

# Important Role of Adrenergic Mechanisms in Acute Glucose Counterregulation Following Insulin-induced Hypoglycemia in Type I Diabetes

## Evidence for an Effect Mediated by Beta-Adrenoreceptors

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### SUMMARY

During hypoglycemia induced by an i.v. insulin infusion for 60 min, rates of plasma glucose (PG) decrease and recovery, PG nadir, and plasma counterregulatory hormone and free fatty acid responses were studied in eight type I uncomplicated diabetic subjects and eight nondiabetic subjects. Each subject was tested three times at two different rates of insulin infusion (25 and 32 mU/m<sup>2</sup>/min): (1) during infusion of saline, (2) during infusion of phentolamine + propranolol (combined alpha,beta-blockade), and (3) during infusion of propranolol alone (isolated beta-blockade) for 150 min. At the time of the studies, the diabetic subjects had been made euglycemic by an overnight i.v. insulin infusion. During infusion of insulin (25 mU/m<sup>2</sup>/min) and saline, the rates of PG decrease and recovery were slower ( $P < 0.01$ ) and PG nadir was delayed in the diabetic subjects. Moreover, their plasma glucagon response was blunted while plasma epinephrine, norepinephrine, growth hormone, and cortisol responses were similar in both groups. Infusion of insulin at 32 mU/m<sup>2</sup>/min caused larger decreases in PG than had been observed when insulin was infused at 25 mU/m<sup>2</sup>/min. Plasma glucagon responses increased in the nondiabetic subjects ( $P < 0.05$ ) but not in the diabetic subjects. However, in the diabetic subjects, plasma epinephrine increased more than in the nondiabetic subjects ( $P < 0.05$ ). There was an inverse correlation between the individual plasma epinephrine responses and the plasma glucagon responses in the diabetic subjects ( $r = -0.72$ ) but not in the nondiabetic subjects. Alpha,beta-adrenergic blockade decreased the plasma glucose nadir and impaired the rate at which normoglycemia was restored in the diabetic subjects ( $P < 0.005$  vs. saline) but not in the nondiabetic subjects. Plasma catecholamine and growth hormone responses were increased and

plasma FFA recovery was suppressed in both groups ( $P < 0.05$  vs. saline), while the cortisol responses were unaltered. During isolated beta-adrenergic blockade, changes in plasma glucose, counterregulatory hormones and FFA were essentially identical to those observed during combined alpha,beta-adrenergic blockade in both groups except that the augmented plasma norepinephrine responses were no longer apparent. Conclusions: although epinephrine is not essential for prompt restoration of normoglycemia in normal man following insulin-induced hypoglycemia, it plays a major role in glucose counterregulation in diabetics who have an impaired glucagon secretion in response to hypoglycemia. These counterregulatory effects of epinephrine are mediated by beta-adrenoreceptors. *DIABETES* 31:641-647, July 1982.

There is considerable evidence that glucagon is the most important hormone involved in acute glucose counterregulation in normal man, and that epinephrine appears to play only a secondary role.<sup>1-3</sup> However, when the release of glucagon is suppressed by an infusion of somatostatin, epinephrine becomes vital in counteracting insulin-induced hypoglycemia.<sup>1,3</sup> Recently, we have shown that the glucagon response to insulin-induced hypoglycemia in patients with type I diabetes is still blunted even after a prolonged period (2-3 wk) of near normal glycemia and that their blood glucose recovery from hypoglycemia remains clearly impaired.<sup>4</sup> Presumably, in such individuals, epinephrine rather than glucagon is the major counterregulatory hormone. The present investigations were therefore undertaken to evaluate the role of adrenergic mechanisms in defense against insulin-induced hypoglycemia in type I diabetes and to establish if this counterregulatory effect is mediated by alpha- and/or beta-adrenoreceptors.

### METHODS AND MATERIALS

**Subjects and protocol.** Informed consent was obtained from eight men with type I diabetes, the clinical features of whom are summarized in Table 1. All patients were in ap-

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TABLE 1  
Clinical features of the patients

| Case | Age (yr) | Diabetes duration (mo) | % Ideal body weight | Body surface area (m <sup>2</sup> ) | ICP* ng/ml |        | MPG* (mmol/L)    |                    | Insulin dose (U/kg/day) |
|------|----------|------------------------|---------------------|-------------------------------------|------------|--------|------------------|--------------------|-------------------------|
|      |          |                        |                     |                                     | 0 min      | 90 min | Fasting          | 90 min after meals |                         |
| 1    | 21       | 36                     | 102                 | 1.86                                | 0.15       | 0.30   | 7.44 ± 0.66 (7)  | 9.00 ± 0.72 (5)    | 0.81                    |
| 2    | 41       | 36                     | 105                 | 1.75                                | 0.10       | 0.25   | 7.44 ± 0.58 (8)  | 9.88 ± 0.61 (9)    | 0.74                    |
| 3    | 26       | 96                     | 102                 | 1.70                                | 0.20       | 0.35   | 7.00 ± 0.72 (7)  | 10.2 ± 0.66 (7)    | 0.85                    |
| 4    | 17       | 60                     | 91                  | 1.74                                | 0.15       | 0.25   | 6.55 ± 0.83 (9)  | 8.55 ± 0.61 (6)    | 0.86                    |
| 5    | 19       | 8                      | 95                  | 1.80                                | 0.25       | 0.40   | 7.83 ± 0.72 (8)  | 10.4 ± 0.88 (7)    | 0.56                    |
| 6    | 15       | 30                     | 100                 | 1.85                                | 0.10       | 0.25   | 6.66 ± 0.44 (9)  | 8.72 ± 0.55 (13)   | 0.94                    |
| 7    | 26       | 12                     | 94                  | 1.89                                | 0.10       | 0.30   | 6.44 ± 0.50 (7)  | 9.22 ± 0.44 (5)    | 0.44                    |
| 8    | 30       | 8                      | 98                  | 1.50                                | 0.20       | 0.45   | 6.05 ± 0.33 (10) | 9.66 ± 0.66 (8)    | 0.56                    |

\* Immunoreactive C-peptide (before and after a mixed meal).

