Elevated endogenous cortisol reduces autonomic neuroendocrine and symptom responses to subsequent hypoglycemia

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McGregor, Veronica P., Salomon Banarer, and Philip E. Cryer. Elevated endogenous cortisol reduces autonomic neuroendocrine and symptom responses to subsequent hypoglycemia. Am J Physiol Endocrinol Metab 282: E770–E777, 2002.—We tested the hypothesis that increased endogenous cortisol secretion reduces autonomic neuroendocrine and neurogenic symptom responses to subsequent hypoglycemia. Twelve healthy young adults were studied on two separate occasions, once after infusions of a pharmacological dose of α-(1–24)-ACTH (100 µg/h) from 0930 to 1200 and 1330 to 1600, which raised plasma cortisol levels to ~45 µg/dl on day 1, and once after saline infusions on day 1. Hyperinsulinemic (2.0 mU·kg⁻¹·min⁻¹) stepped hypoglycemic clamps (90, 75, 65, 55, and 45 mg/dl glucose steps) were performed on the morning of day 2 on both occasions. These markedly elevated antecedent endogenous cortisol levels reduced the adrenomedullary (P = 0.04, final plasma epinephrine levels of 489 ± 64 vs. 816 ± 113 pg/ml), sympathetic neural (P = 0.0022, final plasma norepinephrine levels of 244 ± 15 vs. 342 ± 22 pg/ml), parasympathetic neural (P = 0.0434, final plasma pancreatic polypeptide levels of 312 ± 37 vs. 424 ± 56 pg/ml), and neurogenic (autonomic) symptom (P = 0.0097, final symptom score of 7.1 ± 1.5 vs. 10.6 ± 1.6) responses to subsequent hypoglycemia. Growth hormone, but not glucagon or cortisol, responses were also reduced. The findings that increased endogenous cortisol secretion reduces autonomic neuroendocrine and neurogenic symptom responses to subsequent hypoglycemia are potentially relevant to cortisol mediation of hypoglycemia-associated autonomic failure, and thus a vicious cycle of recurrent iatrogenic hypoglycemia, in people with diabetes mellitus.

epinephrine; norepinephrine; glucagon; diabetes; hypoglycemia-associated autonomic failure

IATROGENIC HYPOGLYCEMIA is the limiting factor in the glycemic management of diabetes (4). It causes recurrent physical morbidity, and often psychosocial morbidity, in most patients with type 1 diabetes mellitus (T1DM) and in many with advanced type 2 diabetes mellitus (T2DM). It sometimes causes chronic disability and even premature death. Furthermore, because it precludes maintenance of true euglycemia over time, iatrogenic hypoglycemia limits full realization of the established microvascular benefits and the potential macrovascular benefits of aggressive glycemic therapy of diabetes (32, 33).

Iatrogenic hypoglycemia is typically the result of the interplay of relative or absolute insulin excess and compromised glucose counterregulation in people with diabetes (5). With respect to compromised defenses against developing hypoglycemia, the concept of hypoglycemia-associated autonomic failure (HAAF) (4, 6, 7) posits that episodes of recent antecedent iatrogenic hypoglycemia cause both defective glucose counterregulation (by reducing the epinephrine response to a given level of subsequent hypoglycemia in the setting of an absent glucagon response) and hypoglycemia unawareness (by reducing the autonomic and the resultant neurogenic symptom responses to a given level of subsequent hypoglycemia), and thus a vicious cycle of recurrent hypoglycemia. There is considerable support for this concept. Recent antecedent hypoglycemia has been shown to shift glycemic thresholds for autonomic (including epinephrine) and symptomatic responses to hypoglycemia to lower plasma glucose concentrations in T1DM (7, 14) and T2DM (29), to impair glycemic defense against hyperinsulinemia in T1DM (7), and to reduce detection of hypoglycemia in the clinical setting in T1DM (24). Perhaps the most compelling support for the concept of HAAF is the finding, in three independent laboratories (3, 8, 13), that hypoglycemia unawareness and, at least in part, the reduced epinephrine component of defective glucose counterregulation are reversible by as few as 2–3 wk of scrupulous avoidance of iatrogenic hypoglycemia in most affected patients.

