Rapid Publication

Perfect Normalization of Excessive Glucagon Responses to Intravenous Arginine in Human Diabetes Mellitus With the Artificial Beta-Cell

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SUMMARY

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 intravenous arginine was studied in both nonobese, hypoinsulinemic non-insulin-dependent (NIDDM) and insulin-dependent diabetic (IDDM) subjects whose blood glucose responses and plasma immunoreactive insulin (IRI) simulated those of healthy subjects with the aid of the artificial beta-cell system that we originally developed.

In both five NIDDM and five IDDM subjects, blood glucose responses and plasma IRI after arginine challenges were made equivalent to those seen in healthy subjects by infusing insulin in response to blood glucose, revealing that previously exaggerated IRG responses were made completely similar to the responses in healthy subjects.

In summary, these results clearly demonstrate that the exaggerated response of A-cell secretion against arginine challenges in insulin-deficient diabetics is secondary to insulin lack, and the perfect normalization of its response is achieved only when both plasma insulin concentration and glycemic control simulate those of healthy subjects. DIABETES 29: 762–765, September 1980.

In the artificial beta-cell system that we originally developed,[1,2] insulin is infused into diabetics to simulate the plasma concentration of insulin seen in healthy subjects, thus establishing glycemic regulation. In this sense, the system is useful not only in the therapy of diabetes mellitus but also as a research tool for the investigation of actions of counterregulatory hormones against insulin on glycemic regulation.

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As far as glucagon secretion is concerned, we showed that in six nonobese non-insulin-dependent and four insulin-dependent diabetics, whose blood glucose responses and plasma insulin concentrations after a 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 healthy subjects.[3]

Since there has been no report on the effect of perfect normalization of glycemic excursion during intravenous arginine stimulation on A-cell hypersecretion in diabetics, the artificial beta-cell system was used in this study to regulate the blood glucose responses and to mimic plasma insulin concentrations of healthy subjects in diabetes mellitus.

MATERIALS AND METHODS

ARTIFICIAL BETA-CELL SYSTEM

Insulin infusion algorithm. Mathematical control theory was applied to derive a relationship between glucose levels and insulin secretion. It is well known that biphasic responses of insulin secretion result from glucose stimulation in the perfused rat pancreas[4] and also with intravenous glucose infusion to normal subjects.[5] The first phase of rapid rise in insulin secretion was assumed to reflect derivative action corresponding to the rate of change in glycemia, while the second phase or slow rise in insulin secretion was assumed to correspond to the proportional action of the absolute glucose concentration. This relationship could be simulated by using a transfer function with a first order delay in both proportional and derivative action. With these considerations, the following equation was proposed:

$$IRI(t) = a \cdot BG(t) + b \cdot \Delta BG(t) + c \qquad (1)$$

where IRI(t) is the concentration of plasma insulin at time t (μU/ml); BG(t) is the concentration of blood glucose at time t (mg/dl); ΔBG(t) is the rate of change in blood glucose concentration computed over 1 min at time t (mg/dl/min); and a, b, and c are individual parameters.

By considering the insulin space, insulin degradation rate, and the diffusion constant to this equation, the insulin

infusion rate (IIR, μU/min) can be expressed as follows:

$$IIR(t) = Kp \cdot BG(t) + Kd \cdot \Delta BG(t) + Kc \qquad (2)$$

where Kp is a coefficient for proportional action, Kd is a coefficient for derivative action, and Kc is a constant for basal insulin secretion.

For the determination of parameters a, b, and c in Eq. (1), a bolus and a constant intravenous glucose infusion or an oral glucose load were carried out in healthy subjects. The numerical relationship between IRI, BG, and ΔBG thus obtained was then investigated by means of a multiple regression analysis. With these procedures, (a, b, c) = (0.1, 0.4, 0) or (1.0, 4.0, −72) were regarded as suitable parameters for i.v. glucose load or oral glucose load, respectively.

SUBJECTS

The responses of nine healthy subjects to i.v. arginine infusion were studied. Subjects' ages ranged from 25 to 35 yr and their weights were within ± 10% of ideal. Five nonobese non-insulin-dependent (NIDDM) and five insulin-dependent (IDDM) diabetic subjects were studied as outpatients or inpatients after an overnight fast. The five NIDDM had weights between −6% and +13% of their ideal body weights and their ages ranged from 48 to 70 yr. All were treated with sulfonylureas. Their durations of disease were between 1 and 20 yr. Four out of five patients had mild to moderate diabetic retinopathy.

Of the five IDDM, ages ranged from 21 to 42 yr and all had been treated with insulin for at least 1 yr. Body weights were between −25% and +9% of ideal. Two patients had diabetic retinopathy.

