Insulin Responses to Selective Arterial Calcium Infusion under Hyperinsulinemic Euglycemic Glucose Clamps: Case Studies in Adult Nesidioblastosis and Childhood Insulinoma

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Abstract. Selective arterial calcium stimulation and hepatic venous sampling (ASVS) for insulin secretion is used as a diagnostic procedure in patients with insulinomas or adult nesidioblastosis. In some of those patients, severe hypoglycemia requiring urgent glucose administration occurs during the procedure. Such glucose administration, however, may affect the results and damage the validity of the test. We report two cases of hyperinsulinemic hypoglycemia, in which ASVS tests were successfully performed under hyperinsulinemic euglycemic glucose clamps. A 40-year-old male with nesidioblastosis developed continual severe hypoglycemia several years after a Billroth II-Braun gastrectomy, and continuous glucose infusion could not be stopped even during ASVS tests. A 9-year-old girl with an insulinoma that showed atypical hypovascularity on imaging examinations had ASVS tests under a glucose clamp for safety. Hyperinsulinemic (>100 µU/ml) euglycemic (>90 mg/dl) clamps were achieved by an artificial endocrine pancreas. The insulin analogue lispro was utilized for clamps and endogenous insulin was measured with an assay that does not cross-react with the analogue. Diagnostically significant responses (more than twofold) of insulin secretion were observed under hyperinsulinemic clamps in both cases. The use of the hyperinsulinemic glucose clamp technique during the ASVS test should be considered for maintaining the safety of some hypoglycemic patients.

Key words: Hypoglycemia, Selective arterial calcium stimulation and hepatic venous sampling, Hyperinsulinemic glucose clamp, Insulinoma, Adult nesidioblastosis

In identifying the causes of hypoglycemia, the most crucial step is to determine whether the hypoglycemia is dependent on an inappropriate autonomous secretion of insulin. Although prolonged fasting is used for this purpose, the development of spontaneous hypoglycemia varies considerably among patients. As a way to demonstrate autonomous insulin secretion under controlled conditions, the hyperinsulinemic glucose clamp technique has been introduced [1–5]. Once the autonomous secretion of insulin is biochemically proved, locating the source of the excessive insulin is the next step. However, the diagnostic sensitivity of imaging examinations for insulinomas, the most common cause of hypoglycemia due to organic hyperinsulinism, is not...
satisfactory. Selective arterial calcium stimulation and hepatic venous sampling (ASVS) for insulin secretion has been developed as a superior method to locate insulinomas [6–9]. The ASVS technique is also used for noninsulinoma pancreatogenous hypoglycemia syndrome, a possible clinical entity within adult nesidioblastosis [10, 11].

In some hypoglycemic patients, severe hypoglycemia occurs frequently. If a hypoglycemic episode happens during a diagnostic test, glucose administration is urgently required. Such glucose administration, however, may cause insulin secretion and damage the validity of the test. In the present study, we successfully performed ASVS tests under hyperinsulinemic euglycemic glucose clamps in two cases of hypoglycemia: adult nesidioblastosis and childhood insulinoma. To distinguish endogenous from exogenous insulin, an insulin analogue was utilized for glucose clamps [12]. This is the first report showing that the glucose clamp technique can be useful in the ASVS test for the safety of patients who frequently suffer from hypoglycemic attacks.