† Mean plasma glucose (mean of the values—number of determinations in parentheses—recorded over 10 days before the first test).

parent good health and were not taking medication other than insulin. No patient had symptoms of peripheral or autonomic neuropathy, and all patients had normal deep-breathing tests, motor and sensory nerve conduction velocity, and fundoscopic examinations. The diabetic subjects admitted to the protocol were selected from patients attending our metabolic unit who had stable, fair-to-good blood glucose control for at least 10 days before the studies while on two daily injections of a mixture of regular and Lente insulin. No symptoms of hypoglycemia had occurred during the week before the studies. On the day before a study, depot insulin (Lente, Novo Industries, Copenhagen, Denmark) was omitted and only regular insulin (Actrapid, Novo Industries) was injected preprandially. About 2 h after dinner (no snack was given after dinner) between 9:30 and 10:00 p.m., an antecubital vein was catheterized (19-gauge butterfly needle) and kept patent with 0.9% NaCl infusion (0.5 ml/min). The patients remained supine in bed until the next morning. This venous access was used to infuse regular insulin (Actrapid) overnight by means of a syringe pump (Model 2681, Harvard Apparatus Co., Millis, Massachusetts) in order to maintain normoglycemia until the beginning of the test (time 0). The rate of insulin infusion, based on measurements of blood glucose every 5–30 min by means of a Dextrometer (Ames Laboratories), was maintained constant from –60 to 60 min.

All studies were begun between 10:00 and 10:30 a.m. after subjects fasted overnight. An antecubital vein of the other arm was catheterized, kept patent with saline infusion, and used for intermittent blood sampling. Following at least a 45-min equilibration period, baseline blood samples were taken at –45, –15, and 0 min. Six different studies were performed. Insulin alone at either 25 mU/m<sup>2</sup>/min or 32 mU/m<sup>2</sup>/min was infused for 60 min to assess the effect of different blood glucose decrements on the counterregulatory hormonal response. To assess the effect of pharmacologically induced alpha- and beta-adrenergic blockade, phenolamine (5 mg over 2 min, followed by 500 µg/min for 150 min, Ciba-Geigy, S.P.A., Milano) and propranolol (3 mg over 3 min, followed by 100 µg/min for 150 min, Imperial Chemical Industries, S.P.A., Caponago, Milano) were infused in the same subjects along with insulin at 25 and 32 mU/m<sup>2</sup>/min. To assess the effect of isolated beta-blockade, propranolol (3 mg over 2 min, followed by 100 µg/min for 150 min) was infused along with insulin (25 and 32 mU/m<sup>2</sup>/min). Each diabetic patient underwent 6 tests (4–6-

day interval), with the exception of case 8, who underwent only 3 studies (saline, alpha,beta-blockade, and beta-blockade during the insulin infusion at 32 mU/m<sup>2</sup>/min).

Eight healthy male volunteers (26–32 yr of age, 28 ± 0.9) who were within 10% of their ideal body weight (Metropolitan Life Insurance Tables, 1959) served as controls. None had a family history of diabetes mellitus or an abnormal glucose tolerance test, and none was taking medication at the time of the study. A diet with at least 250 g carbohydrate/day was recommended during the week before the study. Each subject underwent the same six studies described above at 4–6-day intervals in a single blind randomized order, with the exception of one subject who underwent only three studies (saline, alpha,beta-blockade and beta-blockade during the insulin infusion at 32 mU/m<sup>2</sup>/min).

**Analytical methods.** Blood was drawn at 5–30 min intervals for measurement of plasma glucose, glucagon, epinephrine, norepinephrine, growth hormone, cortisol, and free fatty acids (FFA). The blood samples for glucagon determination were placed in chilled tubes containing 0.05 ml 10% EDTA-Na<sub>2</sub> and 500 U of aprotinin/ml blood. The blood samples for catecholamine assay were collected in iced test tubes containing heparin and sodium metabisulfite (10 mg). Blood for plasma glucose, growth hormone, cortisol, and FFA determination was collected into heparinized tubes. All samples were placed on ice, centrifuged at 4°C at 3000 rpm, the plasma separated and stored at –20°C until the time of assay.

