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Highly Increased Insulin Secretion in a Patient with Postprandial Hypoglycemia : Role of Glucagon-Like Peptide-1 (7–36) Amide

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Abstract. The mechanism(s) of an inappropriate secretion of insulin is poorly understood. We report a case of reactive hypoglycemia associated with an unusually exaggerated insulin secretion. The patient, a 32-year-old man, developed frequent episodes of postprandial hypoglycemia after interferon treatment was begun for chronic type C hepatitis. Oral glucose challenge test confirmed the patient's extremely high plasma IRI response, i.e., more than 1000 μU/ml, and that of plasma C-peptide 56.9 ng/ml at 90 min, followed by symptomatic hypoglycemia (plasma glucose 34 mg/dl) at 240 min. The plasma proinsulin level also was high, but the molar ratio of immuno reactive insulin (IRI)/plasma C-peptide and IRI/proinsulin was within the normal range. Antibodies to insulin or insulin-receptor were negative. Plasma IRI response was apparently greater when the glucose was given orally than when given intravenously. The response of plasma glucagon-like-peptide (GLP)-1 to oral glucose was quite high (from baseline of 45.5 to 303.2 pmol/L) and showed a close parallel with the change in the plasma IRI concentration. The greatly enhanced insulin secretion leading to reactive hypoglycemia in this patient may therefor be attributed to the increased secretion of GLP-1.

Key words: Reactive hypoglycemia, Hyperinsulinemia, Incretine, Glucagon-like-peptide-1, (7–36) amide
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WHILE inappropriate secretion of insulin after meals leading to symptomatic hypoglycemia is not uncommon, its pathophysiological basis is not well understood. The response of plasma insulin to a given rise in plasma glucose has been known to be much greater when the glucose is given orally rather than by intravenous injection, the so-called "incretin effect" [1–4]. This augmentation of insulin secretion is attributed to the secretion of gut hormones having insulinotropic activity, namely, gastric inhibitory polypeptide (GIP) [5–7], glucagon-like peptide-1 (7–36) and amide (GLP-1) [8–10]. GLP-1 is a novel intestinal product of proglucagon [11]. GLP-1 in physiological concentrations strongly stimulates the glucose-induced secretion of insulin and of somatostatin, and inhibits that of glucagon [12]. The net effect of this peptide may be pronounced inhibition of glucose production.

We present a case of symptomatic postprandial hypoglycemia that was attributed to very strong plasma insulin response to the oral ingestion of glucose.

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Case Report

This 32-year-old male theology student was re-

ferred to our Diabetes Center with frequent episodes of postprandial hypoglycemia. He had a childhood history of such hypoglycemic spells especially after eating meals high in carbohydrates. At the age of 20 years, he had undergone a partial gastrectomy with gastroduodenal anastomosis (Billroth I) for duodenal ulcer. At that time, he received a blood transfusion which was complicated by the development of type C hepatitis. Subsequently, at age 31, he received natural interferon-α, Sumiferon® (Sumitomo-Pharmaceutical Co., Osaka), for chronic type C hepatitis 900×10^4 U daily for 6 weeks, reduced to 600×10^4 U three days a week for 6 months.

Following the initiation of interferon therapy he developed sweating, tremor, and dizziness 2 to 4 h after every meal. In a 75 g oral glucose tolerance test (OGTT), his plasma insulin rose to a peak level of 793.2 μU/m*l* at 60 min, and cold sweats, palpitations and lightheadedness developed 120 min after the ingestion of glucose when his plasma glucose level was 56 mg/d*l*. The patient was then referred to our Diabetes Center for further investigation of this exaggerated response of plasma insulin and the concomitant reactive hypoglycemia. On admission, he appeared to be in good health. He was 168 cm tall and weighed 56 kg. There was no family history of diabetes mellitus. The patient did not drink or smoke. Physical examination revealed no abnormalities and his blood pressure was 124/70 mmHg. The relevant laboratory data are shown in Table 1. Ultrasonographic examination and a computed tomographic scan of the abdomen revealed only a small liver cyst.

