The Effect of Short-Term Intravenous Insulin Administration on the Glucagon Response to a Carbohydrate Meal in Adult Onset and Juvenile Type Diabetes*

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Summary. These experiments were designed to determine whether the abnormal glucagon response of diabetics to a glucose meal can be restored to normal by the short-term administration of exogenous insulin in amounts sufficient to produce normal and above normal plasma insulin levels. The immunoreactive glucagon (IRG) response of nine nondiabetics to oral glucose was compared with that of ten juvenile and ten adult type diabetics. In the absence of exogenous insulin, the IRG response of diabetics was strikingly different from the nondiabetics, rising paradoxically, whereas in nondiabetics there was a decline in IRG. When plasma insulin levels were raised to normal by infusion of insulin (0.06 U/kg for 2 hr), the abnormal IRG response of adult type diabetics was not improved; the IRG response of the juvenile type patients was improved, but remained abnormal. Raising plasma insulin briefly to greater than normal concentrations improved the IRG response during the glucose meal in · both groups, but in the adult group total IRG suppression was still only half that of the nondiabetics; in the juvenile type group it was reduced to the nondiabetic level, but at glucose and insulin levels far above those of nondiabetics. The results are compatible with the view that the glucose-sensing function of the A-cells is, at least in part, mediated by or requires insulin. In juvenile diabetics, the abnormality is corrected by raising plasma insulin to above normal levels; adult onset diabetics appear to be less sensitive even to large doses of insulin during a carbohydrate load.

The aetiology of the relative or absolute hyperglucagonaemia reported in most forms of experimental and spontaneous diabetes mellitus [1, 2, 3, 4] is unknown. While the absence of measurable immunoreactive glucagon levels has recently been reported in the plasma of pancreatectomized man by Barnes et al. [5], others have found it to be present even in such patients [6, 7, 8]. In any case, a close correlation between the rise in plasma glucagon levels and increases in plasma ketones, alanine, glycerol and free fatty acid concentrations in insulin-deprived diabetics [9, 10], suggests that glucagon excess in the presence of insulin deficiency contributes to the metabolic abnormalities of uncontrolled diabetes.

It is not clear if in spontaneous diabetes of man the abnormal responses of immunoreactive glucagon (IRG), which include hyperglucagonaemia relative to the fasting hyperglycaemia, an exaggerated rise in immunoreactive glucagon during the infusion of arginine [2], and reduced IRG suppression during a glucose load [3, 4, 11] are entirely the consequence of hypoinsulinaemia, as they seem to be in experimentally induced diabetes [3], or if there is, in addition, a contributing defect in or near the A-cell that is not corrected by restoring plasma insulin levels to normal.

The ability of physiological and supraphysiological quantities of exogenous insulin to correct certain of these A-cell derangements has been studied previously. Insulin given intravenously for short periods of time, in doses that elevated plasma insulin to
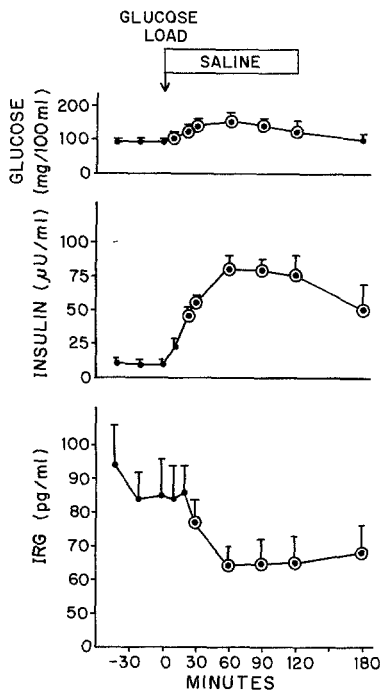

Fig. 1. The mean (± SEM) plasma glucose, insulin, and immunoreactive glucagon (IRG) response to a 100 g glucose load in nondiabetics. ⊙ = p<0.05 vs. baseline

levels of about 50 μU/ml, was shown to lower the fasting plasma IRG concentration of both adult onset and juvenile type diabetics, but only to levels which were still significantly higher than those of nondiabetics rendered comparably hyperglycaemic and insulinaemic [12]; this suggested that raising the plasma insulin level does not restore to normal the A-cell relationship to the steady state plasma glucose level. In other studies, the exaggerated IRG response of juvenile type diabetics to an arginine infusion was restored to normal by physiological increases in the levels of plasma insulin [13, 14], but that of adult type diabetics was not improved by high doses of insulin [14].

