Normalization of the Paradoxic Secretion of Glucagon in Diabetics Who Were Controlled by the Artificial Beta Cell

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SUMMARY

Since it is important to elucidate the precise significance of pancreatic A-cell hypersecretion in the pathogenesis of diabetes mellitus, the change in the immunoreactive glucagon (IRG) response to 100 g oral glucose challenges was studied in diabetics whose blood glucose responses and plasma immunoreactive insulin concentrations (IRI) simulated those in normal subjects with the aid of the artificial beta cell system that we developed originally.

In six nonobese adult-onset and four insulin-dependent diabetics whose blood glucose responses and plasma insulin concentrations after 100 g oral glucose load were made equivalent to those seen in normal subjects by the artificial beta cell, the glucagon release was similar to the response in normal subjects. In one insulin-dependent diabetic with high anti-insulin-binding capacity, the blood glucose response after an oral glucose challenge was not normalized by the artificial beta cell and the glucagon secretion was paradoxically increased. This fact suggested that the paradoxic rise in glucagon, seen in response to an oral glucose load in some diabetics, is secondary to insulin deficiency. DIABETES 28:272-275, April 1979.

It is widely accepted that, in spontaneous or acquired diabetes mellitus, glucagon secretion is abnormal. Not only does the hyperglycemia after an oral glucose load fail to lower plasma immunoreactive glucagon concentrations (IRG) in diabetics,[1] but the hypoglycemia caused by insulin injection fails to stimulate glucagon secretion.[2] In addition, an exaggerated response to aminogenic stimulation with arginine[3] or alanine[4] was observed. Since these pancreatic A-cell dysfunctions were not normalized by the conventional insulin injection treatment, they were thought to be a primary defect of diabetes mellitus.

Recently, Unger's group reported that in juvenile-type diabetics the concomitant administration of insulin could reduce the exaggerated glucagon responses to normal after intravenous arginine[5] or oral glucose loads[6] but required plasma insulin levels well above those found in nondiabetics. However, in those investigations, blood glucose concentrations remained in the hyperglycemic range.

Since it is important to elucidate the precise significance of A-cell hypersecretion in the pathogenesis of diabetes mellitus, we studied the change in IRG responses to 100 g oral glucose challenges in diabetics whose blood glucose levels and plasma immunoreactive insulin concentrations (IRI) were made equivalent to those of normal subjects with the aid of the artificial beta cell system.

MATERIALS AND METHODS

Artificial beta cell system. In the artificial beta cell system that we developed originally,[7] the insulin infusion rate is based on the sum of the proportional (a component related to blood glucose concentration per se) and derivative (a component related to the rate of change in blood glucose concentration) action to blood glucose concentration, because a biphasic response of insulin secretion against the stepwise glucose stimulation was simulated successfully. The characteristics of the system, recognized in clinical applications, are (1) blood glucose responses are controlled on a moment to moment basis, and IRI concentrations simulate those seen in normal subjects (insulin requirements are reduced to about half those given subcutaneously); and (2) hypoglycemia is not a result, because the negative derivative action lessens the insulin infusion rate during the decline of the blood glucose concentration.

Subjects. The response of a group of 11 nondiabetic subjects to a 100 g oral glucose load was studied. Their ages ranged from 25 to 60 yr and their weights were within ±10% of ideal. Six patients with adult-onset and five with insulin-dependent diabetes mellitus were studied as outpatients after an overnight fast. The six adult-onset diabetics had

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weights between −6% and +10% of their ideal body weights, and hyperinsulinemia was not seen after the oral glucose challenge. Their ages ranged from 50 to 67 yr and all were treated with diet only. Of five insulin-dependent diabetics, four had a previous history of ketosis; their ages ranged from 30 to 59 yr, and they had been treated with insulin for at least 3 yr.

Experimental protocols. Patients were studied on two separate days within 2 wk. On a control test day, a 100 g oral glucose load was administered after beginning to monitor the blood glucose concentration in subjects with adult-onset diabetes. In insulin-dependent diabetics, after the initiation of blood glucose monitoring by the method described later, their usual doses and types of insulin (intermediate-acting insulin, 20–28 U in four patients, and regular insulin, 8 U in one patient) were injected subcutaneously. One hour later, 100 g of glucose was administered orally.

