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Differentiation of Insulin Secretion Patterns in Insulinoma

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Abstract
Background In patients with insulinoma, biochemical proof of inappropriately elevated insulin secretion during hypoglycemia is required prior to surgery. Because circulating insulin levels usually vary widely, we have used the combined OGTT-fasting test to define new normative criteria for a retrospective systematic analysis.
Methods We retrospectively analyzed insulin concentrations from OGTT-fasting tests of 64 patients with surgically removed insulinomas. In addition, the response to intravenous somatostatin infusions was estimated. Normative criteria were defined to obtain comparable estimates of insulin concentrations: basal, glucose-stimulated maximum, postglucose plateau, and secretory bursts.
Results Three types of insulin secretion patterns were identified: (1) the autonomous secretion pattern (type 1, N = 17) with basal and post-OGTT plateau insulin concentrations of approximately 50 mU/L, suppression after OGTT by 41%, virtual absence of distinctive secretory bursts, and resistance to somatostatin-mediated suppression (25 %); (2) the inadequate suppression pattern (type 2, N = 28) with moderately elevated basal and post-OGTT insulin concentrations of approximately 20 mU/L, suppression after OGTT by 73%, absence of secretory bursts, and incomplete somatostatin-induced suppression (56 %); (3) the late-burst secretion pattern (type 3, N = 19) with similar basal and post-OGTT insulin concentrations of 17 mU/L, suppression after OGTT by 76%, true insulin bursts of Δ 13 ± 11 mU/L (184%), and nearly complete somatostatin-induced suppression by 64%.
Conclusions By means of a new normative analysis of the combined OGTT-fasting test, three different patterns of insulin secretion can be described in patients with insulinoma: the autonomous secretion type, the inadequate suppression type, and the late-burst secretion type.

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Abbreviations

BG	Blood glucose concentration
GLUT	Glucose transporter protein
MEN	Multiple endocrine neoplasia
NIPHS	Non-insulinoma pancreatogenic hypoglycemia syndrome
OGTT	Oral glucose tolerance test
SNAP	Synaptosomal associated protein
SNARE	SNAP receptor
ZES	Zollinger Ellison syndrome
WDHA	Watery diarrhea hypokalemia achlorhydria syndrome (vipoma)

Introduction

Sensitive short-incubation assays for many hormones allow intraoperative monitoring of circulating hormone levels.

This has become a routine procedure in some endocrine surgery such as surgery for primary hyperparathyroidism but not in patients with pancreatic hyperinsulinism. In the majority of those patients inappropriately suppressed insulin levels during hypoglycemia are the rule [1–4]. Only a subgroup of patients with insulinoma demonstrates grossly elevated insulin levels as well as insulin secretion totally uncoupled to the ambient blood glucose concentration [4, 5]. Furthermore, patients with non-insulinoma pancreatogenic hypoglycemia syndrome (NIPHS) present predominantly postprandial neuroglycopenia rather than fasting hypoglycemia associated with low insulin levels [6–8]. Diagnostic limits for insulin, C-peptide, proinsulin, and insulin surrogate parameters [9] according to the plasma glucose concentration have been thoroughly defined for the standard 72-h supervised fasting test. The positive diagnostic accuracy is close to 100% [1–4, 10, 11]. Due to the use of today's specific and sensitive insulin assays, any detectable circulating insulin during neuroglycopenia already reflects inappropriate hyperinsulinemia [12–14]. Thus, proinsulin should be determined in any patient during neuroglycopenia [15–19]. Because of these facts endocrinologists and surgeons have become alert to interpret the results of fasting tests in more detail. Therefore, in addition to classical diagnostic endpoint analysis, we were especially interested in a systematic analysis of the insulin secretion patterns during the fasting test leading to Whipple's triad. We retrospectively studied patients with insulinoma in comparison to patients with NIPHS and patients with excluded hypoglycemic disorder. We developed normative criteria from combined OGTT-fasting tests and available somatostatin infusion tests [20–22] in representative groups of patients with and without insulinoma. In patients with insulinoma we identified three different insulin secretion patterns: the autonomous, the inadequate suppression, and the late-burst type pattern.

Methods

Patients

Data from 64 patients were analyzed. They were recovered from our local insulinoma registry which contains more than 160 patients, all analyzed before 2000 and thus diagnosed using the same radioimmunoassay for insulin. We included only those patients for whom complete data of a 100-g oral glucose loading performed immediately before onset of the fasting period and an additional exogenous somatostatin infusion test on a separate occasion were available.

Data from 50 control patients, referred with symptoms compatible with hypoglycemia and for whom a hypoglycemic disorder was excluded by means of the combined OGTT-fasting test, were available from the same period before 2000 and analyzed similarly. Somatostatin infusions were not performed in these patients.

Eleven patients with NIPHS with postprandial and less frequently additional fasting hypoglycemia were diagnosed after 2001 and were treated successfully by partial pancreatic resection. These patients received a 75-g oral glucose load and systematic analysis of the combined OGTT-fasting test data was included. However, the standard insulin radioimmunoassay had been replaced by a specific electrochemoluminescence assay system.

The clinical characteristics of investigated patients (gender, age, body mass index, HbA1c, duration of fasting tests, tumor size, and localization) are summarized in Table 1 (mean ± SD; median, range). In all patients the biochemical diagnosis of insulinoma was made by means of a positive pathologic standardized fasting test following the oral ingestion of 100 g of glucose (endogenous suppression test). On a different occasion all patients received a somatostatin suppression test (exogenous suppression) by means of a 3-h somatostatin infusion prior to surgery.

