Counterregulatory Hormonal Responses to Rapid Glucose Lowering in Diabetic Man

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SUMMARY

To define whether rapid rate of fall in blood glucose stimulates counterregulatory hormonal responses in diabetic man, blood glucose in eight hyperglycemic diabetic subjects was rapidly lowered by intravenous insulin administration. Despite precipitous declines in blood glucose, plasma epinephrine and growth hormone remained virtually unchanged. In contrast, norepinephrine and cortisol increased significantly ($P < 0.025$) in the face of hyperglycemia or euglycemia, while glucagon was suppressed ($P < 0.025$). A transient modest fall in mean arterial pressure and a rise in pulse rate were noted. No correlation was observed between glucose disappearance rate or decrement in glucose concentration and the hormonal responses. After sham insulin administration, no change was observed in plasma epinephrine, norepinephrine, and cortisol levels.

These findings suggest that rate of fall in blood glucose per se is not a primary signal for counterregulatory hormonal response. Cortisol but not growth hormone release during falling blood glucose in diabetic subjects can occur despite elevated blood glucose levels. The etiology of norepinephrine and cortisol change is unclear. DIABETES 28:873–877, October, 1979.

The counterregulatory response to hypoglycemia consists of a cascade of neurohormonal responses and metabolic events, involving activation of the sympathetic nervous system, increased secretion of catecholamines, and enhanced release of glucagon, cortisol, and growth hormone. Although it has been generally accepted that these responses are related to the absolute level of blood glucose, controversy exists as to whether the rate of decline in blood glucose is a factor in the initiation of these glucoregulatory events.[1–7] Studies in normal man have shown that, despite rapidly falling blood glucose levels, changes in counterregulatory hormone concentrations occur only when below basal concentrations of blood glucose are achieved.[4] However, these observations may not be applicable to diabetic patients because the glycemic threshold required for this response may be altered as the result of chronic hyperglycemia. Furthermore, studies in diabetic patients showing increased sympathetic nervous system activity, as reflected by changes in galvanic skin resistance during rapid decline in blood glucose,[1] support the concept that significant differences in autonomic responsivity to alterations in blood glucose concentrations may exist between normal subjects and diabetic patients. Nevertheless, the counterregulatory role of adrenomedullary hormones in these events has not yet been elucidated because of the lack of direct assessment of hormonal responses.

This study was designed to directly characterize the sequence of change in the major counterregulatory hormones, namely, catecholamines, glucagon, cortisol, and growth hormone, in diabetic patients during insulin-induced rapid decline in blood glucose, from hyperglycemic to euglycemic levels, a condition not infrequently encountered in the management of the diabetic patient.

MATERIALS AND METHODS

Eight insulin-dependent diabetic subjects (four males and four females) between the ages of 22 and 54 yr, who gave informed consent, participated in this study. The range of duration of diabetes was 4–25 yr. All but two subjects were within 10% of ideal body weight (Metropolitan Life Insurance Company table, 1959). One patient had peripheral atherosclerosis, and early retinopathy was noted in one other patient; none had clinical signs of autonomic neuropathy.

On the day preceding the study, the maintenance dose of insulin was adjusted to allow moderate hyperglycemia without ketonuria. After a 12-h overnight fast, all subjects remained supine for 45 min before the start of base-line ve-