The present studies were designed to compare the effects of exogenous intravenous insulin upon yet another diabetic A-cell abnormality, the abnormal IRG response to oral glucose.

Methods

Ten patients with juvenile type and ten with adult onset diabetes mellitus were studied as outpatients after an overnight fast. Insulin and other medications were omitted on the morning of the tests. Those patients receiving two daily injections of insulin omitted their evening dose on the night prior to the test. Of the juvenile diabetic group, four were male and six were female. Their ages ranged from

17 to 52 and averaged 45 years. Weights ranged from 92 to 115 percent of ideal and averaged 102 ± 3 percent, as determined by the standards of the Metropolitan Life Insurance Company. All required insulin therapy and had diabetes from 2 to 27 years. Of the ten adult type diabetic patients, seven were female. Their ages ranged from 27 to 58 years and averaged 45 years. Their weights ranged from 100 to 172 percent of ideal and averaged 138 ± 9 percent. Seven of the ten were greater than 15 percent of ideal body weight. Three were being treated with insulin and the remaining seven were receiving tolbutamide and/or phenformin therapy. The three patients receiving insulin had been secondary failures on oral hypoglycaemic therapy. All patients had normal hepatic and renal function.

Patients were studied on three separate days within one month. On all three test days, a 100 g oral glucose load was administered subsequent to obtaining baseline blood samples. In one of the three tests a control saline infusion was begun at the time of ingestion of the glucose meal. On another test day, insulin was administered in normal saline as a constant infusion of 0.06 U/kg over a two hour period. On a third experimental day the constant insulin infusion was preceded by a bolus injection of 0.1 U/kg body weight. The order of the three experiments was determined at random.

The response of a group of nine nondiabetic subjects, four males and five females, to the control glucose load was also studied. Their ages ranged from 22 to 33 years and their weights from 94 to 103 percent of ideal.

Blood samples (10 ml) were obtained via 19-gauge butterfly needles inserted in an antecubital vein. Specimens were collected in chilled tubes containing 12 mg EDTA and 1 ml of Trasylol® (500 Kallikrein inhibitor U/ml of blood). They were centrifuged promptly at 4° C. Plasma was separated and stored at −20° C until the time of the hormone assay. Glucagon was assayed by a modification [15] of the previously described radioimmunoassay using antiserum 30K [16]. This assay can distinguish differences of 10 pg/ml with 95% confidence. The variation in this assay between duplicates is less than 5%. Glucagon-like immunoreactivity (GLI) was measured by means of the cross-reacting antiserum 78J. Glucose was measured on a Beckman glucose analyzer by the glucose oxidase method.

