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J Goldman, D Baldwin, A H Rubenstein, D D Klink, W G Blackard, L K Fisher, T F Roe, J J Schnure

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Research Article

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Characterization of Circulating Insulin and Proinsulin-Binding Antibodies in Autoimmune Hypoglycemia

JOSE GOLDMAN, DAVID BALDWIN, ARTHUR H. RUBENSTEIN, DOUGLAS D. KLINK, WILLIAM G. BLACKARD, LYNDA K. FISHER, THOMAS F. ROE, and JOEL J. SCHNURE,
Department of Medicine, University of Chicago, Chicago, Illinois 60637

ABSTRACT Five patients with fasting and(or) postprandial hypoglycemia were found to have insulin antibodies in the absence of previously documented immunization. Studies on the equilibrium-binding of insulin to the autoantibodies revealed two classes of binding sites with association constants and binding capacities analogous to those of insulin antibodies from insulin-treated diabetic patients. Similarly, no consistent differences in these parameters were found in both groups of patients with insulins of bovine, porcine, and human origin. Proinsulin (C-segment directed) antibodies capable of binding bovine or porcine proinsulin were present in 10 of 10 and 9 of 10 insulin-treated diabetics serving as controls, respectively, and, when present, provide incontrovertible evidence of exogenous insulin administration. No such antibodies could be detected in the hypoglycemic patients with autoimmune insulin antibodies.

The kinetics of dissociation of the insulin-antibody complexes were consistent with the existence of two classes of antibody sites. The corresponding dissociation rate constants were large enough to predict that significant amounts of free hormone may be generated by this mechanism and provide a plausible pathogenesis for the hypoglycemia in these patients.

Dr. Rubenstein is an Established Investigator of the American Diabetes Association. Dr. Goldman's present address is Department of Medicine, Henry Ford Hospital, Detroit, Mich. 48202; Dr. Klink, 324 East Wisconsin Avenue, Milwaukee, Wis. 53202; Dr. Blackard, Department of Medicine, Medical College of Virginia, Richmond, Va. 23298; Doctors Fisher and Roe, Division of Endocrinology and Metabolism, Childrens Hospital of Los Angeles, and Department of Pediatrics, University of Southern California School of Medicine, Los Angeles, Calif. 90054; and Dr. Schnure, Endocrine and Metabolic Disease Service, Charles S. Wilson Memorial Hospital, Johnson City, N. Y. 13790. Address reprint requests to Dr. Rubenstein in Chicago.

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INTRODUCTION

The immunogenicity of insulin in humans was first documented by Berson et al. (1) who demonstrated the existence of specific, nonprecipitating antibodies in the serum of insulin-treated diabetic patients. These antibodies have been incriminated in the pathogenesis of some forms of insulin resistance in diabetes mellitus (2). Moreover, hypoglycemia in insulin-treated patients may arise by release of the hormone from circulating insulin-antibody complexes (3). More recently, a number of patients with spontaneous hypoglycemia in association with insulin antibodies have been reported (4–9). An autoimmune origin has been proposed for these antibodies in view of the absence of a history of previous insulin administration (4–9) and the finding of a higher affinity for human insulin (4, 5) in some, but not all of the reported cases (7). The association of spontaneous hypoglycemia with apparent insulin autoantibodies in Graves' disease has also been described (8).

This study is concerned with five patients who presented with a similar syndrome of severe spontaneous hypoglycemia with insulin antibodies in the absence of evidence of previous injection of insulin. The autoimmune hypothesis for the origin of the insulin antibodies was tested by characterization of their association constants and binding capacity and measurement of specific C-segment-directed proinsulin antibodies. A control group of insulin-treated diabetics served as a model of exogenously induced insulin antibodies. Finally, the dissociation rate constants of insulin-antibody complexes were measured to examine the possible role of hormone released from antibody sites in the pathogenesis of this type of hypoglycemia.

METHODS

Case summaries. Patient 1 is a 58-yr-old white woman with a 23-yr history of severe rheumatoid arthritis. At the time that her hypoglycemia was diagnosed, she developed an epi-

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sode of confusion, combativeness, and verbal abusiveness. She was found to have blood glucose of 35 mg/dl, and her symptoms abated completely after intravenous glucose administration. Similar symptoms, associated at times with syncope, had been present for 3 wk. For several months she had experienced terrifying nightmares from which she would wake sweating profusely. During the first day after admission to hospital, interruption of her intravenous infusion of glucose led to a recurrence of hypoglycemia after ≅90 min and blood glucose levels at these times ranged between 20 and 40 mg/dl. Plasma cortisol values were normal, and an oral glucose tolerance test (GTT)[1] showed a diabetic curve (fasting plasma glucose [89]: 1 h, 238; 2 h, 268; 3 h, 122; 4 h, 56; and 5 h, 58 mg/dl) but neither fasting nor reactive hypoglycemia were present. Unusually high levels of serum insulin led to the demonstration of insulin antibodies.