OGTT and standardized supervised fasting test

The fasting test started in the morning after an overnight fast. At time 0 basal venous samples were drawn and the standard oral glucose load was ingested. Thereafter, samples were drawn from an intravenous 0.9% saline line every 30 min for the first 4 h followed by hourly intervals, which were subsequently extended to 2–4 h. Blood glucose was measured immediately and serum was frozen until assayed for serum insulin and C-peptide. If blood glucose levels declined below 40 mg/dl, blood samples were drawn every 5–15 min. The fasting test was terminated after three to six consecutive measurements of biochemical hypoglycemia less than 40 mg/dl, irrespective of symptoms. The mean and median duration of the fasting test in all 64 insulinoma patients was 21 ± 9 h (±SD; median = 14 h, range = 6–46 h). C-peptide data were not used for subsequent calculations and are not shown. The duration of the fasting test in NIPHS patients was 37 ± 21 h (median = 41 h, range = 4–72 h). All control patients fasted for the full 72 h.

Somatostatin infusion test

A somatostatin infusion was started on a separate occasion after an overnight fast. Two teflon catheters were inserted into contralateral veins of both arms for blood sampling and somatostatin infusion. After withdrawal of two basal venous samples for the determination of blood glucose, serum insulin, serum c-peptide, and plasma glucagon, a bolus of

Table 1 Clinical characteristics of 64 patients with insulinoma, 11 patients with NIPHS, and 50 control patients without hypoglycemia

	All insulinomas	Type 1 autonomous	Type 2 inadequate	Type 3 late-burst	NIPHS	Control
N	64	17	28	19	11	50
N (malignant)	9	4	3	2	/	/
Gender (M/F)	22/42	9/8	8/20	6/13	2/9	11/39
Age						
(mean ± SD) (years)	46 ± 16	43 ± 16	49 ± 15	44 ± 16	42 ± 8	39 ± 14
(median; range) (years)	46 (13–76)	46 (13–59)	50 (15–76)	41 (16–66)	41 (29–56)	37 (15–77)
BMI						
(mean ± SD) (kg/m^2)	25.9 ± 4.3	26.2 ± 3.5	26.0 ± 4.6	25.4 ± 4.6	25.0 ± 5.9	24.1 ± 3.6
(median; range) (kg/m^2)	25.4 (16.8–38.4)	25.6 (20.3–32.9)	25.6 (16.8–35.1)	24.5 (19.7–38.4)	23.5 (19.1–39.4)	22.8 (17.1–32.2)
Fasting test duration						
(mean ± SD) (h)	16 ± 9	11 ± 5	16 ± 7	20 ± 11	37 ± 21	72
(median; range) (h)	14 (6–46)	10 (7–24)	15 (6–35)	16 (6–46)	41 (4–72)	/
Tumor size						
Localization (head:body:tail)	18/24/22	2/7/8	5/13/10	11/4/4	/	/
(mean ± SD) (ml)	2.3 ± 3.6	2.7 ± 2.8	2.2 ± 2.7	2.0 ± 5.0	/	/
(median; range) (ml)	1.1 (0.1–22.5)	1.5 (0.2–9.4)	1.2 (0.2–8.2)	0.8 (0.1–22.5)	/	/
HbA1c						
(mean ± SD) (%)	4.6 ± 0.5	4.6 ± 0.5	4.7 ± 0.5	4.3 ± 0.4	5.3 ± 0.4	5.0 ± 0.4

Values are mean ± SD; median (range)

250 μg of somatostatin (synthetic somatostatin-14 acetate, Schwabe-Curamed Co., Karlsruhe, Germany) diluted in 1.0 ml of saline was injected intravenously. At the same time an infusion of 250 μg/h of somatostatin (750 μg diluted in 45 ml of saline with 2 ml of the patient's whole blood added) was started for 3 h by means of a motorized infusion pump. Blood samples were drawn at regular 30–60-min intervals. The test was aborted prematurely in those patients in whom blood glucose dropped below 25 mg/dl in the presence of simultaneous neuroglycopenic symptoms. A clearly detectable suppression of plasma glucagon concentrations with a brisk rebound upon termination of somatostatin infusion reflected an effective infusion of somatostatin in all patients (data not shown). C-peptide concentrations paralleled those of insulin and are not shown.

Data evaluation

Normative data are required in order to calculate descriptive and comparable parameters of insulin secretion from combined OGTT-fasting tests. Individual insulin secretion and corresponding blood glucose curves were visually screened and showed the well-known variability of the absolute insulin concentrations and the duration of the fasting period. Three different patterns of insulin secretion emerged. In the majority of patients an elevated insulin concentration plateau of variable length was detected after the peak glucose-induced insulin secretion despite progredient development of biochemical hypoglycemia. This is the typical secretion pattern of inadequately suppressed insulin secretion in insulinoma. In a subset of these patients, however, the insulin concentration plateau after the glucose-stimulated peak was interrupted at some point by a late burst of insulin secretion. Furthermore, an obvious glucose-stimulated peak was not detected in 25% of the patients with clearly elevated basal insulin concentrations. In these patients constantly elevated insulin concentrations reflected autonomous insulin secretion irrespective of the prevailing blood glucose concentrations. We subsequently calculated the following criteria of insulin secretion during OGTT-fasting tests to further characterize in more detail different types of insulin secretion patterns in patients with insulinoma (see Fig. 1 for a schematic) and defined these as: (1) the autonomous insulin secretion type pattern (type 1), (2) the inadequate suppression type secretion pattern (type 2), and (3) the late-burst-like insulin secretion pattern (type 3).