For comparison within groups, the Student t-test for paired groups was used. The t-test for two groups was employed for comparison between groups. The baseline values were the mean of the three baseline samples. Informed consent of all subjects was obtained.
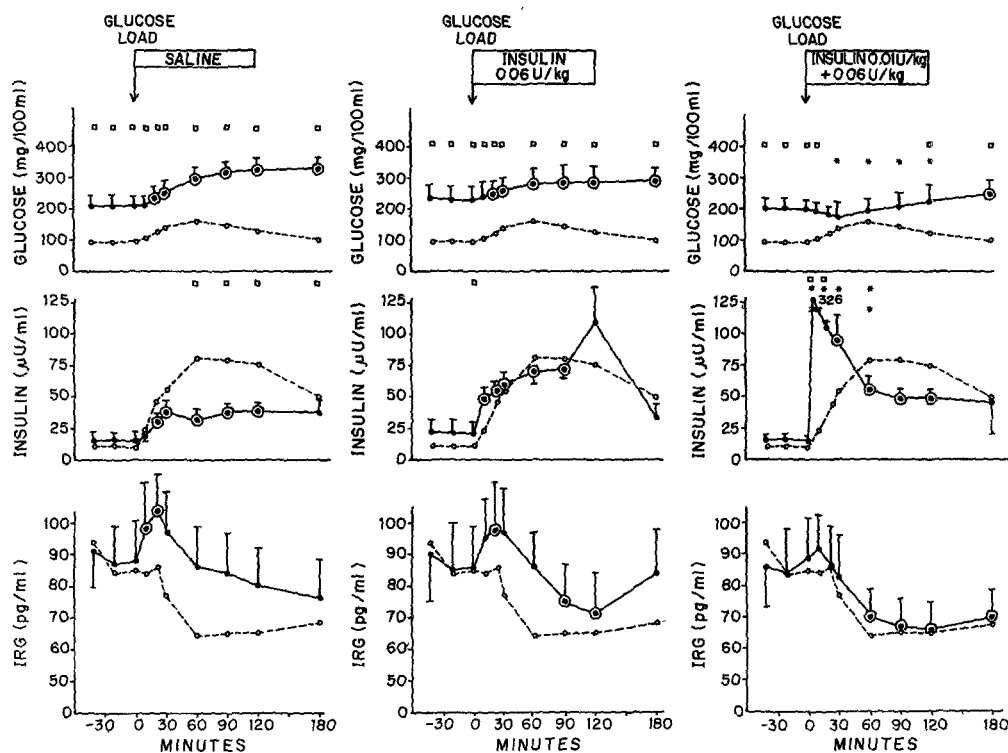
Fig. 2. The mean (± SEM) plasma glucose, insulin, and immunoreactive glucagon response to a 100 g glucose load in adult onset diabetics. Left panel during a saline infusion; middle panel during a constant insulin infusion at a rate of 0.06 U/kg; right panel during constant insulin infusion at a rate of 0.06 U/kg preceded by an IV bolus of 0.1 U/kg. ●—● = diabetics (n = 10); ○----○ = nondiabetics (n = 9); ⊙ = p<0.05 vs. baseline □ = p<0.05 diabetics vs. nondiabetics; * = p<0.05 diabetics with insulin vs. diabetics without insulin

Results

Response of Plasma IRG to Oral Glucose in Nondiabetics

Figure 1 shows the response to a 100 g oral glucose load in nine nondiabetic subjects. The mean plasma glucose concentration rose from a baseline value of 91 ± 1 mg/100 ml to a peak of 157 ± 17 mg/100 ml at one hour. Basal plasma insulin levels averaged 11 ± 1 μU/ml and rose to a peak of 81 ± 12 μU/ml at 30 min. The mean basal plasma IRG level of 88 ± 10 pg/ml declined to a nadir of 64 ± 6 pg/ml at 60 minutes following the glucose load, with a mean maximal decline of 28 ± 5 pg/ml. The mean IRG was significantly below the baseline value at all points from 30 to 180 minutes after the ingestion of the glucose (p<0.05). The mean sum of IRG changes[1] was −105 ± 7 pg/ml (Table 1A).

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[1] The mean sum of the IRG change represents the total area under or over the IRG curve. It is calculated by adding the individual change at each sampling point (be they positive or negative) from the baseline value for each individual subject. The mean (± SEM) for each group was then calculated

Effect of Insulin on the Response of Plasma IRG to Oral Glucose in Adult Onset Diabetes

Figure 2 (left panel) shows the mean response of plasma glucose, insulin, and IRG to a 100 g oral glucose load during an infusion of saline in ten maturity onset diabetics. The mean basal plasma glucose averaged 211 ± 28 mg/100 ml and rose to 327 ± 33 mg/100 ml at 180 min. Plasma insulin could be measured in only seven of the ten adult onset diabetics because of circulating insulin antibodies in three of this group. Insulin rose from a basal level of 19 ± 6 μU/ml to 38 ± 6 at 120 min, and was significantly below that of the nondiabetics at the 30, 60, and 90 min time points (p<0.05). Plasma IRG, which averaged 89 ± 13 pg/ml in the basal state, rose to 104 ± 14 pg/ml at 20 min and later declined to 76 ± 12 pg/ml at 180 min. There were no statistically significant differences from the absolute values of the nondiabetics (Figure 2), but the mean change in the IRG level was significantly different from the nondiabetics at the 20, 30, and 60 min time points (p<0.05). The mean sum of IRG changes was +1 ± 24 pg/ml, significantly greater than in the negative value of the nondiabetics

Table 1. The mean sum of glucose, insulin, IRG and GLI changes in response to a glucose load in diabetics and nondiabetics

