Insulinoma with Normal Plasma Insulin Concentrations and Insulin/Glucose Ratios during Hypoglycemic Episodes

Naoki HATTORI, Takashi ISHIHARA, Yasuhiko SAIKI*, Kunisaburo MORIDERA,
Megumu HINO*, Katsuji IKEKUBO* and Hiroyuki KURAHACHI

A patient with insulinoma had frequent hypoglycemic episodes with normal plasma insulin levels and insulin/glucose ratios. When immunoreactive insulin (IRI) concentrations in this patient were compared among plasma samples with the same C-peptide immunoreactivity (CPR) levels, the concentrations were significantly lower than in control patients with insulinoma and equal to or lower than those of normal subjects. In hepatic venous samples, CPR levels were significantly higher and the IRI/CPR molar ratios were lower than those in a control subject. These results may indicate that normoinsulinemia in this patient could be explained by increased hepatic extraction of insulin.

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Key words: β -cell tumor, hypoglycemia, normoinsulinemia

Introduction

Insulinoma is a pancreatic islet cell tumor that secretes excessive amounts of insulin and frequently causes hypoglycemia. A diagnosis of inappropriate hyperinsulinism can be made based on fasting plasma levels of glucose and insulin after an overnight fast or more prolonged fasting. An insulin ($\mu\text{U/ml}$)/glucose (mg/dl) ratio (I/G ratio) in excess of 0.3 is generally indicative of insulinoma (1). However, insulinomas in patients with normal plasma insulin levels and I/G ratios have been reported (2, 3). We treated a patient with frequent hypoglycemia; diagnosis of insulinoma was made by the recently developed arterial stimulation venous sampling (ASVS) technique (4). The plasma insulin levels and I/G ratios in this patient were both normal despite frequent hypoglycemic episodes. In the present study, we investigated the mechanism of normoinsulinemia in this patient.

Subjects and Methods

Case report (case 1)

A 39-year-old man was admitted to our hospital because of syncope. His plasma glucose was 39 mg/dl (2.2 mmol/l). He fully recovered after intravenous administration of glucose. He had been well until six months earlier when he began to experience typical symptoms of fasting hypoglycemia. His

height was 173 cm and weight 66.5 kg. Biochemical data were unremarkable except for low plasma glucose levels. Thyroid and adrenal functions were normal. Anti-insulin and anti-insulin receptor antibodies were not detected. Provocative tests were performed (Table 1). Overnight fasting test revealed symptomatic hypoglycemia [plasma glucose 38 mg/dl (2.1 mmol/l)] but the insulin level [3.6 $\mu\text{U/ml}$ (25.8 pmol/l)] and I/G ratio (0.95) were normal. Leucine-loading test elicited an abnormal rise in the insulin level.

Abdominal echography, computerized tomography (CT), magnetic resonance image (MRI) and angiography were all negative. Thus, arterial stimulation venous sampling (ASVS) was performed as described by Doppman et al (4). Rather than sampling blood from portal veins which supply each region of the pancreas, blood was taken from hepatic veins after calcium injection as an insulin secretagogue into arteries supplying each region of the pancreas. Catheters were positioned in the right and left hepatic veins for hepatic venous sampling. After femoral arterial puncture, the gastroduodenal, superior mesenteric, splenic and hepatic arteries were catheterized in turn. Calcium gluconate (0.025 $\text{mEq Ca}^{++}/\text{kg}$) was injected into each artery followed by hepatic venous sampling at 0, 0.5, 1, 1.5, 2, and 3 minutes. Calcium injection into the splenic artery but not the other vessels resulted in a seven-fold rise in the insulin concentration at 1 minute [peak value: 562 $\mu\text{U/ml}$ (4,032 pmol/l) vs $28.4 \pm 15.5 \mu\text{U/ml}$ ($204.5 \pm 111.2 \text{ pmol/l}$) in the

From the Departments of Endocrinology and *Nuclear Medicine, Kobe City General Hospital, Kobe

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Reprint requests should be addressed to Dr. Naoki Hattori, the Department of Endocrinology, Kobe City General Hospital, 4-6 Minatojima Nakamachi, Chuo-ku, Kobe 650

Table 1. Provocative Tests

	IRI (μ U/ml)	CPR (ng/ml)	Plasma glucose (mg/dl)
OGTT	5.3→29.5*	2.3→7.0	46→150
Arginine	5.1→45.9	2.2→6.0	34→64
Glucagon	4.0→53.8	2.0→7.2	38→84
Leucine	7.3→63.1	2.8→7.5	48→48

*Basal value→peak value; OGTT, 75g glucose p.o. arginine, 30g d.i.v. glucagon, 1 U i.v. leucine, 200 mg/kg p.o. Conversion factors to SI units: insulin, $\times 7.175$ pmol/l; CPR, $\times 1$ μ g/l; glucose, $\times 0.05551$ mmol/l.

other vessels], suggesting the presence of a tumor in the pancreatic body or tail. At surgery, a 0.9 cm tumor was found in the tail of the pancreas and was resected. Although there was no lymph node swelling or liver metastasis, microscopic examination revealed evidence of malignant insulinoma, i.e., the tumor penetrated the capsule and invaded the parenchymal cells. Postoperatively, the patient had normal fasting plasma glucose and insulin levels.