Calculations

Combined OGTT-fasting test

The following parameters of insulin secretion were calculated (Fig. 1):

a. The basal insulin concentration ($C_{ins\ bas}^{mean}$) was calculated from two or three basal samples before oral glucose ingestion.
b. The maximal glucose-stimulated insulin concentration ($C_{ins\ max}^{mean}$) was calculated as the mean from the absolute maximum level after oral glucose ingestion, the preceding and the following insulin levels, separated by 30 min each.
c. The mean plateau insulin concentration ($C_{ins\ plat}^{mean}$): Some time after the glucose-induced maximum ($C_{ins\ max}^{mean}$), the onset of an insulin concentration plateau ($C_{ins\ plat}^{0}$) of largely variable length was identified. From subsequent individual insulin concentrations,

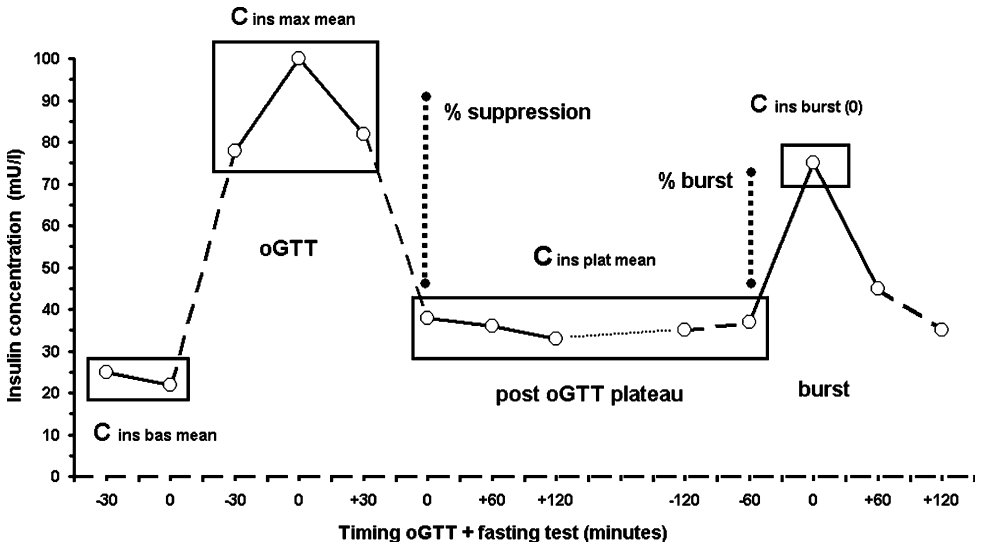

Fig. 1 Schematic of normative description of insulin concentrations from OGTT-fasting tests in patients with insulinoma. For details of the calculation of $C_{ins\ bas}^{mean}$ $C_{ins\ max}^{mean}$, $C_{ins\ plat}^{mean}$, $C_{ins\ burst}^{0}$ refer to the subsection "Calculations"

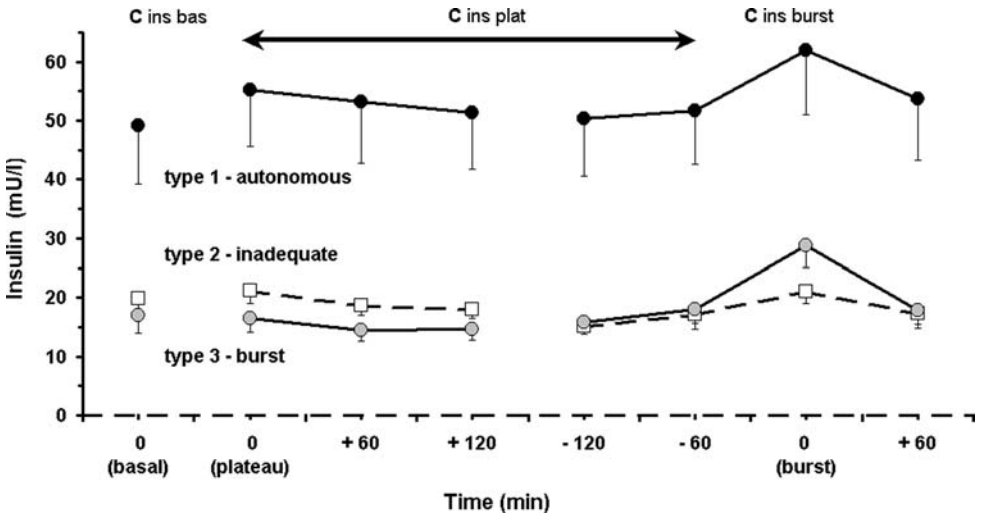

Fig. 2 Absolute normative insulin concentrations from combined OGTT-fasting tests in different insulin secretion patterns of insulinoma. Data are given as mean ± SEM. The maximal glucose-induced insulin secretion ($C_{ins\ max}^{mean}$) is not shown. For *p* values refer to Table 2

a moving average was calculated ($C_{ins\ plat}^{0}$, $C_{ins\ plat}^{+60}$, $C_{ins\ plat}^{+120}$, $C_{ins\ plat}^{+180}$, etc.) and an overall plateau mean value was set to 100% ($C_{ins\ plat}^{mean}$) (Fig. 2).

d. Identification of a late burst of insulin concentration ($C_{ins\ burst}^{0}$): A secretory burst of insulin secretion was identified and defined when the insulin concentration between two measurements during the plateau phase increased by at least 50% above $C_{ins\ plat}^{mean}$. Such a peak became $C_{ins\ burst}^{0}$.
e. The percentage increase of late-burst insulin secretion was calculated as the ratio of the burst ($C_{ins\ burst}^{0}$) and the mean plateau insulin concentration ($C_{ins\ plat}^{mean}$).

By means of mathematical normative description the autonomous secretion pattern type 1 was found in 17 patients (27%). In these patients insulin suppression from the peak glucose-induced maximum to the mean plateau concentration was less than 50% and no significant burst greater than 150% of $C_{ins\ plat}^{mean}$ was detected during the plateau phase. In the majority of patients (73%, $N = 47$), typical inadequate, incomplete suppression after the glucose-induced peak was evident. These patients could be separated into a group of 28 patients (44%) with the true inadequate suppression type 2 secretion pattern without any significant burst secretion as defined in "Calculations" subsection above. In a subset of these patients ($N = 19$, 30%), inadequate suppression was interrupted by a true significant burst (late-burst secretion pattern type 3) which exceeded the mean plateau concentration by more than 50%.