	$\Sigma\Delta$Glucose[1] mg/dl/180 min	$\Sigma\Delta$Insulin[1] μU/ml/180 min	$\Sigma\Delta$IRG[1] pg/ml/180 min	$\Delta\Sigma$GLI[1] ngeq/ml/120 min
	A. No Insulin			
Nondiabetics	+242 ± 51[a]	+307 ± 49	−105 ± 27	+1.933 ± 0.755
Adult onset diabetics	+459 ± 63[b]	+100 ± 24[c]	+1 ± 24[c]	+1.642 ± 0.906
Juvenile diabetics	+1061 ± 97[c]	−	+133 ± 37[c]	+1.565 ± 0.383
	B. Insulin (0.06 U/kg/2 h)			
Adult onset diabetics	+270 ± 81	+292 ± 45[a]	−8 ± 22[b]	+1.265 ± 0.517
Juvenile diabetics	+689 ± 129[cd]	−	− 27 ± 26[bd]	+1.280 ± 0.594
	C. Insulin (0.1 U/kg bolus + 0.06 U/kg/2 h)			
Adult onset diabetics	+72 ± 84	+617 ± 49[cd]	−60 ± 25[d]	+0.306 ± 0.337
Juvenile diabetics	+423 ± 101[d]	−	−89 ± 18[d]	+0.495 ± 0.364

[a] = SEM
[b] = $p < 0.05$ vs. nondiabetics
[c] = $p < 0.01$ vs. nondiabetics
[d] = $p < 0.01$ vs. same patients without insulin
[1] The $\Sigma\Delta$ glucose, insulin, IRG and GLI represent the sum of the changes (both positive and negative) of these substances from the mean of the three baseline values. A sum for each individual subject is calculated and from these individual sums a mean ± SEM for the group was calculated
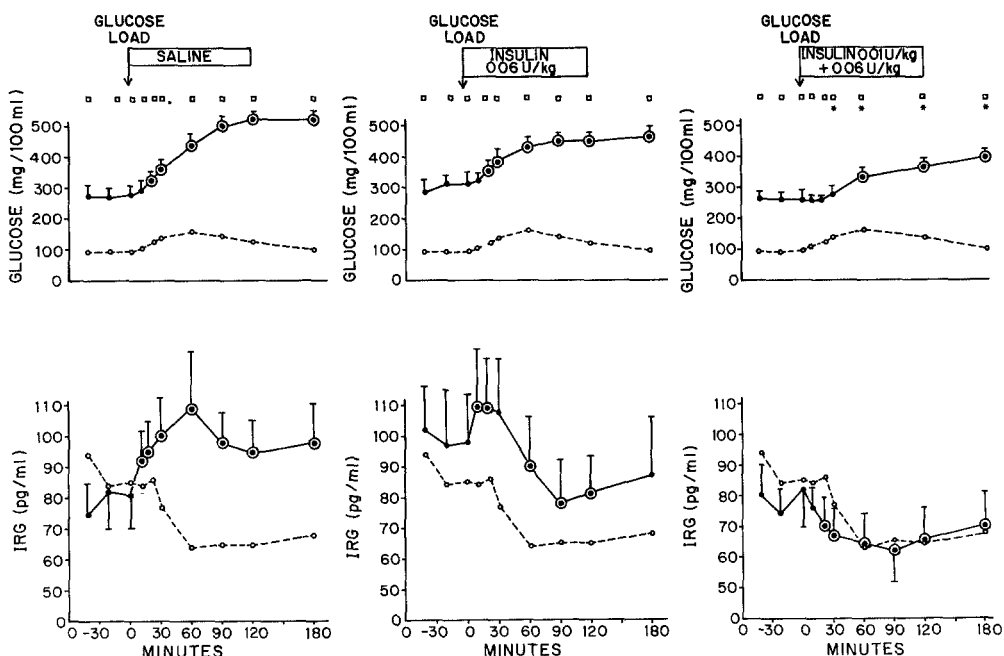

Fig. 3. The mean (± SEM) plasma glucose and immunoreactive glucagon response to a 100 g glucose load in juvenile type diabetics. Left panel during a saline infusion; middle panel during a constant insulin infusion at a rate of 0.06 U/kg, right panel during a constant insulin infusion at a rate of 0 06 U/kg preceded by a bolus of 0.1 U/kg. ●——● = diabetics (n = 10), o----o = nondiabetics (n = 9); ⊙ = $p < 0.05$ vs. baseline; □ = $p < 0.05$ diabetics vs nondiabetics; * = $p < 0.05$ diabetics with insulin vs. diabetics without insulin

(p<0.01) (Table 1A) and reflected the initial rise and late decline of plasma IRG following oral glucose.