Plasma glucose was determined by Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California). Glucagon was determined by radioimmunoassay with 30 K antiserum<sup>5</sup> kindly supplied by Dr. Unger (Dallas). Plasma catecholamines were determined by a sensitive and specific fluorimetric method as previously reported.<sup>6</sup> Plasma growth hormone was determined by radioimmunoassay.<sup>7</sup> Plasma cortisol was measured by Mattingly's fluorimetric method.<sup>8</sup> FFA were measured by the Dole and Meinertz method.<sup>9</sup> Immunoreactive C-peptide was determined before and 90 min after a mixed meal (297 Kcal, 19% protein, 38% lipid, 43% carbohydrate) according to the method of Beischer et al.<sup>10</sup>

**Statistical methods.** All data are expressed as mean ± SEM. Paired and, when appropriate, unpaired Student's *t* tests were used for comparison between the means.<sup>11</sup> Correlation coefficients were calculated by the least square method.<sup>11</sup>

TABLE 2  
Glucose control before the studies

|  |              | MBG*<br>(mmol/L)            | Basal insulin<br>requirement to<br>keep blood glucose<br>normal over the 60<br>min before the test<br>(mU/m <sup>2</sup> /min) | Basal plasma glucose before the test<br>(mmol/L) |           |           |
|--|--------------|-----------------------------|--|--|-----------|-----------|
|  |              |                             |  | -45 min  | -15 min   | 0 min     |
| Insulin infusion rate<br>(25 mU/m <sup>2</sup> /min) (N = 7) | Saline       | 5.53 ± 0.20<br>(15.9 ± 1.1) | 6.6 ± 2.1  | 4.2 ± 0.1  | 4.2 ± 0.1 | 4.3 ± 0.1 |
|  | α,β-Blockade | 5.26 ± 0.24<br>(16.5 ± 1.0) | 6.6 ± 2.1  | 4.3 ± 0.1  | 4.2 ± 0.1 | 4.3 ± 0.1 |
|  | β-Blockade   | 5.41 ± 0.28<br>(16.3 ± 1.5) | 6.6 ± 2.1  | 4.2 ± 0.1  | 4.3 ± 0.1 | 4.2 ± 0.1 |
| Insulin infusion rate<br>(32 mU/m <sup>2</sup> /min) (N = 8) | Saline       | 5.05 ± 0.23<br>(17.2 ± 1.2) | 6.5 ± 1.8  | 4.5 ± 0.1  | 4.4 ± 0.1 | 4.3 ± 0.1 |
|  | α,β-Blockade | 5.10 ± 0.18<br>(15.3 ± 1.6) | 6.5 ± 1.8  | 4.5 ± 0.1  | 4.4 ± 0.1 | 4.4 ± 0.1 |
|  | β-Blockade   | 5.32 ± 0.24<br>(17.2 ± 1.6) | 6.5 ± 1.8  | 4.4 ± 0.1  | 4.4 ± 0.1 | 4.4 ± 0.1 |

\* Mean blood glucose (Dextrometer) recorded during the overnight feedback insulin infusion. The mean number of the determinations is given in parentheses.

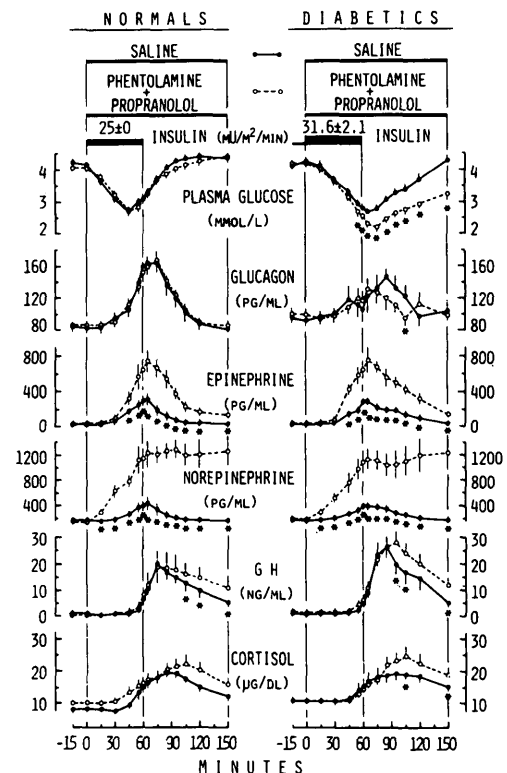
## RESULTS

**Baseline plasma glucose concentrations and insulin requirements (Table 2).** Mean blood glucose concentrations of the diabetic subjects and the amounts of insulin infused overnight prior to each study were comparable.

**Responses to infusion of insulin at 25 mU/m<sup>2</sup>/min in normal and diabetic subjects (Figure 1).** The mean basal plasma glucose of the diabetic subjects (4.27 ± 0.09 mmol/L) was not statistically different from that of the control subjects (4.16 ± 0.08 mmol/L, P = NS). During infusion of insulin, the plasma glucose of the diabetic subjects reached a nadir at 70 min (2.66 ± 0.12 mmol/L), which was not significantly different from the nadir of the nondiabetic subjects (2.72 ± 0.08 mmol/L, P = NS). However, the nadir in the nondiabetic subjects occurred earlier at 45 min. Thus, the rate of plasma glucose decrease, as calculated from baseline value to the nadir, was significantly slower in the diabetic subjects (0.0222 ± 0.0027 mmol/L/min) than in nondiabetic subjects (0.0327 ± 0.0011 mmol/L/min, P < 0.005). Plasma glucose increased to values not significantly different from basal values at 130 min in the diabetic subjects (3.88 ± 0.15 mmol/L) and at 85 min in the nondiabetic subjects (4.05 ± 0.04 mmol/L). Thus, the rate of plasma glucose recovery (from the nadir to the value which was no more statistically different from the baseline) was significantly slower in the diabetic subjects (0.0235 ± 0.0033 mmol/L/min) than in the nondiabetic subjects (0.0333 ± 0.0022, P < 0.05).