On the day of the experiment, patients were connected with an artificial beta cell system. Blood was continuously drawn from an antecubital vein by means of a dual lumen catheter. Two silicon tubes fixed in a plug were inserted into a cannula (Medicut no. 16, Aloe Medical). One tube was used for infusing heparinized saline (100 U/ml) at a rate of 1.0 ml/min. Another tube withdrew the mixture of blood and heparinized saline into the AutoAnalyzer at a rate of 0.16 ml/min. Blood glucose concentration was measured by means of a modification of the GOD-PAP method (Boehringer-Mannheim, Ca. no. 16115). The time delay between blood withdrawal and readout of blood glucose was 4 min. The optical density was registered through the A-D converter on the recorder and at the same time was put into the microcomputer system. Then, the system forecasted the blood glucose concentration 4 min in the future with the aid of a hyperbolic tangent curve to which the last 10 data points were fitted. The system then calculated the insulin infusion rate according to the algorithm. Calculated insulin infusion rate was transferred to the serial numbers of pulses in the pump driver circuit that drove the pulse motor of the minipump (Nikkiso, Japan). Insulin was infused intravenously via a peripheral vein of the forearm. Body weight and the parameters determining the insulin infusion rate were put into the microcomputer system arbitrarily according to the condition and insulin secretory ability of the subject. Blood samples for hormone determinations were obtained from the antecubital vein of the opposite arm.

Immunoreactive insulin concentrations in plasma were measured by the method of Hales and Randle.[8] Plasma glucagon was assayed by radioimmunoassay using antiserum 30 K.[9]

For comparison within groups, the Student's *t* test for paired groups was used.

RESULTS AND DISCUSSION

In normal subjects, IRG decreased substantially after the oral glucose load from the prestimulated level of 132.0 ± 13.6 (mean ± SEM) pg/ml to a minimum of 90.2 ± 9.6 pg/ml at 90 min and 93.0 ± 9.0 pg/ml at 120 min, respectively, which were significantly lower than the basal level ($p < 0.05$) (Figure 1, lower panel).

When insulin was not infused in six adult-onset diabetics, blood glucose concentrations rose from the prestimulated level of 150.0 ± 16.0 mg/100 ml to 271.0 ± 17.5, 337.5 ± 23.0, 351.7 ± 29.6, and 330.7 ± 40.1 mg/100 ml at 30, 60, 90, and 120 min after the glucose load, respectively. Plasma IRI increased gradually to the level of 34.8 ± 7.5 μU/ml at 120 min. In all six patients, IRG was not suppressed at all but rose despite pronounced hyperglycemia. Mean (±SEM) IRG increased from 133.5 ± 17.8 at 0 min to 222.5 ± 31.2, 260.3 ± 58.5, 335.8 ± 82.9, and 268.3 ± 58.4 pg/ml at 30, 60, 90, and 120 min, respectively, as shown in Figure 1.