The intravenous administration of glucagon-free insulin at a constant rate of 0.06 U/kg over two hours (Figure 2, middle panel) raised peripheral plasma insulin to levels not significantly different from the nondiabetics. Plasma glucose rose from

a baseline level of 232 ± 34 mg/100 ml to 289 ± 46 mg/100 ml 180 min after the ingestion of the glucose load. IRG increased to 98 ± 15 pg/ml at 20 min from a baseline of 87 ± 14 pg/ml and later declined to 71 ± 13 pg/ml at 120 min. The mean change in IRG differed significantly from those of the nondiabetics at the 20, 30, and 60 min time points (p<0.05) and were not significantly improved

from the response without any insulin (Figure 2, left panel). The mean sum of IRG changes was +8 ± 22 pg/ml, significantly different from the response in the nondiabetics (p<0.05) and not significantly different from the response of adult diabetics without the insulin infusion (Table 1B), and again reflected an early rise and late decline in plasma IRG levels.

When a 0.1 U bolus of insulin per kg of body weight followed by a 0.06 U/kg infusion of insulin was administered with the oral glucose load, mean plasma insulin rose to a peak of 326 ± 23 μU/ml at 10 min (Figure 2, right panel). Plasma glucose rose from 199 ± 31 mg/100 ml to a peak of 250 ± 36 mg/100 ml at 180 min. Insulin and glucose levels were both significantly greater than those of the nondiabetics during the first 30 min; mean plasma IRG rose slightly, but not significantly, during the first 20 minutes and then declined by 19 ± 8 pg/ml to a nadir of 66 ± 9 pg/ml at 120 min. The mean sum of IRG changes was −60 ± 25 pg/ml, about half that of nondiabetics (N.S.), but less than that of the diabetics without insulin (p<0.01) (Table 1C).

As an index of insulin-induced improvement in IRG response, the difference between the IRG response without exogenous insulin and with the maximal dose of insulin was calculated for each diabetic patient[2]. It averaged 59 ± 17 pg/ml in these patients. This improvement in IRG was a consequence of a reduction in the early IRG rise and an increase in the subsequent IRG decrement. There were no differences in any of these experiments between the responses of obese and nonobese diabetics.

Effect of Insulin on the Response of Plasma IRG to Oral Glucose in Juvenile Type Diabetics

Figure 3 (left panel) shows the response of juvenile type diabetics to a 100 g glucose load in the absence of infused insulin. Basal plasma glucose concentration was 273 ± 22 mg/100 ml and rose to 525 ± 26 mg/100 ml at 120 min. IRG rose from 79 ± 11 pg/ml to a peak of 109 ± 17 pg/ml at 60 min and remained significantly above baseline values throughout the 180 min (p<0.05). The mean sum of IRG changes was + 133 ± 37 (Table 1A).

When insulin was infused at a constant rate of 0.06 U/kg of insulin at the time of glucose ingestion (Figure 3, centre panel), presumably raising peripheral plasma insulin to the physiological levels observed in adult type diabetics receiving the same

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[2] The arithmetic sum of the mean IRG change with and without the administration of exogenous insulin

insulin dose (Figure 2, centre panel), plasma glucose · concentration rose from 314 ± 26 mg/100 ml to a level of 457 ± 27 mg/100 ml at 120 min. IRG rose slightly above the baseline value of 99 ± 16 pg/ml during the first 30 min; thereafter it declined by 21 ± 4 pg/ml to 78 ± 14 pg/ml at 90 min. While this was a statistically significant improvement (p<0.05), the mean decrease in IRG remained significantly less than that of nondiabetics at 10, 20, 30, and 60 min (p<0.05). The mean sum of IRG changes was − 29 ± 26 pg/ml, significantly less than the nondiabetics (p <0.05), but greater than in the untreated state (p <0.01) (Table 1B).

When the insulin infusion was preceded by a bolus of 0.1 U of insulin/kg (Figure 3, right panel), presumably raising peripheral plasma insulin to levels observed in the experiments in adult type diabetics given the same insulin dose (Figure 2, right panel), glucose rose from 258 ± 30 mg/100 ml to 397 ± 29 mg/100 ml at 180 min. Mean plasma IRG declined from the very start of the insulin infusion, reaching levels not significantly different from the nondiabetics, although glucose levels (and presumably the insulin levels) were significantly higher. The mean sum of IRG changes was −89 ± 18 pg/ml, not different from nondiabetics and significantly below the response of these patients without insulin (p < 0.01) (Table 1C).