The mediator(s) and mechanism(s) of HAAF are unknown (6). Davis and colleagues [Davis et al. (10, 11) and Galassetti et al. (16)] have suggested that the cortisol response to antecedent hypoglycemia mediates HAAF. That suggestion was based on their findings that prior cortisol infusion mimics the phenomenon (10) and that the absence of a cortisol response to prior hypoglycemia (in patients with primary adrenocortical...
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failure) minimizes the phenomenon (11). It has been supported by their finding that antecedent exercise, which releases cortisol, reduces many responses, including autonomic (but not symptomatic) responses, to subsequent hypoglycemia (16). However, we found prior exercise to have a substantially more limited impact on the responses to subsequent hypoglycemia (22).

The basic features of the experimental designs of the relevant studies (10, 11, 16, 22) are similar: an intervention, such as saline, hypoglycemia, cortisol infusion, or exercise, on day 1 and measurement of responses to hyperinsulinenic hypoglycemia on day 2. From a clinical and pathophysiological perspective, the end points directly relevant to HAAF are the adrenomedullary (plasma epinephrine), sympathetic neural (plasma norepinephrine, muscle sympathetic nerve activity), and neurogenic (autonomic) symptom responses to hypoglycemia (6, 7). The parasympathetic neural response (plasma pancreatic polypeptide) is of interest because it is the third component of the autonomic response, but it is not known to have an important role in glucose counterregulation per se or in the perception of hypoglycemia (5). The glucagon response is also of interest, because glucagon is a key counterregulatory hormone; however, the glucagon response to hypoglycemia is typically absent in T1DM (5) and advanced T2DM (29). Growth hormone and cortisol are also involved in defense against prolonged hypoglycemia (5).

There is consensus that day 1 hypoglycemia reduces adrenomedullary epinephrine (10, 11, 18), sympathetic neural norepinephrine (10, 11, 18), muscle sympathetic neural activity (10, 11), neurogenic symptom (11, 18), pancreatic polypeptide (10, 11, 18), and glucagon (10, 11, 18) responses to day 2 hypoglycemia in healthy subjects. Growth hormone responses have been found to be reduced (10, 11) or unaltered (18). Cortisol responses have also been found to be reduced (11, 18) or unaltered (10). Davis et al. (10) found that cortisol infusions [which raised plasma cortisol concentrations to ~32 μg/dl (~885 nmol/l)] on day 1 reproduced most of these effects: reduced epinephrine, norepinephrine, muscle sympathetic nerve activity, pancreatic polypeptide, and glucagon responses to hypoglycemia on day 2. Effects on symptomatic responses were not reported. Galassetti et al. (16) reported that two bouts of exercise [which raised plasma cortisol concentrations to ~21 μg/dl (~580 nmol/l) and ~16 μg/dl (~440 nmol/l)] on day 1 also reduced the epinephrine, norepinephrine, muscle sympathetic nerve activity, pancreatic polypeptide, and glucagon responses to hypoglycemia on day 2. Symptom responses were not reduced. Growth hormone, but not cortisol, responses were also reduced. In contrast, McGregor et al. (22) found that two bouts of exercise [which raised plasma cortisol concentrations to ~17 μg/dl (~470 nmol/l) and ~17 μg/dl (~470 nmol/l)] reduced the epinephrine response to hypoglycemia on day 2 by only ~30%; the norepinephrine, neurogenic symptom, pancreatic polypeptide, and glucagon responses were unaltered. Growth hormone, but not cortisol, responses were also reduced. Given these discrepancies, coupled with the fact that hypoglycemia raises plasma cortisol concentrations to only ~25 μg/dl (~690 nmol/l) (7, 10, 18, 22), we determined the impact of maximal stimulation of endogenous cortisol secretion by infusions of a pharmacological dose of ACTH during the day on responses to hypoglycemia the following morning in healthy subjects. Our primary hypothesis was that increased antecedent endogenous cortisol secretion, as opposed to antecedent cortisol infusion (10), reproduces all of the key components of HAAF: reduced adrenomedullary, sympathetic, neural, and neurogenic symptom responses to subsequent hypoglycemia. A secondary hypothesis was that increased antecedent endogenous cortisol secretion reduces pancreatic polypeptide, glucagon, and growth hormone, but not cortisol, responses to subsequent hypoglycemia. Although it would not establish the point, confirmation of our primary hypothesis is critical to the possibility that increased cortisol secretion mediates HAAF (10, 11, 16).

