Studies on the Etiology of "Brittle Diabetes"

Relationship Between Diabetic Instability and Insulinogenic Reserve

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SUMMARY

To clarify possible etiologic mechanisms for brittleness of diabetic control, a relationship between the degree of diabetic instability and insulinogenic reserve or insulin-binding capacity of plasma IgG was studied in 46 insulin-treated diabetics attending the outpatient clinic. Evaluation of insulinogenic reserve was based on elevations of plasma C-peptide immunoreactivity (CPR) during the oral glucose tolerance test (OGTT). The degree of instability was quantified by the standard deviations (S.D.) of 10 values of fasting blood glucose, which were determined for the last six months while subjects were attending a hospital as outpatients. An inverse correlation was evident between residual B-cell secretory capacity and blood glucose regulatory instability ($r = -0.69$, $p < 0.005$), but there was no consistent relationship of the insulin-binding capacity to the degree of diabetic instability ($r = 0.15$, $p > 0.05$). Furthermore, pancreatic A-cell functions were investigated in seven unstable and seven stable diabetics, classified according to their S.D. values. Although immunoreactive glucagon (IRG) responses to an infusion of arginine were observed, plasma IRG did not rise in unstable diabetics during insulin-induced hypoglycemia, in which condition IRG in stable diabetics rose significantly. In contrast, plasma cortisol responses to the insulin-induced hypoglycemia were demonstrated in both diabetic groups. Plasma CPR did not decrease in unstable but did in stable diabetics following the insulin injection. The comparison of unstable diabetic patients with more stable ones on the basis of clinical data, such as means of age, duration of diabetes mellitus, duration of insulin therapy, and dose of insulin, revealed no significant difference.

The total lack of insulinogenic reserve results inevitably in loss of the automatic regulation of circulating insulin levels, which seems to be one of the essential factors for causing the hyperlability of diabetic control. The pancreatic A-cell dysfunction is also attributable in part to the metabolic variability in brittle diabetes.

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As etiologic factors for metabolic instability are observed in certain diabetic patients, various candidates have been considered. These include the following: dysfunction of plasma-protein transport and of hepatic and peripheral tissue metabolism of insulin,[1] overinsulinization ("Somogyi effect"),[2] lack of insulinogenic reserve,[3] insulin antibodies,[4] higher diurnal HGH levels,[5,6] and abnormal response of plasma glucagon to hypoglycemia.[7] However, despite the numerous reports through the years, the data so far do not seem to permit a firm conclusion as to a cause of the brittleness because of the difficulties to define and measure diabetic instability and its biochemical and hormonal correlates.

Several attempts have been made by means of analysis of blood and urinary glucose data to assess instability of diabetes. But some of the indices[8-10] proposed so far are too laborious to apply to outpatients.

The difficulty in assessing insulinogenic reserve in insulin-treated diabetics because of the infeasibility of measuring circulating immunoreactive insulin (IRI) levels in the presence of endogenous antibodies to in-

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sulin has been another obstacle to the search for the causes of this state. The availability of an assay of C-peptide has enabled the estimation of endogenous insulin production in diabetic patients in whom circulating insulin antibodies and exogenous insulin therapy interfere with the insulin immunoassay.

In the present study we investigated secretory capacity of the pancreatic B-cells in insulin-treated diabetics by assessing changes in the concentrations of plasma C-peptide immunoreactivity (CPR) in response to oral glucose and the relationship between the insulinogenic reserve thus determined and the instability of the diabetes, which was quantified by standard deviation of mean for 10 values of fasting blood glucose of each patient. Furthermore, abnormalities in the secretion of glucagon and cortisol, major counterregulatory hormones against insulin in regulating glucose homeostasis, were also studied in unstable diabetics. In addition, suppressibility of the secretory function of pancreatic B-cells during a hypoglycemic state induced by insulin was explored in these patients.

MATERIAL AND METHODS

Subjects

Forty-six insulin-requiring patients attending the outpatient diabetes clinic of the Second Department of Internal Medicine, Osaka University Hospital, and 15 normal subjects were included in the study. The patients, with a range of 21 to 71 years of age, constituted a broad spectrum of diabetes, from the very stable to the extremely unstable, and all received one or two daily injections of Lente or Rapitard (mixture of pork and beef insulin, Novo, Denmark) for longer than one year. The duration of diabetes varied from one to 25 years. Patients with renal insufficiency were not included in the study. The controls, with ages ranging from 20 to 63 years, had normal blood glucose response during oral glucose tolerance test or a blood glucose concentration of lower than 110 mg./100 ml. at two hours after a meal. None of the control subjects had a family history of diabetes mellitus. All subjects were within 10 per cent of their ideal body weight, calculated from Jones' equation.[11]