EXPERIMENTAL PROTOCOLS

Patients were studied on two separate days within 2 wk. On a control test day, 0.5 g/kg of 10% arginine solution was infused in 30 min, 24 h after the last administration of sulfonylureas in NIDDM or 48 h after the last s.c. injection of insulin (Lente Insulin, 32 U in two patients; Rapitard Insulin, 20 or 30 U in three patients) in IDDM, respectively.

On the day of an attempt to normalize glycemic response, patients were connected to the 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 0.05 ml/min. Another tube withdrew the mixture of blood and heparinized saline into the AutoAnalyzer (Technicon) at a rate of 0.1 ml/min. Blood glucose concentration was measured by means of a modified method of the GOD-PAP method (Boehringer-Mannheim, Cat. no. 16115). The time delay from blood withdrawal to the 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.[1,2] The system then calculated the insulin infusion rate according to the algorithm. The calculated insulin infusion rate was transferred to the serial numbers of pulses in the group driver circuit that drove the pulse motor of the micropump (Nikkiso, Japan). Insulin was infused intravenously via a peripheral vein of the forearm. The rate of insulin infusion was expressed as times basal (B = 225 μU/kg/min). Body weight and parameters a, b, and c were put into the microcomputer system arbitrarily.

It took about 2 h to reduce the blood glucose to euglycemia by the system; euglycemia was then maintained for at least 1 h before the initiation of arginine infusion.

Blood samples for hormone determinations were obtained from the antecubital vein of the opposite arm. Immunoreactive insulin concentrations (IRI) in plasma were measured by the method of Hales and Randle.[6] Plasma glucagon (IRG) was assayed by radioimmunoassay using antiserum 30K.[7]

The Student's *t* test for paired groups was used for statistical analysis.

RESULTS

In normal subjects, blood glucose increased gradually during arginine stimulation from the prestimulated level of 101.2 ± 2.3 mg/dl (mean ± SEM) to the peak level of 128.9 ± 5.1 mg/dl at 30 min. Plasma IRI showed biphasic response during arginine infusion in which the first peak was seen at 5 min (56.0 ± 7.0 μU/ml) and the second peak was recognized at 30 min (80.2 ± 12.0 μU/ml). Plasma IRG increased substantially during arginine infusion from 100.3 ± 14.3 pg/ml to 273.3 ± 58.8 pg/ml at 30 min (Figure 1). When insulin was not infused in five NIDDM, blood glucose concentration rose slightly from the prestimulated level of 239.2 ± 19.5 mg/dl to 277.6 ± 19.8 mg/dl at 45 min. Plasma IRI increased only slightly. Plasma IRG rose rapidly to 358.0 ± 25.3 pg/ml at 45 min. The mean IRGs obtained at 45, 60, 90, and 120 min were all statistically different from those of healthy subjects. In these five patients, normalization of blood glucose response was achieved with the artificial beta-cell system by using the parameters a, b, and c as 1.0, 4.0, and −72, respectively. The amount of insulin infused during 30 min of arginine infusion was 1.88 ± 0.38 U (1.11–3.21 U). The mean plasma IRI was raised gradually to 105.5 ± 24.2 μU/ml at 30 min. The mean IRI value obtained at 5 min was significantly lower and those obtained at 45 and 60 min were higher than the IRI value of healthy subjects. As far as the IRG response was concerned, insulin administration perfectly normalized the glucagon secretion. The peak level of IRG showed 256.8 ± 31.6 pg/ml. At every time point, no statistical differences were obtained between IRG levels of diabetics with insulin infusion and those of healthy subjects (Figure 1).

All five IDDM showed ketosis at 48 h after s.c. insulin injection. With arginine infusion in such circumstances, blood glucose concentrations rose gradually from 298.8 ± 32.2 mg/dl to 339.4 ± 30.7 mg/dl at 45 min. The IRG response was remarkably exaggerated, increasing from the prestimulated level of 228.8 ± 49.4 pg/ml to 648.6 ± 79.6 pg/ml at 30 min, then gradually decreasing to 276.0 ± 63.2 pg/ml at 120 min after the initiation of arginine infusion. These responses at all sampling time points were significantly higher than those of healthy subjects. When these five IDDM were challenged with arginine infusion under the control of the artificial beta-cell system (parameters, a = 1.0, b = 4.0, c = −72), blood glucose rose from the prestimulated level of 75.2 ± 1.4 mg/dl to 103.8 ± 5.1 mg/dl at 30 min. Insulin was infused in response to both blood glucose concentra-