Patients and Methods

Patients

1st case

A 40-year-old man was referred to our hospital because of repeated severe hypoglycemia. He had been found to have Arnold-Chiari malformation and Klippel-Feil syndrome. At 34 years old, he underwent a laparoscopic selective vagotomy (gastric stapling with posterior truncal vagotomy) for recurrent duodenal ulcers. However, repeated vomiting occurred thereafter, so he had a pyloroplasty 4 months after the vagotomy and a Billroth II-Braun gastrectomy 9 months after the vagotomy. At the age of 38 years, which was 3 years and 8 months after the gastrectomy, the patient began having frequent neuroglycopenic episodes, starting with a loss of consciousness during bathing after a meal. He was admitted to another hospital, where glucose was continuously infused because the discontinuation of infused glucose resulted in hypoglycemia below 50 mg/dl (sometimes even under 20 mg/dl) of plasma glucose within 2–3 hours. Repeated simultaneous measurements of plasma glucose and serum insulin levels during hypoglycemic episodes [ratios of insulin (µU/ml) to glucose (mg/dl) were 0.54 ± 0.18 (mean ± SD; n = 6)], taken together with non-suppressed levels of C-peptide, suggested that the hypoglycemia resulted from endogenous hyperinsulinism, although those data were obtained within 2–3 hours after sugar or glucose was administered either orally or intravenously. On admission to our hospital, the patient was 163 cm tall and weighed 56.3 kg. Physical examination revealed no significant abnormalities except a short neck and surgical scars in the abdomen. Laboratory data disclosed normal pituitary, thyroidal and adrenal functions. Serum calcium and gastrin were normal. Insulin antibodies were absent. Imaging examinations including ultrasonography, computerized tomography and magnetic resonance imaging were negative for pancreatic tumors. Although ASVS was planned, probable hypoglycemia during the procedure was a concern. Therefore, ASVS was performed under a hyperinsulinemic euglycemic glucose clamp.

2nd case

A 9-year-old girl had a generalized convulsion and became unresponsive early one morning. The convulsion stopped within a few minutes and she recovered consciousness soon. Her past history was unremarkable except for a tonsillectomy. On arrival at our hospital, the patient, who was 140 cm tall and weighed 39 kg, had clear consciousness and no neurological abnormalities. However, her plasma glucose level was 32 mg/dl and her serum insulin level was 13 µU/ml. After she was hospitalized, fasting and postprandial hypoglycemia under 50 mg/dl of plasma glucose was frequently observed. Insulin antibodies were absent. A hyperinsulinemic, sequentially euglycemic and hypoglycemic clamp test using lispro-insulin [12] was performed. Computerized tomography and magnetic resonance imaging revealed a tumor of 3.5 cm in diameter in the pancreatic head. Because the tumor appeared hypovascular, which is an atypical finding of insulinomas, ASVS was planned and performing the procedure under a glucose clamp was chosen for the safety of the patient.

Study procedures were performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from either the patient (1st case) or the parents of the patient (2nd case).
Glucose clamp procedures

Hyperinsulinemic glucose clamps were performed with an artificial endocrine pancreas (model STG-22, Nikkiso, Tokyo, Japan) [13]. Following a 10-min priming period, the insulin analogue lispro (Humalog, Eli Lilly, Indianapolis, IN) was infused continuously at a rate of 1.12 mU/kg/min and glucose concentrations in arterialized venous blood were maintained at a target level of 90 mg/dl by glucose infusion via the artificial pancreas. In a hyperinsulinemic, sequentially euglycemic and hypoglycemic clamp test [12, 14], the target glucose level was switched from 90 to 45 mg/dl after a 60-min clamped euglycemic period. An artificial endocrine pancreas was also employed, without insulin infusion, to maintain blood glucose levels during surgery with the target glucose level set at 100 mg/dl.

Arterial stimulation and venous sampling technique

Arterial stimulation and venous sampling was performed according to procedures described previously [6] with minor modifications. Calcium gluconate 8.5% was injected as a bolus at a dose of 0.025 mEq Ca\(^{2+}\)/kg body weight into each selectively catheterized artery; the arteries were the superior mesenteric artery (SMA), gastroduodenal artery (GDA), proper hepatic artery (PHA), and the proximal and distal parts of the splenic artery (SpA) in the 1st case and the inferior pancreaticoduodenal artery (IPDA), superior pancreaticoduodenal artery (SPDA), PHA, and proximal SpA in the 2nd case. Blood samples for the determination of endogenous insulin were obtained from the right hepatic vein.