The degree of diabetic instability was quantified by the standard deviation (S.D.) of 10 values of fasting blood glucose or by the difference between the maximum and minimum value among them. Blood specimens for determination of these blood glucose concentrations were obtained most recently for a

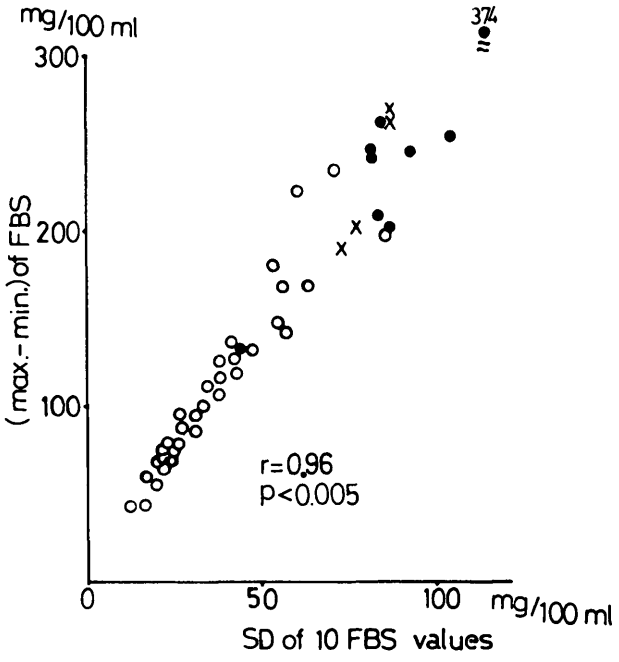

FIG. 1. Correlation between the S.D. of 10 values of fasting blood glucose and the maximum difference among them in insulin-treated diabetics. Blood specimens for determination of these blood glucose values were obtained most recently during a six-month period prior to OGTT while subjects were attending a hospital as outpatients. o represents a patient with plasma CPR response to oral glucose (ΔCPR > 0.2 ng./ml.). x and • represent patients showing no discernible plasma CPR rise (ΔCPR $\leq$ 0.2 ng./ml.) during OGTT from the basal level of higher (x) or lower (•) than 1 ng./ml.

period of six months prior to an oral glucose tolerance test described below while subjects were treated at a hospital as outpatients. A dose of insulin was adjusted so as to achieve good control based on each doctor's judgment during the period. As demonstrated in figure 1, the S.D. values of the 10 FBS concentrations range from 17.3 to 115 mg./100 ml., with a mean value of 50.8 mg./100 ml. and standard deviation of 27.6 mg./100 ml. The stable (D I) and unstable (D II) patients were selected from the diabetics according to their S.D. values; the S.D. values of D I were near or smaller than the mean of S.D. minus one standard deviation, 23.2 mg./100 ml., and those of D II were larger than the mean plus one standard deviation, 78.4 mg./100 ml. Both groups consist of seven patients (four women and three men each) whose clinical details are described in tables 1 and 2. Patients whose retinal changes progressed to large hemorrhages or proliferative stage were not included in either group.

Oral glucose tolerance test. After an overnight fast, all 46 diabetic and six healthy subjects were given 100

TABLE 1

Clinical features of the subjects

Diabetic group	Number of patients	Weight index* (%)	Age (yr.)	Duration of DM (yr.)	Insulin therapy duration (yr.)	Insulin therapy dose (U./day)	BUN mg./100 ml.
D I	7 (3)						
Mean ± S.D.		94.2 ± 8.8	52.0 ± 16.4	13.3 ± 6.9	9.0 ± 5.6	25.7 ± 10.0	17.7 ± 6.2
Range		85.9 ~ 106	24 ~ 71	3 ~ 20	2 ~ 19	12 ~ 40	10 ~ 26
D II	7 (2)						
Mean ± S.D.		87.6 ± 10.1	46.3 ± 19.5	9.0 ± 3.7	8.9 ± 4.0	34.3 ± 7.6	16.6 ± 3.8
Range		66.1 ~ 95.9	20 ~ 70	4 ~ 15	3 ~ 15	28 ~ 48	10 ~ 21

( ): no. of juvenile-onset (< 30) diabetics.
*Body weight divided by the ideal weight, calculated according to Jones' equation.

gm. of glucose orally. Blood samples were obtained at 0, 30, 60, 90, 120, and 180 minutes for determination of blood glucose and plasma CPR. Insulin-binding capacity of plasma IgG was determined in the fasting blood samples obtained from 41 of the patients. CPR secretion in response to oral glucose (Σ ΔCPR) was expressed as the area under the plasma CPR curve above the fasting CPR level from 0 to 180 minutes.