Somatostatin test

The following parameters of insulin secretion were calculated from the basal insulin secretion before onset of somatostatin infusion ($C_{ins\ som}^{bas}$):

a. the insulin concentration at the end of somatostatin infusion ($C_{ins\ som}^{180}$),
b. the suppression rate of insulin concentration induced by somatostatin as the percentage of $C_{ins\ som}^{180}/C_{ins\ som}^{bas}$.

Analytical methods

Blood glucose levels were measured immediately with a Beckman Glucose Analyzer (Beckman Instruments Inc., Fullerton, CA, USA), which in 2001 was replaced by the HemoCue Glucose Analyzer (glucose dehydrogenase method; HemoCue AB, Aengelholm, Sweden).

Because all patients with an insulinoma as well as the control subjects were diagnosed before the year 2000, it was assured that the reported radioimmunoassay data were comparable. Serum insulin concentrations were assayed by radioimmunoassay (RIA) (RIA100, Phadebas Co., Uppsala, Sweden, distributed in Germany by Pharmacia Co., Erlangen, Germany). Precision was 13% for low (3 mU/L) and 5% for high (90 mU/L) serum levels. The intra-assay coefficient of variance (CV) was 9% and the limit of detection was 3 mU/L. Recovery (1.5–60 mU/L added) was 96% (CV = 9%) and linearity was 99% (CV = 5%). Cross-reactivity with human proinsulin was 40% and less than 0.2% with C-peptide. The normal fasting range of the insulin assay in normal subjects was 4–10 mU/L.

Serum C-peptide (RIAgnost HC-Peptid, Behring-Werke, Marburg, Germany) and plasma glucagon concentrations (Linco Res. Inc., St.Charles, MO, USA) were assayed by means of standard commercial RIA kits.

Serum insulin concentrations in patients with NIPHS diagnosed after 2001 were measured by means of a specific electrochemoluminescence immunoassay (ECLIA) run on a modular immunoanalyzer E170 (Elecsys Insulin®, Roche Diagnostics Co., Mannheim, Germany) without any proinsulin crossreaction.

Statistical methods

The unpaired two-sided *t* test with the assumption of heterogeneous variances (Microsoft Excel functions) was used for statistical analysis. *P* values of 0.05 were chosen to detect statistically significant results. Data are given as mean ± SD and, when appropriate, as median (range). Mean ± SEM are used in the figures.

Results

According to calculated normative data of insulin secretion during a standardized supervised OGTT-fasting test, three different types of insulin secretion patterns were identified. The characteristics of the patients are summarized in Table 1. No significant differences were seen between the secretion patterns with respect to the median of age (46 years; range = 13–76 years), BMI (25.4 kg/m^2; range = 16.8–38.4 kg/m^2), duration of fasting test (14 h; range = 6–46 h), HbA1c (4.6 ± 0.5%), and tumor size (1.1 ml; range = 0.1–22.5 ml). Patients with insulinoma were older and more obese compared with the control group (median age = 37 years, range = 15–77 years; BMI = 22.8 kg/m^2, range = 17.1–32.2 kg/m^2). Age, gender, and female preponderance were no different in the group of NIPHS patients (median age = 41 years, range = 29–56 years; BMI = 23.5 kg/m^2, range = 19.1–39.4 kg/m^2).

A malignant insulinoma with liver and/or lymph node metastases was seen in nine patients (15%) with no predominant preference in any of the secretion pattern groups. The tumors of all patients were evenly distributed over the regions of the pancreas. However, autonomous type 1 tumors were rare in the head, but mostly found in the body or tail. In contrast, late-burst type 3 tumors were predominantly found in the head. The body and tail of the pancreas comprised most of the inadequate suppression type 2 tumors (Table 1).

Insulin concentrations during OGTT-fasting tests

The most elevated basal insulin concentrations ($C_{ins\ bas}$) were seen in the autonomous type 1 pattern (49 ± 41 mU/L), being significantly higher compared with type 2 and type 3 secretion patterns. The basal insulin concentrations of these two patterns were less than one half of the type 1 pattern (20 ± 14 and 17 ± 12 mU/L), but still two to three times higher than in control patients (8 ± 4 mU/l) and in patients with NIPHS (5 ± 3 mU/L; Table 2). Maximal glucose-stimulated insulin concentrations ($C_{ins\ max}$) were not significantly different among the three groups of

Table 2 Normative insulin concentrations (details in Calculations subsection and Fig. 1) from combined OGTT-fasting tests in different insulin secretion patterns of insulinoma (autonomous type 1, inadequate suppression type 2, late-burst type 3), in patients with NIPHS and in patients without hypoglycemia (control)

	Type 1 (N = 17)	Type 2 (N = 28)	p vs. 1[a]	Type 3 (N = 19)	p vs. 1[a]	Control (N = 50)	NIPHS (N = 11)
OGTT-fasting test							
$C_{ins\ bas}$ (mU/L)	49.2 ± 40.9	20.0 ± 13.9	0.0012	17.0 ± 12.4	0.0031	8.1 ± 4.5	4.9 ± 2.9
$C_{ins\ max}^{mean}$ (mU/L)	96.1 ± 62.5	99.4 ± 74.3	n.s.	95.4 ± 66.8	n.s.	103.7 ± 67.1	107.2 ± 69.0
$C_{ins\ max}$ / $C_{ins\ bas}$ (factor)	2.3 ± 0.9	6.4 ± 6.0	0.008	6.9 ± 6.6	0.008	13.7 ± 8.8	26.6 ± 24.5
$C_{ins\ plat}^{mean}$ (mU/L)	54.1 ± 43.1	18.0 ± 9.1	0.0001	15.9 ± 7.8	0.0009	4.4 ± 1.5	3.2 ± 1.3
Suppression ratio							
$C_{ins\ max}$ / $C_{ins\ plat}$ (%)	41.3 ± 18.7	73.4 ± 17.1	<0.0001	75.8 ± 19.0	<0.0001	94.4 ± 2.7	96.3 ± 2.2
$C_{ins\ burst}^{0}$ (mU/L)	61.9 ± 45.0	21.1 ± 10.9	<0.0001	28.7 ± 16.1	0.0049	/	/
$C_{ins\ burst}$ / $C_{ins\ plat}$ (%)	116 ± 18	117 ± 15	n.s.	184 ± 41	p vs. 2/1 0.00001	/	/
Median (range) (%)	123 (68–143)	117 (86–145)		178 (149–315)			
BG bas (mg/dl)	48 ± 17	51 ± 19		61 ± 18	p vs 2/1 0.042	80 ± 9	72 ± 12
BG low (mg/dl)	32 ± 6	28 ± 8		30 ± 7		48 ± 9	38 ± 10

