Hypoglycemia in response to glucose and glucagon in insulinoma patients with a negative prolonged fast: Functional and morphological properties

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ABSTRACT. A negative 72-h fast is usually considered to preclude the diagnosis of insulinoma. The aim of this study was to describe the functional and morphological properties of two exceptional patients with an insulinoma who had exhibited pre-operatively a negative 72-h fast. Despite the ability of tumor cells to turn off insulin secretion in response to low plasma glucose during 72 h of fasting, hyperinsulinemic hypoglycemia occurred in both patients in response to stimulation by classical secretagogues. Pre-operatively, both patients underwent oral and *iv* glucose challenge tests and *iv* glucagon stimulation test. Insulin secretion was rapidly stimulated by these secretagogues to an exaggerated extent and thereby caused hypoglycemia due to an insulin mass effect. In contrast to the common functional features during suppression and stimulation tests, the tumors differed widely with regard to insulin and proinsulin response to calcium during ASVS tests and morphological properties. In patient 1, the immunohistochemical proinsulin distribution pattern resembled that of normal β-cells, i.e. the staining was restricted to the perinuclear area; insulin and proinsulin were not stimulated by calcium during the ASVS test. In patient 2, the proinsulin staining pattern was abnormal, i.e. proinsulin was also found in the periphery of tumor cells; insulin and proinsulin were stimulated by calcium. We conclude that normal or exaggerated rather than defective glucose sensing may explain hypoglycemia in these exceptional insulinoma patients. Different functional characteristics of these tumors can be correlated with distinct morphological properties.
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INTRODUCTION

Hypoglycemia in the fasting state is the classical clinical manifestation of patients with insulinoma because insulin secretion cannot be appropriately and reliably downregulated in response to low blood glucose by tumor cells (1). It is well known that hypoglycemia in some patients with insulinoma may occur in the fasting as well as in the post-prandial period (1). However, the findings of hypoglycemia occurring exclusively in the post-prandial period and a documented negative 72-h fast in patients with insulinomas are extremely uncommon. To the best of our knowledge, only 3 patients with a documented negative 72-h fast have been reported in the literature (2-4). In a series of 170 patients with insulinoma who underwent a supervised fast at the Mayo Clinic between 1976 and 2000, two patients were observed to have a negative 72-h fast (5). Therefore, a negative 72-h fast is often considered to preclude the diagnosis of an insulinoma.

Over the last 3 yr, we observed two patients with histologically confirmed insulinomas who fasted without experiencing hypoglycemia for 72 h. Thus, tumor cells of these patients preserved characteristic features resembling normal β-cells, i.e. the ability to downregulate insulin secretion in response to low blood glucose. Nevertheless, insulin-mediated hypoglycemia occurred in both patients following stimulation with different secretagogues. Thus, the ability to suppress insulin secretion in response to low plasma glucose concentrations did not prevent these patients from suffering from hyperinsulinemic hypoglycemia. We felt that the mechanism of hypoglycemia in these exceptional insulinoma patients would be worthy of further study.

MATERIAL AND METHODS

Patients

Both patients were hospitalized for the evaluation of hypoglycemic disorders and gave informed consent for further investigation of their conditions. In both patients, kidney and liver function tests were normal and insulin antibodies were not detectable.

Patient 1: An 80-yr-old woman was admitted for evaluation of a suspected hypoglycemic disorder when a glucose concentration of 1.5 mmol/l was measured after she caused a car accident. This episode occurred 2 h after lunch. She complained of several episodes of unconsciousness, disorientation, and falls during the preceding five yr. Retrospectively, these episodes could be assigned to recurrent hypoglycemic events. During the evaluation, no hypoglycemic symptoms could be provoked throughout the supervised 72-h fast. However, hypoglycemia could be provoked by oral and *iv* glucose administration indicating exclusively reactive hypoglycemia in this patient. A selective arterial calcium stimulation and hepatic venous sampling (ASVS) test was negative, but a computed tomography (CT) scan disclosed a pancreatic tumor of 1.7 cm in diameter in the body of the pancreas. This tumor was positive by octreotide scintigraphy (i.e., SS-receptor positive) and could be laparoscopically removed by enucleation. The histological findings revealed an insulinoma displaying densely packed, relatively uniform tumor cells with strong immunohistochemical staining for insulin. Postoperatively performed oral and *iv* glucose challenge tests provoked no more hypoglycemia. After 2 yr follow-up, no hypoglycemic episodes reoccurred. Parts of the results of this case study were published previously (6).