Arginine test. After an overnight fast, 30 gm. of L-arginine hydrochloride diluted in 300 ml. of water was infused at a constant rate for 30 minutes in the cubital vein in D I and D II patients and 10 normal subjects. Blood was taken from the contralateral cubital vein 15 and 0 minutes before the infusion and at 10, 20, 30, 45, 60, and 90 minutes after the infusion was begun. Blood glucose, plasma CPR, and immunoreactive glucagon (IRG) levels were determined.

Insulin tolerance test, ITT. After an overnight fast, D I and D II patients and 10 normal subjects reported to a test room at around 9 a.m. and rested comfortably in the supine position for one hour. At approximately 10 a.m., monocomponent Actrapid insulin (pork insulin, Novo, Denmark) was injected intravenously as a bolus in the subjects. To attain comparable blood glucose nadirs in all groups, 0.1 U. per kilogram body weight was administered to the normals and 0.2 or 0.3 U. per kilogram body weight to the diabetics, depending upon their fasting blood glucose levels. Blood was taken from a contralateral cubital vein catheter connected to a three-way stopcock, kept patent with an infusion of saline, at 15 and 0 minutes before and 10, 20, 30, 45, 60, 90, and 120 minutes after the injection. Blood glucose, plasma CPR, IRG, and cortisol levels were measured. All medications were omitted on the morning of the test day. In most cases, the three tests were carried out in this order within one month.

For the determination of plasma IRG concentration, a portion of all blood samples was transferred into tubes containing EDTA and lyophilized Trasylol, an inhibitor of proteolysis, in amounts sufficient to provide 500 kallikrein inactivator units per milliliter of blood. The samples were centrifuged as soon as possible and the plasma was separated and preserved at −20° C. until analyzed. Blood glucose was assayed by the Hoffman method[12] as applied to the AutoAnalyzer. Plasma IRG was measured radioimmunologically with antiglucagon serum specific for pancreatic glucagon, AGS 18 (donated by Dr. P. P. Foá). The antibody reacts predominantly to the carboxyl terminal of the glucagon molecule[13] and binds

TABLE 2

B-cell reactivity and insulin antibody titer in stable and unstable diabetics

Diabetic groups	10 FBS values S.D.	10 FBS values Max.·diff.	10 FBS values Mean	Σ ΔCPR ng.·min./ml.	Insulin-binding capacity μU./ml.
D I	21.7 ± 5.2	74.3 ± 18.6	132 ± 27.4	405 ± 252	951 ± 1,094
Range	12.2 ~ 27.6	43 ~ 100	90 ~ 176	105 ~ 727	268 ~ 3,340
D II	90.9 ± 13.5	248.3 ± 60.9	189 ± 57.5	5 ± 18	1,614 ± 1,610
Range	78.6 ~ 115.0	197 ~ 374	138 ~ 300	−14 ~ 44	245 ~ 4,785
D I vs. D II p <	0.005	0.005	0.05	0.005	N.S.

insignificant amounts of gut glucagon-like immunoreactivity when this is present in low concentrations and only 5 ~ 8 per cent when present in concentrations of 1,000 pg./ml. or higher.[14] Technical details for the radioimmunoassay are identical to those described previously[13] except for the following minor modification: 0.4-ml. portions of rabbit antiglucagon serum diluted in 0.04 M phosphate buffer pH7.4, containing 1 per cent bovine serum albumin and 0.1 per cent of NaN_3 ("buffer"), were incubated at 4° C. with 0.2 ml. of glucagon standard or unknown samples and 20 pg. of ^{125}I-glucagon (purchased from Hoechst, Japan) in 0.1 ml. After 48 hours, 0.2 ml. of pooled human serum or "buffer" and 1.0 ml. of 25 per cent polyethylene glycol were added to each tube, which was centrifuged at 3,000 rpm for 15 minutes after agitation. The supernatant fluids were decanted, and radioactivity of the precipitates was measured in an automatic gamma counter; this assay system can detect as little as 6 pg. of glucagon. As against the findings of Cresto et al.,[15] we could not detect such a high titer of circulating endogenous antibodies to glucagon as to interfere with the assay in D I and D II patients, judging from radioactivity in the precipitate of the sample blank, which was incubated similarly except for the lack of adding a glucagon antibody.