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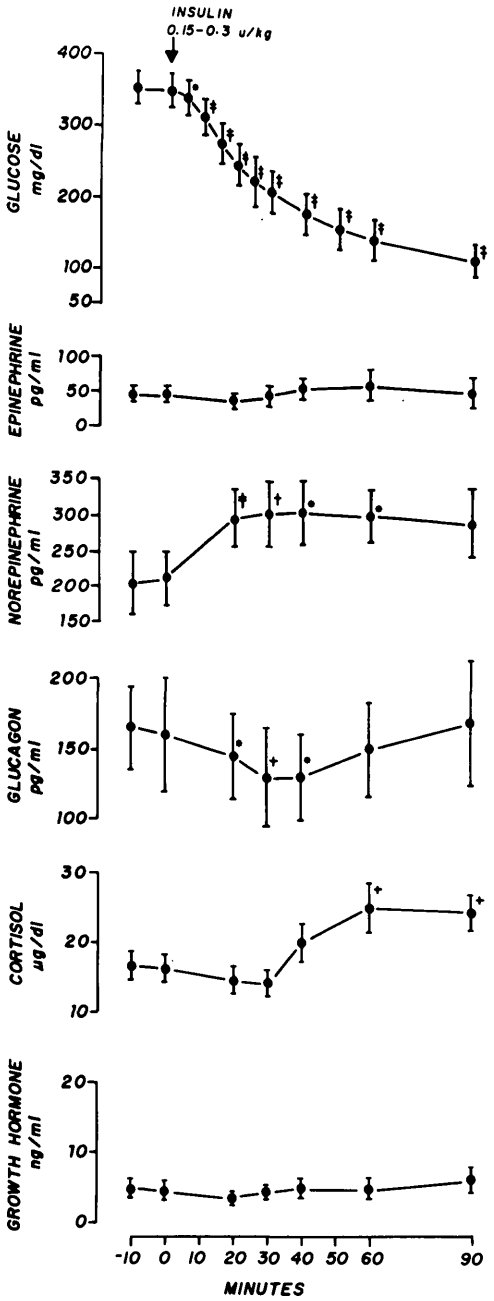

FIGURE 1. Plasma glucose, epinephrine, norepinephrine, glucagon, cortisol, and growth hormone levels (mean ± SEM) in eight diabetic subjects after i.v. administration of insulin 0.15–0.3 U/kg. *P < 0.05; †P < 0.025; ‡P < 0.005. (P values represent significant differences from the mean base-line level.)

nous sampling via an indwelling 19-gauge needle in an antecubital vein. Crystalline insulin (Lilly), 0.15–0.3 U/kg body wt, or normal saline for sham control in four subjects was then given as an i.v. bolus, and 0.3-ml blood samples were obtained for plasma glucose determination by the glucose oxidase method[8] at 5- and 10-min intervals for 90 min. Blood samples for cortisol and growth hormone were collected in chilled heparinized tubes, centrifuged at 4°C, and frozen within 15 min until assayed by previously described methods.[8] Blood samples for glucagon were collected in 0.05 M benzamidine and stored in a similar fashion until assayed.[9] The intra- and interassay coefficients of variation of glucagon assay, using 30 K antiserum, were 1.5% and 5% at the level of 300 pg/ml. Samples for plasma catecholamines were collected in heparinized tubes containing 4 mM reduced glutathione and 5 mM EGTA, pH 5.5, and stored at −80°C until assayed by a modification of the radioenzymatic method of Passon and Peuler.[10] Incubation is done in duplicate in a total volume of 275 µl, containing 200 µl of unknown plasma sample, 90 mM Tris, 10 mM EGTA, 27 mM $MgCl_2$, 2.9 mM reduced glutathione, 6 µCi tritiated S-adenosyl-methionine (Searle-Amersham), and 15 µl modified, purified rat liver catechol-O-methyltransferase (COMT),[11] pH 8.2. The internal standards are based on the increments of activity generated from the added catecholamines of known concentration (50, 100, 200, 500, 1000 pg/tube) to the individual patient's base-line plasma. The average increments of activity are 16,100 cpm/ng epinephrine and 13,300 cpm/ng norepinephrine. The intra-assay coefficients of variation were 6.7% for epinephrine at 90 pg/ml and 5.2% for norepinephrine at 320 pg/ml. The interassay coefficients of variation were 9.3% for epinephrine and 9.6% for norepinephrine at concentrations of 39 and 250 pg/ml, respectively. The mean supine plasma catecholamine levels (± SD) derived from 50 consecutive normal volunteers were 35 ± 26 pg/ml for epinephrine and 204 ± 103 pg/ml for norepinephrine. Blood volume was replaced by an equal amount of normal saline. Blood pressure and pulse rate were monitored at 10-min intervals. Mean arterial pressure was calculated by adding one-third of pulse amplitude to diastolic pressure.