Patient 2: A 67-yr-old man complained of recurrent hypoglycemic episodes for more than ten yr. He noticed diaphoresis, dizziness, and blurred vision during these episodes which occurred exclusively in the post-prandial period. Two episodes with loss of consciousness required medical assistance due to inability to ingest food. A prolonged fast was performed twice but no hypoglycemic symptoms occurred during 72 h of fasting. However, hypoglycemia was demonstrated following oral and *iv* glucose administration. A tumor in the head of the pancreas was detected by magnetic resonance imaging. This tumor was negative by octreotide scintigraphy. During ASVS, plasma insulin levels in the hepatic vein increased 171-fold after calcium injection into the gastroduodenal artery, confirming that the tumor was insulin secreting. The patient was referred to surgery and a tumor of 2.7 cm in diameter in the head of the pancreas was removed. The histological findings revealed an insulinoma. A postoperatively performed *iv* glucose challenge test did not provoke further hypoglycemia. After 9 months' follow-up, no hypoglycemic episodes reoccurred.

Prolonged fast

Both patients were hospitalized for supervised 72-h fasts. Timed collection of blood for the determination of venous plasma glucose, insulin, and C-peptide was performed in 4-to-6-h intervals. The criterion to end the fast before 72 h was the occurrence of neuroglycopenic symptoms, as described previously (7).

Oral glucose tolerance test (OGTT)

After an overnight fast, 75 g glucose dissolved in 3 dl water were ingested within 10 min. Blood samples for the determination of plasma glucose, C-peptide, insulin, and proinsulin concentrations were collected from both patients before (t=0) and at 30, 60, 90, and 120 min after the administration of glucose.

Iv glucose tolerance test (IVGTT)

After an overnight fast, 0.5 g glucose per kilogram body weight in a 50% glucose solution were injected *iv* over 30 sec. Plasma glucose, C-peptide, insulin, and proinsulin concentrations were determined in the blood samples obtained before (t=0) and at 1, 3, 10, 20, 40, and 60 min after the glucose injection.

Glucagon stimulation test

In the fasting state, 1 mg glucagon was injected *iv*. Plasma glucose, C-peptide, insulin, and proinsulin were determined in the blood samples collected before (t=0) and at 6, 30, and 60 min after the glucagon injection.

ASVS

The procedure was performed as previously described (8). A sampling catheter was placed transfemorally in the right hepatic vein close to its junction with the inferior vena cava. After full standard angiography, the proper hepatic, the gastroduodenal, the splenic, and the superior mesenteric arteries were catheterized. In each artery, calcium gluconate (0.025 milliequivalents Ca^{++} per kg body weight) was injected. Blood was sampled from the right hepatic vein before (t=0) and at 30, 60 and 120 sec after the intra-arterial injection of calcium. A more than 2-fold rise in insulin levels within 30-120 sec after injection of calcium indicates the localisation of an insulin-secreting tumor in the vascular territory of the artery stimulated (in contrast to no response from normal β-cells). Plasma proinsulin was determined in the samples obtained following calcium stimulation in the artery supplying the tumor (indicated by the maximum increase in insulin concentrations) and a control artery (no increase in insulin concentrations). From each artery, the baseline sample and the sample with the highest plasma insulin value following calcium injection was selected for the additional determination of proinsulin. In patient 1, the artery supplying the tumor was defined retrospectively from the localization of the insulinoma by CT.