Plasma C-peptide was determined by a C-peptide radioimmunoassay kit[16] (Daiichi Radioisotope Labs, Tokyo). A modification of the double-antibody radioimmunoassay method of Morgan and Lazarow[17] was used for this assay. Synthetic human connecting peptide was used for preparation of the standard and of rabbit anti-C-peptide serum. Tyrosylated human connecting peptide was iodinated for preparation of labeled C-peptide. In this assay system, 0.2 ml. of unknown sample or standard solution was required. The sensitivity of this assay system was 39 pg. per tube—i.e., 0.195 ng./ml. plasma, calculated as the 95 per cent confidence limit of the blank.

This method is not only specific for C-peptide but also detects human proinsulin. Furthermore, the reported concentrations of plasma immunoreactive C-peptide are estimates rather than exact amounts, since patients who have endogenous insulin antibodies and some endogenous insulin secretory reserves may have substantial amounts of proinsulin sequestered in the circulation in a form bound to insulin antibodies. In addition, our assay system seemed to be affected by nonspecific factor(s) to some extent, since the plasma CPR level was 0.46 ng./ml. in a pancreatectomized patient. Plasma cortisol levels were determined with the radioimmunoassay kit[18] purchased from Eiken Immunochemical Labs, Tokyo. Insulin-binding capacity of plasma IgG was measured by the method of Christiansen.[19] Statistical analysis was performed by the Student *t*-test.

RESULTS

As shown in figure 1, the S.D. of 10 fasting blood sugar (FBS) levels was well correlated with the maximum difference among the 10 values ($r = 0.96$, $p < 0.005$). Accordingly, only the S.D. value was employed thereafter as an index for expressing the degree of diabetic instability. The relationship between the secretory capacity of pancreatic B-cells, quantified by Σ ΔCPR in oral glucose tolerance test, and the S.D. value is demonstrated in figure 2. All but one of the patients with no demonstrable CPR response during OGTT had an S.D. value greater than 75 mg./100 ml., while those with large values of Σ

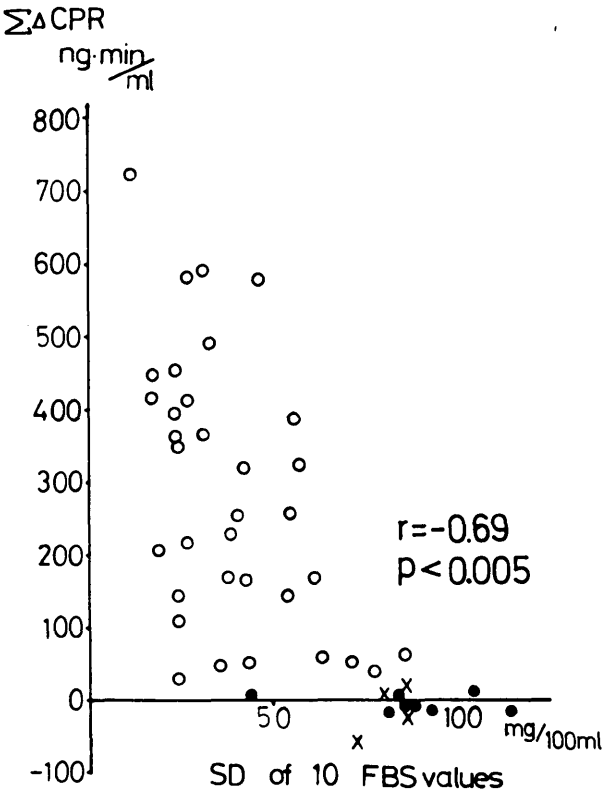

FIG. 2. Correlation between the incremental area of plasma CPR response curve during OGTT (Σ ΔCPR) and the S.D. of 10 FBS values. Σ ΔCPR was expressed as the area under the plasma CPR curve above the fasting CPR level from 0 to 180 minutes. Symbols indicating the subjects are the same as in figure 1.

ΔCPR showed small values of S.D. These two parameters were inversely correlated ($r = -0.69$, $p < 0.005$). Thus, a negative correlation between insulin secretory ability and blood glucose regulatory instability was evident. On the other hand, no correlation was found between insulin-binding capacity of plasma IgG and the S.D. value, as shown in figure 3 ($r = 0.15$, $p > 0.05$).

