A Case of Autoimmune Insulin Antibody Syndrome Associated with Polymyositis, Empty Sella and Apparent High Urinary Output of Immunoreactive Insulin

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Abstract. Patients with autoimmune insulin antibody are characterized by hypoglycemic attacks and antibodies to insulin in serum without prior insulin administration. In the present report, a patient with hypoglycemia due to autoimmune insulin antibody associated with primary empty sella syndrome and polymyositis appeared to have high urinary immunoreactive insulin (IRI) in the face of normal urinary C peptide. Consequently, the urinary IRI / C peptide ratio was apparently high. The amelioration of hypoglycemic attacks and polymyositis by prednisolone treatment was accompanied by the disappearance of the antibodies and complete normalization of the urinary IRI and IRI / C peptide ratio. No comparable rise in the urinary IRI and IRI / C peptide ratio was observed in the patients with other disorders studied. Glucose clamp and glucose tolerance study showed decreased sensitivity to exogenous or newly secreted insulin, prolonged half disappearance time of serum insulin, and normal disappearance of blood glucose. These results were consistent with the idea that autoantibodies buffered the effect of exogenous or newly secreted insulin and maintained a relatively constant level of serum free insulin which was not high enough when a large amount of glucose was loaded, but was too high after prolonged fasting, which eventually caused hypoglycemic attacks.

Key words: Urinary IRI, Autoimmune insulin antibodies, Glucose clamp, Primary empty sella syndrome, Polymyositis, Anti-pituitary antibodies. (Endocrinol Japon 39: 307-313, 1992)

PATIENTS with autoimmune insulin antibody, first reported in 1970 by Hirata *et al.* [1], are characterized by frequent attacks of hypoglycemia associated with high affinity-antibodies to insulin in the patient's serum, without a prior history of insulin administration. Hypoglycemic attacks and anti-insulin autoantibodies are transient and regress within several years. More of these patients are reported in Japan [2] than in other countries

[3-5] and are sometimes associated with previous intake of drugs containing sulfhydryl groups such as methimazole, alpha-mercaptopyruvate, glutathione, and D-penicillamine [6, 7]. Since the autoantibodies interfere with radioimmunoassays and insulin is retained in the serum, a marked increase in serum immunoreactive insulin is observed in the face of normal or slightly increased serum C peptide. Although a high serum level of free insulin is a possible cause of hypoglycemia, other mechanisms can be hypothesized [8]. These include the following two. Anti-idiotypic antibodies to anti-insulin antibodies may be produced in the patients. These antibodies may bind

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to the insulin receptor and mimic the insulin action, causing hypoglycemic attacks. The other hypothesis is that anti-insulin antibodies bind to the insulin-receptor complex and interfere with the dissociation of the complex, which leads to continuation of the insulin action and hypoglycemia. Here we report a rare case of hypoglycemic attacks due to autoimmune insulin antibodies, associated with polymyositis and primary empty sella syndrome. In this patient, an apparent increase in urinary immunoreactive insulin (IRI) was observed. Additionally, the results of glucose clamp and glucose tolerance test suggested inappropriately high serum free insulin as a cause of hypoglycemia.

Case Report

A 64-year-old man was referred to us on February 16th, 1990 to examine the cause of hypoglycemia. In the morning of April 28th, 1989, he had convulsions, lost consciousness and was carried to a physician's office. His blood glucose level was 1.8 mM and the administration of glucose restored him. In order to examine the cause of hypoglycemia, he was transferred to another hospital. On admission, he was diagnosed as having polymyositis. Fever, atrophy and weakness of the proximal muscles of upper and lower extremities, accelerated erythrocyte sedimentation rate, positive C-reactive protein in the serum, increase in serum gamma globulin, serum glutamic oxaloacetic transaminase, creatine phosphokinase, and lactic acid dehydrogenase, and myogenic