Assays

Venous blood samples were drawn into tubes containing sodium fluoride for the determination of plasma glucose which was measured by the glucose oxidase technique (Beckman Analyzer; Beckman, Fullerton, CA, USA). Parallel samples were allowed to clot, centrifuged, and sera removed for determination of β-cell peptides. Insulin concentrations were determined by a highly specific two-site enzyme-linked immunosorbent assay (DAKO Ltd., Cambridgeshire, UK). Cross-reactivity of this assay was 0.3% for human proinsulin and 0% for human C-peptide. Insulin concentrations in samples obtained during the ASVS test and the prolonged fast in patient 2 were determined with a solid-phase radioimmunoassay (CIS Bio international, Oris Industries, Gif-Sur-Yvette, France) with a cross-reactivity of 14% for proinsulin and 0.1% for C-peptide. C-peptide was determined by a solid-phase, chemiluminescent enzyme immunoassay (Immulite C-peptide, DPC, Los Angeles, CA, USA). Proinsulin was measured by a two-site enzyme linked immunosorbent assay (DAKO Ltd., Cambridgeshire, UK). The reported reference interval in healthy individuals is 2 to 6 pmol/l. Measurement of β-hydroxybutyrate was performed by a enzymatic method as described previously (9).

Pathology

Tumor tissue was fixed in 10% phosphate-buffered formalin and embedded in paraffin according to standard protocols. Both insulinomas were classified according to the World Health Organization

(WHO) classification. Immunohistochemistry was performed on 4 µm sections mounted on Superfrost plus slides (Fisher Scientific, Pittsburgh, USA) as described (10). In short, the sections were de-paraffinised and rehydrated by passing through xylene and a graded series of ethanol. Antigen retrieval was performed for 20 min at 98 C in 0.01 M sodium citrate buffer (pH 6.4) in a microwave oven. After pretreatment in 0.3% hydrogen peroxide for 20 min and conditioning with 0.75 % normal horse serum for another 30 min, the sections were incubated for 1 h at room temperature with a 1:600 dilution of the anti-proinsulin antibody clone 1G4 (Novacastra Laboratories, Newcastle, UK) in TBS containing 4% non-fat dry milk. The sections were then washed in TBS and incubated with biotinylated horse anti-mouse IgG followed by avidin-peroxidase conjugate using the Vectastain ABC elite kit (Vector Laboratories, Burlingame, CA, USA). The chromogenic reaction was carried out using 3-3' diaminobenzi-dine followed by nickel/cobalt amplification. Nuclear Fast Red (Row-ley Biochemical, Danvers, MA) was used for counterstaining.

RESULTS

Prolonged fast

Both patients fasted without the appearance of hy-poglycemic symptoms for 72 h. Laboratory results of the fast are summarised in Table 1. Plasma glucose at the termination of the fast was 2.7 mmol/l in patient 1, and 3.5 mmol/l in case 2. In both patients, insulin and C-peptide levels were adequately lowered in re-sponse to falling plasma glucose values. In both pa-tients, the appearance of ketonuria +++ (Ketostix®) was documented. Suppression of insulin secretion during the fast was also reflected by an appropriate increase in β-hydroxybutyrate to 3007 µmol/l in case 1 (reference for individuals without hyperinsulinae-mia at the end of the prolonged fast is greater than 2700 µmol/l).

OGTT

The OGTT results are shown in Figure 1 (left panels). In both patients, neuroglycopenic symptoms occurred

following oral glucose administration. Patient 1 was disoriented and perspiring 90 min after glucose inges-tion at a plasma glucose concentration of 2.1 mmol/l (concomitant insulin concentration, 357 pmol/l). In case 2, plasma glucose concentration fell to 0.5 mmol/l 120 min after the glucose load (concomitant insulin concentration, 306 pmol/l). At this time, confu-sion, disorientation and tachycardia were noted, but the patient recovered promptly following oral glucose administration. Insulin secretion was stimulated in both patients to an exaggerated extent. Insulin levels rose from 20 and 26 pmol/l at baseline, to 3517 and 4657 pmol/l, respectively, within 30 min following the oral glucose intake (mean insulin concentration was 493 pmol/l in 24 healthy individuals, and 561 pmol/l in 8 insulinoma patients). Ninety min after the glucose challenge, plasma glucose concentration was 2.1 mmol/l in both patients. At this glucose value, insu-lin concentrations were 357 pmol/l and 775 pmol/l in case 1 and 2, respectively. Whereas patient 1 re-covered from hypoglycemia spontaneously, plasma glucose decreased further to 0.5 mmol/l in patient 2. Proinsulin concentrations increased from 14 pmol/l at baseline to a maximum of 44 pmol/l 30 min after the glucose ingestion in case 1, and from 42 pmol/l to 173 pmol/l in case 2.