METHODS AND MATERIALS

Subjects. Twelve healthy young adults gave their informed consent to participate in this study, which was approved by the Washington University Human Studies Committee (Institutional Review Board) and conducted at the Washington University General Clinical Research Center (GCRC). Three of these adults were women, and nine were men. Their mean (±SD) age was 24.3 ± 5.2 yr, and their mean body mass index was 23.7 ± 3.7 kg/m².

Experimental design. Subjects were studied on two consecutive days on two separate occasions, separated by ≥2 wk, in random sequence: 1) infusions of α-(1–24)-ACTH (cosyn- tropin, Organon, West Orange, NJ), 100 μg/h, from 0930 to 1200 and from 1330 to 1600 on day 1 and hyperinsulinenic (2.0 mU·kg⁻¹·min⁻¹, 12.0 pmol·kg⁻¹·min⁻¹) stepped hypoglycemic clamps (hourly steps at 90, 75, 65, 55, and 45 mg/dl, 5.0, 4.2, 3.6, 3.1, and 2.5 mmol/l) on the morning of day 2; 2) saline infusions on day 1 and identical hyperinsulinenic stepped hypoglycemic clamps on day 2.

Before entry into the study, all potential subjects were screened to assure that they met the inclusion criteria: good health on the basis of medical history and physical examination and a normal hematocrit, fasting plasma glucose concentration, and electrocardiogram. On the α-(1–24)-ACTH and saline days (day 1), the subjects reported to the GCRC at ~0830, and a line was inserted into an antecubital vein. α-(1–24)-ACTH (100 μg/h) or saline was infused from 0930 to 1200 and again from 1330 to 1600. Blood samples were obtained at 0900, 0930, 1000, 1100, 1200, 1300, 1330, 1400, 1500, and 1600. A snack was provided at 1200. On the following day (day 2), the subjects reported to the GCRC, after an overnight fast, at ~0800. An intravenous line (for insulin and glucose infusions) and a line in a hand vein (with that hand kept in an ~55°C Plexiglas box for arterialized venous blood sampling) were inserted, and electrocardiogram leads and a vital signs monitor (Propaq Encore, Protocol Systems, Beaverton, OR) were attached. The subjects remained supine throughout the study. After 30 min of supine rest and starting at ~0900, regular insulin was infused in a dose of 2.0 mU·kg⁻¹·min⁻¹ (12.0 pmol·kg⁻¹·min⁻¹) from 0 through 300 min. Glucose (20%) was infused at variable rates on the basis of plasma glucose measurements with a glucose monitor.
oxidase method (Yellow Springs Analyzer 2, Yellow Springs Instruments, Yellow Springs, OH) every 5 min to maintain plasma glucose concentrations at target levels of 90, 75, 65, 55, and 45 mg/dl (5.0, 4.2, 3.6, 3.1, and 2.5 mmol/l) in hourly steps (28). Arterialized venous samples for analytes (given in Analytical methods) other than glucose and symptom scores were obtained at 30-min intervals throughout the experiment. Heart rates and blood pressures were recorded at 30-min intervals; the electrocardiogram was monitored throughout.