electromyographic changes in proximal muscle supported this diagnosis. His fasting blood glucose level was 1.7 mM, the serum insulin level was more than 2300 pM and the serum C peptide level was 1.4–2.8 nM (Table 1). Abdominal ultrasonography, computed tomography, and angiography showed no evidence of pancreatic tumor. Prednisolone therapy was effective in preventing the hypoglycemia, lowering the serum insulin level and improving the polymyositis. On February 16th, 1990, he was referred to our hospital in order to examine the cause of hypoglycemia. The patient had never received such medications as insulin, antithyroid drug, glutathione, or D-penicillamine all of which are known to elicit antibodies to insulin. Physical examination revealed an alert man in apparently good health but with mental retardation. His height was 155 cm, weight 49 kg, pulse rate 78/min, and blood pressure 120–70 mmHg. Lung auscultation disclosed coarse crackles on inspiration at the lung bases. Atrophy and weakness of proximal muscles of the upper and lower extremities were evident. Laboratory findings showed normal results in the blood cell count, blood chemistry, and urinalysis except that slight normocytic normochromic anemia (hemoglobin level of 11.1 g/dl) was found. Chest roentgenogram revealed reticulonodular shadows in both lung bases which was compatible with the presence of pulmonary fibrosis, frequently associated with polymyositis. HLA types were A26, B37, BW75, CW3, CW6, DR9 and DQW9, none of which has been reported to be preferentially associated with Japanese insulin autoimmune syndrome [2]. Anti-nuclear, anti-Jo1, and anti-islet

Table 1. Results of 75 g oral glucose tolerance test

May, 1989						
Time (min)	0	30	60	90	120	
BG (mM)	1.7	4.4	5.7	7.2	8.4	
IRI (pM)	>2300	>2300	>2300	>2300	>2300	
CPR (nM)	1.4	1.6	2.0	1.9	2.8	
February, 1990						
Time (min)	0	30	60	90	120	180
BG(mM)	2.5	4.6	5.5	9.1	8.2	6.2
IRI (pM)	18.6	84.6	79.6	424	224	142
CPR (nM)	0.10	0.23	0.26	1.2	1.1	0.73

Results of 75 g oral glucose tolerance tests performed in May, 1989 and February, 1990 are shown. BG, Blood glucose; IRI, Immunoreactive insulin; CPR, C peptide.

Table 2. Clinical data

Autoantibodies			
Islet cell antibody	–	Anti-insulin antibody	48.4%
		IgG kappa light chain	72 %
Islet cell surface antibody	–	lambda light chain	28 %
Anti-nuclear antibody	–	Anti-insulin receptor antibody	–
Anti-JoI antibody	–	Anti-pituitary antibody	
Anti-microsomal antibody	–	Anti-ATT20 antibody	+
Thyroid test	–	Anti-GH3 antibody	+
Results of glucose clamp study and intravenous glucose tolerance test			
t1/2 of blood glucose	44.7 min (normal<57.8 min)		
t1/2 of serum insulin	13.4 min (normal: 4.28±1.23*)		
SIP	8.9×10 ⁻⁵ dl/min per μU/ml (normal: 7.9×10 ⁻⁴ [12], 5.5×10 ⁻⁴ [13], 4.9×10 ⁻⁴ [14])		
HLA type			
A26, B37, BW75, CW3, CW6, DR9, DQW9			

Anti-pituitary antibodies were determined using ATT20 cells (cultured mouse ACTH producing pituitary tumor cells) and GH3 cells (cultured rat GH producing pituitary tumor cells [9]. *, Mean ± SD for 9 normal subjects; t1/2, Half life; SIP, Glycemia independence of clamp-based insulin sensitivity index [10].

cell antibodies were negative. Antibodies to pituitary cells (ATT20 cells, mouse ACTH secreting tumor cells; GH3 cells, rat growth hormone secreting tumor cells) [9] were positive (Table 2). Anti-insulin antibody was 6.5% which was within the normal range (less than 10%). Antibodies to insulin receptors determined three times during the admission were all negative. Serum levels of thyroid hormone, adrenal cortical and medullary hormones, and pituitary hormones were all normal in spite of the presence of partial empty sella syndrome; herniation of suprasellar cistern into anterior sella turcica, that was demonstrated by brain computed tomography and magnetic resonance imaging. Urinary 17-OHCS, 17-KS, urinary cortisol, and the results of various provocative tests for pituitary hormones were normal. A diagnosis of polymyositis was made on the basis of physical and laboratory findings (as described above and in Fig. 1), electromyographic findings which revealed short duration and low amplitude potentials in proximal muscles of the extremities, and from responses to prednisolone therapy. Figure 1 demonstrates the inflammatory indexes, serum levels of the myogenic enzymes, and various indexes of insulin autoimmune syndrome throughout the hospital course. Exacerbation of polymyositis at the end of March, 1990 was accompanied by