IVGTT

Results of the IVGTT are shown in Figure 1 (middle pan-els). Neuroglycopenic symptoms occurred in patient 1 180 min after *iv* glucose injection at a venous plasma glu-cose concentration of 1.8 mmol/l (concomitant insulin concentration, 302 pmol/l), whereas patient 2 became neuroglycopenic when plasma glucose concentration was 1.4 mmol/l (concomitant insulin concentration, 7035 pmol/l) within 60 min following glucose injection. In both patients, *iv* glucose administration was followed

Table 1 - *Plasma glucose, insulin, C-peptide, β-hydroxybutyrate concentrations, and urine Ketostix® during the supervised 72-h fast in two patients with insulinoma without hypoglycemic symptoms during these tests (nd=not done).*

	Baseline	24 h	48 h	72 h
Patient 1 (80-yr-old woman, BMI 24 kg/m^2)				
Plasma glucose (mmol/l)	4	4.2	2.8	2.7
Insulin (pmol/l)	nd	15	<3	<3
C-peptide (pmol/l)	390	270	230	310
Urine Ketostix®	-	++	++	+++
β-hydroxybutyrate (µmol/l)	-	-	-	3005
Patient 2 (67-yr-old man, BMI 27 kg/m^2)				
Plasma glucose (mmol/l)	4	4.8	3.9	3.5
Insulin (pmol/l)	76	83	42	42
C-peptide (pmol/l)	721	714	423	230
Urine Ketostix®	-	-	++	+++
β-hydroxybutyrate (µmol/l)	-	-	-	nd

BMI: body mass index.