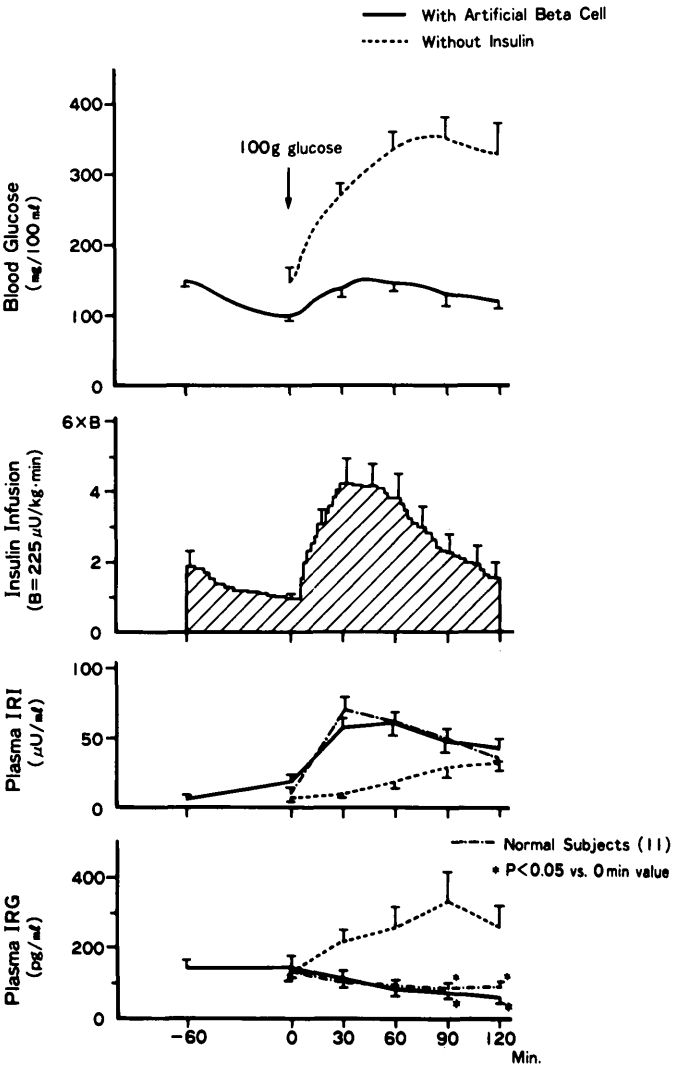

FIGURE 1. The mean (±SEM) blood glucose, plasma IRI, and plasma IRG responses after a 100 g oral glucose load in six adult-onset diabetics under artificial beta cell control (closed line) or without insulin infusion (open line). Insulin infusion pattern of artificial beta cell control is also depicted. The mean (±SEM) plasma IRI and IRG responses after a 100 g oral glucose load in 11 normal subjects are depicted also.

In these six patients, normalization of blood glucose responses was achieved with the artificial beta cell system by increasing the rate of insulin infused as blood glucose increased (Figure 1). Mean amount of insulin infused during the 2 h after administration of the glucose load was only 4.5 U. Mean plasma IRI was raised to 58.3 ± 6.2 μU/ml at 30 min and to 61.3 ± 5.6 μU/ml at 60 min, respectively, which simulated IRI response seen in normal subjects (Figure 1). As far as the IRG response was concerned, insulin administration significantly blunted the glucagon response from the prestimulated level of 150.0 ± 25.4 pg/ml to 77.2