The clinical characteristics of the stable and the unstable diabetics classified according to their S.D. values are shown in table 1. The number of juvenile-onset diabetics included in each group was nearly comparable (3/7 in D I and 2/7 in D II). Furthermore, there were no significant differences between the two groups in their mean values of weight index, age, duration of diabetes mellitus, duration of insulin therapy, and dosage of insulin. However, it is interesting that the duration of insulin therapy was almost similar to the duration of diabetes mellitus in the D II group, while 4.3 years' difference was noted between the two durations in the D I group; in other words, insulin therapy was instituted immediately after the onset of the disease in most patients of D II,

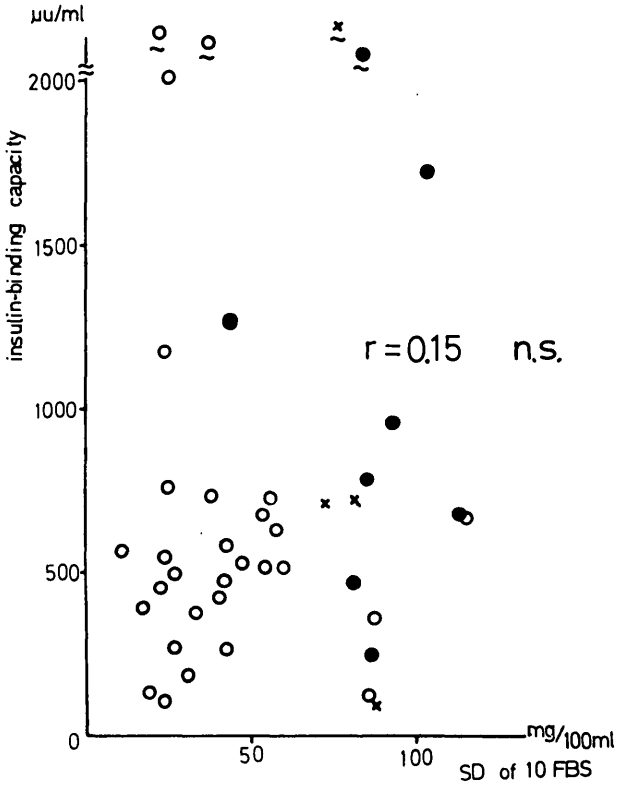

FIG. 3. Correlation between insulin-binding capacity of plasma IgG and the S.D. value. Symbols indicating the subjects are the same as in figure 1.

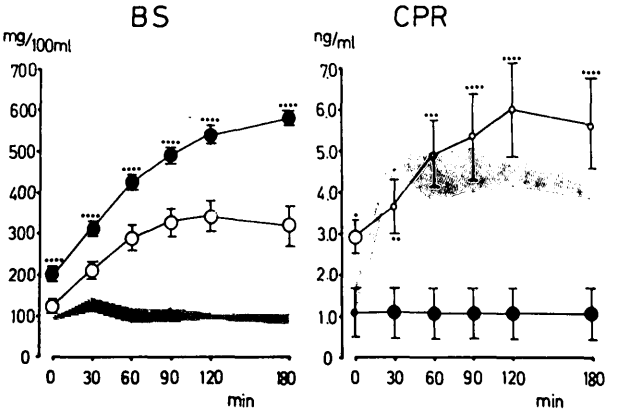

FIG. 4. Blood glucose and plasma CPR responses to oral glucose in D I (o) and D II (•) patients. D I and D II represent stable and unstable diabetics, respectively. See text about details of D I and D II. Values are mean ± S.E.M. The large symbol indicates significant difference from the corresponding normal value, which is expressed as shaded area (M ± S.E.M.). Dots represent a significant difference between the values of two diabetic groups: one dot, $p < 0.05$; two dots, $p < 0.025$; three dots, $p < 0.01$; four dots, $p < 0.005$.

whereas it was started several years after the onset in D I. D I and D II were not selected on the basis of their "metabolic instability" but rather specifically on their particular S.D. levels, which in itself explains why their S.D. levels were significantly different, as shown in table 2. In addition, significant differences in mean values of fasting blood glucose and of Σ ΔCPR were noted, and the mean insulin-binding capacity of plasma IgG tended to be higher in D II but not significantly different from that in D I.

Figure 4 shows mean blood glucose and plasma CPR responses to oral glucose in D I and D II. Both the mean fasting blood glucose levels and the responses to glucose ingestion were significantly higher at all times in D II than in D I. Furthermore, the blood glucose level kept rising until the end of the experiment in D II, but it tended to fall after reaching a peak at 120 minutes in D I. The mean CPR concentration was low at fasting and did not change significantly during OGTT in D II. On the contrary, in D I it was increased from 2.92 ± 0.42 ng./ml. (mean ± S.E.M.) at the basal to a peak value, 6.12 ± 1.13 ng./ml., at 120 minutes, which was significantly greater than the basal level ($p < 0.005$). To investigate abnormalities in other endocrine functions presumably responsible for diabetic brittleness, plasma IRG and cortisol responses were studied in these patients. Changes in plasma IRG, CPR, and blood glucose concentrations in response to an intravenous infusion of arginine in D I and D II are illustrated in

