Effects of Acute Insulin Withdrawal and Administration on Plasma Glucagon Responses to Intravenous Arginine in Insulin-dependent Diabetic Subjects

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SUMMARY

To assess further the role of insulin in the abnormal alpha-cell dysfunction found in human diabetes mellitus, the effects of acute insulin withdrawal and administration on plasma glucagon responses to intravenous arginine were studied in eight insulin-dependent diabetic subjects. Arginine infusions (30 gm. over 30 minutes) were performed during and at one and four hours after discontinuation of a 14-hour insulin infusion (1.5 U. per hour), which had rendered the subjects euglycemic, and on another occasion before and one and four hours into a five-hour infusion of insulin (1.5 U. per hour). During the last hour of the 14-hour infusion, glucagon responses to arginine (area under the curve, nanograms per milliliter per minute) were similar to those found in normal subjects (10.3 ± 0.8 vs. 9.0 ± 0.8, respectively). After discontinuation of the insulin infusions, glucagon responses increased progressively ($p < 0.01$) to values (16.8 ± 1.2) that exceeded those of normal subjects by four hours ($p < 0.01$). These were similar to results found in the same subjects studied when their diabetes was in less than optimal control (14.9 ± 1.3). Infusion of insulin under these conditions progressively decreased glucagon responses to arginine to values (9.6 ± 0.8; $p < 0.01$) that, at four hours, were similar to those of normal subjects and to values found at the end of the 14-hour infusion of insulin in the same diabetic individuals.

These results demonstrate a rapid effect of insulin on glucagon responses to arginine and suggest that the abnormal responses seen in diabetes mellitus are the immediate result of insulin deficiency. Since abnormal glucagon responses to glucose in diabetes are not as readily corrected by insulin, the mechanisms underlying the abnormal responses to these two stimuli may differ. DIABETES *25*:955-60, October, 1976.

Excessive glucagon responses during intravenous infusion of arginine occur in human diabetes mellitus,[1-10] but the cause of this abnormality is poorly understood. Ohneda et al.[8] have reported that chronic oral sulfonylurea therapy normalized these responses in adult-onset diabetics, and more recently it was found that prolonged (14-hour) infusions of insulin at a rate as low as 1 U. per hour corrected responses in juvenile-onset, insulin-dependent diabetics.[9] These observations suggest that the abnormal glucagon responses to arginine observed in diabetes mellitus may be a consequence (either direct or indirect) of insulin lack. The present investigation was therefore undertaken to assess this question further by studying the evolution in plasma glucagon responses to arginine after acute withdrawal and administration of insulin: Arginine-induced glucagon secretion was studied after acute withdrawal of insulin from juvenile-onset, insulin-dependent diabetic subjects whose responses had been normalized by means of prolonged insulin infusion and, also, during infusion of insulin in the same individuals when their glucagon responses were excessive in association with less than optimal diabetic control.

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SUBJECTS AND METHODS

Subjects. Informed consent was obtained from eight men (aged 21 to 33 years) with juvenile-onset, insulin-dependent diabetes and from 15 apparently healthy nondiabetic subjects (11 men and four women, aged 18 to 28 years) with no family history of diabetes. Individuals in each group were within 10 per cent of their ideal body weight (96 ± 2 and 99 ± 2 per cent [mean ± S.E.M.], respectively, for diabetic and normal subjects) as assessed by Metropolitan Life Insurance Company tables. None of the diabetic subjects was acutely ill or ketotic at the time of testing.

Protocol. All studies were begun between 7 and 8 a.m. with subjects in the postabsorptive state. Antecubital veins were cannulated bilaterally with 19-gauge butterfly needles and kept patent by slow infusions of 0.45 per cent saline solution; one arm was used for blood sampling and the other for administration of drugs. At least one-half hour was allowed for equilibration before collection of initial baseline specimens. Normal subjects were given a constant infusion of arginine (30 gm. over 30 minutes), and the experiment was terminated after 60 minutes.