Control subjects

As a control, five patients with insulinoma (cases 2–6, Table 2) and 50 normal subjects (22 men and 28 women, aged 16–72 years) were investigated for fasting plasma glucose (FPG), immunoreactive insulin (IRI), C-peptide immunoreactivity (CPR) and the ratios of these levels. In addition, as a control for ASVS results, hepatic venous sampling was performed in a 36-year-old woman who was suspected of having a pancreatic tumor, although no tumor was detected.

Extraction of tissues

The tumor obtained at operation was frozen at -30°C . The frozen tissues were homogenized manually on ice in 65% acid-ethanol which had been acidified to pH 2.8 with phosphoric acid (5). The extract was submitted for hormone assays after dilution and gel chromatography.

Gel chromatography

The tissue extract was applied onto a Biogel P30 (1 $\times$ 50 cm) column and eluted with 0.04 mol/l sodium phosphate buffer (pH 7.4) containing 0.1% BSA, 0.1 mol/l NaCl and 7.5 mmol/l EDTA. The column was calibrated with various molecular weight markers.

Assays

Plasma proinsulin levels were measured by radioimmunoassay developed in Mitsubishiyuka BCL laboratory. Plasma samples (1 ml) were added onto a Sep-Pak C_{18} column and extracted with 60% CH_3CN . The normal proinsulin value in the fasting state was 0.032 ± 0.006 μ g/l (3.6 ± 0.7 pmol/l) ($n=30$). Plasma IRI and CPR levels were measured by commercially available radioimmunoassay kits. The assay for proinsulin was highly specific to proinsulin and the cross-reactivities to the

Table 2. Clinical Characteristics in Patients with Insulinoma

Case	Sex	Age (yr)	FPG (mg/dl)	IRI (μ U/ml)	CPR (ng/ml)	Tumor (size, location, malignancy)
1	M	39	38	3.6	2.0	0.9 cm, tail, malignant
2	F	62	38	13.8	1.9	0.8 cm, body, benign
3	M	67	42	23.8	2.5	1.0 cm, head, malignant
4	M	17	30	20.8	4.2	1.9 cm, tail, benign
5	M	46	53	19.6	3.2	3.0 cm, tail, benign
6	F	53	24	10.0	1.8	1.0 cm, head, benign

FPG: fasting plasma glucose, IRI: immunoreactive insulin, CPR: C-peptide immunoreactivity.

other related peptides including insulin, A chain, B chain, C-peptide and glucagon were all less than 0.01%. The assay for insulin (Dainabot, Tokyo, Japan) was also specific for insulin: the cross-reactivities were not detected up to 10^7 pg/ml for proinsulin and CPR, and up to 10^6 pg/ml for glucagon, secretin and gastrin. On the other hand, the assay for CPR (Daiichi Radioisotope, Tokyo, Japan) measured proinsulin with the cross-reactivity of 25% but no cross-reactivity was observed with insulin. The data were expressed as mean $\pm$ SD and analyzed by Student's *t* test.

Results

Plasma IRI/FPG and CPR/FPG ratios during 4 hypoglycemic episodes in case 1 and 15 hypoglycemic episodes in 5 control patients with insulinoma are shown in Fig. 1. Plasma IRI/FPG

Fig. 1. IRI (μ U/ml)/FPG (mg/dl) and CPR (ng/ml)/FPG (mg/dl) ratios in hypoglycemic episodes in case 1 (●) and other insulinomas (○). Dotted areas indicate mean $\pm$ 2SD of those in normal subjects. Conversion factors for IRI (immunoreactive insulin): $\times 7.175$ pmol/l, CPR (C-peptide immunoreactivity): $\times 1$ μ g/l, FPG (fasting plasma glucose): $\times 0.05551$ mmol/l.