figure 5. No significant difference in the mean fasting plasma IRG levels between these groups was demonstrated. The mean peak values of plasma IRG during the arginine test in D I, D II, and normal groups were 368 ± 50.6, 411 ± 44, and 340 ± 33 pg./ml., respectively, among which no significant difference was noticed. D II showed no discernible increase in plasma CPR level during the arginine test, while D I responded clearly to arginine, and the increment in plasma CPR was significant at 10, 30, 45, and 60 minutes, as determined by paired *t*-test. The mean blood glucose levels tended to keep increasing in D II until the end of the experiment, as they did during OGTT. As shown in figure 6, following the intravenous injection of insulin, the mean blood glucose level

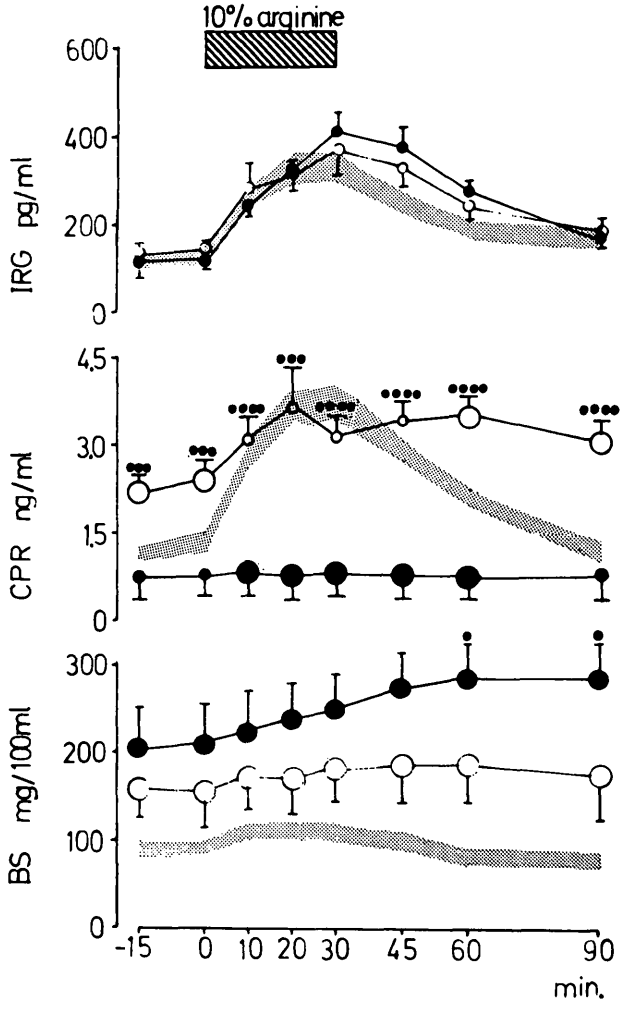

FIG. 5. Blood glucose, plasma CPR, and IRG responses to an infusion of arginine in D I and D II patients. The ways of expressing the subjects and statistical significance are the same as in figure 4. Values are mean ± S.E.M.