The diabetic subjects were randomly studied twice within a 10-day period. On one occasion, their usual insulin regimen was decreased slightly (by 10 to 20 per cent) the day before testing to render their control less than optimal. On the other occasion, they were managed solely by means of subcutaneous regular insulin for one to two days before the study, as previously described[11] (to deplete residual stores of long-acting insulin), and subsequently infused with regular insulin (monocomponent, Eli Lilly Co., Indianapolis, Indiana) at a rate averaging 1.5 U. per hour for 14 hours before beginning the test to render them normoglycemic. On both occasions, three arginine infusions (30 gm. over 30 minutes) were performed in sequence beginning at 0, 105, and 285 minutes. On the day on which the diabetics' control had been rendered less than optimal and their fasting plasma glucose levels averaged 287 ± 13 mg. per 100 ml., a five-hour insulin infusion (1.5 U. per hour) was begun after the first arginine infusion at minute 45 so that the subsequent infusions of arginine were performed one and four hours into the period of the insulin infusion. On the other day, when the diabetics had been rendered normoglycemic by means of the prolonged infusions of insulin, the insulin infusion was discontinued after the initial arginine infusion at minute 45 so that the subsequent arginine infusions were performed at one and four hours after acute withdrawal of insulin.

Plasma glucose and glucagon levels were determined at 10-to-15-minute intervals, as previously described.[12] Serum insulin levels[6] were determined only in the control subjects. All results are expressed as the mean ± S.E.M. Total glucagon responses to arginine were determined by computer calculation of areas under the curve for the 45-minute interval after initiation of each infusion of arginine after subtracting baseline values (values immediately prior to each infusion of arginine). Two-tailed, paired Student's *t* tests were used to examine data obtained in the diabetic subjects (who served as their own control); unpaired *t* tests were used on differences in results from nondiabetic and diabetic subjects.

RESULTS

Effect of acute insulin administration on glucagon responses to arginine in insulin-dependent diabetics (table 1, figures 1 and 2). Before infusion of insulin, the diabetic subjects were markedly hyperglycemic (figure 1) and their basal glucagon levels were slightly, but not significantly, higher than those found in normal subjects (table 1). In this state of less than optimal diabetic regulation, glucagon responses to arginine in the diabetics significantly exceeded responses of normal subjects in terms of both absolute circulating levels ($p < 0.05$ to < 0.01) and areas under the curve ($p < 0.01$), as shown in figure 2.

Infusion of insulin at a rate of 1.5 U. per hour resulted in a gradual decline in plasma glucose levels to 133 ± 16 mg. per 100 ml. at the end of the experiment (figure 1). This rate of insulin infusion was chosen because preliminary experiments in normal subjects indicated that it would produce maximal circulating levels of only 30 to 40 μU. per milliliter—values within the physiologic range expected to approximate responses seen during infusion of arginine in nondiabetic subjects. Indeed, in the control subjects in this study, insulin levels rose from basal values of 12 ± 2 μU. per milliliter to values of 32 ± 4, 39 ± 4, and 42 ± 5 μU. per milliliter at 10, 20, and 30 minutes, respectively, during infusion of arginine. Unfortunately, insulin levels were not measured in the diabetic subjects because of the unavailability at the time of a method for determining insulin in the presence of insulin antibodies, which would most likely render circulating levels of free insulin lower than those found when similar amounts of

TABLE 1

Plasma glucagon responses to intravenous arginine (30 gm. over 30 minutes) in normal and insulin-dependent diabetic subjects before (control) and after prolonged (14-hour) infusion of insulin and four hours of acute insulin deprivation

Subjects	Plasma glucagon (pgs. per milliliter)*							
	−30	−15	0	10	20	30	45	60
				(minutes)				
Normal (N = 15)	61 ± 6	59 ± 6	59 ± 6	280 ± 17	331 ± 20	359 ± 21	101 ± 11	62 ± 9
Diabetics (N = 8)								
(control)	87 ± 11	86 ± 10	87 ± 11	392 ± 39	481 ± 43	587 ± 61	278 ± 33	125 ± 16
p†<	N.S.	N.S.	N.S.	0.05	0.01	0.01	0.01	0.01
On insulin								
(14-hour infusion)	46 ± 11	47 ± 12	45 ± 11	281 ± 35	379 ± 32	391 ± 34	116 ± 24	54 ± 16
p†<	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
p‡<	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Off insulin								
(4 hours)	—	100 ± 14	109 ± 15	478 ± 43	571 ± 51	634 ± 67	354 ± 41	165 ± 27
p†<		0.05	0.05	0.01	0.01	0.01	0.01	0.01
p‡<		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
p§<		0.01	0.01	0.01	0.01	0.01	0.01	0.01