All results are expressed as mean ± SEM. Glucose disappearance rate (Kg) was calculated from the slope of least square analysis of $\log_e$ plasma glucose with time from 10 to 35 min.[12] Statistical analysis was performed by paired two-tailed Student's *t* test.

RESULTS

The mean of base-line plasma glucose was 350 ± 24 mg/dl. After insulin administration, glucose concentration fell precipitously and reached mean levels of 210 ± 30, 145 ± 30, and 117 ± 24 mg/dl at 30, 60, and 90 min, respectively (Figure 1). Decrements in plasma glucose concentration after i.v. administration of insulin in each diabetic patient are shown in Table 1. Mean Kg was 2.19 ± 0.3% per minute. All subjects had lowest plasma glucose levels (none below 53 mg/dl) at 90 min, except subject no. 8, whose glucose level reached a nadir at 80 min.

TABLE 1
Decrements in plasma glucose concentration from the base-line glucose levels following i.v. administration of insulin in eight diabetic subjects

Subject no.	Base-line plasma glucose (mg/dl)	Decrements in plasma glucose (mg/dl)		
		30 min	60 min	90 min
1	345	174	242	256
2	304	146	194	213
3	365	123	183	220
4	272	125	161	189
5	348	124	181	198
6	490	85	161	226
7	381	216	302	328
8	291	128	216	232
Mean ± SEM	350 ±24	140 ±14	205 ±17	233 ±15

TABLE 2
Responses of blood pressure and pulse rate following a bolus of i.v. insulin in eight diabetic subjects (mean ± SEM)

	Min									
	−10	0	10	20	30	40	50	60	80	90
Systolic blood pressure (mm Hg)	114 ±4	114 ±3	117 ±4	115 ±3	111 ±4	109 ±6	108 ±5	111 ±4	110 ±5	111 ±5
Diastolic blood pressure (mm Hg)	73 ±4	73 ±3	70 ±4	69 ±3	67 ±4	69 ±4	65* ±4	71 ±4	66 ±4	67 ±4
Mean arterial pressure (mm Hg)	86 ±4	86 ±3	86 ±4	84 ±3	82 ±3	82 ±4	79* ±4	84 ±4	81 ±3	82 ±3
Pulse rate (beats/min)	73 ±5	74 ±5	74 ±5	81† ±5	80‡ ±5	83* ±4	84 ±4	86† ±6	88* ±5	85* ±7

* $P < 0.05$
† $P < 0.02$
‡ $P < 0.005$

Mean changes in plasma glucose, catecholamines, glucagon, cortisol, and growth hormone after i.v. administration of insulin in diabetic subjects are shown in Figure 1. The mean base-line plasma epinephrine and norepinephrine were 46 ± 11 and 212 ± 39 pg/ml, respectively. Despite the rapid fall in plasma glucose, plasma epinephrine remained virtually unchanged. However, in subject no. 7 (Table 1), plasma epinephrine rose to 408 pg/ml at 90 min, when plasma glucose was 53 mg/dl. Similarly, in subject no. 8, plasma epinephrine increased to 1524 and 1885 pg/ml when plasma glucose reached 59 and 64 mg/dl at 80 and 90 min, respectively. Therefore, their 90-min catecholamine levels were not included in the mean calculation (Figure 1). Plasma norepinephrine rose significantly to 298 ± 42 pg/ml by 20 min ($P < 0.005$) and remained elevated throughout the 60-min period ($P < 0.05$) (Figure 1).

After insulin administration, plasma glucagon level declined from the base line of 160 ± 42 to 145 ± 32 pg/ml ($P < 0.05$) by 20 min, reached the nadir of 129 ± 35 pg/ml ($P < 0.025$) at 30 min, and returned to base line by 90 min (Figure 1). A rebound in glucagon levels to 327 pg/ml was observed at 80 min in subject no. 8.