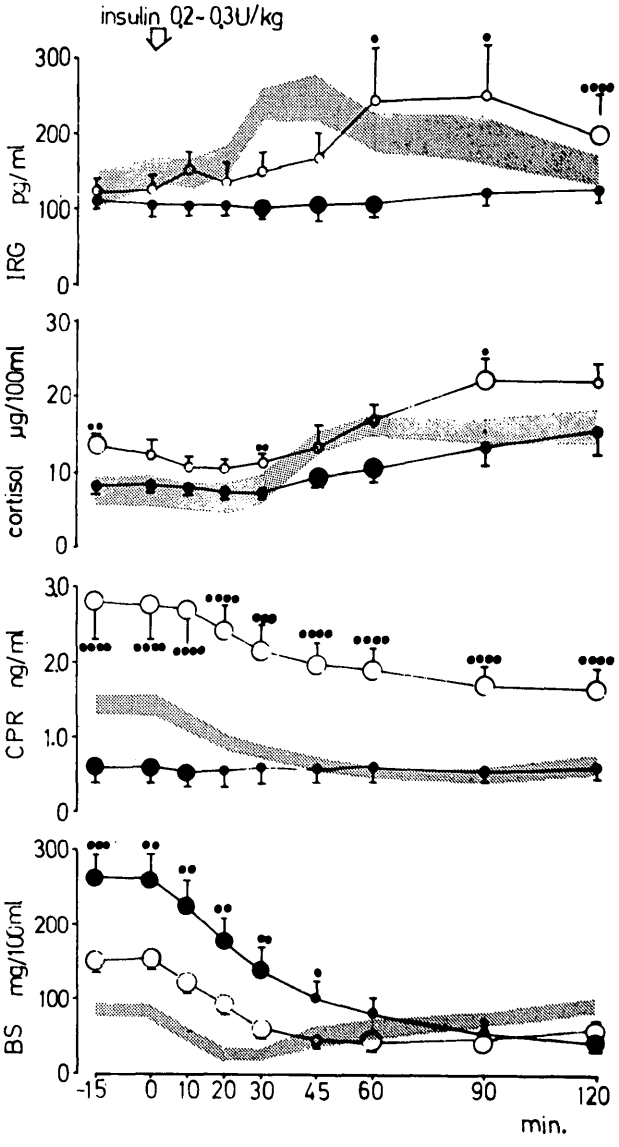

FIG. 6. Blood glucose, plasma CPR, cortisol, and IRG responses to an intravenous injection of insulin as a bolus; 0.1 U. and 0.2 or 0.3 U. per kilogram body weight of MC Actrapid were injected intravenously as a bolus in normals and diabetics, respectively. The ways of expressing the subjects and statistical evaluation are the same as in figure 4. Values are mean ± S.E.M.

declined in each group, reaching a nadir of 27 ± 3 mg./100 ml. at 30 minutes in normals, 40 ± 4 mg./100 ml. at 60 minutes in D I, and 48 ± 9.1 mg./100 ml. at 120 minutes in D II. No decline in plasma CPR level was observed in D II, but it decreased further from the basal level both in D I and in normals following the insulin injection. Nadirs of plasma CPR level in D II, D I, and normals were 96.7 ± 4.6 per cent, 60.5 ± 7.1 per cent, and 35.4 ± 3.5 per cent of

the corresponding basal levels, respectively, and these decrements were statistically significant ($p < 0.025$) except for that of D II. The mean IRG concentrations were increased significantly in response to insulin-induced hypoglycemia in D I and normals but not in D II. The maximum value of 236 ± 31 pg./ml. was attained at 45 minutes in normals, that of 270 ± 72 pg./ml. at 60 minutes in D I, which was significantly greater than the corresponding basal levels, and that of 127 ± 16 pg./ml. at the end of the experiment in D II. The values of D II were significantly smaller than those of normals ($p < 0.01$) and of D I ($p < 0.05$), while there was no significant difference between D I and normals. In contrast to plasma IRG, the mean plasma cortisol response to hypoglycemia was demonstrated in D II. The plasma cortisol value at 120 minutes was significantly greater than the basal value, tested by paired comparison ($p < 0.05$). Except for the values at a few points, the mean concentrations of plasma cortisol were not significantly different among the three groups.

DISCUSSION

No one argues the idea that excessive fluctuation of blood glucose levels despite apparently optimal therapeutic measures characterizes patients with unstable diabetes. However, controversy has continued as to the way blood glucose variability is quantified for assessing the degree of diabetic instability. The S.D. of 10 fasting blood glucose values or the difference between their maximum and minimum values employed in this study is a measure of the blood glucose changes resulting from day-to-day variation in response to a certain therapy but not a measure of within-day variability. There is an opinion[9] that quantification of blood glucose variability should include two elements: day-to-day and within-day changes. Since the two types of variabilities are so related as to make various degrees of interdependence possible and the subjects in the present study were limited to outpatients from whom several blood specimens could not easily be obtained within a day in general, we chose our own measure for quantifying diabetic instability. Though our measure was obtained on the basis of a relatively small number of fasting blood glucose determinations under less standardized conditions, patients could be studied over more extended periods by the use of these practical clinical data instead of those derived from the laborious continuous blood glucose monitoring.[8,9] This was one of the merits in our measure besides its simplicity. As shown in figure 1, the S.D. of the 10 FBS values clearly correlated with the difference between their maximum and minimum values. This fact suggests that two out of the 10 determinations did not happen to differ extremely from one another, with a relatively small fluctuation of the remaining values, but all of the 10 values fluctuated so widely as to be proportional to the extent of their maximum difference.