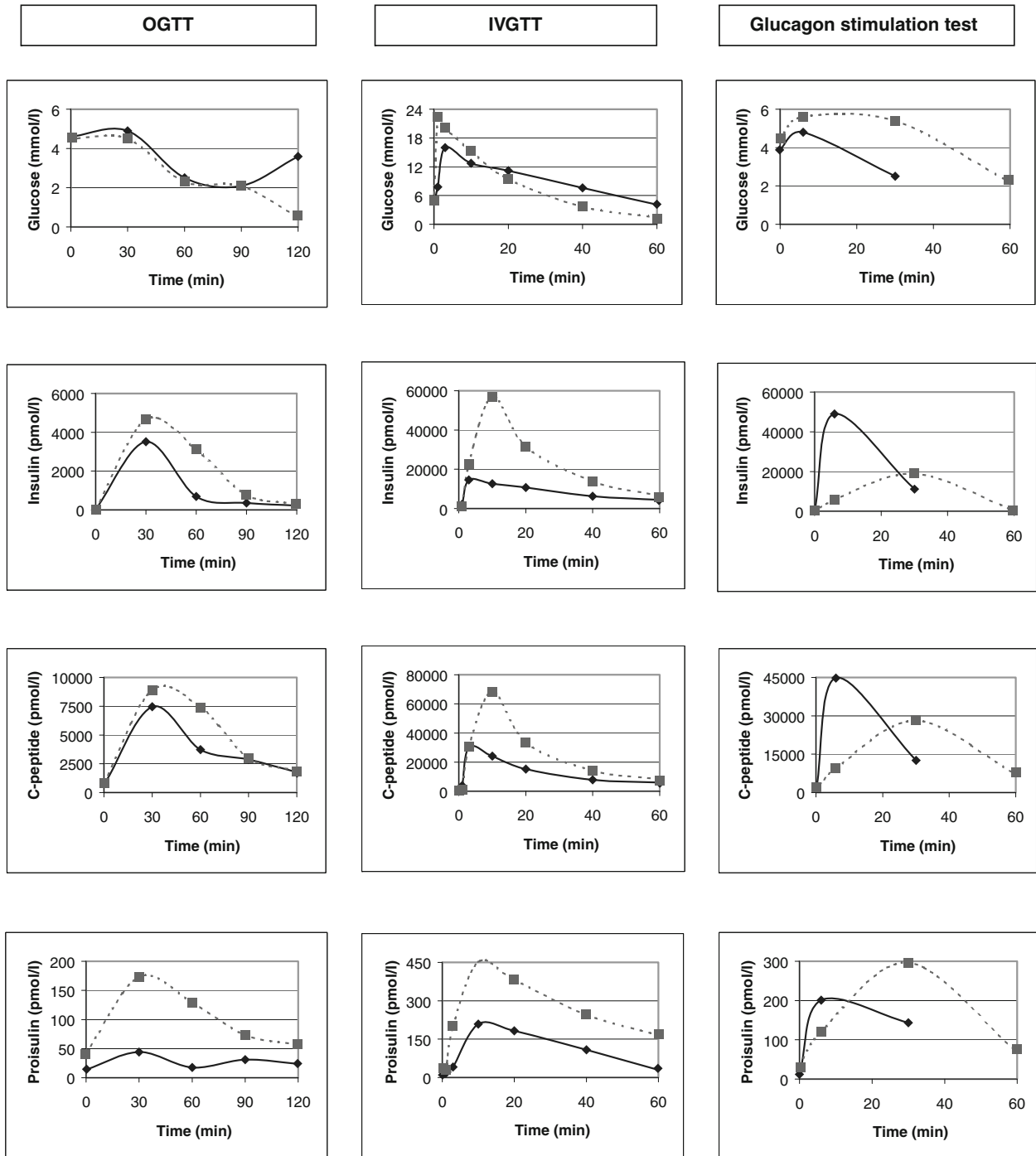| OGTT	IVGTT	Glucagon stimulation test

Figure 1 - *Stimulation tests. Oral glucose tolerance test (OGTT) (left panels), iv glucose tolerance test (IVGTT) (middle panels), and glucagon stimulation test (right panels) in patient 1 (continuous line) and patient 2 (dotted line). Venous plasma glucose, insulin, C-peptide, and proinsulin concentrations are shown after oral ingestion of 75 g glucose, iv injection of 0.5 g glucose per kg body weight, and after intravenous injection of 1 mg glucagon at time "0".*

by a rapid and marked stimulation of insulin secretion. Maximum insulin concentration in patient 1 was 14'590

pmol/l 3 min after the glucose injection, and 56'835 pmol/l in patient 2 10 min after the glucose administra-

tion (post-operatively, maximum insulin concentrations were 1108 and 706 pmol/l, respectively). Proinsulin concentration was higher in patient 2 than in patient 1 (baseline 36 *vs* 13 pmol/l and maximum level after oral glucose 452 *vs* 209 pmol/l, respectively).

Glucagon stimulation test

The results of the glucagon stimulation test are shown in Figure 1 (right panels). *Iv* administration of 1 mg glucagon elicited hypoglycemia in both patients. The tests were stopped at plasma glucose concentrations of 2.5 mmol/l, 30 min after the glucagon injection in patient 1 and at plasma glucose concentration of 1.8 mmol/l 90 min after the glucagon administration in patient 2, respectively. Both neuroglycopenic and adrenergic symptoms were noted. Concomitant insulin concentrations were 11095 and 354 pmol/l, respectively. In contrast to the oral and *iv* glucose challenge tests, the glucagon-induced increase in insulin concentration was higher in patient 1 than in patient 2 (increase from 32 pmol/l at baseline to a maximum of

48'940 pmol/l in patient 1 and from 23 pmol/l to a peak concentration of 19'010 pmol/l in patient 2 (post-operatively, peak insulin concentration was 566 pmol/l in patient 1). Proinsulin increased to 201 pmol/l in patient 1 compared to 297 pmol/l in patient 2.