Data are given as mean ± SD or median (range) as indicated

[a] Exact p values calculated for statistically significant differences of secretion type 2 and type 3 versus type 1

patients with insulinoma and compared with control patients and patients with NIPHS. Dependent upon the magnitude of the basal insulin concentrations, the mean ratio of the maximal glucose-stimulated insulin concentrations above basal levels was a factor of 2 in the autonomous type 1 pattern compared with a factor of 6–7 in both of the other patterns of insulin secretion. In control subjects a factor of 14 and in patients with NIPHS a factor of 27 was found. Likewise, the suppression rate of $C_{ins\ max}$ to $C_{ins\ plat}^{mean}$ (Table 2) was only 41% in type 1 patients and significantly lower ($p < 0.0001$) compared with 73% in the type 2 pattern and 76% in the type 3 pattern. The suppression rate was 94% in control subjects and 96% in patients with NIPHS.

The absolute insulin concentrations are shown in Figure 2 for all three groups of secretion patterns. The figure includes the basal ($C_{ins\ bas}$) and the plateau phases from its onset at time 0 ($C_{ins\ plat}^{0}$), with a moving average at hourly intervals ($C_{ins\ plat}$) until the onset of the burst secretion ($C_{ins\ burst}^{0}$). The glucose-stimulated maximum ($C_{ins\ max}$) is not shown. Continuously elevated mean insulin concentrations of 50–60 mU/L are seen during the entire fasting test in patients with the autonomous type 1 pattern. At $C_{ins\ burst}^{0}$ the increase seen in patients with the late-burst type 3 pattern (29 ± 16 mU/L) reflects a near doubling of the plateau insulin concentration with a calculated $C_{ins\ plat}^{mean}$ of 16 ± 8 mU/L (Table 2). In both, the type 2 inadequate suppression pattern and the type 1 autonomous pattern the corresponding values at $C_{ins\ burst}^{0}$ of 21 ± 11 mU/L and 62 ± 45 mU/L were not significantly increased above the calculated mean plateau concentrations ($C_{ins\ plat}^{mean}$) because no individual bursts as defined were seen in the profiles of individual patients.

The ratios of insulin concentrations in relation to the mean plateau insulin concentrations $C_{ins\ plat}^{mean}$ set to 100% are shown in Figure 3 for the inadequate suppression type 2 pattern and the late-burst type 3 pattern. Although similar in magnitude with regard to absolute levels of $C_{ins\ bas}$ and $C_{ins\ plat}$, in the late-burst type 3 secretion pattern a doubling of the mean plateau at $C_{ins\ burst}^{0}$ by a mean 84 ± 41% increase above plateau levels is seen ($\Delta = 12.9 \pm 10.6$ mU/L). In the type 2 secretion pattern no such bursts were detected with a calculated maximal increase above plateau levels of 17 ± 15% ($\Delta = 3.1 \pm 3.5$ mU/L). This magnitude was similarly seen in the type 1 pattern (not shown) with a calculated increase of 16 ± 18% ($\Delta = 6.2 \pm 5.8$ mU/L).

Insulin concentrations during somatostatin infusion

Comparable ranges of the basal insulin concentrations in all three groups of insulin secretion patterns were found at baseline before onset of somatostatin infusions (Table 3). The resistance of insulin suppression to physiologic suppressive maneuvers such as fasting seen in patients with the autonomous type 1 secretion pattern was similarly evident during exogenous suppression by means of somatostatin infusion. In the type 1 secretion pattern the mean and the final insulin concentrations during and after somatostatin infusion decreased by 25% from mean 54 mU/L to mean 37 mU/L (Table 3). This suppression was comparable to the rate of 41% suppression induced by fasting after glucose-induced stimulation. In contrast, patients with type 2 and type 3 secretion patterns during fasting tests showed significantly lower basal insulin concentrations of mean 24 mU/L and mean 16 mU/L, which were suppressed to values near 6 mU/L by 56% ($p = 0.0004$ vs. type 1) and to 5 mU/L by 64% ($p < 0.0001$ vs. type 1) at the end of somatostatin infusions as summarized in Table 3. The