Patient 2 is a 43-yr-old white woman of Latin-American ancestry. For 1 yr she had noted episodes of dizziness, tremulousness, and diaphoresis occurring 3.5-4 h after meals, on one or two occasions daily. Milder attacks also occurred before breakfast. The symptoms could be relieved by ingesting sugar-containing foods. An oral GTT showed a diabetic pattern with frank reactive hypoglycemia (fasting plasma glucose [70]: 30 min, 165; 1 h, 209; 2 h, 222; 3 h, 125; 4 h, 61; and 5 h, 41 mg/dl). An 1,800-calorie diet and 250 mg chlorpropamide daily were initiated by a private physician, but the latter medication had to be discontinued because of worsening hypoglycemia. The patient's mother was an insulin-requiring diabetic who routinely gave her a monthly intramuscular injection of vitamin B-12. Insulin administration was denied. Fasting and stimulated insulin levels were markedly elevated in the double antibody radioimmunoassay leading to the suspicion that insulin antibodies might be present. Their presence was subsequently confirmed. The patient has been described in detail (10).

Patient 3, a 3-yr-old boy, was a product of a full-term pregnancy and cesarean section. Symptoms of hypoglycemia were not noted in the neonatal period. Between the ages of 10 and 23 mo he developed several bouts of ketotic hypoglycemia characterized by lethargy, and by one episode of seizure associated with a blood glucose of 13 mg/dl and an insulin level of 4 μU/ml. Remission of symptoms followed intravenous glucose. During this period he showed an appropriate hyperglycemic response to glucagon administration and had normal cortisol levels. At 25 mo several episodes of severe hypoglycemia with ketosis occurred. Lethargy, diaphoresis, and thready pulse characterized these episodes, and they invariably led to coma unless intravenous glucose was administered. Insulin levels of 254 μU/ml were demonstrated at this time. At age 2.5 yr, the hypoglycemic attacks were more severe and frequent. Leucine administration failed to elicit hypoglycemia, and a standard intravenous tolbutamide test resulted in a blood glucose value of 42 mg/dl at 30 min. During these two tests, spuriously high serum insulin levels of >1,000 μU/ml were detected and led to the demonstration of insulin antibodies. An oral GTT was normal (fasting plasma glucose [65]: 30 min, 113; 1 h, 131; 2 h, 69; 3 h, 55; 4 h, 53; and 5 h, 55 mg/dl). There was no evidence of exogenous insulin administration, and at least one hypoglycemic episode occurred in the hospital under circumstances where relatives could not have been present.

Patient 4, a 26-yr-old white woman, developed postprandial hypoglycemia, during her fifth pregnancy. Her blood glucose was 28 mg/dl during one such episode. Fasting hypoglycemia was also documented on one occasion. There was a history of grand mal epilepsy, but the seizure activity was unrelated to hypoglycemia. Renal glycosuria and ketonuria were present during her pregnancy, but no hyperglycemia was noted. An oral GTT showed a fasting plasma glucose (74): 1 h, 120; 2 h, 77; 3 h, 55; 4 h, 57; and 5 h, 49 mg/dl, but the corresponding serum "insulin values" ranged from 425 to 600 μU/ml. Circulating insulin antibodies were found. There was no history of previous insulin therapy or evidence of surreptitious self-administration of hormone. Plasma glucose responses to tolbutamide and glucagon were normal.

Patient 5, is a 48-yr-old white woman with a history of intermittent fasting hypoglycemia over a period of 15 yr. Severe hypoglycemia with coma developed on several occasions with blood sugar levels of 13 and 25 mg/dl. The oral GTT was nondiabetic but showed reactive hypoglycemia (fasting plasma glucose [98]: 30 min, 172; 1 h, 124; 2 h, 105; 3 h, 44; 4 h, 65; and 5 h, 79 mg/dl). Spuriously high levels of serum insulin as a result of the presence of insulin antibodies were found. After being lost to follow up, the patient was readmitted to a local hospital comatose and hypoglycemic. Residual brain damage after restoration of normoglycemia made admission to a nursing home necessary.