Table 1. Insulin concentrations of serum samples containing insulin analogues determined by the Elecsys and Abbott assays

<table>
<thead>
<tr>
<th>Serum</th>
<th>Assay</th>
<th>Lithium</th>
<th>Aspart</th>
<th>Aspart</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(-)</td>
<td>1/5</td>
<td>1/25</td>
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<tr>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>Elecsys</td>
<td>36</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Abbott</td>
<td>41</td>
<td>&gt;900</td>
<td>270</td>
</tr>
<tr>
<td>#2</td>
<td>Elecsys</td>
<td>7.2</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Abbott</td>
<td>7.7</td>
<td>&gt;900</td>
<td>300</td>
</tr>
</tbody>
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Each insulin analogue (lispro or aspart) was added to sera (#1 and #2) in a concentration of approximately 1500 µU/ml. Each of those samples was then diluted with the identical serum. Data are means of duplicate measurements (µU/ml).

Measurements

For determining endogenous insulin concentrations in EDTA-plasma from subjects under hyperinsulinemic glucose clamps with the insulin analogue lispro, an electrochemiluminescence immunoassay (Elecsys Insulin, Roche Diagnostics, Mannheim, Germany) was employed. This immunometric assay using monoclonal antibodies reportedly has excellent analytical reproducibility (the interassay coefficient of variance 2.2–2.7%) and does not cross-react with lispro-insulin in a diluent [15]. We confirmed, by assaying serum samples to which insulin analogues were added at various concentrations, that the Elecsys assay does not react with either lispro- or aspart-insulin (NovoRapid, Novo Nordisk A/S, Bagsværd, Denmark) and those analogues do not interfere with the Elecsys results of intrinsic human insulin measurements (Table 1). In addition, this automated assay system gives results in 18 minutes once prepared samples are set in the measuring apparatus, which enabled us to evaluate data while a glucose clamp was in process. To ascertain “total” insulin levels (endogenous insulin plus the analogue) in peripheral venous blood during glucose clamps, another insulin assay was used (Insulin RIABEAD II, Abbott Japan, Tokyo, Japan). A comparison of working curves demonstrated that this assay detects human insulin and lispro-insulin equally well (unpublished data from Eli Lilly Japan K.K., Kobe, Japan). This was also confirmed by our dilution-recovery studies (Table 1). The C-peptide level was measured by radioimmunoassay (C-peptide RIA Shionogi II, Shionogi, Osaka, Japan).
Pathology

Paraffin sections of formalin-fixed pancreatic tissues were subjected to hematoxylin-eosin staining and immunohistochemical examinations. Commercially available antibodies for insulin (Dako, Glostrup, Denmark; at dilution of 1:200), glucagon (Nichirei, Tokyo, Japan; kit dilution), somatostatin (Dako; 1:10) and pancreatic polypeptide (Dako; 1:500) were used. Bound primary antibodies were detected with the avidin-biotin-peroxidase complex technique.

Results

1st case

At the beginning of the hyperinsulinemic glucose clamp, the blood glucose concentration was 65 mg/dl, so that glucose had to be infused (5.6–8.0 mg/kg/min for >10 min) before the insulin priming. The glucose level was clamped after approximately 45 min and was maintained at the target concentration of 90 (87–91) mg/dl throughout the following ASVS test; the glucose infusion rate was 4.7–7.6 mg/kg/min. Peripheral “total” insulin levels were 98 µU/ml before the ASVS test and 90 µU/ml after the ASVS test. Under the condition of that hyperinsulinemic glucose clamp, twofold or more increases of endogenous insulin concentrations in the right hepatic vein were observed when calcium was infused into the SMA, GDA, and the proximal and distal parts of the SpA; the responses were marked for the SMA and distal SpA stimuli and less marked for the GDA and proximal SpA stimuli (Fig. 1A). Calcium infusion into the PHA did not cause an insulin increase. Arteriograms taken during the ASVS procedure revealed no tumor stain in the pancreas.

The patient underwent a distal pancreatectomy with his blood glucose level controlled by an artificial endocrine pancreas. Intraoperative ultrasonography detected no tumor in the pancreas. Glucose infusion (4.7–9.9 mg/kg/min before the glucose level was clamped and 1.2–3.2 mg/kg/min after the glucose level was clamped) stopped when blood vessels supplying and draining the pancreatic body and tail were ligated. The distal pancreatectomy was performed to the level of the superior mesenteric vein. After the pancreatic resection, the blood glucose level gradually (over approximately 150 min) increased to 170 mg/dl toward the end of the surgery.