The differences between the mean IRG response of each of these patients without insulin and with the maximal insulin dose, an index of the effect of insulin-mediated improvement in IRG response, averaged 220 ± 36 pg/ml in this group. This was significantly greater than the 59 ± 17 pg/ml insulin-mediated improvement in the IRG response of adult diabetics (p<0.001).

Effect of Insulin on the Response of Glucagon-like Immunoreactivity (GLI) to Oral Glucose in Diabetics and its Influence on IRG Results

To determine the influence of changes in GLI, approximately 2% of which crossreacts in the glucagon assay, GLI was measured in the same plasma specimens. During a glucose tolerance test without an insulin infusion, adult type diabetics exhibited a rise in plasma GLI from a baseline level of 2.0 ± 0.2 ng equiv/ml to 2.5 ± 0.3 ng equiv/ml at 120 min (p<0.05), a pattern that was virtually identical with that of nondiabetics (Figure 4A). The mean GLI response was unaffected by the constant infusion of insulin. However, with a bolus injection of insulin preceding the infusion, GLI failed to rise significantly.

In juvenile diabetics without insulin infusion,

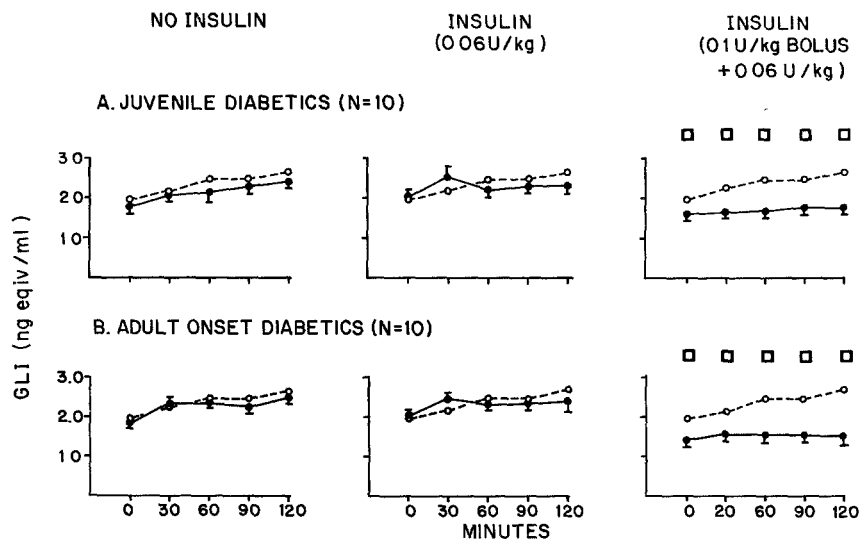Fig. 4. The mean (± SEM) plasma GLI (glucagon-like immunoreactivity) response to a glucose load in juvenile and adult onset diabetics. Left panel during a saline infusion; middle panel during a constant insulin infusion at a rate of 0.06 U/kg; right panel during a constant insulin infusion at a rate of 0.06 U/kg preceded by a bolus of 0.1 U/kg. ●—● = diabetics (n = 10); ○---○ = nondiabetics (n=9); □ = $p < 0.05$ diabetics vs. nondiabetics

GLI rose from 1.9 ± 0.1 ng equiv/ml in the basal state to 2.4 ± 0.3 ng equiv/ml ($p<0.05$), not significantly different from the nondiabetic pattern (Figure 4B). The mean maximal rise in GLI was 0.7 ± 0.2 ng equiv/ml. This GLI pattern was not significantly altered by constant infusion of insulin, although the increment at 120 min and the mean maximal GLI rise were somewhat less. When a bolus of insulin preceded the infusion, the initial rise in GLI was significantly reduced ($p<0.05$) and the mean maximal rise was only 0.3 ± 0.1 ng equiv/ml, significantly less than in the nondiabetics ($p<0.05$).

Assuming 2% crossreactivity between GLI and the 30K antiserum used to assay IRG, a change of 1 ng equiv/ml of the former would be reflected by a 20 pg/ml change in the IRG values of the 30K assay. Comparison of the changes in Figure 4A and 4B and those in Figure 1, 2, and 3 indicate that the IRG changes observed in these studies cannot be attributed to changes in GLI.