Methods

The plasma concentration of proinsulin was determined after extracting the plasma samples on a Sep-Pak cartridge (Waters Associates, Milford, MA, USA) by a double antibody RIA with a specific antiserum that does not cross-react with insulin or C-peptide [12]. Plasma GLP-1 [7–36] amide was measured by the method described previously [13]. In brief, the plasma was extracted with 67% ethanol and lyophilized samples or standard GLP-1 [7–36] amide were incubated for 24 h with anti-GLP-1 serum followed by the addition of ^{125}I-labeled GLP-1 [1–37] which was iodinated by the lactoperoxidase method and purified by HPLC. Separation of the bound and free peptides was done with dextran coated charcoal. This antibody recognizes not only GLP-1 [7–36] amide but also the major proglucagon fragment and GLP-1 [1–36] amide. To assess the biphasic pattern of insulin secretion and the peripheral sensitivity to insulin concomitantly, we performed a hyperglycemic (200 mg/d*l*) clamp test with an artificial endocrine pancreas (Nikkiso STG-22, Nikkiso Co., Tokyo). The insulin sensitivity index (ratio of glucose infusion rate/plasma IRI) was high during a steady-state hyperglycemia in this patient.

Table 1. Laboratory data on admission

Urinalysis		Blood chemistry		Serology	
Protein	(−)	TP	6.8 g/d*l*	HBsAg	(−)
Glucose	(−)	Alb	3.9 g/d*l*	HBsAb	(−)
		GOT	36 KU	HCVAb	(+)
CBC		GPT	53 KU		
WBC	2900/mm^3	LDH	131 mU/m*l*	Thyroid Hormones	
RBC	462×10^4/mm^3	ALP	216 IU	fT3	4.58 pg/m*l*
Hb	14.3 g/d*l*	r-GTP	21 mU/m*l*	fT4	1.26 ng/d*l*
Hct	41.3 %	TTT	1.0 KU	TSH	0.4 μU/m*l*
Plt	17.3×10^4/mm^3	ZTT	11 KU		
		ChE	0.86 ΔpH		
		T-Chl	143 mg/d*l*	Insulin Ab	1.3 %
		TG	94 mg/d*l*	Insulin receptor Ab	(−)
		BUN	11.7 mg/d*l*		
		Cre	0.9 mg/d*l*		
		HbA1c	4.3 %		

Results

We found that prolonged fasting for up to 42 h did not evoke hypoglycemia in this patient and the ratio of plasma IRI/glucose remained within the normal range during fasting. Repeated OGTT confirmed the patient's unusually high plasma insulin response, ie, more than 1000 μU/ml, and that of plasma C-peptide (56.9 ng/ml) at 90 min . He developed symptomatic hypoglycemia (plasma glucose 34 mg/dl) 240 min after ingesting the glucose. The plasma proinsulin concentration was also high, the molar ratios of IRI/plasma C-peptide and IRI/proinsulin were 0.38 and 242, respectively, at the peak plasma concentration of insulin. OGTT was repeated after the subcutaneous injection of atropine sulfate, 0.5 mg. While the pattern of the plasma glucose curve was little changed, the peak values for plasma IRI (739.1 μU/ml) and of plasma C-peptide (35.5 ng/ml) were clearly blunted as compared with the results of testing without atropine. In contrast, an intravenous glucose tolerance test (IVGTT) and the hyperglycemic clamp test revealed a marked attenuation of the insulin response, peaking at 200 μU/ml and 182 μU/ml, respectively, even though the plasma glucose concentration was comparable to those seen with OGTT. The plasma concentrations of IRI and GLP-1 changed in a parallel direction; GLP-1 immunoreactivity increased markedly from a baseline of 45.5 to 303.2 pmol/L during OGTT, and in the atropine-treated OGTT from 29.6 to 182.7 pmol/L, while it showed no increase in the IVGTT (from 51.6 to 31.8 pmol/L).