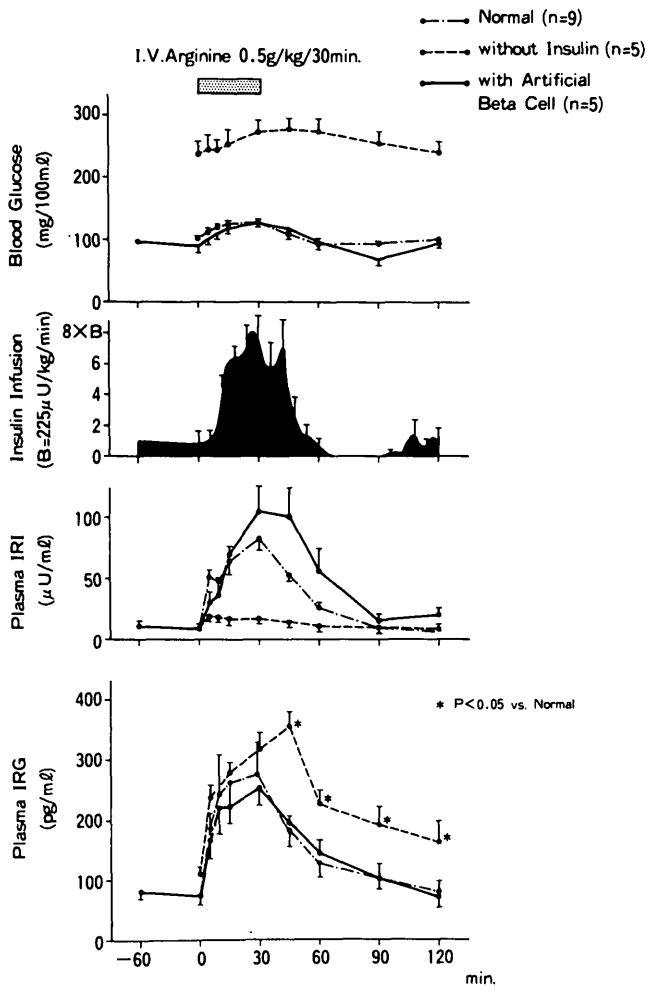

FIGURE 1. The mean (±SEM) blood glucose, plasma IRI, and plasma IRG responses during and after 30-min arginine infusion in 5 non-insulin-dependent diabetics using the artificial beta-cell (closed line) or without insulin infusion (open line). The insulin infusion pattern of artificial beta-cell control is depicted in the panel. The mean (±SEM) plasma IRI and IRG responses in 9 healthy subjects are also depicted (broken line).

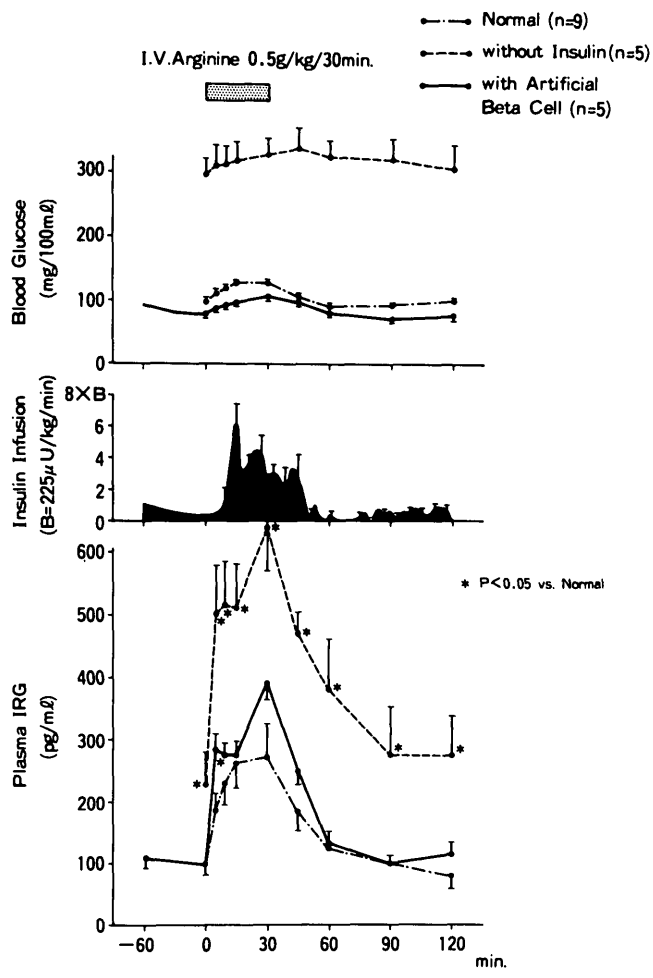

FIGURE 2. The mean (±SEM) blood glucose and plasma IRG responses during and after 30-min arginine infusion in 5 insulin-dependent diabetics using the artificial beta-cell (closed line) or without insulin infusion (open line). Insulin infusion pattern of artificial beta-cell control is depicted in the panel.

tion and the rate of change in blood glucose concentration. The peak of mean insulin infusion rate was 6.0 ± 1.4 times B (B = 225 μU/kg/min) at 15 min, because from 10 to 15 min the mean rate of change in blood glucose was large, about +1.6 mg/dl/min. The mean amount of insulin infused for 30 min was 1.17 ± 0.25 U (0.61–1.64 U). Mean IRG was 96.2 ± 14.8 pg/ml before arginine challenge and 287.2 ± 21.2, 274.2 ± 19.6, 273.2 ± 23.9, 391.7 ± 26.5, 252.0 ± 24.1, and 130.8 ± 22.1 pg/ml at 5, 10, 15, 30, 45, and 60 min, respectively. Except for the 5 min value, none were significantly different from those of healthy subjects (Figure 2).