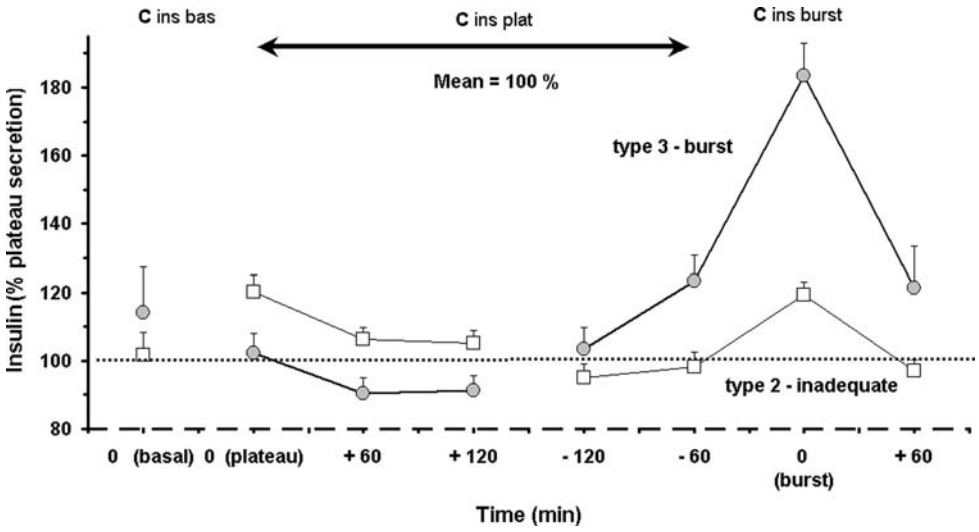

Fig. 3 Percentage of $C_{ins\ bas}^{mean}$, $C_{ins\ plat}^{0,\ +60,\ +120,\ \ldots}$, and $C_{ins\ burst}^{0}$ in relation to the overall mean plateau insulin concentration $C_{ins\ plat}^{mean}$ in insulinoma patients with inadequate suppression type 2 and late-burst type 3 secretion patterns. For p values refer to Table 2

Table 3 Insulin and blood glucose concentrations during a 3-h somatostatin infusion in different insulin secretion patterns of insulinoma (autonomous type 1, inadequate suppression type 2, late-burst type 3)

	Type 1 ($N = 17$)	Type 2 ($N = 28$)	p vs. 1 [a]	Type 3 ($N = 19$)	p vs. 1 [a]
$C_{ins\ som}^{bas}$ (mU/L)	53.6 ± 44.4	23.6 ± 23.1	0.005	16.4 ± 9.5	0.001
$C_{ins\ som}^{180}$ (mU/L)	37.4 ± 32.5	5.9 ± 3.6	0.0002	5.3 ± 4.2	0.0027
Suppression ratio					
$C_{ins\ som}^{bas}/C_{ins\ som\ som}^{180}$	25.5 ± 18.5	55.7 ± 26.7	0.0002	63.6 ± 18.8	<0.0001
BG bas (mg/dl)	49 ± 13	54 ± 22	n.s.	58 ± 16	n.s.
BG som (180) (mg/dl)	35 ± 11	64 ± 43	0.0031	77 ± 34	0.001

Values are mean ± SD

[a] Exact p values calculated for statistically significant differences of secretion type 2 and type 3 versus type 1

absolute levels of insulin concentrations during somatostatin infusions for all three different types of insulin secretion patterns are shown in Figure 4. Because of the magnitude of nearly suppressed insulin concentrations, significant differences could not be detected between type 2 and type 3 secretion patterns.

As expected from the insulin concentrations, differences in the course of blood glucose concentrations during somatostatin infusions were detected and are shown in Table 3. Because plasma glucagon concentrations (not shown) were evenly suppressed in all patients, the blood glucose concentrations in autonomous type 1 patients decreased from a basal level of 49 ± 13 mg/dl well into the hypoglycemic range of 35 ± 11 mg/dl (Fig. 5). These glucose levels were significantly lower compared with increasing blood glucose concentrations in the late-burst type 3 patients (77 ± 34 mg/dl), with the highest insulin suppression rates during somatostatin infusions and in patients with inadequate suppression type 2 (64 ± 43 mg/dl).

Discussion

Pancreatic insulinoma—the most frequent, functionally active neuroendocrine tumor of the gastrointestinal tract—is a unique tumor as evident from its clinical and biochemical secretory characteristics. In contrast to other neuroendocrine GEP tumors such as gastrinoma, glucagonoma, or vipoma, an insulinoma is benign in 85–90% of patients, and, except for the rare malignant insulinoma and MEN1-associated tumors, patients are usually cured after surgery. The tumor in most patients is small, most often less than 2 cm in diameter, and frequently positive imaging results are not achieved. The secretory behavior of insulinomas is different from other GEP tumors. The diagnosis of a functionally active GEP tumor is suspected on the grounds of well-described clinical syndromes, e.g., ZES, WDHA syndrome, or the glucagonoma syndrome. Based upon the patient's history and individual symptoms, the diagnosis is biochemically established upon demonstration

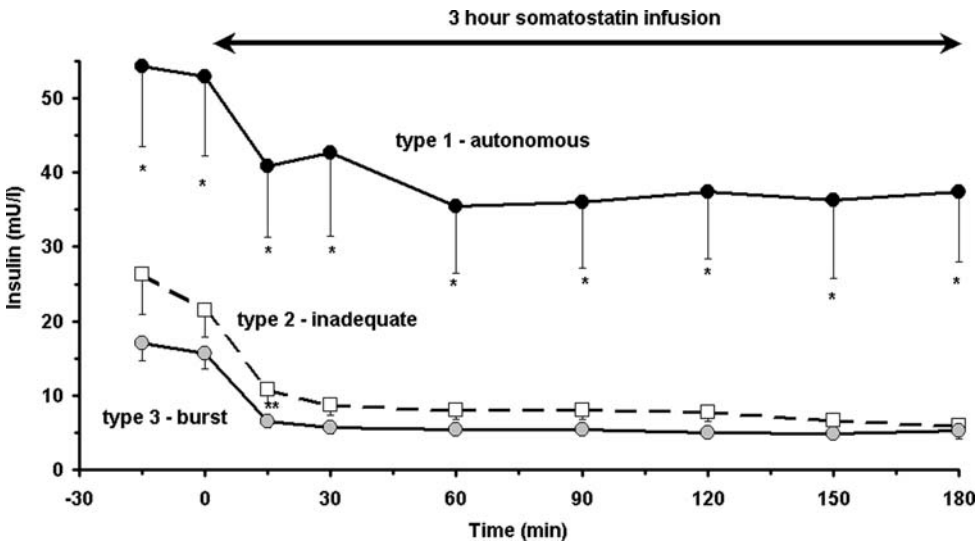

Fig. 4 Insulin concentrations during a 3-h somatostatin infusion in different insulin secretion patterns of insulinoma according to OGTT-fasting test results (*$p < 0.05$ type 1 vs. types 2 + 3; **$p < 0.05$ type 2 vs. type 3)