Although basal plasma glucagon levels in the diabetic subjects (93 ± 8 pg/ml) were not significantly different from those of the nondiabetic subjects (83 ± 7 pg/ml, P = NS), the plasma glucagon responses during hypoglycemia in the diabetic subjects were blunted compared with those observed in the nondiabetic subjects. A statistically significant increase in plasma glucagon was observed only at 85 and 95 min (146 ± 10 and 132 ± 18 pg/ml, P < 0.05 vs. basal) in the diabetic subjects. In contrast, plasma catecholamine, growth hormone, and cortisol responses were similar in both groups. Basal FFA values of the diabetic subjects (561 ± 13 meq/L) and of the nondiabetic subjects

(554 ± 24 meq/L, P = NS), as well as the plasma FFA nadirs following infusion of insulin (336 ± 12 in diabetic subjects vs. 336 ± 18 meq/L in control subjects, P = NS), were similar. Plasma FFA levels returned to basal values by 85 min in both groups and subsequently increased to peak values of 1034 ± 68 and 959 ± 43 meq/L at 150 min in the diabetic and nondiabetic subjects, respectively.



**FIGURE 1.** Effect of insulin infusion (25 mU/m<sup>2</sup>/min) for 60 min plus saline or alpha,beta-blockade on plasma glucose and counterregulatory hormone response in seven normals and seven type I diabetics. In diabetics, additional 6.6 ± 2.1 mU/m<sup>2</sup>/min insulin was infused (insulin requirement to keep blood glucose normal during the 60 min before the test). \*P < 0.05 vs. saline.

**Effect of combined alpha- and beta-adrenergic blockade (Figure 1).** When insulin was infused along with phentolamine plus propranolol in the diabetic subjects, plasma glucose concentrations decreased to a lower nadir ( $2.22 \pm 0.11$  vs.  $2.66 \pm 0.12$  mmol/L,  $P < 0.005$ ) and increased at a slower rate ( $0.0138 \pm 0.0022$  vs.  $0.0235 \pm 0.0033$  mmol/L/min,  $P < 0.001$ ) than when insulin alone was infused. Despite this more profound and prolonged hypoglycemia, there was no significant increase in plasma glucagon. In contrast, in the nondiabetic subjects, combined adrenergic blockade had no effect on plasma glucose and glucagon responses.

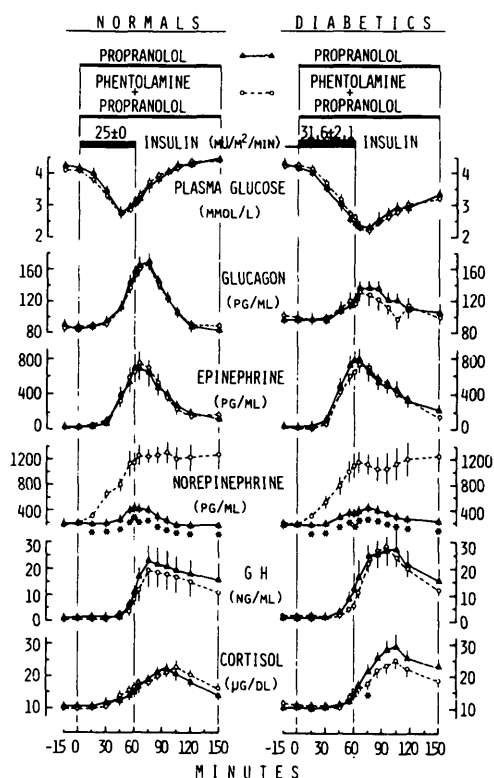
Combined adrenergic blockade increased plasma catecholamine and growth hormone responses in both groups. Cortisol responses were unaltered in the nondiabetic subjects. However, in the diabetic subjects, values at 105 and 150 min were greater than those observed during administration of insulin alone. Basal plasma FFA values and nadirs were unaltered in both groups. However, the increase above basal values that had been observed when insulin alone was infused did not occur in both groups during combined adrenergic blockade (diabetic subjects  $499 \pm 23$ ; nondiabetic subjects  $463 \pm 29$  at 150 min,  $P < 0.05$ ).

**Effect of isolated beta-adrenergic blockade (Figure 2).** In both diabetic and nondiabetic subjects, plasma glucose, FFA, glucagon, epinephrine, growth hormone, and cortisol responses during isolated beta-adrenergic blockade were not significantly different from those observed during combined alpha- and beta-adrenergic blockade. However, the

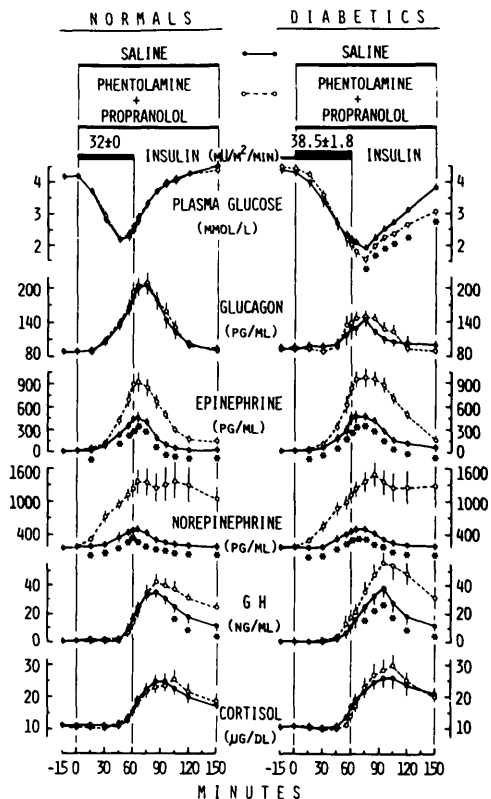
increased plasma norepinephrine responses observed during combined alpha- and beta-adrenergic blockade were no longer evident during isolated beta-adrenergic blockade.