In the hyperglycemic clamp test, we observed biphasic secretion of insulin. The peripheral sensitivity to insulin was considered normal as judged by the insulin sensitivity index (glucose infusion rate/steady-state plasma insulin=0.06). When the glucose infusion was stopped, the plasma concentrations of counterregulatory hormones all increased reciprocally as the plasma level of glucose declined.

Discussion

Our patient had a long history of symptomatic postprandial hypoglycemia. Interestingly, these episodes were aggravated after the administration of

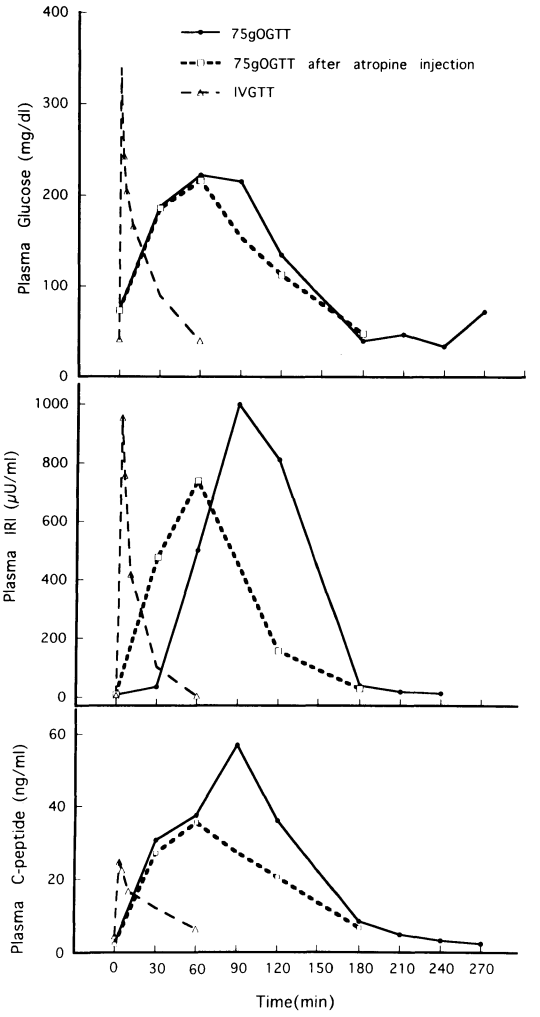

Fig. 1. Response of plasma glucose concentration (upper), IRI (middle), plasma C-peptide (lower) during intravenous glucose tolerance test (IVGTT), oral glucose tolerance test (OGTT) and OGTT after atropine injection.

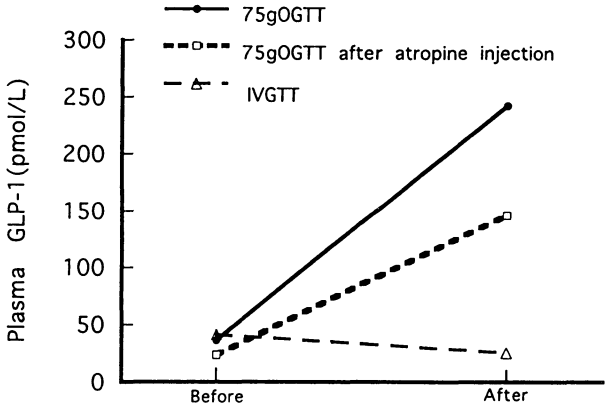

Fig. 2. Change in plasma glucagon like peptide-1 (GLP-1) before and after intravenous glucose tolerance test (IVGTT), oral glucose tolerance test (OGTT) and OGTT after atropine injection.