The resected pancreas weighed 43 g. Serial sections (5-mm thickness) of the fixed tissue, as well as ultrasonography of the resected pancreas soaked in saline, failed to detect any tumorous lesions. Histological examinations of the fixed tissue revealed normal islets within 1.5 cm from the cut end, but more distantly, increased numbers of various-sized and irregular-shaped islets were found (Fig. 2A). Ductuloinsular complexes (Fig. 2B) and insulin-positive cells budding off the duct epithelium (Fig. 2C) were also observed.

For 6 weeks after the surgery, the blood glucose of the patient stayed in the range from euglycemia to mild hyperglycemia, although insulin administration of 22–37 U/day was transiently required while intravenous high calorie infusion was carried out. For the follow-
ing 2 weeks, whereas blood glucose levels increased (up to \( \approx \)200 mg/dl) in the daytime through the evening, early morning fasting hypoglycemia (40–60 mg/dl) was observed several times. The administration of the alpha-glucosidase inhibitor voglibose was started, which caused postprandial hyperglycemia to improve and fasting hypoglycemia to disappear. Although the patient stopped taking the inhibitor after 18 months, neither relapse of hypoglycemia nor development of overt diabetes has been observed during a 24-month follow-up period after the surgery (fasting plasma glucose, 89 mg/dl; serum insulin, 2.0 µU/ml; HbA1c, 5.9%).

2nd case

A hyperinsulinemic, sequentially euglycemic and hypoglycemic clamp test demonstrated that endogenous insulin secretion was not suppressed even during hypoglycemia in this patient. During euglycemia vs. hypoglycemia, peripheral venous insulin concentrations determined by the Elecsys assay were 14.2–16.0 vs. 13.9–15.3 µU/m and C-peptide concentrations were 2.3–2.6 vs. 2.2–2.4 ng/ml. When a hyperinsulinemic glucose clamp was performed with the ASVS test, the blood glucose was clamped within 30 min and maintained throughout the procedure (82–94 mg/dl) with glucose infusion of 4.5–9.3 mg/kg/min. The peripheral “total” insulin level was 100 µU/ml before the ASVS but increased up to 270 µU/ml at the end of the procedure. The simultaneous C-peptide level increased from 1.9 to 7.9 ng/ml. Selective calcium infusion into the IPDA and SPDA evoked a significant increase of endogenous insulin concentrations in the right hepatic vein (Fig. 1B). Angiograms of those arteries showed a weak tumor stain but no definite feeding vessels.

At surgery, continuous monitoring of blood glucose by the artificial endocrine pancreas was carried out. Glucose was infused (1.0–2.3 mg/kg/min) only during palpation of the tumor, and the blood glucose increased to 150–170 mg/dl within 20 min after the tumor resection. A solid encapsulated tumor of 4 × 3.5 × 3.3 cm in the head of the pancreas was entirely removed. Histologically, the tumor was diagnosed as an islet cell tumor. Tumor cells were positive for insulin, weakly positive for somatostatin, and negative for glucagon and pancreatic polypeptide by immunohistochemical staining. The patient has had no evidence of hypoglycemia during a follow-up period of 18 months.

Discussion

We experienced two cases of organic hyperinsulinemic hypoglycemia, in which the hyperinsulinemic glucose clamp technique was able to be employed for preventing hypoglycemia during ASVS tests. Both cases presented here, however, are rather exceptional representatives of organic hyperinsulinism. Nesidioblastosis is extremely rare in adults, while insulinoma
is relatively rare in children.