**Responses to infusion of insulin at 32 mU/m<sup>2</sup>/min in normal and diabetic subjects (Figures 3, 4, and 5).** During this infusion of insulin, plasma glucose concentrations decreased slower in the diabetic subjects than in the nondiabetic subjects ( $0.0322 \pm 0.0016$  vs.  $0.0450 \pm 0.0016$  mmol/L/min,  $P < 0.001$ ) (Figure 3). The nadir of the diabetic subjects occurred later (75 min) than did that of the nondiabetic subjects (45 min) and was lower ( $1.88 \pm 0.07$  vs.  $2.16 \pm 0.08$  mmol/L,  $P < 0.01$ ). At 150 min, the plasma glucose concentration of the diabetic subjects was still below basal values ( $3.83 \pm 0.09$  vs.  $4.37 \pm 0.12$  mmol/L,  $P < 0.05$ ), whereas concentrations of the nondiabetic subjects had returned to basal values by 95 min. Thus, the recovery rate of plasma glucose was slower in the diabetic subjects (calculated from the nadir to 150 min) than in the nondiabetic subjects (calculated up to 95 min) ( $0.0255 \pm 0.0011$  vs.  $0.0355 \pm 0.0033$  mmol/L/min,  $P < 0.02$ ).

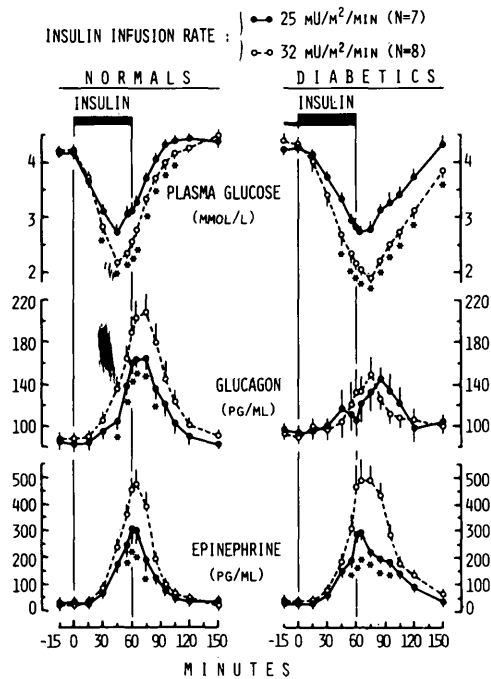
Plasma glucagon increased significantly in the diabetic subjects at 65 min from  $91 \pm 8$  to  $132 \pm 16$  pg/ml,  $P < 0.025$ , and remained significantly elevated at 75 and 85 min. However, the response was less than that observed in the nondiabetic subjects. The mean delta increase in the diabetic subjects (calculated as the difference between the peak and the basal value) was 47% of that of the nondia-



**FIGURE 2.** Effect of combined alpha, beta- and isolated beta-blockade on plasma glucose and counterregulatory hormone response during insulin infusion ( $25 \text{ mU/m}^2/\text{min}$ ) for 60 min in seven normals and seven type I diabetics. In diabetics, additional  $6.6 \pm 2.1 \text{ mU/m}^2/\text{min}$  insulin was infused (insulin requirement to keep blood glucose normal during the 60 min before the test). \* $P < 0.05$  vs. alpha, beta-blockade.



**FIGURE 3.** Effect of insulin infusion ( $32 \text{ mU/m}^2/\text{min}$ ) for 60 min plus saline or alpha, beta-blockade on plasma glucose and counterregulatory hormone response in eight normals and in eight type I diabetics. In diabetics, additional  $6.5 \pm 1.8 \text{ mU/m}^2/\text{min}$  insulin was infused (insulin requirement to keep blood glucose normal during the 60 min before the test). \* $P < 0.05$  vs. saline.