*mean ± S.E.M.
†p vs. normal subjects.
‡p vs. control.
§p vs. on insulin (14-hour infusion).

insulin are infused into normal subjects. Transient decreases in the rate of fall of plasma glucose levels were observed during periods of arginine administration after one and four hours of the insulin infusion. This acute administration of insulin diminished glucagon responses to arginine. At one hour into the insulin-infusion period, glucagon responses were slightly less than those observed before the infusion, but these differences, both in terms of absolute circulating levels (figure 1) and incremental areas under the curve (figure 2), were not significant. Nonetheless, these responses were now not significantly different from those found in normal subjects (figure 2). At four hours into the insulin infusion, glucagon responses were significantly diminished compared with responses before insulin, both on an absolute basis (figure 1) and in terms of incremental areas under the

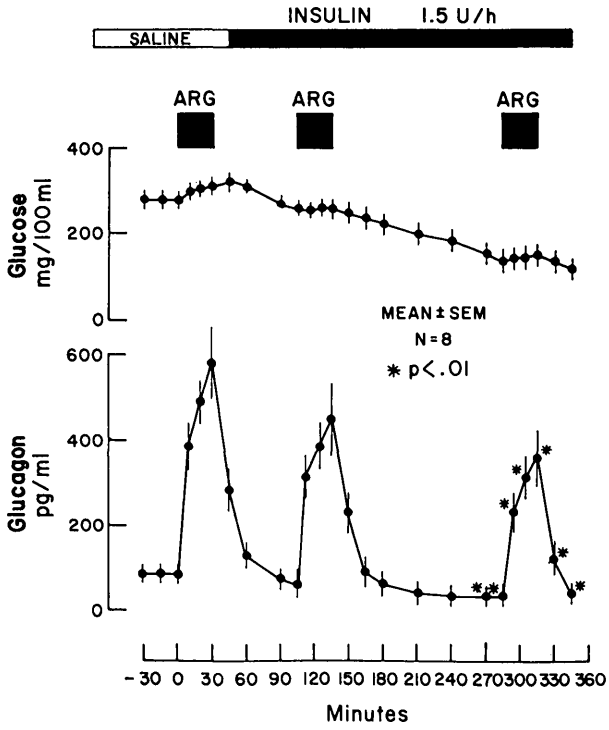

FIG. 1. Sequential effects of acute insulin infusion on plasma glucagon responses to arginine in poorly controlled juvenile-onset, insulin-dependent diabetic subjects.

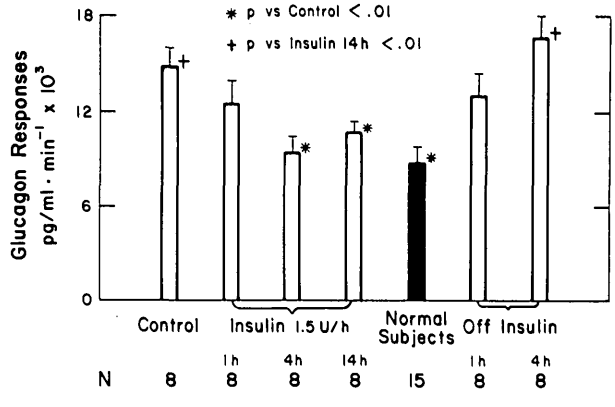

FIG. 2. Comparison of total glucagon responses to arginine (incremental area under the curve during the 45-minute period after initiation of arginine infusion) in nondiabetic subjects and in juvenile-onset, insulin-dependent diabetic subjects infused with insulin and withdrawn from insulin for various periods.

curve (figure 2), and were similar to those of normal subjects. Basal plasma glucagon levels (levels immediately prior to infusion of arginine) were also diminished significantly ($p < 0.01$) at this time. Thus, acute administration of insulin was able, within four hours, to normalize previously excessive glucagon responses to arginine in poorly controlled insulin-dependent diabetics.