During the first 30-min period, plasma cortisol tended to fall from the base line of 16.2 ± 2 μg/dl (Figure 1). A significant increase in cortisol levels was observed at 60 and 90 min, with concentrations of 25 ± 3.7 and 24.4 ± 2.7 μg/dl, respectively ($P < 0.025$). Although the rise of plasma cortisol level at 40 min was not significant when compared with the base-line level, it was significantly different from the 30-min level ($P < 0.025$). At the peak cortisol response (60 min), the plasma glucose concentration ranged between 75 and 329 mg/dl, with a mean of 245 ± 30 mg/dl.

Base-line growth hormone was 4.6 ± 1.3 ng/ml and remained unchanged throughout the study (Figure 1), except in subject no. 8, whose growth hormone rose to 46 ng/ml at 80 min when plasma glucose reached 59 mg/dl.

Blood pressure and pulse rate responses after i.v. insulin administration in diabetic subjects are shown in Table 2. No significant change in systolic blood pressure was observed throughout the study, while a slight but significant decline in diastolic blood pressure and mean arterial pressure was noted at 50 min. A significant rise in pulse rate was observed by 20 min ($P < 0.005$) with the mean increment of 8 ± 2 beats/min.

There was no significant correlation between either glucose disappearance rate, or decrement in, or absolute level of glucose concentration, and increment in norepinephrine or cortisol levels; nor was there any correlation between the changes in blood pressure or pulse rate and plasma epinephrine or norepinephrine.

Mean plasma glucose, epinephrine, norepinephrine, and cortisol levels after sham insulin administration in four diabetic patients (subjects no. 1,2,6,8) are shown in Table 3. There was virtually no significant change in these para-

TABLE 3
Plasma glucose epinephrine, norepinephrine, and cortisol responses to sham i.v. insulin administration in four diabetic subjects (mean ± SEM)

	Min								
	−10	0	10	20	30	40	50	60	90
Plasma glucose* (mg/dl)	365 ±55	360 ±53	365 ±56	364 ±59	353 ±54	359 ±58	350 ±55	351 ±56	354 ±59
Epinephrine (pg/ml)	24 ±3	25 ±5	21 ±1	21 ±5	25 ±6	18 ±5	18 ±6	26 ±8	25 ±8
Norepinephrine (pg/ml)	187 ±48	202 ±51	230 ±76	239 ±63	214 ±55	213 ±59	188 ±43	197 ±48	182 ±44
Cortisol (μg/dl)	12.9 ±2.6	13.3 ±1.4	11.9 ±1.8	11.4 ±1.2	10.7 ±1.5	10.1 ±1.1	10.1 ±1.0	10.3 ±1.5	10.8 ±1.9

* Plasma glucose levels at 5-min intervals are not shown.

meters throughout 90 min when compared with their baseline levels. Similarly, there was no change in blood pressure and pulse rate throughout the procedures. Significant differences in cortisol levels between insulin-induced glucose lowering and the sham studies were noted at 40 ($P < 0.05$), 60, and 90 min ($P < 0.01$), respectively, when comparing the groups or when each subject acted as his own control. No significant differences in plasma epinephrine and norepinephrine were observed between the experiments.