Table 2. Hyperglycemic clamp

Time	(min)	0	5	10	60	90	120	140	160	180	200	220	280	320
Plasma glucose	(mg/dl)	76	96	141	212	208	204	180	137	105	78	60	56	61
IRI	(μU/ml)	8.9	100.2	80.5	100.5	125.3	182.0	133.9	92.8	53.5	28.7	20.6	12.4	9.4
HGH	(ng/ml)						0.1>			0.1>	0.2	0.2	3.4	9.9
Cortisol	(μg/dl)						8.8			10.5	9.2	9.3	26.1	24.5
Glucagon	(pg/ml)						66.0			47.0	55.0	84.0	125.0	112.0

Glucose infusion was discontinued at 120 min (indicated by a broken line).

interferon for chronic hepatitis type C. While interferon has been reported to cause autoimmune thyroiditis [14], our patient showed no increase in circulating antiinsulin or antiinsulin-receptor antibodies. The metabolic effects of interferon are not fully understood. It has been reported that the administration of human leukocyte interferon to healthy subjects reportedly impairs glucose tolerance, despite the augmented insulin response, which is compatible with the development of insulin resistance [15]. Whether the increased secretion of insulin in our patient was related to interferon therapy remains to be clarified. Gastrectomy in this patient was followed by a Billroth I reconstruction. Consequently gastric emptying remained unchanged largely as seen in the pattern of the plasma glucose curve after OGTT. The unusually exaggerated insulin response to oral ingestion of glucose cannot therefore be attributed to the gastrectomy itself in this case. An insulinoma seemed unlikely because of a lack of history of fasting hypoglycemia and the presence of normal IRI/glucose ratios during a 42 h fast. Also a CT scan and ultrasonographic study of the pancreas showed no abnormalities. On a molar basis, plasma proinsulin was less than 0.5% of the peak value of IRI during OGTT, thus excluding the possibility of hyperproinsulinemia. Abnormal insulin is also unlikely because of the normal IRI/plasma C-peptide molar ratio and the unusual presentation of postprandial hypoglycemia. The response of counterregulatory hormones was also considered to be normal. The major finding in the present study was that plasma insulin and GLP-1 concentrations exhibited a parallel change both in direction and degree, suggesting that GLP-1 functioned as an incretin and contributed to the marked increase in postprandial insulin response and reactive hypoglycemia. Indeed, the response of both hormones was highest after the administration of oral glucose, and was slightly attenuated following atropine administration before the oral ingestion of glucose, and was lowest during IVGTT. The secretion of GLP-1 was recently reported to be under cholinergic neural modulation [16], which would be compatible with the above observation. The response of GLP-1 to oral glucose administration (from 45.5 to 303.2 pmol/L) in this case seems very high. In our assay, the normal fasting level of GLP-1 was 38.8 ± 2.7 pmol/L (mean ± SEM) in 12 healthy subjects. Unfortunately, because we do not have enough data on the normal response during OGTT, we compared our results with those of other investigators. For instance, during 75 g OGTT, plasma GLP-1 rose from a baseline of 15.6 ± 6.0 to a peak of 48.4 ± 4.0 pmol/L in 5 normal subjects as reported by Kreymann *et al.* [8], from 60.9 ± 6.9 to 84.4 ± 4.4 pmol/L in 10 healthy subjects as found by Takahashi *et al.* [17], and from 40 to about 90 pmol/L in 9 normal controls as reported by Nauck *et al.* [18]. In gastrectomized patients with the dumping syndrome, the plasma GLP-1 concentration increased from 10 ± 3 to 85 ± 17 pmol/L [7]. In another report, it attained a maximum of 291.4 ± 61.4 pmol/L (pancreatic + intestinal GLP-1) [19] following oral glucose ingestion. These reported values for the plasma GLP-1 concentrations are in most comparable among research reports, while some differences probably reflect the specificity of the antisera used. Our antiserum cross-reacted with other molecular species that had no insulinotropic activity or less [eg, the major proglucagon fragment, GLP-1 (1–36) amide, or GLP-1 (1–37) amide [12]].

Our method may therefore have overestimated the concentration of GLP-1 (7–36) amide, a potent

insulinotropic peptide in mediating the incretin effect. Even considering this possibility, the response of plasma GLP-1 was very strong in our patient.

Because of the limited number of plasma samples, we did not determine the plasma level of the other insulinotropic hormone, GIP. Although GLP-1 is a more potent insulin secretagogue than GIP [8], both hormones interact additively in normal man [18]. The marked increase in insulin secretion observed in this patient may therefore reflect the combined insulinotropic action of these hormones.

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