increased frequency and degrees of hypoglycemic attacks with a concomitant increase in anti-insulin antibodies to 48.4%. Total and free serum IRI after overnight fasting were 129 and 35.9 pM, respectively. Fasting serum IRI determined by the usual double antibody method (108 pM) was disproportionally increased compared to low serum C peptide (0.10 nM). Urinary IRI determined on the same day was markedly increased to 11200 pmol/day, whereas the mean ± SD of 16 normal individuals showed 42.1 ± 17.5 pmol of IRI per day (Fig. 1). However simultaneous measurement of urinary C peptide was within the normal range. Prednisolone (60 mg/day) treatment ameliorated polymyositis and hypoglycemic attacks with simultaneous decreases in autoantibodies to insulin, and urinary and serum IRI levels without altering the urinary C peptide level. Before starting prednisolone, an intravenous glucose tolerance test and a glucose clamp study [10] were performed (Table 2). In these studies, the half disappearance time of blood glucose was 44.7 min (normal value was less than 57.8 min), the half disappearance time of serum IRI [11] was 13.4 min (normal value was from 3.00 to 5.75 min), and the increase in the blood glucose disposal rate in response to increased insulin infusion was blunted. The insulin sensitivity index independent of the

Fig. 1. Serial determinations of inflammatory indexes, serum levels of myogenic enzymes, and various indexes of insulin autoimmune syndrome during the hospital course. ESR, Erythrocyte sedimentation rate; FBG, Fasting blood glucose; IRI, Immunoreactive insulin; CPR, C peptide; Ab, Antibodies.

Fig. 2. Scatchard analysis and time course of insulin binding to antibodies. Scatchard analysis of [¹²⁵I] labeled human insulin-binding to antibodies is shown. Time course of the binding at 4°C is shown in the inset. Time course of binding was performed by incubating the patient's immunoglobulin with 50 pM of [¹²⁵I] labeled human insulin in the absence (total binding) (○---○) or presence (nonspecific binding) (×---×) of 0.5 μM unlabeled human insulin. Specific binding (●---●) was calculated by subtracting nonspecific binding from total binding. Specific binding of [¹²⁵I] insulin to immunoglobulin obtained from a normal subject is also shown (■---■).

glycemic value (SIP) [10] was 8.9×10^{-5} dl/min per $\mu\text{U/ml}$, whereas normal values in three different reports were 7.9×10^{-4} [12], 5.5×10^{-4} [13], and 4.9×10^{-4} [14], suggesting that the patient was 6–9 times less sensitive to exogenous insulin. The results of a 75 g oral glucose tolerance test showed a low fasting level and gradual increase in blood glucose. Blood glucose levels were more than 7.8 mM after 120 min and the results were compatible with an impaired glucose tolerance pattern (Table 1).

With goat anti-human immunoglobulin, the patient's antibodies were determined as IgG which consisted of a 72% kappa light chain and a 28% lambda chain (Table 2). The antibodies displayed two sets of binding sites when analyzed by Scatchard analysis (Fig. 2). Higher affinity sites displayed an insulin binding affinity constant of $2.2 \pm 0.4 \times 10^{10}$ l/mol and a maximal insulin binding capacity of 0.42 ± 0.02 nmol/l serum, whereas those of lower affinity sites were $1.2 \pm 0.9 \times 10^7$ and 104 ± 84 , respectively (mean $\pm$ SD of three determinations).

Methods

The measurement of antibodies to insulin was performed as described before [15]. In brief, serum was deprived of insulin by treatment with acid dextran coated charcoal after acidification, followed by neutralization and polyethylene glycol precipitation of immunoglobulin after the addition of a trace amount of [^{125}I] labeled human insulin. [^{125}I] Insulin in the precipitate was expressed as a percentage of total [^{125}I] insulin. For

the determination of the class of immunoglobulin and light chain-type, anti-human immunoglobulin with heavy chain specificity and light chain specificity was used for the immunoprecipitation of [^{125}I] labeled human insulin-antibody complex. Total and free IRI in serum were determined as described before [16]. Urinary IRI and C peptide were determined by radioimmunoassays as described before [17].