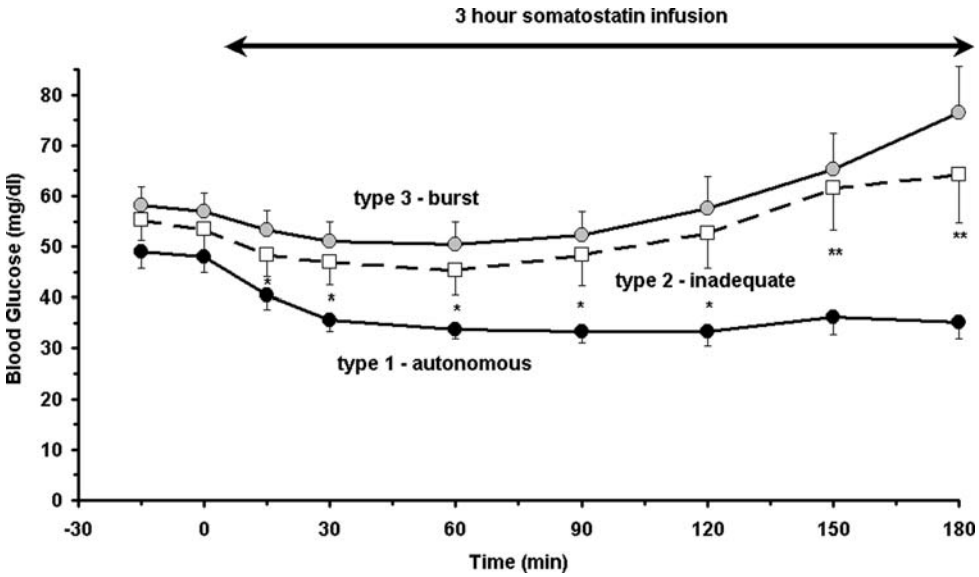

Fig. 5 Blood glucose concentrations during a 3-h somatostatin infusion in different insulin secretion patterns of insulinoma according to OGTT-fasting test results (*$p < 0.05$ type 1 vs. type 3; **$p < 0.05$ type 1 vs. type 2)

of grossly elevated circulating hormone levels in the range of several hundred to several thousand units as pg/ml. Classical suppression tests are not required and not well established.

In patients with insulinoma a positive diagnosis is rarely associated with grossly exaggerated basal insulin levels independent of the prevailing blood glucose concentration. This is due to the tight coupling between blood glucose and circulating insulin levels. Physiologically, the insulin concentration changes briskly during the immediate postprandial state and the late postabsorptive state by a factor of 10–20 or even more. In patients with autonomous insulin secretion, a constantly elevated secretion rate would cause fasting and/or spontaneous hypoglycemia as a result of the failure to shut off insulin scerection. Likewise, glucose-unresponsive insulin secretion with insufficient postprandial insulin release in response to a meal or an oral glucose load would cause early hyperglycemia in patients with autonomous insulin secretion.

Proof of biochemical hypoglycemia with simultaneous inadequate and incomplete insulin suppression is regularly required in all patients with insulinoma as the basis of any surgical intervention. The supervised fasting test has been established as the standard endogenous suppression test for insulinoma [1, 2, 4, 11, 23]. Over the decades thresholds and cutoff values have been developed and amended to prove or discard the diagnosis of insulinoma in the presence of hypoglycemia [1, 4]. The fasting test, however, does not investigate the physiologic postprandial stimulation of insulin secretion and, more important, its subsequent suppression during the postabsorptive phase. Reluctance to routinely combine a glucose load or a test meal with the fasting test may be due to the notion that postprandial hypoglycemia may not be a real disease per se except for reactive adaptations as found in insulin-resistant obesity, early type-2 diabetes, or after gastric surgery [24]. Since description of the first patients with NIPHS [6–8] and the characteristic postprandial neuroglycopenia, the diagnostic workup of patients with a suspected hypoglycemic disorder has changed. We recently reported [8] that these patients also may demonstrate late fasting hypoglycemia, typically associated with detectable insulin levels that are inappropriately elevated in the presence of true biochemical hypoglycemia. To manage difficult diagnostic situations, Service [9] proposed additional criteria as insulin surrogates in insulinoma such as plasma β-hydroxybutyrate, plasma free fatty acids, and the plasma glucose response to intravenous glucagon at the end of the prolonged fast.

Based on these considerations, we questioned whether we could retrospectively detect typical insulin secretion patterns in patients with insulinoma. We analyzed the data from combined OGTT-fasting tests, the classical endogenous suppression test. Furthermore, we evaluated data from an exogenous suppression test, the somatostatin infusion test. We found three distinct insulin secretion patterns derived from basic data such as the insulin concentration during the combined OGTT-fasting test. The patterns of insulin secretion during endogenously induced suppression were paralleled by similar patterns of exogenous suppression induced by somatostatin infusion and the entire range from virtual resistance to almost near-normal complete suppression was found.