Materials. A control group of 10 insulin-requiring diabetics was randomly chosen from the Endocrinology Clinic of the University of Chicago. The affinity constants, binding capacities, and dissociation rate constants of serum insulin antibodies were measured by procedures which have been reported in detail (11). Peptides were labeled with ^{125}I (Industrial Nuclear Co., St. Louis, Mo.) as described by Freychet et al. (12). Free and total insulin and C-peptide immunoreactivities were measured by the procedures of Nakagawa et al. (13) and Kuzuya et al. (14), respectively. To determine specific proinsulin (C-segment directed) antibodies, insulin antibodies were first removed by adsorption to insulin coupled covalently to Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). The coupling procedure used cyanogen bromide-activated Sepharose 4B as described by Shapiro et al. (15). Bovine, porcine, and human insulins, and bovine and porcine proinsulins and C-peptides were a generous gift from Dr. Ronald E. Chance of Eli Lilly and Co., Indianapolis, Ind.

RESULTS

The pertinent clinical features of the five patients with hypoglycemia are summarized in Table I. Neither a history of insulin therapy nor evidence of surreptitious self-administration of insulin could be elicited in these patients, despite a high level of suspicion by the medical staff. Typically, the finding of high levels of serum insulin by the double antibody radioimmunoassay led to the suspicion and demonstration of insulin antibodies. In this type of radioimmunoassay, the second antibody is specific for the animal-raised insulin antibody used in the assay, and therefore the ^{125}I-insulin bound to the human insulin antibody of the serum sample will not be precipitated. As a result, less radioactivity is measured in the precipitate and spuriously higher insulin values are obtained from the standard curve.

Insulin antibodies. Fig. 1 shows representative data from competitive binding experiments in which labeled bovine and human insulins were each incubated with the patient's serum in the presence of varying concentrations of either cold bovine or human insulin. No

[1] *Abbreviation used in this paper*: GTT, glucose tolerance test(s).

TABLE I
Autoimmune Hypoglycemia: Clinical Presentation

	Patient	Age	Sex	Hypoglycemia		Hyperglycemia-fasting	GTT		Reference
				Fasting	Postprandial		Diabetic	Late hypoglycemia	
Literature cases*	1	52	M	+	+	−	+	+	(4)
	2	42	M	−	+	−	+	+	(5)
	3	3 d	F	+	−	−	−	−	(6)
	4	52	F	+	−	−	+	+	(7)
	5	59	M	−	+	−	+	+	(9)
	6	77	M	−	+	−	+	+	(9)
	7	23	F	+	−	−	+	−	(8)
Newly studied patients	1	58	F	+	+	−	+	−	
	2	43	F	+	+	−	+	+	
	3	10 mo	M	+	−	−	−	−	
	4	26	F	+	+	−	−	+	
	5	48	F	+	+	−	−	+	

* Summary of patients reported in the literature.

consistent differences in the ability of the two species of insulin to compete with the labeled hormones for antibody binding sites could be demonstrated in either group of patients, thereby indicating that the hypoglycemic patients did not necessarily have antibodies with a higher affinity for human insulin. Similarly, exogen-