Although nesidioblastosis is originally a term denoting the histological picture of disseminated proliferation of islet cells arising from pancreatic ducts, as a clinical entity it appears to be heterogeneous [16]. As to adult nesidioblastosis, the concept of noninsulinoma pancreaticogénous hypoglycemia syndrome has been proposed [10, 11]. The clinical manifestations of this syndrome include reactive hyperinsulinemic hypoglycemia after meal ingestion, no hypoglycemia in prolonged fasting, stimulated insulin secretion in ASVS, and a pancreas with the absence of insulin-producing tumors but showing histological features of islet cell hyperplasia and nesidioblastosis. Recently, it has been reported that the syndrome occurs frequently after Roux-en-Y gastric bypass surgery, presumably owing to increased levels of glucagon-like peptide-1 [17]. In our 1st case, hypoglycemia appeared a few years after a Billroth II-Braun gastrectomy and soon became severe and persistent. Although those episodes were so frequent that it was practically impossible to determine whether they were autonomous hypoglycemia or reactive hypoglycemia, there seemed to be some characteristics of reactive hypoglycemia in the incipient episodes. Therefore, this case might be a type of adult nesidioblastosis after alimentary tract alterations which could cause exaggerated secretion of the incretin hormone. The most important point in the present study is that diagnostically significant insulin responses for ASVS tests were observed even under hyperinsulinemic glucose clamps in those hypoglycemic patients. Normal insulin secretion is suppressed not only by hypoglycemia but also by hyperinsulinemia itself. These negative feedback systems are disrupted in patients with insulinoma, and the lack of inhibition of insulin secretion under hyperinsulinemic glucose clamps with euglycemia [1–5] as well as hypoglycemia [12, 14] is used for the biochemical diagnosis of insulinomas. Although the ASVS technique was developed for the localization of insulinomas, the detection of insulinomas or nesidioblastosis by ASVS is essentially biochemical; a positive test implies that pathological beta-cells “paradoxically” responding to in vivo calcium infusion exist downstream of the artery, although the mechanism remains unknown [18]. There is a case report of insulinoma in which a small tumor was detected by contrast-enhanced ultrasonography, and the only biochemical evidence for abnormal insulin secretion was a positive ASVS test [19]. In the present study, both in a patient with adult nesidioblastosis and in a child with an insulinoma, insulin responses to intra-arterial calcium infusion occurred under standard euglycemic clamps with physiological hyperinsulinenia (≈100 µU/ml), indicating that exogenous hyperinsulinemia does not interfere with the abnormal insulin responsiveness in those organic hyperinsulinemic diseases.

When applying the hyperinsulinemic glucose clamp technique to the ASVS test, we employed an insulin analogue as exogenous insulin and measured specifically endogenous insulin using an assay that does not detect the analogue. This particular strategy may not be necessary if C-peptide is used as an indicator of endogenous insulin secretion. Although it is reported that C-peptide concentrations in the hepatic vein of patients with insulinomas are stimulated in ASVS tests [18], no diagnostic criterion using the C-peptide level is available at present. The longer half life of C-peptide compared with that of insulin seems to be unfavorable for the ASVS test. Finally, we wish to emphasize the clinical implications of the glucose clamp technique during the ASVS test for performing the diagnostic procedure while maintaining the safety of patients. Insulin secretion stimulated by calcium infusion may trigger severe hypoglycemia. Once hypoglycemia occurs, a bolus of glucose infusion may not necessarily be sufficient for terminating sustained hypoglycemia. Urgent glucose administration itself, or continuous glucose infusion without the hyperinsulinemic clamp technique, may stimulate insulin secretion, affecting the results and damaging the validity of the test. In fact, a case was reported in which the test was abandoned because of a hypoglycemic episode following calcium injection [7]. In our 1st case, we adopted the clamp technique because continuous glucose infusion for preventing hypoglycemia could not be stopped. In the 2nd case, judging from peripheral “total” insulin and C-peptide levels, it seemed that there was a marked induced response of endogenous insulin during the test; therefore it is reasonable to assume that the clamp technique must have protected the patient from hypoglycemia. Hereafter, hyperinsulinemic euglycemic glucose clamps during the performance of ASVS tests should be considered for maintaining the safety of some hypoglycemic patients, especially those whose hypoglycemic episodes are frequent and severe.
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References