Discussion

The results confirm earlier reports concerning the abnormal IRG response in diabetics to an oral glucose load [4, 11]. In juvenile diabetics, in the absence of exogenous insulin, there was a sustained paradoxical rise in IRG following the oral glucose load. In adult diabetics, in the absence of exogenous insulin, there was a biphasic IRG pattern consisting of an early rise and a late decline.

In juvenile type diabetics a constant insulin infusion, begun at the time of glucose ingestion, which presumably raised plasma insulin levels to those of the nondiabetics, converted the IRG pattern to

a biphasic form consisting of an initial rise and a late decline. When the infusion of insulin was preceded by an initial bolus injection of insulin, the IRG response was "normalized"; the initial paradoxical IRG was abolished and a total IRG response similar to that of nondiabetics was produced. However, this "normalization" was achieved at glucose and, presumably, insulin levels well above those which in nondiabetics were associated with the same degree of IRG suppression.

In adult type diabetics, the constant infusion, beginning at the time of glucose ingestion, raised insulin levels almost as high as in the nondiabetics, but failed to change the abnormal biphasic IRG pattern. With a large intravenous dose of insulin, which raised peripheral plasma insulin to almost twice normal for a brief period, the IRG levels, although significantly improved, still declined only half as much as they did in nondiabetics. This lack of significant insulin-mediated improvement, even at above normal peripheral insulin levels, confirms earlier studies [14, 17].

The GLI response to glucose in both types of diabetics without exogenous insulin did not differ from that of nondiabetics. While the effect of insulin on the GLI response remains uncertain, it seems clear that GLI changes played no role in the qualitative response of IRG described above.

These results can be interpreted as evidence of decreased sensitivity of an insulin-requiring glucose-sensing function of the A-cells of juvenile diabetics. Alternatively, as proposed by Weir [18], they could reflect a lack of endogenously produced insulin surrounding the A-cells in the islets of juvenile diabetics. Physiological insulinaemia may not be sufficient to restore the A-cell response to a glucose load to that of the nondiabetic islet, i.e. the A-cell "defect"

not correctable by insulin replacement may represent a "within-islet" insulin deficiency [11, 12]. It is noteworthy that the above normal insulin levels induced by the administration of high doses of insulin by bolus plus constant infusion reduced the mean sum of IRG change of juvenile diabetics by a total of 220 pg/ml below those in the untreated state, about the same as the mean IRG change of nondiabetics which, with lower insulin and glucose increments, averaged 239 pg/ml below those of the untreated juvenile diabetics. This implies that suppression of diabetic hyperglucagonaemia in juvenile diabetics by means of exogenous insulin requires circulating insulin levels which, for the non-islet tissues, constitute a marked excess and impose the danger of hypoglycaemia.

As for the adult type diabetics, the findings here reinforce the previous report [16] of the difficulty in producing A-cell suppression during a carbohydrate load even with with large doses of insulin. For although the insulin was given only for short periods of time and the total change in IRG following the oral glucose was not significantly different from normal, total IRG suppression was only half that of the nondiabetics. This fact again raises the possibility of a defect in A-cell function in maturity onset diabetics independent of both hypoinsulinaemia or within-islet insulin lack. Since seven of the patients in the adult-onset group were obese this may reflect a form of "insulin resistance" of the obese, maturity onset diabetic A-cell. Reports by Patel et al. [19] and Baetens et al. [20] of a reduction in the number of somatostatin-containing islet cells in ob/ob mice may perhaps provide a clue as to the aetiology of the abnormal A-cell function, particularly since hyperglycaemia has been shown to stimulate release of pancreatic somatostatin [21, 22, 23], a possible local suppressor of glucagon [24, 25].

However, the foregoing data, as well as evidence that insulin suppresses glucagon release from gastric A-cells [26] and glucagon-secreting tumours [27], strongly implicates insulin, perhaps locally secreted insulin, as a major determinant of the normally negative pancreatic A-cell response to a glucose meal.

It must be remembered that in these studies all patients were hyperglycaemic and relatively insulin deficient, and that insulin was administered for a rather short period of time. It is possible that different results might be obtained if these studies were done after a more prolonged period of insulin administration with resulting normalization of plasma glucose levels.

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