Discussion

A rare case of hypoglycemic attacks due to autoimmune insulin antibodies associated with polymyositis and primary empty sella syndrome was reported. The patient apparently had increased urinary immunoreactive insulin (11200 pmol/day) which was accompanied by the exacerbation of hypoglycemic attacks and autoimmune insulin antibodies. The increase in urinary IRI was not due to increased pancreatic secretion, because the urinary C peptide level was normal. Among other disorders examined, urinary IRI was increased in patients with proximal renal tubular defect, chronic hepatitis, liver cirrhosis, and insulinoma (Table 3). However, these values were far below that in the present case. It was strange that the serum level of free insulin was not so high as that speculated from high urinary IRI. As a result, the apparent renal clearance of insulin was extremely high. One possibility is that antibody secreted in urine interfered with the radioimmunoassay of urinary IRI. Another possibility is that insulin-antibody complex was excreted into the urine resulting in high urinary IRI. The

Table 3. Urinary output of insulin and C peptide in various disorders

Subjects	Age (years old)	IRI (pmol/day)	CPR (nmol/day)	IRI/CPR ($\times 10^{-3}$)
Normal (N=16)*	54 $\pm$ 15	42.1 $\pm$ 17.5	26.1 $\pm$ 5.6	1.6 $\pm$ 0.6
CLD (N=5)*	55 $\pm$ 32	97.5 $\pm$ 64.1	38.3 $\pm$ 29.4	3.5 $\pm$ 2.4
Insulinoma	64	31.6	25.4	1.2
(N=2)	52	323	30.8	11
PRTD (N=1)	46	1860	11.4	160
Present A	64	11200	26.2	430
case B		52.5	33.6	1.6

Daily urinary output of insulin and C peptide was determined in 16 normal subjects (8 males and 8 females), 5 patients with chronic liver disease (CLD) (2 males and 3 females), 2 female patients with insulinoma, a male patient with proximal renal tubular defect (PRTD) due to multiple myeloma presenting Fanconi's syndrome, and present case before (A) and after (B) prednisolone treatment.

*, Mean $\pm$ SD are shown.

third possibility is that renal tubular reabsorption of insulin was impaired. However, we did not evaluate renal tubular function at that time and we do not have any data supporting this idea. As we did not expect to obtain such results, we did not further characterize urinary IRI to ensure that the urinary IRI reflected intact insulin itself.

Another unique observation in this patient is that the serum level of IRI determined by the usual double antibody method was not high in spite of the presence of autoimmune insulin antibodies. Although we did not further pursue the reasons for this unusual observation, it is possible that the antibodies in radioimmunoassays can bind to the autoantibody-insulin complex due to the recognition of different epitopes of the insulin molecule in these two antibodies, which consequently resulted in normal serum IRI levels. Alternatively, if the autoimmune antibodies have considerably lower affinity with insulin than the antibodies used in radioimmunoassays, the conventional double antibody method gives normal serum IRI values in the presence of the autoimmune insulin antibodies.

In order to further understand the mechanism of hypoglycemia in this patient, a glucose clamp study, an oral glucose tolerance test, and an intravenous glucose tolerance study were performed. These results were compatible with the following ideas. Due to the antibodies present in the patient's serum, the patient was resistant to either exogenous insulin (indicated by a low SIP value) or to newly secreted insulin (indicated by impaired glucose tolerance) which action was

buffered by binding to the antibodies. The disappearance of the serum insulin was blunted because the insulin bound to the antibody was not metabolized as quickly as free insulin. However, serum free insulin, which was in equilibrium with antibody-bound insulin, was high enough to keep the glucose disposal rate within the normal range. In other words, the change in serum free insulin in response to the changes in serum total insulin was not so great when the antibody was present, as that when the antibody was absent. This free insulin concentration was not high enough when the large amount of glucose was loaded, but was too high when the patient was kept fasting and the glucose disposal rate was not decreased after a long fast, which eventually caused hypoglycemic attacks. However, this does not exclude the other possible mechanisms [8] for the hypoglycemia.

Autoimmune disorders sometimes coexist in one patient. We previously reported that patients with primary empty sella syndrome frequently showed positive anti-pituitary antibodies, regardless of the presence or absence of endocrine abnormality [18]. The antibodies did not seem to result from destruction of the pituitary gland, but could be the cause of the disorder. In the present case, primary empty sella syndrome was accompanied by the positive anti-pituitary antibodies. Together with the fact that primary empty sella syndrome was associated with polymyositis and autoimmune insulin antibodies, this finding supported the theory that the primary empty sella syndrome in the present case was caused by an autoimmune process.

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