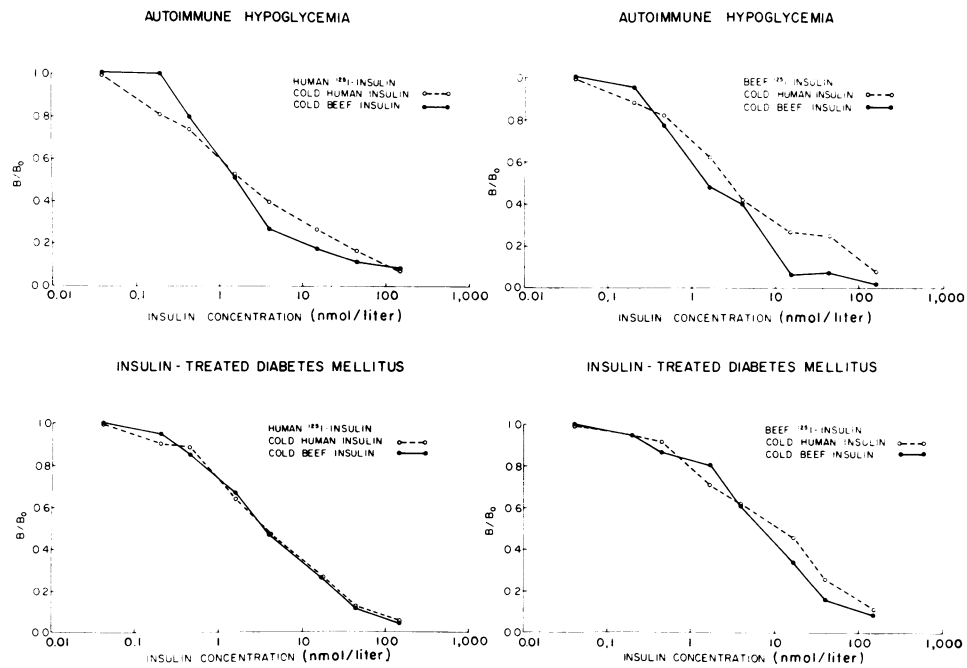

FIGURE 1 Cross-competition binding studies with insulin antibodies from autoimmune hypoglycemic subjects and insulin-treated diabetic patients. Sera diluted 1:10 were incubated with 50 pM human or bovine ^{125}I-insulin in the presence of varying concentrations of cold human (dashed line) or bovine (solid line) insulin. Barbital-albumin buffer (sodium barbital 7 mM, sodium acetate 12 mM, sodium choloride 130 mM, albumin 0.5%) (pH 7.5) was used, and incubations were carried out at 37°C for 2 h. Separation of free and bound insulin was accomplished by the addition of 0.1% (final concentration) carrier bovine gamma globulin and 12.5% (final concentration) polyethylene glycol. The sera used in this study correspond to patient 1 of the autoimmune hypoglycemia group and patient 7 of the insulin-requiring diabetic group. B/B_0 is the ratio between bound ^{125}I-insulin (B) and the ^{125}I-insulin bound in the absence of cold insulin B_0.

ously induced antibodies in insulin-treated diabetics did not consistently show higher affinities for beef or, in experiments not shown in Fig. 1, pork insulin.

Direct evidence regarding this point was derived from a detailed characterization of the antibody binding parameters. Scatchard plots of the insulin-antibody binding data showed bimodal distributions in all hypoglycemic and diabetic patients (Fig. 2). Two classes of antibodies characterized by the high-affinity:low-capacity and low-affinity:high-capacity sites were inferred from these plots. The association constants, binding capacities, and free energies of association for bovine, porcine, and human insulin in both groups of patients are shown in Tables II and III. No consistent differences in these parameters with regard to any species of insulin are apparent, and the overlap of values is complete for the hypoglycemic and diabetic patients. There is thus no consistent increase in affinity for human insulin in the antibodies of patients with hypoglycemia, and quantitative differences in antibody binding capacity cannot account for this syndrome.

Results of kinetic studies on the dissociation of insulin-antibody complexes indicate that the data can be fitted with two exponential functions, in agreement with the two classes of antibody sites demonstrated in the equilibrium-binding measurements. The first order dissociation rate constants for each class of antibodies are given in Table IV. Again, complete overlap in the values of these parameters in both groups of patients was found.

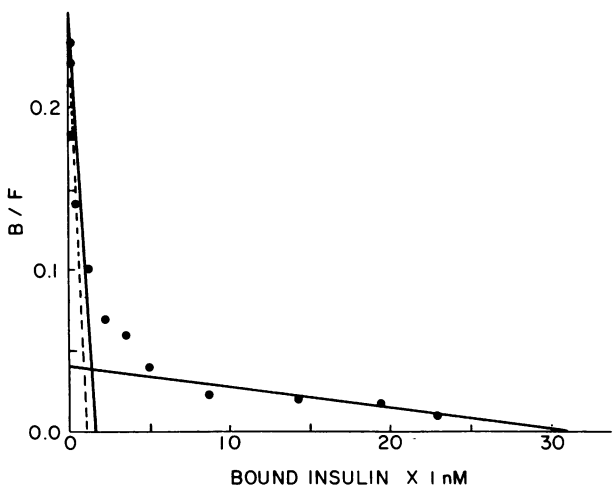

FIGURE 2 Scatchard plot of a representative binding assay of serum insulin antibodies. Final serum dilution, assay conditions, and separation of free (F) and bound (B) insulin were as described in Fig. 1. Nonspecific binding was defined as bound insulin not displaced from the serum binding sites at micromolar concentrations of cold insulin. Homologous species of labeled and cold insulins were used in each assay. Solid lines represent the least squares' linear fit of the binding data. The dashed line is the least squares' linear fit of the points related to the high-affinity antibody sites after subtraction of the contribution of the low-affinity antibody sites to the total binding in the corresponding range of insulin concentration. Association constants and binding capacities were calculated from the slopes and abscissa intercepts (16), respectively.