DISCUSSION

It is widely accepted that excessive glucagon responses during intravenous infusion of arginine occur in human diabetes mellitus.[8–10] Raskin et al.[11] have reported that the infusion of supraphysiologic amounts of insulin blunted glucagon responses to arginine in IDDM, but not in NIDDM. Then, in IDDM, Gerich et al.[12] reported that by 4-h infusion of insulin at the rate of 1.5 U/h, previously excessive glucagon secretion to arginine could be reduced to a degree indistinguishable from that observed in normal subjects. Ohneda et al.[13] showed that chronic oral sulfonylurea therapy normalized these responses in NIDDM.

The present studies were undertaken to characterize the mechanism underlying excessive glucagon responses to arginine by mimicking the blood glucose response of normal subjects as closely as possible in diabetics with the aid of an artificial beta-cell system. The characteristic of the system that we had originally developed, i.e., insulin infusion rate determined to simulate the peripheral IRI levels of healthy subjects in response to blood glucose, establishes perfect glycemic normalization.[1–3]

It was found that in both nonobese NIDDM and IDDM, previously excessive glucagon responses to arginine could be perfectly normalized by appropriate intravenous insulin infusion. In the preliminary study (data not shown) three nonobese NIDDM, included in this series of study, were challenged by arginine infusion in the euglycemic state maintained by the artificial beta-cell system, with the parameters being a = 0.1, b = 0.4, and c = 0, respectively. In all three cases, blood glucose rose gradually from the prestimulated levels (80–98 mg/dl) to the peak values (168–202 mg/dl) at 30 min during arginine infusion. Maximal insulin infusion rate by the artificial beta-cell was only 2.3–2.8

times B and obtained IRI was less than 34 μU/ml at 30 min during arginine infusion. It was revealed that IRG values were not statistically different from those without insulin infusion.

In the data of IDDM subjects shown in this paper, only the mean IRG value at 5 min after initiation of arginine infusion was statistically higher than in the healthy subjects. Estimating from the results in NIDDM with the artificial beta-cell, IRI values obtained at 5 min in IDDM might be lower than in healthy subjects. These data clearly demonstrate that an exaggerated response of A-cell secretion against arginine challenge in hypoinsulinemic diabetes mellitus is secondary to insulin deficiency, and the normalization of its response is achieved only when both plasma insulin concentration and glycemic control simulate those of healthy subjects.

The mechanism of action of peripherally infused insulin on glucagon secretion is hard to explain, because even though peripheral plasma insulin concentration is normalized, the level of insulin around the insular A-cell might be far below those observed within the islets of healthy subjects.

The inconsistency between the present results about NIDDM and those from Unger's group[11,14-16] may be attributed to the characteristics of the patients. Contrary to obese hyperinsulinemic diabetics, all cases studied in the present report were nonobese and hypoinsulinemic; plasma IRI was maintained at a low level before and during arginine infusion (Figure 1). Because no subclassifying characteristics other than obesity are well enough established at the present time to differentiate within NIDDM,[17] the final conclusion on the effect of insulin on glucagon secretion should be clarified after the careful examination of obese hyperinsulinemic NIDDM.

In the artificial beta-cell system, insulin is infused by sensing the blood glucose and not arginine itself. It is of interest that parameters (a = 1.0, b = 4.0, c = −72), which were suitable in simulating the IRI response to oral glucose load, were also effective in mimicking the insulin secretion in response to arginine infusion, although the values at 5 min after arginine infusion were statistically lower than in healthy subjects. One might therefore agree that the parameters used were proven to be permissive for regulating blood glucose after meals containing proteins as well as carbohydrate.

In summary, with the aid of the artificial beta-cell system in nonobese, hypoinsulinemic NIDDM, and in IDDM whose blood glucose responses and plasma insulin concentrations after arginine were made equivalent to those seen in healthy subjects, the glucagon secretion was completely similar to the response in healthy subjects. These findings are consistent with our earlier report on the normalization of the paradoxic secretion of glucagon during oral glucose load in both nonobese NIDDM and IDDM who were controlled by the artificial beta-cell.[3]

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