ASVS

In patient 1 the ASVS test was falsely negative, i.e. there was no increase in hepatic venous plasma insulin after intra-arterial calcium stimulation (Fig. 2, 1A). In patient 2, a 117-fold increase in insulin concentration after calcium injection into the gastroduodenal artery (GDA) indicated an insulinoma supplied by the GDA (Fig. 2, 2A). In addition, a 6-fold calcium-induced increase in proinsulin was observed. No increase in insulin and proinsulin was found after calcium stimulation of the control arteries in both patients.

Pathology

The neoplasm of patient 1 showed the classical histology of well differentiated pancreatic endocrine
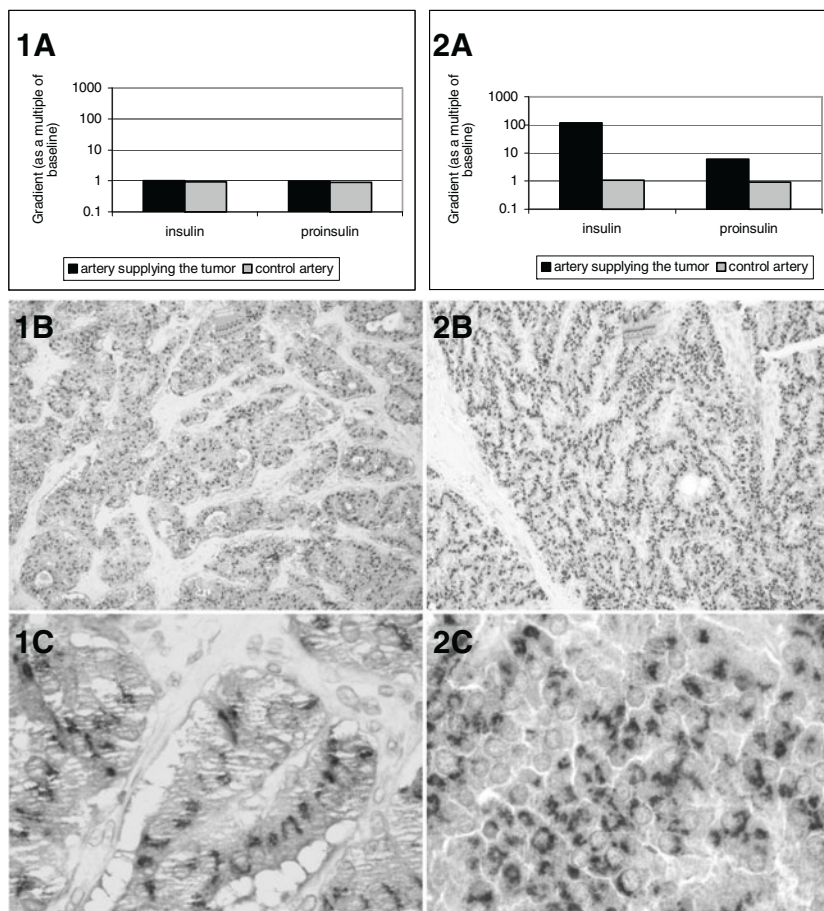

Figure 2 - *Functional and structural findings. Results of selective arterial calcium stimulation tests and immunohistochemical findings in patient 1 (1A-1C) and patient 2 (2A-2C). No increase in plasma insulin and proinsulin concentrations after calcium injection into the artery supplying the tumor and control artery was observed in patient 1 (1A). Proinsulin staining was restricted to the perinuclear area in patient 1 (1B and 1C). There was a 117-fold increase in plasma insulin and 6-fold increase in proinsulin after calcium injection into the artery supplying the tumor in patient 2, and no increase in insulin and proinsulin after calcium stimulation into the control artery (2A). Proinsulin staining was present in the periphery of the tumor cells in patient 2 (2B and 2C).*

