine preoperatively and improved response after operation need further explanation. This adenoma seemed to release only small amounts of insulin to these stimuli, but to have a somewhat higher basal rate of insulin secretion. In the uninvolved pancreas the secretion of the insulin might be suppressed either by the existing hypoglycemia or by the unknown humoral substance released from the tumor tissue. For the maximal response of insulin for these stimuli, a normal level of blood sugar is known to be necessary (Turner et al., 1971; Turner et al., 1973). The nature of the humoral substance is speculative, but may possibly alter glucose metabolism (Suda et al., 1965) which is closely related to insulin secretion (Ashcroft et al., 1972).

An interesting observation is that secretin provoked much higher level of insulin preoperatively than when the same lot number of secretin was injected postoperatively. The insulin may be released from adenoma since the amount of insulin provoked by secretin exceeded the normal range. On the other hand, secretin was shown by a separate investigation to require normal level of blood sugar for the maximum release of insulin in normal pancreas. The reason why only secretin provoked excessive insulin is not known, but secretin might have different mode of action on insulin secretion from other insulin secretagogues.

Regarding insulin-antagonistic hormones, plasma levels of glucagon, cortisol, growth hormone, thyroxine and urinary excretion of VMA were all within normal range. It is probable that hypoglycemia in a case of insulinoma may be gradual in onset and does not serve as stimuli for the release of these antagonistic hormones. This is in contrast to insulin tolerance test where marked reduction of blood sugar occurs within 60 min. When this subject was given predonison, 20 mg/day for two weeks, fasting blood sugar did not rise above 65 mg/100 ml with a rise of IRI up to 55 μU/ml. Injection of long-acting ACTH for 3 days also did not increase fasting blood sugar. These facts suggest that the increase of insulin-antagonistic hormones would be accompanied by a rise of insulin with the consequense of no rise of blood sugar.

The removed adenoma contained 14.7 U per gram tissue of biological insulin. As immunoreactive insulin, the adenoma contained 10 U more insulin. The difference between immunological and biological activity of insulin content may be explained by the presence of proinsulin. However, gel filtration of serum revealed a single peak of immuno-reactive regular insulin showing that circulating proinsulin is minimal if present.

2) unpublished observation, Harano, Y. & Y. Shigeta.
The excised adenoma contained cells with electron dense spherical granules. Creutzfeldt recently classified insulinoma into 4 types based on the ultrastructural appearances (Creuzfeldt et al., 1973). Atypical diffuse granules-containing insulinoma is classified as type 3. Negativity for aldehyde-fuchsin-staining and the degree of insulin content were also similar to their description. Diagnosis of insulinoma was difficult to make morphologically, but was established by the insulin content of the excised adenoma in the present case.

A positive vascular staining on celiac angiography and a number of qualitative abnormalities of insulin secretion led us to the final diagnosis.

References