Plasma-free and total insulin and C-peptide. Average values of plasma glucose and free and total insulin and C-peptide occurring during a standard oral GTT are presented in Table V. Peak values of free insulin in one of the patients (No. 4) were very high and were associated with symptomatic hypoglycemia 240 min after the administration of glucose. In the other three patients in whom free insulin concentrations were measured, the absolute values were not markedly elevated, but in two patients, the peak values occurred late at 120 min and remained elevated above levels obtained in healthy individuals during an oral GTT. The high levels of total C-peptide (Table V) in patient 2 correspond to proinsulin bound to insulin antibodies through its A- and B-chain antigenic determinants.

Proinsulin (C-segment-directed) antibodies. The sera of hypoglycemic patients failed to show specific binding of bovine and(or) porcine proinsulins. Similarly, no specific binding of bovine and porcine C-peptides could be demonstrated (Table VI). In constrast, the group of insulin-treated diabetics showed significant levels of specific binding of these peptides. Sera from all 10 patients bound bovine proinsulin, and in 9 of 10 patients porcine proinsulin was also bound. Only two of these diabetic patients had antibodies which bound bovine C-peptide, while four patients bound porcine C-peptide (Table VI).

DISCUSSION

We have described five patients who presented with fasting and(or) postprandial hypoglycemic episodes. When their serum insulin levels were measured by immunoassay, spuriously high values as a result of the presence of insulin antibodies were found. Seven other patients with similar clinical and laboratory features have been described (4–9). In all of these cases, the more conventionally recognized causes of hypoglycemia were excluded, and a differential diagnosis between factitious and autoimmune hypoglycemia remained.

An autoimmune origin for the insulin antibodies in patients with this syndrome was initially suggested by the lack of evidence of previous insulin administration (4–9). A similar situation also pertains to the five hypoglycemic patients described in this series. Despite many months of observation and a high level of suspicion regarding the possibility of exogenous insulin injection, no evidence to support this diagnosis was forthcoming. The only possible exception in this regard was the second patient, who had received monthly treatments of intramuscular vitamin B_{12}. These injections had been administered by the patient's mother, who is an insulin-

TABLE II
Insulin Antibodies in Autoimmune Hypoglycemia

Patient	Insulin	High-affinity site			Low-affinity site			Total capacity
		Ka	Capacity	ΔG°	Ka	Capacity	ΔG°	
		1/10⁹M	*U/liter*	*Kcal/mol*	*1/10⁷M*	*U/liter*	*Kcal/mol*	*U/liter*
1a*	Beef	1.96	0.12	−13.2	2.70	1.81	−10.5	1.93
	Pork	1.04	0.26	−12.8	1.60	4.01	−10.2	4.27
	Human	1.66	0.20	−13.0	0.50	13.00	−9.5	13.20
1b*	Beef	6.50	0.02	−13.9	12.00	0.24	−11.5	0.26
	Pork	9.69	0.02	−14.1	0.39	14.38	−9.3	14.40
	Human	3.40	0.04	−13.5	0.34	10.38	−9.3	10.42
2	Beef	0.47	2.86	−12.3	1.20	9.10	−10.0	11.96
	Pork	1.49	0.57	−13.0	18.00	1.10	−11.7	1.67
	Human	3.26	0.54	−13.5	3.00	3.88	−10.6	4.42
3	Beef	0.69	3.84	−12.5	1.20	13.11	−10.0	16.95
	Pork	2.80	1.15	−13.4	0.39	37.25	−9.3	38.40
	Human	1.98	1.22	−13.2	0.92	12.95	−9.8	14.17
4	Beef	2.08	9.43	−13.2	1.80	23.57	−10.3	33.00
	Pork	2.79	0.14	−13.4	0.88	9.50	−9.8	9.64
	Human	3.10	0.05	−13.5	1.00	3.71	−9.9	3.76
5	Beef	0.72	3.82	−12.6	2.80	7.28	−10.6	11.10
	Pork	2.04	0.80	−13.2	0.35	14.30	−9.3	15.10
	Human	4.22	0.47	−13.9	4.50	3.66	−10.8	4.13

* Separate determinations on the same patient carried out at a year interval.

requiring diabetic. The possibility that insulin-contaminated syringes might have been used in this patient cannot be excluded with certainty. However, the absence in her serum of C-segment-directed proinsulin antibodies, which might have arisen in response to the small amounts of proinsulin present in commercial insulin preparations, renders this possibility less likely (*vide infra*).