tumors with trabecular and pseudoacinar growth patterns. Because of the focal angioinvasion, the tumor was classified as well differentiated pancreatic endocrine tumor with uncertain clinical behavior. The MIB-1 proliferation index of 2% was not elevated. The tumor of patient 2 was predominantly trabecular with small solid areas. Both tumors were immunohistochemically positive for insulin in all tumor cells. Reactions for glucagon, SS, gastrin, vasoactive intestinal peptide, pancreatic polypeptide, serotonin and substance P were all negative. As in non-neoplastic β-cells, the main proinsulin distribution pattern in both tumors was a perinuclear polar staining, corresponding to a localization in the Golgi apparatus (11). While the staining was homogeneous throughout the tumor of patient 1 (Fig. 2, 1B and 1C), the small solid areas of tumor 2 showed focally a diffuse cytoplasmatic staining (Fig. 2, 2B and 2C).

DISCUSSION

The present report represents the first detailed functional and morphological description of two patients with histologically confirmed insulinomas who fasted without the occurrence of hypoglycemic symptoms for 72 h. Insulin secretion was adequately suppressed during the fast in both patients. Nevertheless, hypoglycemia due to inappropriately elevated insulin concentrations occurred following administration of classical secretagogues. *Iv* glucose and glucagon administration resulted in excessive increases in insulin concentrations up to 56'835 and 48'940 pmol/l, respectively. At such insulin concentrations, hepatic and peripheral insulin extraction is limited since insulin clearance by insulin receptors and possibly, also by other mechanisms, is over-saturated and the half-life of insulin may therefore increase. This can be derived from Figure 1, illustrating that at peak insulin concentrations, the half-life of insulin appeared to be markedly prolonged (far exceeding 5 min) whereas C-peptide concentration decreased according to its expected half-life of around 30 min. Considering the IVGTT, C-peptide, as a marker reflecting insulin secretion, reached its maximum level within 5 to 10 min following glucose injection and decreased thereafter according to its half-life. This suggests that tumor cells had depleted their insulin-storing vesicles within 5 to 10 min after glucose injection, and insulin secretion declined despite plasma glucose values above the normal range, i.e. during the min preceding hypoglycemia. Thus, excessively high insulin concentrations may explain the development of hypoglycemia in these patients. Insulin clearance rates of insulin and C-peptide have been reported to be normal in patients with insulinoma. However, this

may not always be true, particularly for insulinomas secreting excessive amounts of insulin which result as expected in decreased insulin clearance.

Because the tumor cells of these two patients shared functional properties with normal β-cells (i.e. the ability to down regulate insulin secretion in response to low ambient glucose levels), we wondered whether the tumors would also demonstrate characteristic structural features of normal β-cells. In normal β-cells, proinsulin immunolabelling is restricted to the perinuclear region, i.e. to the Golgi apparatus and the associated immature granules. As shown in Figure 2, the staining pattern of proinsulin in the tumor of patient 1 was similar to that of normal β-cells, i.e. predominantly in the perinuclear Golgi area (Fig. 2, 1B and 1C). These findings fit with the functional data of patient 1, i.e. the tumor cells (similar to normal β-cells) were unresponsive to calcium administration during the ASVS test (Fig. 2, 1A). Among 29 patients with hypoglycemic disorders undergoing ASVS at our hospital, patient 1 was the only one with a false negative ASVS test. In patient 2, the immunohistochemical distribution pattern of proinsulin was focally clearly different from normal β-cells, i.e. proinsulin staining was found in the whole cytoplasm extending to the periphery of the tumor cells, indicating that mature secretory granules contained proinsulin (Fig. 2, 2B and 2C). The focally abnormal proinsulin distribution pattern observed in patient 2 is consistent with the increase in proinsulin following calcium administration during the ASVS test (Fig. 2, 2A). Apparently, calcium stimulated exocytosis of vesicles containing both insulin and proinsulin. The tumor of patient 2 was highly sensitive to an acute increase in extracellular calcium, resulting in a 117-fold increase in insulin concentration, representing the most pronounced increase among all patients with hypoglycemic disorders undergoing ASVS at our hospital. As shown in Figure 1, the tumor of patient 2 (tumor size 27 mm) was more responsive to glucose resulting in higher insulin concentrations after oral and intravenous glucose administration when compared to patient 1 (tumor size 17 mm). In contrast, the smaller tumor of patient 1 seemed to be more responsive to glucagon. Nevertheless, the increase in proinsulin was more pronounced in patient 2 as compared to patient 1 during the glucose challenge tests as well as following the administration of glucagon (Fig. 1). This finding is also in line with the described morphological features. We conclude that even within the rare entity of glucose-sensitive insulinomas with a negative prolonged fast, a