Fig. 2. Relationship between CPR and IRI concentrations in case 1 (●) and other insulinomas (○) in the plasma samples collected randomly during the daytime. Dotted areas indicate mean $\pm$ 2SD of IRI concentrations in the plasma samples (at least 6) with the same CPR levels in normal subjects.

ratios in control patients with insulinoma were significantly higher than those in normal subjects, and exceeded 0.3 during 13 of the 15 hypoglycemic attacks (87%), whereas those in case 1 were within normal limits. In contrast, plasma CPR/FPG ratios in case 1 were significantly higher than those in normal subjects. There was not a significant difference in the CPR/FPG ratio between case 1 and the other control patients with insulinoma. Plasma proinsulin levels in case 1 were elevated [$0.35 \pm 0.01 \mu\text{g/l}$ ($39 \pm 1 \text{ pmol/l}$)] [normal: $0.032 \pm 0.006 \mu\text{g/l}$ ($3.6 \pm 0.7 \text{ pmol/l}$)] and the plasma proinsulin/insulin molar ratio was 1.1.

The relationship between CPR and IRI in plasma samples collected randomly during the daytime is shown in Fig. 2. When IRI concentrations in case 1 were compared among plasma samples with the same CPR levels, IRI was significantly lower than in the control patients with insulinoma and equal to or lower than that in normal subjects.

The amounts of IRI, CPR and proinsulin in the tumor extract were $57.1 \text{ nmol/g tissue}$, $14.3 \text{ nmol/g tissue}$ and $1.7 \text{ nmol/g tissue}$, respectively. Gel filtration study revealed that the insulin in the tumor extract was eluted at the position of authentic insulin.

The CPR levels in hepatic venous samples collected before Ca injection for ASVS in case 1 ($6.1 \pm 1.0 \mu\text{g/l}$) were significantly higher than those in the control subject who underwent ASVS ($0.86 \pm 0.09 \mu\text{g/l}$). IRI/CPR molar ratios in hepatic venous samples in case 1 (0.03 ± 0.01) were significantly lower than those in the control subject (0.11 ± 0.01).

Discussion

The diagnosis of insulinoma is generally made by demon-

strating fasting hypoglycemia and inappropriately elevated plasma IRI levels. Indeed, patients with insulinoma, except for case 1 in this study, showed that plasma IRI/FPG ratios during hypoglycemic episodes were all significantly higher than those in normal subjects. However, insulinomas with normoinsulinemia and normal IRI/FPG ratios as in case 1 have been reported (2, 3), although the mechanism is unclear. There are several possibilities to be considered. First, insulinoma secretes insulin in short bursts, causing wide fluctuations in plasma IRI levels. This is unlikely in the present case because the plasma CPR level was elevated during all hypoglycemic episodes indicating hypersecretion of insulin despite peripheral normoinsulinemia. Secondly, patients with insulinoma often have elevated plasma proinsulin levels (2) and there is a possibility that the proinsulin cross-reacts with the CPR assay. Indeed proinsulin levels during hypoglycemic episodes in case 1 were significantly higher than those in normal subjects. Although the CPR assay cross-reacts with proinsulin by 25%, the estimated CPR value by the cross-reactivity of proinsulin was $0.09 \mu\text{g/l}$ ($0.35 \mu\text{g/l} \times 0.25$), far less than the actual value. In addition, hyperproinsulinemia does not cause hypoglycemia because of its low bioactivity (6) and the tumor extract contained a sufficient amount of insulin. Thirdly, the insulinoma in case 1 may secrete abnormal insulin which is easily broken down. We could not exclude this possibility, although the molecular weight of insulin determined by gel filtration seemed to be similar to that of authentic insulin.

Finally, we observed that peripheral IRI concentrations in patients with insulinoma, other than case 1, were significantly higher than those in normal subjects when plasma samples with the same CPR levels were compared. This indicates that the peripheral insulin which escaped from hepatic extraction was increased due to the down-regulation of hepatic insulin receptors (7). However, peripheral IRI concentrations in case 1 were the same or lower than those in normal subjects when plasma samples with the same CPR levels were compared, suggesting that the removal of the secreted insulin by the liver increased. The IRI/CPR ratios in hepatic venous samples also support this hypothesis. Insulin in the hepatic venous samples is considered unextracted insulin after the first pass through the liver. Hypersecretion of insulin may lead to increased unextracted insulin by the liver and the IRI/CPR ratios in the hepatic venous samples are expected to increase. The CPR levels in case 1 were significantly higher than those in the control subject but the IRI/CPR ratios were lower. Therefore, it is suggested that although insulin is excessively secreted in case 1, most of it is metabolized in the liver resulting in normal peripheral insulin levels. Although insulin receptors in insulinomas are expected to decrease on the down regulation model (7), an increase in insulin receptors was reported in a patient with insulinoma (8). This could explain the frequent hypoglycemia with normal insulin levels. We do not know whether the patient had a peculiar type of insulinoma or this situation commonly exists in the early stage of insulinoma before down-regulation of insulin receptors occurs.

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