The autonomous type 1 secretion pattern with blunted glucose-induced stimulation of insulin secretion clearly demonstrated the most elevated insulin concentrations which were incompletely suppressed by either fasting (41%) and/or somatostatin (26%). On the other hand, in the

majority of patients (inadequate suppression type 2 and late-burst type 3), maximal glucose-induced insulin concentrations were suppressed by nearly 75% during the fasting period but were still inadequately elevated during the postglucose plateau phase until termination of the fasting test in the presence of true biochemical hypoglycemia. In a subgroup of these patients (type 3 late-burst pattern), a burst evident from a mean near doubling of the mean plateau insulin concentration was detected at some point during the late phase of the fasting test. By definition a burst was considered to be significant only if the increase in insulin concentration was greater than 50% of the calculated mean plateau level in order to group the patient into the burst pattern. These two groups of patients with insulinoma (type 2 and type 3) were similarly sensitive to near complete inhibition by somatostatin (suppression rates of 56% and 64%, respectively) into the range of 5 mU/L, with an established detection limit of the insulin radioimmunoassay used at 3 mU/L. Hence, during somatostatin infusions the blood glucose concentrations rose to levels well above the basal level which was in contrast to rapid development of neuroglycopenic symptoms in autonomous type 1 patients. Lack of a suppressive action of somatostatin in these latter insulinomas could not be explained with an insufficient dose of the infusion rate which was 100 times the dose necessary to mimick physiologically induced somatostatin concentrations in plasma [20, 21]. We do not promote the use of routine intravenous somatostatin infusions during the diagnostic workup of individual insulinoma patients. The response to exogenous somatostatin, however, endorses the validity of identified different insulin secretion patterns in response to endogenous suppression by means of fasting.

Immediate clinical implications regard intraoperative monitoring of circulating insulin concentrations [5, 25–27]. Only in the minority of patients (25% of our patients) with the typical autonomous type 1 secretion pattern and unregulated, continuously elevated insulin concentrations can a decrease of insulin secretion after removal of a tumor be expected. In the majority of patients with inadequate suppression or late-burst patterns, however, the true and relevant circulating insulin levels are likely to be masked during the conditions of general anesthesia, with fluctuations of the glycemia and possible fluid administrations containing glucose, lactate, or catecholamines.

Our description of distinguishable insulin secretion patterns may represent the phenotypical expression of reported differences at the ultrastructural level [28, 29]. Three decades ago, Creutzfeldt et al. [28] published obvious ultrastructural characteristics with respect to the presence or absence of typical and atypical secretory granula in tumor cells of 30 insulinomas. A classification into four different types of insulinoma (types I–IV) was proposed. This classification was later modified by Berger et al. [29] who investigated insulinoma tissues of 12 patients with the differentiation of two subtypes based on morphologic and functional criteria. A trabecular arrangement of tumor cells was associated with typically granulated B cells, a moderate elevation of proinsulin-like material extractable from the tumor, and an almost complete in vivo suppressibility of circulating insulin by somatostatin and diazoxide infusions. On the other hand, medullary arrangement of tumor cells was associated with poorly granulated B cells, atypically granulated secretory cells, increased extractable proinsulin-like material, and virtual resistance to somatostatin infusions. Despite evident heterogeneity of human insulinomas at the histologic, ultrastructural, and functional secretory levels, the molecular or genetic basis of the oncogenesis of sporadic insulinoma and its transformation into a malignant insulinoma is poorly understood. Only in patients with multiple endocrine neoplasia (MEN1) and related insulinoma has the detection of the tumor suppressor gene or its absence through mutations led to a more detailed understanding of the pathogenesis [30].

In most nonendocrine cells translated proteins are continuously transported to the cell membrane by bulk flow mechanisms, the constitutive pathway [31] of protein secretion. Endocrine cells, however, have adopted a complex regulated pathway [32] with involvement of regulatory signaling peptides (SNAPs), vesicular recognition surface proteins, and plasma membrane fusion proteins with receptor function (SNAREs). Heterogeneity of the secretorial behavior of insulinomas can be to the result of several factors and potential sites of defects such as (1) disturbance of the cycles controlling cell growth, proliferation, and apoptotic cell death [33]; (2) an overexpression of oncogenes causing transformation of normal B cells into hyperplastic and abnormal insulin-producing tumor cells [34]; (3) aberrations in the expression of somatostatin receptors as a regulatory factor of insulin secretion; (4) aberrations in the secretory pathway of insulin due to alterations in the formation and fusion of secretory granula [35–38]; (5) lack of appropriate glucose-sensing devices such as the absence of GLUT2 transcripts [39]. However, endocrine tumor cells with contained hormone secretion may have lost the complex expression of the machinery needed for regulated hormone release. Thus, multiple mechanisms could be malfunctional in endocrine tumor cells and hence provide the basis for a heterogeneous phenotypical expression of the secretory behavior of typically welldifferentiated insulinomas.

Experienced centers that treat a considerable number of patients with hypoglycemic disorders usually are aware of a likely insulinoma when insulin concentrations are rather low but clearly detectable despite the presence of

biochemical hypoglycemia. Predominant circulating proinsulin, rapid removal of insulin through the first pass across the liver, or sporadic insulin hypersecretion of the tumor may be possible reasons. Our normative criteria derived from combined OGTT-fasting tests suggest the existence of three typical different insulin secretion patterns with differentiation of true autonomous insulin secretion, inappropriately suppressed insulin secretion, and sporadic burst-like insulin secretion. These do not challenge the criteria developed for the standard fasting test used in the diagnosis of hypoglycemic disorders. Rather than promoting an entirely new approach, we recommend a combined OGTT-fasting test in every patient in whom a hypoglycemic disorder is suspected. The increasing diagnosis of NIPHS patients strongly supports this procedure.

In conclusion, we identified three different patterns of insulin secretion in patients with insulinoma in response to inhibitive maneuvers such as fasting and somatostatin infusion. By means of normative criteria, typical insulin secretion patterns may easily identify those patients who have an insulinoma when the circulating insulin levels are barely detectable. Whether those three secretion patterns may be correlated with different cellular defects of aberrant insulin secretion at the transcriptional and/or translational molecular level has to be further elucidated.

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