considerable structural and functional heterogeneity may be encountered. The tumor of patient 1 exhibited all clinical and morphological features of healthy β-cells, i.e. a negative 72 h fast and a negative ASVS test. In contrast, some foci of tumor cells in patient 2 were morphologically distinct from normal β-cells, i.e. disclosed a positive staining for proinsulin in the periphery of cells, a finding characteristic for insulinoma. In common with most insulinomas (distinct form the tumor in patient 1), the tumor of patient 2 was responsive to calcium during the ASVS test.

The central clinical issue in insulinoma patients, tumor-induced hypoglycemia, may be viewed, at least theoretically, as a problem of defective glucose sensing because most of these tumors do not reliably turn off insulin secretion in response to falling ambient glucose concentrations during a fast. Such a paradigm did not, however, account for the development of hypoglycemia in our two patients who, following stimulation with classical β-cell secretagogues, released enormous amounts of insulin causing hypoglycemia. Thus, in response to low ambient glucose concentrations during the fast, a normal glucose sensing of these particular tumor cells allowed an appropriate downregulation of insulin secretion, resulting in negative prolonged fasts. In contrast, qualitatively normal but quantitatively exaggerated, rather than defective, glucose sensing of tumor cells resulted in hypoglycemia due to a release of enormous amounts of insulin in response to glucose and glucagon.

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REFERENCES

1. Service FJ. Hypoglycemic disorders. N Engl J Med 1995, 332: 1144-52.

2. Connor H, Scarpello JH. An insulinoma presenting with reactive hypoglycemia. Postgrad Med J 1979, 55: 735-8.

3. Sjoberg RJ, Kidd GS. Case report: a glucose responsive insulinoma- implication for the diagnosis of insulin secreting tumors. Am J Med Sci 1992, 304: 164-7.

4. Power L. A glucose-responsive insulinoma. JAMA 1969, 207: 893-6.

5. Service FJ, Natt N. The prolonged fast. J Clin Endocrinol Metab 2000, 85: 3973-4.

6. Wiesli P, Spinas GA, Pfammatter T, Krahenbuhl L, Schmid C. Glucose-induced hypoglycemia. Lancet 2002, 360: 1476.

7. Wiesli P, Brandle M, Schwegler B, Lehmann R, Spinas GA, Schmid C. A plasma glucose concentration below 2.5 mmol L-1 is not an appropriate criterion to end the 72-h fast. J Intern Med 2002, 252: 504-9.

8. Doppman JL, Miller DL, Chang R, Gorden P, Eastman RC, Norton JA. Intraarterial calcium stimulation test for detection of insulinomas. World J Surg 1993, 17: 439-43.

9. Moore JJ, Marcus M, Sax SM. Kinetic assay of beta-hydroxybutyrate in plasma with a COBAS-BIO centrifugal analyzer. Clin Chem 1982, 28: 702-3.

10. Perren A, Weng LP, Boag AH, et al. Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol 1999, 155: 1253-60.

11. Roth J, Komminoth P, Heitz PU. Topographic abnormalities of proinsulin to insulin conversion in functioning human insulinomas. Comparison of immunoelectron microscopic and clinical data. Am J Pathol 1995, 147: 489-502.