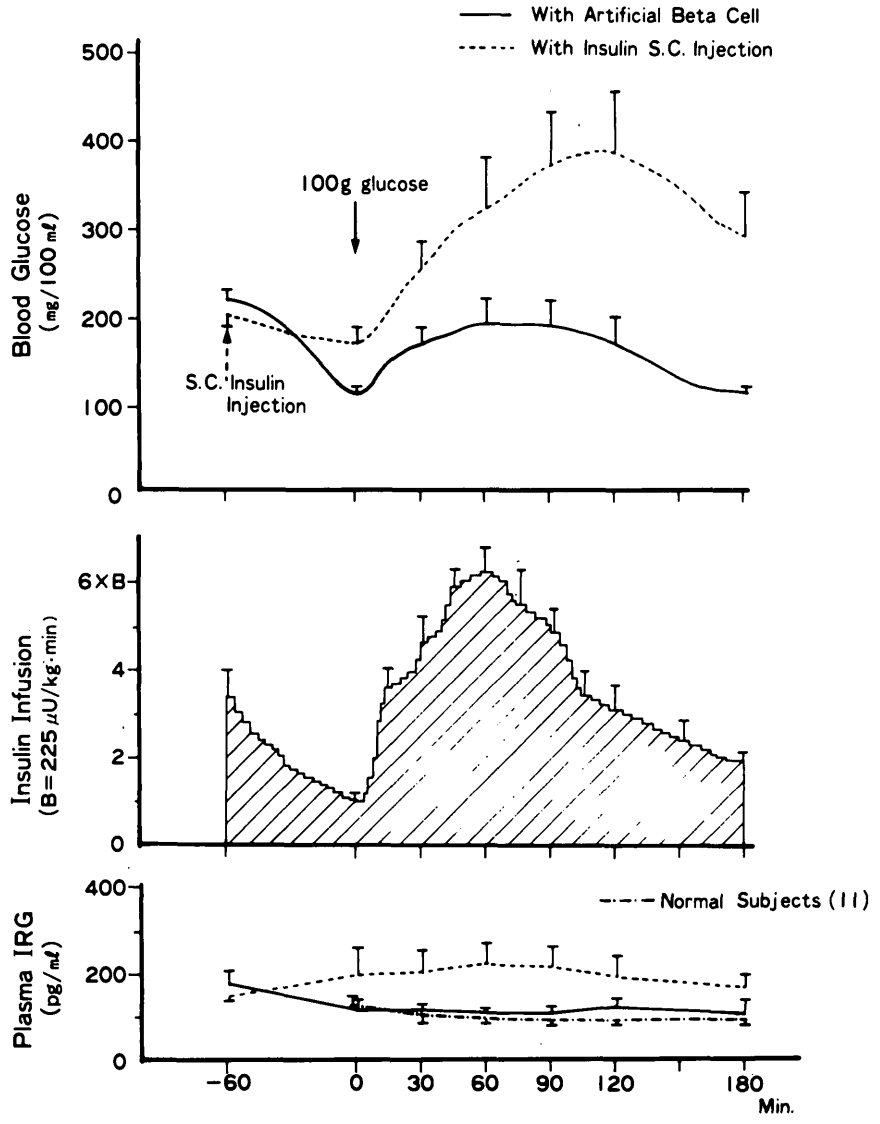

FIGURE 2. The mean (±SEM) blood glucose and plasma IRG responses after a 100 g oral glucose load in five insulin-dependent diabetics under artificial beta cell control (closed line) or with subcutaneous insulin injection (open line). Insulin infusion pattern of artificial beta cell is also depicted.

± 15.3 at 90 min ($p < 0.05$) and to 66.0 ± 12.9 pg/ml at 120 min ($p < 0.05$), values about the same as in nondiabetics.

In five insulin-dependent diabetics, even though the usual subcutaneous insulin injection was given 1 h before the oral glucose challenge, blood glucose concentrations rose gradually from 168.2 ± 8.6 to 253.2 ± 32.0, 322.8 ± 54.9, 370.6 ± 60.2, 384.2 ± 71.7, and 290.8 ± 52.9 mg/100 ml at 30, 60, 90, 120, and 180 min, respectively (Figure 2). The IRG response was not suppressed but tended to rise although differences were not significant statistically. Mean (±SEM) IRG was 195.6 ± 60.0 just before the 100-g glucose intake and was 200.2 ± 50.9, 219.4 ± 47.1, 215.6 ± 46.6, 192.0 ± 40.6, and 167.5 ± 15.3 pg/ml at 30, 60, 90, 120, and 180 min, respectively (Figure 2).

Of five insulin-dependent diabetics who were challenged with 100 g of oral glucose under control of the artificial beta cell system, one failed to normalize the blood glucose because of high insulin antibody levels; the others showed normalized responses. The mean total insulin infused over the 3-h period was 9.1 U. Mean (±SEM) IRG was 114.0 ± 7.8 pg/ml before glucose challenges and was 115.6 ± 7.8, 109.0 ± 4.1, 105.8 ± 7.8, and 117.0 ± 19.9 pg/ml at 30, 60, 90, and 120 min, respectively. These values were not significantly different from the prestimulated value (Figure 2), but in four patients they were clearly lower after glucose administration than in the control state using subcutaneous insulin. In the one patient with high anti-insulin–binding capacity, plasma IRG increased during experimental period.

In summary, our results suggest that physiologic insulin concentrations restore the A-cell response to an oral glucose load in hypoinsulinemic diabetics to that of the normal subjects if strict blood glucose regulation is achieved. Kerner and Pfeiffer[10] also examined glucagon secretion in diabetics during oral glucose loads by giving insulin with their artificial pancreas. But glucagon suppression occurred only 120 min after the start of the test. The total quantities of insulin infused for 3 h amounted to 21.2 ± 3.2 U, which was around three times as much as ours, suggesting that the plasma insulin concentrations were far above the physiologic levels. The explanation for the different results obtained in the two laboratories is not known.

There would be three possibilities of insulin action explaining the normalization of glucagon suppressibility in diabetes mellitus. Firstly, in hypoinsulinemic diabetes, the glucoregulatory function of the A cell may be normal if

insulin is infused to simulate the concentrations seen in normal subjects. But even when the peripheral vein, insulin concentration is normalized, it is hard to know whether the local concentration of insulin in the vicinity of the A cells is enough or not. Secondly, exaggerated increments of plasma immunoreactive gastric inhibitory polypeptide (GIP) after ingestion of glucose have been demonstrated in diabetic subjects by Dupre et al.[11] This fact suggests that the rise of plasma IRG may be dependent on the glucagonotropic action of GIP, which might be expressed in the presence of a defective insulin response. Thus, it can be suggested that adequate insulin infusion may inhibit secretion of GIP, lowering the secretion of IRG indirectly. Thirdly, it might be suggested that the paradoxic elevations of plasma IRG seen in hypoinsulinemic diabetics could be due to the increment of extrapancreatic glucagon-like immunoreactivity. But, this might play only a small role since trace amounts of insulin should suppress the secretion of extrapancreatic glucagon.[12]

We would like to suggest that inadequate response of A-cell secretion is secondary to insulin deficiency.

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