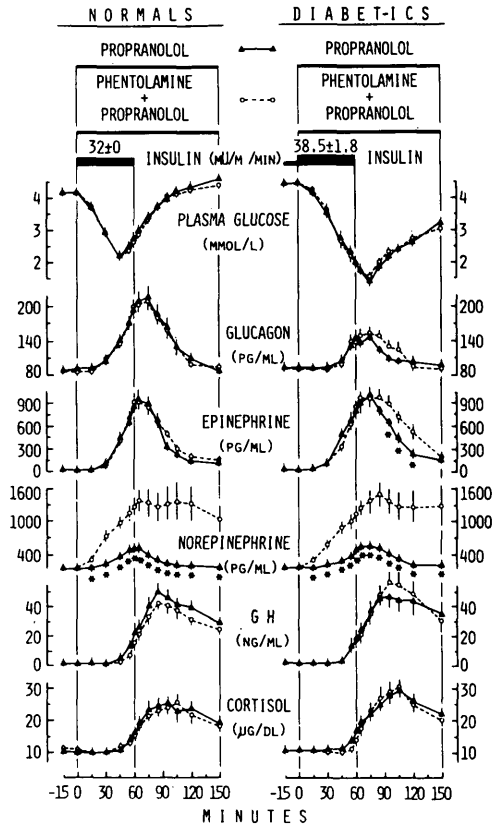


**FIGURE 4.** Effect of insulin infusion at two rates plus saline in normals and in type I diabetics on plasma glucose, glucagon, and epinephrine response. In diabetics, additional  $6.6 \pm 2.1$  or  $6.5 \pm 1.8$  mU/m<sup>2</sup>/min insulin was infused during the tests of insulin infusions at the rates of 25 and 32 mU/m<sup>2</sup>/min, respectively (insulin requirement to keep blood glucose normal during the 60 min before the test). \* $P < 0.05$  vs. 25 mU/m<sup>2</sup>/min.

betic subjects ( $P < 0.025$ ). Despite the lower plasma glucose values during the 32 mU/m<sup>2</sup>/min insulin infusion, the plasma glucagon response of the diabetic subjects did not differ significantly from that observed during the 25 mU/m<sup>2</sup>/min ( $P = NS$ ). In contrast, there was a twofold increase in plasma glucagon responses in the nondiabetic subjects ( $P < 0.05$ ) (Figure 4).

Plasma epinephrine increased more in the diabetic subjects than in the nondiabetic subjects (based on areas under the curve,  $26,427 \pm 3,597$  vs.  $17,933 \pm 2,401$  pg/ml<sup>-1</sup> · min<sup>-1</sup>;  $P < 0.05$ ). There was an inverse correlation between the increments in plasma glucagon and increments in plasma epinephrine during both insulin infusions in the diabetic subjects ( $r = -0.72$ ,  $P < 0.01$ ) but not in the nondiabetic subjects. Plasma norepinephrine, cortisol, and growth hormone responses were not significantly different in both groups. Basal plasma FFA values of diabetics ( $553 \pm 22$  meq/L) were not statistically different from those of nondiabetic subjects ( $555 \pm 18$  meq/L). In both groups the plasma FFA nadirs ( $228 \pm 21$  meq/L in diabetic subjects and  $294 \pm 18$  meq/L in nondiabetic subjects) as well as the rebound above baseline ( $1066 \pm 64$  meq/L in diabetic subjects and  $996 \pm 40$  meq/L in nondiabetic subjects) at 150 min were comparable.

**Effect of combined alpha- and beta-adrenergic blockade (Figure 3).** Combined adrenergic blockade did not alter plasma glucose responses in the nondiabetic subjects. In the diabetic subjects, however, the plasma glucose nadir was lowered ( $1.55 \pm 0.11$  vs.  $1.88 \pm 0.07$  mmol/L,  $P < 0.005$ ) by adrenergic blockade, and the rate of recovery of plasma glucose was further impaired ( $0.0018 \pm 0.0008$  vs.  $0.0255 \pm 0.0011$  mmol/L/min,  $P < 0.005$ ). In



**FIGURE 5.** Effect of combined alpha,beta- and isolated beta-blockade on plasma glucose and counterregulatory hormone response during insulin infusion (32 mU/m<sup>2</sup>/min) for 60 min in eight normals and in eight type I diabetics. In diabetics, additional  $6.5 \pm 1.8$  mU/m<sup>2</sup>/min insulin was infused (insulin requirement to keep blood glucose normal during the 60 min before the test). \* $P < 0.05$  vs. alpha,beta-blockade.

both groups, although plasma glucagon and cortisol responses were unaltered, plasma epinephrine, norepinephrine, and growth hormone responses were increased. Basal plasma FFA values and their nadirs were comparable in diabetic and nondiabetic subjects. Adequacy of adrenergic blockade was indicated by the fact that in both groups no increase above basal values was observed (diabetic subjects  $443 \pm 36$  vs.  $556 \pm 25$  meq/L; nondiabetic subjects  $448 \pm 16$  vs.  $563 \pm 12$  meq/L).

**Effect of beta-adrenergic blockade (Figure 5).** During the infusion of insulin and propranolol, plasma glucose, FFA, glucagon, epinephrine, cortisol, and growth hormone responses of both the diabetic and nondiabetic subjects were not significantly different from those observed during combined alpha- and beta-adrenergic blockade. However, in both groups the augmented plasma norepinephrine responses observed during combined adrenergic blockade were no longer evident.

## DISCUSSION

This study was undertaken to investigate the role of the adrenergic mechanisms in acute glucose counterregulation in diabetic man. Patients with type I diabetes have a blunted plasma glucagon response to insulin-induced hypoglycemia.<sup>12-18</sup> The results of the present study indicate that in such individuals catecholamines become essential for prompt restoration of normoglycemia and that this effect is mediated by beta-adrenoreceptors.