The present study revealed that the degree of diabetic instability was inversely correlated with the residual B-cell secretory activity in insulin-treated diabetics, which was estimated by measuring changes in plasma CPR concentrations in response to oral glucose. Although it has been hypothetically postulated that the lack of insulin secretory ability might account in part for hyperlability of diabetic control, there has been no scientific evidence to prove this except for one report[3] in which insulinogenic reserve was determined by assessing changes in IRI concentrations in insulin-treated diabetics. Our results confirmed and extended these findings of Cremer[3] by improving the methodologic difficulty in evaluation of the secretory capacity of the pancreatic B-cell in the patient with endogenous antibodies to insulin. The effect of these antibodies in the C-peptide assay is not generally appreciated. However, the antibodies, which are produced in response to exogenous insulin, bind human proinsulin and greatly prolong its presence in the circulation.[20] The antibody-bound proinsulin is measured as CPR by our C-peptide immunoassay.[21] Block et al.[22] have found in their insulin-requiring diabetic patients that over 80 per cent of plasma CPR is actually proinsulin. This is likely to be the case in our patients. Therefore, the CPR values of the patients cannot be compared directly to those of the controls. However, the incremental area of plasma CPR over the baseline during OGTT may be predominantly composed of C-peptide and can be used as an index assessing insulinogenic reserve more accurately in contrast to the total area of plasma CPR.

As shown in figure 2, it was evident that insulinogenic reserve was preserved to varying degrees in our insulin-requiring patients. It is well known that the supply of insulin is adjusted on a minute-to-minute basis to the body's need: an additional insulin release in response to hyperglycemia modulates blood glucose increase and decrease, or discontinuation of insulin secretion[23,24] under a hypoglycemic state prevents excessive blood glucose decrease. This automaticity of insulin secretion might be one of the

most important factors responsible for keeping blood glucose concentrations within a small fluctuation in the normal, nondiabetic state. As shown in figures 4-6, the pancreatic B-cells of D I had enough capacity to respond both to such stimuli as glucose and arginine and to the suppression due to insulin-induced hypoglycemia. On the other hand, in D II patients whose pancreatic B-cell responded neither to the stimuli nor to the suppressor, probably because of total lack of insulinogenic reserve, blood insulin concentrations depended totally on an exogenous supply of insulin irrespective of their body's need. Such unphysiologic concentrations, in turn, might lead to wide fluctuations in values for blood glucose in accordance with the variability of the body's need for insulin. However, this is not to imply that insulinogenic reserve exclusively accounts for varying degrees of diabetic instability or stability. Pancreatic A-cell dysfunction may also play some role in derangement of blood glucose regulation in unstable diabetics, especially in prevalence of recurrent hypoglycemia.

Symptomatic insulin-induced hypoglycemia did not stimulate IRG secrection in D II but did in D I, whereas the infusion of arginine evoked a clear rise of plasma IRG levels in both groups. These results are similar to the findings of Gerich et al.[25] in juvenile-type diabetics. The possibility that the lack of plasma IRG rise during hypoglycemia in D II was due to an inadequate and sluggish fall of the blood glucose level and to too early termination of the experiment cannot be ruled out in the present study, but it might be reasonable to presume that in these patients the pancreatic A-cells were also insensitive to hypoglycemia to some extent, since plasma cortisol response was observed under the same hypoglycemic state. It is not yet certain whether extrapancreatic glucagon[14,26,27] contributes to the abnormalities in plasma IRG responses observed in the unstable diabetics.

We have, as yet, little information on whether abnormal cortisol secretion exists in unstable diabetics. Our limited data did not reveal any plasma cortisol pattern characteristic of brittle diabetes. Abnormal cortisol secretion, even if it exists, is not an essential pathogenetic factor for the hyperlability of diabetes, judging from the report of Feldman et al.[28] concerning the role of cortisol in the counterregulation of insulin-induced hypoglycemia. Since the integrity of the sympathoadrenomedullary axis is essential for the counterregulatory response to intracellular glucopenia in man,[29] determinations of plasma levels of pressor amines are necessary for clarifying the etiologic factors responsible for diabetic instability. The present study could not demonstrate any correlation between plasma insulin-binding capacity and the degree of diabetic instability. However, this fact does not necessarily deny the etiologic role of insulin antibodies in loss of diabetic stability. Affinity, rather than capacity, of the antibodies is reported[4] to be more important for buffering changes in free insulin concentration, which influences the dynamics of insulin action. Therefore, the drawing of conclusions on this matter needs to wait until the affinity constant of insulin-binding antibodies of our patients is determined.

In conclusion, the total lack of insulinogenic reserve is most likely to be the fundamental etiologic factor in diabetic brittleness. Loss of the residual B-cell function leads to loss of the automatic regulation of circulating insulin levels. This loss of automaticity causes failure of prompt compensation for either hyperglycemia or hypoglycemia, resulting in wide fluctuations of blood glucose levels. Inappropriate glucagon secretion may also be responsible for diabetic instability.

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