Analytical methods. Plasma insulin (20), glucagon (12), pancreatic polypeptide (17), growth hormone (27), and cortisol (15) were measured with radioimmunoassays. Plasma epinephrine and norepinephrine were measured with a single isotope derivative (radioenzymatic) method (30). Serum nonesterified fatty acids (19), blood β-hydroxybutyrate (25), lactate (21), and alanine (2) were measured with enzymatic methods. Symptoms of hypoglycemia were quantitated by asking the subjects to score (0, none, to 6, severe) each of 12 symptoms: six neurogenic symptoms (adrenergic: sweaty, hungry, and tingling) and six neuroglycopenic symptoms (difficulty thinking/confused, tired/drowsy, weak, warm, faint, and dizzy) on the basis of our published data (34).

Statistical methods. Data in this manuscript are reported as means ± SE except where the SD is specified. Data were analyzed by general linear model repeated-measures analysis of variance. P values <0.05 were considered to indicate statistical significance.

RESULTS

ACTH or saline on day 1. Infusions of α-(1–24)-ACTH raised plasma cortisol concentrations (means ± SE) from 13.2 ± 2.7 μg/dl (365 ± 75 nmol/l) at 0930 to 36.0 ± 3.6 μg/dl (995 ± 100 nmol/l) at 1200 and to 44.8 ± 3.1 μg/dl (1,235 ± 85 nmol/l) at 1600 (Fig. 1). Corresponding plasma cortisol levels during saline infusions were 14.1 ± 1.9 μg/dl (390 ± 50 nmol/l) at 0930, 10.5 ± 1.4 μg/dl (290 ± 40 nmol/l) at 1200, and 10.2 ± 1.1 μg/dl (280 ± 30 nmol/l) at 1600 (Fig. 1).

![Fig. 1. Plasma cortisol concentrations (means ± SE) before and during α-(1–24)-ACTH (●) or saline (○) infusions on day 1 and on the morning of day 2.](image)

Hyperinsulinemic stepped hypoglycemic clamps on day 2. Plasma insulin concentrations were raised comparably (to ~120 μU/ml, 720 pmol/l) to induce hypoglycemia, and plasma C-peptide concentrations decreased comparably (to <0.2 ng/ml, <0.1 nmol/l) during hypoglycemia on day 2 after α-(1–24)-ACTH or saline infusion on day 1 (Fig. 2). Target plasma glucose concentrations were achieved during the hyperinsulinemic stepped hypoglycemic clamps (Fig. 3); final plasma glucose concentrations were 47 ± 1 mg/dl (2.6 ± 0.1 mmol/l) on the day after α-(1–24)-ACTH and 46 ± 1 mg/dl (2.6 ± 0.1 mmol/l) on the day after saline. The glucose infusion rates required to maintain the hypoglycemic clamps were higher (P = 0.0245) on the day after α-(1–24)-ACTH (Fig. 3); the final glucose infusion rates were 4.1 ± 0.6 mg·kg⁻¹·min⁻¹ (23 ± 3 μmol·kg⁻¹·min⁻¹) on the day after α-(1–24)-ACTH and 2.9 ± 0.6 mg·kg⁻¹·min⁻¹ (16 ± 3 μmol·kg⁻¹·min⁻¹) on the day after saline.

Plasma epinephrine responses to hypoglycemia on day 2 were reduced (P = 0.0004) after α-(1–24)-ACTH on day 1 (Fig. 4). The final plasma epinephrine concentrations were 489 ± 64 pg/ml (2,670 ± 350 pmol/l) on the day after α-(1–24)-ACTH and 816 ± 113 pg/ml (4,450 ± 620 pmol/l) on the day after saline. Plasma norepinephrine responses to hypoglycemia on day 2 were also reduced (P = 0.0022) after α-(1–24)-ACTH on day 1 (Fig. 4). The final plasma norepinephrine concentrations were 244 ± 15 pg/ml (1.44 ± 0.09 nmol/l) on the day after α-(1–24)-ACTH and 342 ± 22 pg/ml (2.02 ± 0.13 nmol/l) on the day after saline.