In the present study, a blunted glucagon response in diabetic subjects was accompanied by impaired recovery of plasma glucose concentrations from hypoglycemia, which was more evident during infusion of insulin at 32 mU/m<sup>2</sup>/min than at 25 mU/m<sup>2</sup>/min. Since the response of the other counterregulatory hormones to insulin-induced hypoglycemia seemed appropriate in the diabetic subjects, the impaired glucose recovery of the diabetic subjects could be accounted for by the deficient response in glucagon. The recent observation<sup>1,3</sup> that, in normal subjects, a selective suppression of the plasma glucagon response to insulin-induced hypoglycemia causes a greater decrease in plasma glucose and a severe impairment of recovery despite the normal response of the other counterregulatory hormones is consistent with this hypothesis. Since the diabetic subjects in our study also had a slower rate of blood glucose decrease and a delayed glycemic nadir, a role of a prolonged biologic activity of the insulin due to circulating anti-insulin antibodies cannot be excluded.

At both insulin infusion rates (25 and 32 mU/m<sup>2</sup>/min), combined alpha- and beta-adrenergic blockade (infusion of phentolamine and propranolol) caused more profound hypoglycemia and a further impairment in restoration of normoglycemia in the diabetic subjects, but did not alter plasma glucose responses in the nondiabetic subjects. These results thus suggest that in the diabetic subjects, catecholamines were at least partially compensating for the impaired plasma glucagon response. The fact that there was a highly significant inverse correlation between the residual plasma glucagon response and the plasma epinephrine responses in the diabetic subjects provides additional support for this concept. Thus, our results indicate that, in contrast to normal man,<sup>1-3</sup> in patients with type I diabetes, epinephrine appears to be the major acute glucose counterregulatory hormone and that glucagon, due to deficient A-cell responses during hypoglycemia, plays a minor role.

Isolated beta-adrenergic blockade resulted in the same impairment of glucose counterregulation in the diabetic subjects as did combined alpha- and beta-adrenergic blockade. These observations suggest that all of the adrenergic effects on glucose counterregulation could be solely accounted for by a beta-receptor mechanism. Our results are similar to those recently reported by Popp et al.,<sup>18</sup> who found that beta-adrenergic blockade attenuated recovery from insulin-induced hypoglycemia in type I diabetic subjects. Furthermore, our data are in accordance with those of Gerich et al.<sup>19</sup> who showed that the hyperglycemic action of epinephrine is beta-mediated in juvenile diabetics, and with those of Rizza et al.,<sup>20</sup> suggesting the same conclusion in normal subjects.

In the present study, during combined alpha- and beta-adrenergic blockade, plasma epinephrine and norepinephrine concentrations were markedly increased, whereas during isolated beta-adrenergic blockade, only plasma epinephrine concentrations were increased. These observations are consistent with the recent suggestions that beta-adrenergic blockade reduces the plasma clearance of epinephrine<sup>21</sup> and that pre-synaptic alpha-adrenergic blockade facilitates the release of norepinephrine.<sup>22</sup> Since these increases occurred in both the diabetic and nondiabetic subjects, and the latter had no changes in plasma glucose responses during adrenergic blockade, it is un-

likely that different degrees of hypoglycemia were responsible.

The increase in growth hormone did not differ statistically from that observed during alpha- and beta-blockade. This suggests that the adrenergic inhibition of growth hormone release in response to hypoglycemia is beta-mediated,<sup>23</sup> and that this effect overcomes the inhibition of growth hormone response to insulin-induced hypoglycemia reported during blockade of alpha-adrenoreceptors.<sup>23-25</sup>

Our observations regarding glucose counterregulation are pertinent to current attempts to optimize glucose control in insulin-dependent diabetes with intensified insulin regimens, either by multiple daily subcutaneous insulin injections or by continuous subcutaneous insulin infusions. These regimens may increase the risk of more severe or more frequent hypoglycemic reactions. Since catecholamines appear to be the primary hormonal defense against hypoglycemia in patients with insulin-dependent diabetes, care should be taken when using drugs that interfere with the hyperglycemic actions of catecholamines.