Previous studies (4, 5, 7, 8) have attempted to solve the diagnostic dilemma of factitious vs. an autoimmune basis for the development of insulin antibodies by performing competition studies in which radiolabeled porcine and bovine insulin were displaced from specific antibody sites by unlabeled porcine, bovine, and(or) human insulin. The displacement curves obtained with antibodies from these hypoglycemic patients have been interpreted as indicating a higher affinity for human insulin. However, the differences noted in the competition experiments were small, and detailed control studies were not made with antibodies generated in response to the administration of therapeutic preparations of insulin. Contrary to these previous reports, competition experiments and direct measurements of the association constants and binding capacities of insulin antibodies from the hypoglycemic patients and the control group of insulin-treated diabetics demonstrated no consistent differences for bovine, porcine, and human insulins. Therefore, no distinction between autoimmune and factitious hypoglycemia would seem to be possible on this basis.

Commercial preparations of mixtures of bovine-porcine insulin have small and variable amounts of contaminating bovine and porcine proinsulin (17–20). The sequences of the C-segments of the precursor molecules differ from each other and from their human counterpart to a much larger extent than the insulin A and B chains (18, 21, 22) and have been shown to be potent antigens in both experimental animals (23–26) and humans (27–30). A survey of insulin-treated diabetic patients demonstrated specific proinsulin antibodies in 80% of the cases (29). Our results have confirmed and extended this finding that C-segment-directed proinsulin antibodies were detected in the sera of 100 and 90% of the insulin-treated diabetics tested with bovine and porcine proinsulins, respectively. In addition, bovine and porcine C-peptide specific antibodies were present in the sera of 20 and 40% of the diabetic patients, respectively. The reason for the higher frequency of proinsulin-directed antibodies cannot be accounted for by the insulin A- and B-chain antigenic determinants in the proinsulin molecule, because these antibodies were completely removed from the sera by adsorption to insulin coupled covalently to Sepharose beads. Conformation-dependent antigenic determi-

TABLE III

Insulin Antibodies in Diabetics Treated with Insulin

Patient	Insulin	High-affinity site			Low-affinity site			Total capacity
		Ka	Capacity	ΔG°	Ka	Capacity	ΔG°	
		1/10⁹M	*U/liter*	*Kcal/mol*	*1/10⁷M*	*U/liter*	*Kcal/mol*	*U/liter*
1	Beef	0.56	2.14	−12.4	1.30	16.55	−10.1	18.69
	Pork	1.10	0.95	−12.8	1.10	22.80	−10.0	23.75
	Human	0.39	2.01	−12.2	5.30	5.97	−10.9	7.98
2	Beef	2.20	0.87	−13.2	3.80	13.10	−10.7	13.97
	Pork	2.90	0.81	−13.4	2.10	10.15	−10.4	10.96
	Human	4.40	0.53	−13.7	2.50	7.00	−10.5	7.53
3	Beef	25.60	0.08	−14.7	6.20	1.47	−11.0	1.55
	Pork	17.80	0.10	−14.5	48.00	0.24	−12.3	0.34
	Human	12.40	0.11	−14.3	28.00	0.32	−12.0	0.43
4	Beef	1.36	4.68	−12.9	2.60	22.60	−10.5	27.28
	Pork	1.80	3.10	−13.1	1.30	40.20	−10.0	43.30
	Human	1.28	4.32	−12.9	0.86	62.30	−9.8	66.62
5	Beef	1.29	0.45	−12.9	0.72	20.04	−9.7	20.49
	Pork	0.27	0.89	−12.0	0.46	17.23	−9.4	18.12
	Human	1.50	0.22	−13.0	4.00	3.98	−10.8	4.20
6	Beef	6.22	0.08	−13.9	0.11	23.02	−8.6	23.10
	Pork	2.09	0.08	−13.2	0.09	14.42	−8.4	14.50
	Human	3.49	0.08	−13.5	0.46	6.72	−9.4	6.80
7	Beef	7.34	0.28	−14.0	0.20	37.32	−8.9	37.60
	Pork	20.50	0.07	−14.6	0.90	15.43	−9.9	15.50
	Human	4.16	0.47	−13.7	0.80	17.53	−9.8	18.00
8	Beef	0.65	4.28	−12.5	0.35	86.49	−9.3	90.77
	Pork	0.26	4.20	−11.9	0.13	30.20	−8.7	34.60
	Human	0.70	1.50	−12.5	0.44	32.90	−9.4	34.40
9	Beef	0.21	1.26	−11.8	1.78	9.22	−10.3	10.48
	Pork	0.10	2.26	−11.4	0.42	17.24	−9.4	19.50
	Human	0.95	0.47	−12.7	0.45	16.00	−9.4	16.47
10	Beef	7.52	1.73	−11.2	1.84	2.65	−10.3	4.38
	Pork	6.40	0.51	−11.1	0.60	12.22	−9.6	12.77
	Human	6.78	1.33	−11.1	0.31	13.99	−9.2	15.32