Neurogenic symptom responses to hypoglycemia on day 2 were reduced (P = 0.0097) after α-(1–24)-ACTH on day 1 (Fig. 5). The final neuroglycopenic symptom scores were 7.1 ± 1.5 on the day after α-(1–24)-ACTH and 10.6 ± 1.6 on the day after saline. However, neuroglycopenic symptom responses to hypoglycemia on day 2 were not reduced significantly (P = 0.5786) after α-(1–24)-ACTH on day 1 (Fig. 5).
Symptom scores were 6.3 ± 2.2 on the day after α-(1–24)-ACTH and 7.5 ± 2.0 on the day after saline.

Plasma glucagon responses to hypoglycemia on day 2 were not reduced (P = 0.3397) after α-(1–24)-ACTH on day 1 (Fig. 6). The final plasma glucagon concentrations were 108 ± 18 pg/ml (31 ± 5 pmol/l) on the day after α-(1–24)-ACTH and 98 ± 9 pg/ml (28 ± 3 pmol/l) on the day after saline. Plasma pancreatic polypeptide responses to hypoglycemia on day 2 were reduced (P = 0.0434) after α-(1–24)-ACTH on day 1 (Fig. 6). The final plasma pancreatic polypeptide concentrations were 312 ± 37 pg/ml (75 ± 9 pmol/l) on the day after α-(1–24)-ACTH and 424 ± 56 pg/ml (101 ± 13 pmol/l) on the day after saline.

Plasma growth hormone responses to hypoglycemia on day 2 were reduced (P < 0.0001) after α-(1–24)-ACTH on day 1 (Fig. 7). The final plasma growth hormone concentrations were 16.3 ± 2.2 ng/ml (720 ± 100 pmol/l) on the day after α-(1–24)-ACTH and 23.1 ± 2.8 ng/ml (1,020 ± 120 pmol/l) on the day after saline. On the other hand, the plasma cortisol response to hypoglycemia on day 2 was not reduced significantly (P = 0.8126) on day 2 after α-(1–24)-ACTH on day 1 (Fig. 7). The final plasma cortisol concentrations were 22.2 ± 2.1 μg/dl (610 ± 60 nmol/l) on the day after α-(1–24)-ACTH and 24.2 ± 1.6 μg/dl (670 ± 45 nmol/l) on the day after saline.

Serum nonesterified fatty acid concentrations and blood β-hydroxybutyrate levels were suppressed comparably during hyperinsulinemic hypoglycemia on day 2 after α-(1–24)-ACTH or saline on day 1 (Table 1). β-Hydroxybutyrate levels were slightly lower (P = 0.0390) at the end of hypoglycemia on the day after α-(1–24)-ACTH (43 ± 6 μmol/l) than on the day after

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**Fig. 3.** Plasma glucose concentrations and glucose infusion rates (means ± SE) before and during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH (●) or saline (○) infusions on day 1.

**Fig. 4.** Plasma epinephrine and norepinephrine concentrations (means ± SE) before and during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH (●) or saline (○) infusions on day 1.

**Fig. 5.** Neurogenic (autonomic) and neuroglycopenic symptom scores (means ± SE) before and during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH (●) or saline (○) infusions on day 1.

**Fig. 6.** Plasma glucagon and pancreatic polypeptide concentrations (means ± SE) before and during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH (●) or saline (○) infusions on day 1.

**Fig. 7.** Plasma growth hormone concentrations and growth hormone infusion rates (means ± SE) before and during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH (●) or saline (○) infusions on day 1.
During hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH (●) or saline (○) infusions on day 1.

Saline (67 ± 11 µmol/l). Blood lactate responses to hypoglycemia on day 2 were not reduced (P = 0.4201) after α-(1–24)-ACTH on day 1 (Table 1). Blood alanine concentrations were also similar on both occasions (Table 1).