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#### REFERENCES

- Rizza, R. A., Cryer, P. E., and Gerich, J. E.: Role of glucagon, catecholamines, and growth hormone in human glucose counterregulation: effects of somatostatin and combined  $\alpha$ - and  $\beta$ -adrenergic blockade on plasma glucose recovery and glucose flux rates following insulin-induced hypoglycemia. *J. Clin. Invest.* 64:62-71, 1979.
- Clarke, W. L., Santiago, J. V., Thomas, L., Ben-Galim, E., Haymond, M. W., and Cryer, P. E.: Adrenergic mechanisms in recovery from hypoglycemia in man: adrenergic blockade. *Am. J. Physiol.* 236:147-52, 1979.
- Gerich, J., Davis, J., Lorenzi, M., Rizza, R., Bohannon, N., Karam, J., Lewis, S., Kaplan, R., Schultz, T., and Cryer, P.: Hormonal mechanisms of recovery from hypoglycemia in man. *Am. J. Physiol.* 236:380-85, 1979.
- Bolli, G., Calabrese, G., De Feo, P., Compagnucci, P., Zega, G., Angeletti, G., Cartechini, M. G., Santeusano, F., and Brunetti, P.: Lack of glucagon response in glucose counterregulation in type I (insulin dependent) diabetics: absence of recovery after prolonged optimal insulin therapy. *Diabetologia* 22:100-105, 1982.
- Faloon, G., and Unger, R.: Glucagon. In *Methods of Hormone Radioimmunoassay*. Jaffe, B., and Behrman, H., Eds. New York, Academic Press, 1974, pp. 317-30.
- Bolli, G., Cartechini, M. G., Compagnucci, P., Malvicini, S., DeFeo, P., Angeletti, G., Santeusano, F., and Brunetti, P.: Effect of metabolic control on urinary excretion and plasma levels of catecholamines in diabetics. *Horm. Metab. Res.* 11:493-97, 1979.
- Peake, G.: Growth hormone. In *Methods of Hormone Radioimmunoassay*. Jaffe, B., and Behrman, H., Eds. New York, Academic Press, 1974, pp. 103-21.
- Mattingly, D.: A simple fluorimetric method for the estimation of 11-hydroxycorticoids in human plasma. *J. Clin. Pathol.* 15:374-79, 1964.
- Dole, V. P., and Meinertz, H.: Microdetermination of long-chain fatty acids in plasma and tissues. *J. Biol. Chem.* 235:2595-99, 1960.
- Beischer, W., Keller, L., Maas, M., Schiefer, E., and Pfeiffer, E. F.: Human C-peptide, part I: radioimmunoassay. *Klin. Wochenschr.* 54:709-16, 1976.
- Armitage, P.: *Statistical Methods in Medical Research*. Oxford and Edinburgh, Blackwell, 1971, pp. 116-26, 147-66.
- Gerich, J., Langlois, M., Noacco, C., Karam, J. H., and Forsham, G. H.: The effect of beta-adrenergic blockade on the response to insulin-induced hypoglycemia in man. *Am. J. Physiol.* 236:147-52, 1979.

- P. H.: Lack of glucagon response to hypoglycemia in diabetics: evidence for an intrinsic pancreatic alpha-cell defect. *Science* 182:171-73, 1973.
- <sup>13</sup> Benson, J. V., Jr., Johnson, D. G., Palmer, J. P., Werner, P. L., and Ensink, J. W.: Glucagon and catecholamine secretion during hypoglycemia in normal and diabetic man. *J. Endocrinol. Metab.* 44:459-64, 1977.
- <sup>14</sup> Reynolds, C., Molnar, G. D., Horwitz, D. L., Rubenstein, A. H., Taylor, W. F., and Jiang, N. S.: Abnormalities of endogenous glucagon and insulin in unstable diabetes. *Diabetes* 26:36-45, 1977.
- <sup>15</sup> Campbell, L. V., Kraegen, E. W., and Lazarus, L.: Defective blood glucose counterregulation in diabetics in a selective form of autonomic neuropathy. *Br. Med. J.* 2:1527-29, 1977.
- <sup>16</sup> Ohneda, A., Watanabe, K., Horigome, K., Sakai, T., Kai, J., and Oikawa, S.: Abnormal response of pancreatic glucagon to glycemic changes in diabetes mellitus. *J. Clin. Endocrinol. Metab.* 46:504-10, 1978.
- <sup>17</sup> Viberti, G. C., Keen, H., and Bloom, S. R.: Beta blockade and diabetes mellitus: effect of oxprenolol and metoprolol on the metabolic, cardiovascular and hormonal response to insulin-induced hypoglycemia in insulin-dependent diabetics. *Metabolism* 29:873-79, 1980.
- <sup>18</sup> Popp, D. A., Shah, S. D., and Cryer, P. E.:  $\beta$ -adrenergic mechanisms in hypoglycemic glucose counterregulation and posthypoglycemic hyperglycemia in insulin dependent diabetes mellitus. *J. Clin. Invest.* 69:315-26, 1982.
- <sup>19</sup> Gerich, J., Lorenzi, M., Tsalikian, E., and Karam, J.: Studies on the mechanism of epinephrine-induced hyperglycemia in man. Evidence for participation of pancreatic glucagon secretion. *Diabetes* 25:65-71, 1976.
- <sup>20</sup> Rizza, R., Cryer, P., Haymond, M., and Gerich, J.: Adrenergic mechanisms for the effect of epinephrine on glucose production and clearance in man. *J. Clin. Invest.* 65:682-89, 1980.
- <sup>21</sup> Cryer, P., Rizza, R., Haymond, M., and Gerich, J.: Epinephrine and norepinephrine are cleared through beta-adrenergic but not alpha-adrenergic mechanisms in man. *Metabolism* 29(Suppl. 1):1114-18, 1980.
- <sup>22</sup> Cryer, P. E., Haymond, M. W., Santiago, J. V., and Shah, S. D.: Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated emodynamic and metabolic events. *N. Engl. J. Med.* 295:573-77, 1976.
- <sup>23</sup> Blackard, W. G., and Heidingsfelder, S. A.: Adrenergic receptor control mechanism for growth hormone secretion. *J. Clin. Invest.* 47:1407-14, 1968.
- <sup>24</sup> Imura, H., Kato, Y., Ikeda, M., Morimoto, M., and Yawata, M.: Effect of adrenergic-blocking or -stimulating agents on plasma growth hormone, immunoreactive insulin and blood free fatty acid level in man. *J. Clin. Invest.* 50:1069-79, 1971.
- <sup>25</sup> Ježová-Repčėková, D., Klimeš, I., Jurčovičová, J., and Vigaš, M.: Effect of adrenergic receptor blockade on cortisol and GH response to insulin-induced hypoglycemia in man. *Int. J. Clin. Pharmacol. Biopharm.* 17:64-67, 1979.