nants have been demonstrated to exist in synthetic polypeptide systems and naturally-occurring proteins (31–33). Such antigenic determinants will interact with the corresponding antibody sites only if the specific antigenic conformation is present regardless of the fact that identical amino acid sequences exist in the nonantigenic conformational state. The C-segment of proinsulin has an unordered (34) but fixed conformation which differs from the free C-peptide in its three-dimensional structure, thus accounting for the differences in the binding of proinsulin and free C-peptide to C-segment-directed antibodies.

The absence of specific proinsulin-directed antibodies in this series of hypoglycemic patients constitutes strong evidence against self-administration of insulin and supports an autoimmune origin for their insulin antibodies. However, it must be stressed that although the presence of bovine-porcine C-segment antibodies provides incontrovertible evidence for exogenous insulin administration, their absence does not prove that the antibodies arose endogenously. For instance, injections of highly purified monocomponent insulin, infrequent injections with low doses of insulin, or use of the intravenous route of administration may result in the formation of insulin, but not proinsulin antibodies.

The dissociation rate constants in both groups of patients indicate that the release of free hormone from the

TABLE IV
Dissociation Kinetics of Insulin-Antibody Complexes

Patients	First order dissociation rate constant	
	High-affinity site	Low-affinity site
	(per min) × 10^3	*(per min) × 10^2*
Autoimmune hypoglycemia		
1a*	0.91	3.46
1b*	3.43	3.34
2	4.61	1.50
3	3.46	0.58
4	3.79	0.56
5	2.00	0.81
Insulin-treated diabetics		
1	1.20	1.15
2	6.45	0.88
3	2.53	5.73
4	0.90	1.61
5	1.70	1.12
6	3.80	1.36
7	5.92	1.47
8	8.29	1.08
9	3.78	0.74
10	14.70	6.56

* Separate determinations on the same patient carried out at a year interval.

pool of antibody-bound insulin can proceed at maximal rates of 0.09–1.47 and 0.56–6.56%/min, for the high- and low-affinity antibodies, respectively (Table IV). If the pool of antibody-bound insulin is large enough, substantial amounts of free hormone can be released by this mechanism (35). Unlike insulin that is secreted by the pancreas, a hormone dissociating from circulating insulin-antibody complexes is not under feed-back control and may thus give rise to hypoglycemic episodes. A similar mechanism has been invoked to explain the recurrent hypoglycemia and absence of insulin requirements in one ketosis-prone diabetic (36). Because of the first order character of the dissociation reaction, the absolute amount of insulin released per unit of time from the pool of bound hormone will be directly proportional to the concentration of bound hormone. It is possible that the higher frequency of these episodes in the autoimmune group of patients may be accounted for by insulin dissociating from its antibodies at times when their blood sugar is within the normal range as compared with the raised glucose values which usually prevail in diabetic patients. In addition, factors such as insulin resistance and secretion of contrainsulin hormones may also determine whether or not hypoglycemia will occur at a particular time. Furthermore, an enhanced output of insulin in the presence of insulin antibodies has been documented in vitro with isolated rat islet tissue (37). This circumstance may contribute to the hypoglycemic syndrome, although clearly the secreted insulin would be bound by antibodies, and dissociation would still be required for its hypoglycemic action. Also, the rapid binding of newly secreted insulin by circulating antibodies may prevent the immediate action of the hormone and thus result in delayed meta-