Effect of acute insulin withdrawal on glucagon responses to arginine (table 1, figures 2 and 3). Since the above results suggested that the excessive glucagon responses to arginine observed in insulin-dependent diabetic individuals might be an immediate consequence of insulin deficiency, studies were undertaken to determine the time course for the appearance of these responses after acute withdrawal of insulin. The same diabetic subjects studied above were rendered normoglycemic by means of a prolonged (14-hour) infusion of insulin averaging 1.5 U. per hour. During the last hour of this infusion, glucagon responses to arginine administration were similar to those of normal subjects and significantly ($p < 0.01$) lower than those observed in the same subjects when their diabetes was less than optimally regulated (table 1 and figure 2).

Discontinuation of the insulin infusion resulted in a progressive rise in plasma glucose levels, which reached 316 ± 11 mg. per 100 ml. at the end of the experiment. Administration of arginine at one and four hours after discontinuation of the insulin infusion appeared to accelerate the rise in plasma glucose levels. Acute deprivation of insulin increased glucagon responses to arginine as well as basal glucagon levels (figure 3). At one hour after discontinuation of the insulin infusion, glucagon levels at 150 and 180 minutes were significantly ($p < 0.01$) higher than those at comparable response times to arginine when insulin was being infused; incremental areas under the curve were also greater, but this difference was not significant. At four hours after discontinuation of the insulin infusion, basal glucagon levels and glucagon responses exceeded those observed during infusion of insulin both on an absolute basis (figure 3) and in terms of incremental areas under the curve (figure 2). These values also exceeded those of normal subjects and were similar to those observed when the diabetic subjects were under less than optimal control (table 1). Thus, unequivocally abnormal glucagon responses to arginine developed within four hours of acute withdrawal of insulin—about the time required for insulin to normalize glucagon responses to arginine under the present experimental conditions.

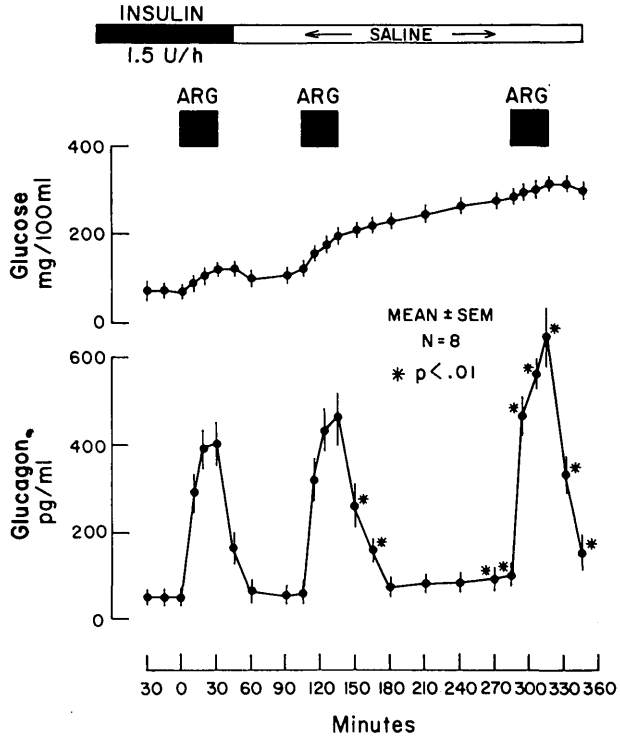

FIG. 3. Sequential effects of acute insulin withdrawal on plasma glucagon responses to arginine in juvenile-onset, insulin-dependent diabetic subjects maintained normoglycemic by means of prolonged infusion of insulin.

DISCUSSION

The present studies were undertaken to characterize further the mechanism underlying excessive glucagon responses to arginine found in human diabetes mellitus by studying the sequential changes in such responses during acute insulin withdrawal and during insulin replenishment in juvenile-onset, insulin-dependent diabetics. It was found that, within four hours of insulin deprivation, glucagon responses became abnormally excessive compared both with responses seen in nondiabetic individuals and with those in the same subjects studied after prolonged infusion of insulin. Also, it was found that, by four hours of infusion of insulin, previously excessive glucagon responses to arginine could be normalized to a degree indistinguishable from that observed after the 14-hour infusion of insulin at a similar rate in the same subjects. These results thus demonstrate a rapid effect of insulin on the human diabetic alpha-cell response to arginine and provide further evidence that insulin lack

is responsible (either directly or indirectly) for the excessive glucagon responses to this agent found in diabetes mellitus. Nevertheless, these results do not necessarily indicate that other abnormalities of glucagon secretion found in human diabetes mellitus occur on a similar basis. For example, the rapid correction of abnormal glucagon responses to arginine in the present study is in sharp contrast with the relative inability of much larger quantities of exogenous insulin to normalize inappropriate glucagon responses to glucose in diabetic individuals.[13-15]