DISCUSSION

This study demonstrates that in diabetic man there is virtually no adrenomedullary response, as indicated by the lack of plasma epinephrine change, associated with precipitous fall in plasma glucose from moderate hyperglycemia to euglycemia. The patients, who became relatively hypoglycemic, had marked increases in plasma epinephrine at a time when plasma glucose concentration clearly fell at a slower rate. This finding, confirming the observations in normal man,[2,4] indicates that rate of decline is not a stimulus for activation of the sympatho-adrenomedullary axis. Preliminary reports by other investigators[13] indicated small increases in plasma epinephrine and norepinephrine during an insulin-induced fall in glucose from 95 to 59 mg/dl within a 40-min period in both normal subjects and diabetic patients; yet the calculated glucose disappearance rate in that study was less than that in this and other studies.[4] Thus, the change of glucose concentration approaching a hypoglycemic threshold, rather than the rate of fall per se, may be a signal triggering increased adrenomedullary activity. It is of interest that a large magnitude in epinephrine response noted in two of our patients, at a time when plasma glucose was 53 and 59 mg/dl, respectively, is in contrast with the findings previously reported in the normal subjects.[3,14–16] In the latter studies, changes of plasma epinephrine reported at glucose levels of 50–60 mg/dl were much smaller than those observed in our patients; furthermore, it has been shown that the magnitude of epinephrine response was closely related to the degree of hypoglycemia.[3]

In contradistinction to epinephrine response, norepinephrine levels in our diabetic patients rose significantly during the rapid phase of glucose disappearance. However, no association was noted between plasma glucose decrement or glucose disappearance rate and response of plasma norepinephrine. Although there was no correlation between hemodynamic responses and plasma catecholamines in this study, a modest decline in blood pressure and an increase in pulse rate associated with insulin administration were comparable to those reported previously by Gundersen and Christensen.[17] These investigators have shown that insulin administration itself in diabetic subjects (in the absence of hypoglycemia) resulted in decrement in plasma volume accompanied by elevation in plasma norepinephrine, presumably as a consequence of the activation of baroreceptor mechanisms. Changes of galvanic skin resistance, as described previously,[1] may also be related to these hemodynamic events. It is unlikely that the sustained norepinephrine increases in these diabetic patients were due to experimental stress since no consistent change was observed during sham experiments.

Our data, in agreement with findings in normal man,[4] fail to support the concept that rapid fall in glucose does stimulate the release of pancreatic glucagon.[7] In fact, despite the precipitous fall in plasma glucose, a significant decline in glucagon levels was observed in all patients. Although glucose concentration is known to be a primary signal modulating glucagon secretion, it is possible that insulin itself, as administered in this experiment, suppressed glucagon release.[18]

The finding of enhanced cortisol secretion in diabetic patients during the fall in blood glucose, in the face of hyperglycemia, confirms the observation previously made by Sönksen,[19] but is in contrast with that observed in normal subjects.[4,6,20] Since ACTH release precedes that of cortisol by about 15 min,[21] the initial burst of ACTH secretion could have occurred during the rapid phase of glucose disappearance, suggesting that the rapid fall of blood glucose was indeed responsible for activation of the adrenocortical response. However, the magnitude of cortisol increment is unrelated to the decrement in glucose or the glucose disappearance rate. The altered adrenocortical response seen in this study may be due to derangement of intrahypophyseal glucose metabolism as a result of relative insulin lack.[22] It is unlikely that such cortisol response in this study is related to noradrenergic activity, because plasma cortisol levels in normal man are unaffected by norepinephrine infusion, achieving a blood level fourfold greater than in our diabetic patients.[23] However, decreased plasma volume as a consequence of insulin administration may possibly be a responsible stimulus for cortisol release. Experimental procedure is again unlikely to be a stimulus for such cortisol response, since cortisol levels under similar conditions were virtually unchanged.

During the rapid phase of glucose lowering in our diabetic patients, there was virtually no growth hormone response. These findings are similar to those seen in normal subjects during the precipitous fall in blood glucose after a period of experimentally induced hyperglycemia.[4] Although it is acknowledged that the response of growth hormone to hypoglycemic stress in diabetic patients is highly variable,[24] the present findings do not support the concept that rapid rate of fall in blood glucose stimulates growth hormone release.[5]

In summary, these observations fail to substantiate that the rate of decline in blood glucose is a signal for the initiation of glucocounterregulatory hormone response. Furthermore, since the rise of norepinephrine and cortisol associated with rapidly falling glucose levels was not related to glucose disappearance rate or glucose decrement, the hemodynamic responses as a consequence of insulin administration may be a major stimulus for noradrenergic activity and adrenocortical response.

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