TABLE V
Plasma Glucose, Insulin, and C-Peptide Concentrations during Oral GTT

Patient	min	Time							
		0	30	60	90	120	180	240	300
1	Plasma glucose, *mg/100 ml*	89	—	238	—	268	122	56	58
	Free insulin, *μU/ml*	13	—	59	—	106	47	22	17
	Total insulin, *μU/ml*	121	—	141	—	184	119	91	97
	Free C-peptide, *ng/ml*	1.0	—	3.5	—	5.2	3.0	1.2	1.0
2	Plasma glucose, *mg/100 ml*	70	165	209	—	222	125	61	41
	Free insulin, *μU/ml*	5	16	44	—	74	38	14	7
	Total insulin, *μU/ml*	1,297	1,759	2,846	—	2,982	2,809	1,979	1,414
	Total C-peptide, *ng/ml**	16.2	20.2	25.1	—	28.9	25.7	25.0	23.0
3	Plasma glucose, *mg/100 ml*	65	113	131	121	69	55	53	55
	Free insulin, *μU/ml*	4	10	31	30	14	8	6	6
	Total insulin, *μU/ml*	1,033	1,252	1,142	1,302	941	1,067	932	874
	Free C-peptide, *ng/ml*	0.4	1.9	3.8	3.5	1.3	0.6	0.2	0.2
4	Plasma glucose, *mg/100 ml*	48	81	120	86	57	42	28	—
	Free insulin, *μU/ml*	102	384	460	468	412	442	390	—
	Total insulin, *μU/ml*	4,200	6,208	7,031	6,644	5,779	5,796	5,485	—
	Free C-peptide, *ng/ml*	0.03	2.4	1.6	1.2	0.3	0.2	0.2	—

* There was insufficient serum to perform free C-peptide determinations on this patient.

TABLE VI
Specific C-Segment-Directed Antibodies

Patient		Proinsulin		C-peptide	
		Bovine	Porcine	Bovine	Porcine
Autoimmune hypoglycemia	1	−	−	−	−
	2	−	−	−	−
	3	−	−	−	−
	4	−	−	−	−
	5	−	−	−	−
Insulin-treated diabetes mellitus	1	+	+	−	+
	2	+	+	+	+
	3	+	+	−	−
	4	+	+	−	−
	5	+	+	−	+
	6	+	−	−	−
	7	+	+	−	+
	8	+	+	+	−
	9	+	+	−	−
	10	+	+	−	−

bolic clearance of carbohydrates during a GTT with an apparent diabetic profile. It should be appreciated that the theoretical possibilities outlined above have not been unequivocally proven to be present in our patients. However, many of the studies were conducted at times when the patient was not having hypoglycemic reactions. Obviously, further studies are required to provide convincing data regarding the pathogenesis of the hypoglycemia in patients with this syndrome.

With the increased use of insulin for treating maturity-onset diabetes as a consequence of the University Group Diabetes Program study (38, 39), it is likely that a population of diabetics with substantial beta-cell reserve and significant insulin-antibody titers will exist. A note of caution seems in order in view of the possibility of hypoglycemia resulting from dissociation of insulin-antibody complexes in such patients.

At present, the etiology of the syndrome of autoimmune hypoglycemia is unknown. Circulating antibodies directed against pancreatic islet cells have been demonstrated in the majority of patients with juvenile-onset diabetes (40), and insulin-receptor antibodies have been identified in a group of patients with marked insulin resistance (41). Specific antibodies to thyroid (42), adrenal, and ovarian cells (43) occur in patients with hypofunction in these glands. Similarly, autoantibodies to a wide variety of other body constituents such as lipoproteins (44), erythrocyte membranes (45), hepatic cell constituents (46, 47), platelets (48), etc., have been recognized, but definitive information concerning the causes of these conditions is still not available. Also, glucagon autoantibodies have been demonstrated to occur in the absence of known specific immunization (49). In this context it seems reasonable to accept the possibility that antibodies to insulin may arise spontaneously. However, because of the widespread availability of insulin, and our inability to specifically exclude exogenous administration, absolute proof of either of these alternatives is not forthcoming with currently available methods. Irrespective of the initial immunogenic stimulus, it is clear that the development of insulin antibodies in these patients may result in severe and even life-threatening hypoglycemic episodes. Furthermore, in all conditions associated with autoantibodies, the common denominator is a loss of immune tolerance resulting in the synthesis of self-directed antibodies and(or) the development of self-directed, delayed hypersensitivity. In this sense the syndrome of hypoglycemia with associated insulin antibodies fulfills the criteria defining autoimmunity and, as true for all autoimmune diseases, it remains to be proven whether the primary pathogenic mechanism is exogenous or endogenous in origin.

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