The mechanism of action of insulin and the reason for its different effects on glucagon responses to glucose and arginine are unclear. Josefsberg et al.[10] suggested that insulin deficiency per se was not the immediate cause for excessive glucagon responses to arginine because certain conditions associated with insulin deficiency, such as hereditary isolated growth hormone deficiency, familial dwarfism with high plasma immunoreactive growth hormone, and constitutional short stature with lean body, were not accompanied by excessive glucagon responses to arginine. Rather, these authors postulated that metabolic stresses secondary to the insulin deficiency of diabetes might necessitate enhanced secretion of a catabolic hormone such as glucagon. Nevertheless, the demonstration that insulin can normalize glucagon release from islets of experimentally diabetic rats in vitro[16,17] and the rapid correction observed in the present study argue for a more direct effect of insulin on the alpha cell.

The differential effect of insulin on inappropriate glucagon responses to arginine and glucose observed in human diabetes suggests that these abnormalities may result from different causes. Excessive glucagon responses to arginine appear to be secondary to insulin lack. However, while insulin deficiency may magnify abnormal glucagon responses to glucose, it may not necessarily be the primary cause. Attempts to normalize glucagon responses to glucose in diabetic individuals by infusing pharmacologic quantities of insulin for up to six hours have proved only partially successful.[13-15] This contrasts with the rapid (within four hours) and complete normalization of abnormal responses to arginine observed in the present study with relatively small amounts of insulin and with the correction by insulin of abnormal glucagon secretion in animals made insulin-deficient and diabetic experimentally.[16-18] It has been postulated that inappropriate glucagon responses to glucose in human diabetes may be the result of an abnormal alpha cell glucoreceptor.[4,15,19] This concept is supported by the findings of selective impaired suppression of plasma glucagon by glucose (completely normal suppression by elevated plasma free fatty acid levels but not by glucose plus pharmacologic amounts of insulin[15]) and of the failure of the diabetic alpha-cell to respond appropriately to hypoglycemia as well as hyperglycemia.[4]

Current understanding of alpha-cell function and the actions of insulin suggests a mechanism consistent with this hypothesis that could explain how modest doses of insulin might completely correct glucagon responses to arginine in human diabetes but only partially correct abnormal glucagon responses to glucose at higher doses. Recent evidence indicates the presence of separate alpha-cell receptors for glucose and arginine[20,21] through which these agents affect glucagon release. Additionally, changes in intracellular cyclic adenosine 3′, 5′-monophosphate (cAMP) are thought to modulate glucagon release,[22-25] since elevation of alpha-cell cAMP by epinephrine stimulates basal glucagon release in vitro[22] and administration of other agents known to elevate intracellular cAMP levels, such as isoproterenol[23,24] and theophylline,[25] also augment glucagon secretion. Although not assessed in islet tissue as yet, insulin has been reported to lower intracellular cAMP levels in other tissues.[26] Accordingly, insulin lack may result in elevated alpha-cell cAMP levels, and part of the excessive glucagon secretion found in human diabetes may be due to high intracellular cAMP. Acute administration of insulin could normalize glucagon responses to arginine by lowering intracellular cAMP. It should be pointed out that insulin administered in vivo, as in the present study, would most likely affect the flux of potassium, phosphorus, and perhaps calcium, and thus the ability of insulin to influence glucagon release might also involve these factors. An action of insulin on cAMP or on flux of ions could also explain the partial correction of inappropriate glucagon responses to glucose by insulin, but complete correction would not occur if there were a defective glucoreceptor. Thus, two mechanisms may be operative in the derangements of alpha-cell function in human diabetes: altered cAMP metabolism secondary to insulin lack and an abnormal glucoreceptor.

NOTE ADDED IN PROOF

Since submission of this manuscript, Raskin et al. (Diabetes 25:227-29, 1976) have reported that infusion of supraphysiologic amounts of insulin blunted glucagon responses to arginine in juvenile-onset but not in adult-onset diabetic subjects.

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