Heart rate (P = 0.2984), systolic blood pressure (P = 0.3606), and diastolic blood pressure (P = 0.1986) responses to hypoglycemia on day 2 were unaltered by α-(1–24)-ACTH on day 1 (Table 2).

**DISCUSSION**

These data indicate that markedly increased endogenous cortisol secretion reduces autonomic neuroendocrine and neurogenic symptom responses to subsequent hypoglycemia in healthy humans. Two 2.5-h infusions of a pharmacological dose of α-(1–24)-ACTH raised plasma cortisol concentrations to ~45 µg/dl (~1,240 nmol/l), 4.5-fold higher than the levels during saline infusion. During hyperinsulinemic stepped hypoglycemia on the day after α-(1–24)-ACTH infusions, compared with the day after saline infusions, adrenomedullary (plasma epinephrine), sympathetic neural (plasma norepinephrine), and parasympathetic neural (plasma pancreatic polypeptide) responses to hypoglycemia were reduced. As a result of the reduced autonomic responses [presumably the reduced adrenomedullary and sympathetic neural responses (34)], neurogenic (autonomic) symptom responses to hypoglycemia were also reduced. The reduced autonomic responses, specifically the reduced epinephrine response (5), was reflected biologically in that higher rates of glucose infusion were required to maintain the final hypoglycemic step (despite the fact that the glucagon response was not reduced). Notably, in contrast to the effects of antecedent hypoglycemia (7, 11, 14, 18, 24, 29), reduced neurogenic symptom responses to hypoglycemia after cortisol elevations per se, i.e., those produced by infusion of the hormone (10) or those produced by exercise (16, 22), have not been reported previously. Furthermore, although some effect, perhaps cortisol elevations, of antecedent exercise has been reported to reduce adrenomedullary, sympathetic neural, and parasympathetic neural (but not neurogenic symptom) responses to subsequent hypoglycemia in one study (16) but only the adrenomedullary response in another study (22), endogenous cortisol elevations per se have not been shown previously to reduce adrenomedullary, sympathetic neural, parasympathetic neural, and neurogenic symptom responses to subsequent hypoglycemia.

**Table 1. Serum nonesterified fatty acid and blood β-hydroxybutyrate, lactate, and alanine concentrations during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH or saline on day 1**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>-30</th>
<th>-15</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>240</th>
<th>270</th>
<th>300</th>
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<tbody>
<tr>
<td>Nonesterified fatty acids, µmol/l</td>
<td>After saline</td>
<td>339 ± 52</td>
<td>351 ± 50</td>
<td>322 ± 50</td>
<td>132 ± 22</td>
<td>72 ± 8</td>
<td>68 ± 10</td>
<td>65 ± 12</td>
<td>53 ± 12</td>
<td>56 ± 10</td>
<td>68 ± 10</td>
<td>80 ± 15</td>
<td>78 ± 13</td>
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<tr>
<td>After ACTH</td>
<td>532 ± 59</td>
<td>479 ± 53</td>
<td>475 ± 50</td>
<td>219 ± 77</td>
<td>93 ± 12</td>
<td>73 ± 6</td>
<td>64 ± 13</td>
<td>74 ± 12</td>
<td>64 ± 13</td>
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<td>β-Hydroxybutyrate,µmol/l</td>
<td>After saline</td>
<td>91 ± 12</td>
<td>80 ± 9</td>
<td>75 ± 6</td>
<td>63 ± 11</td>
<td>63 ± 8</td>
<td>82 ± 11</td>
<td>56 ± 10</td>
<td>48 ± 11</td>
<td>55 ± 12</td>
<td>59 ± 13</td>
<td>79 ± 12</td>
<td>58 ± 11</td>
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<tr>
<td>After ACTH</td>
<td>153 ± 31</td>
<td>123 ± 23</td>
<td>118 ± 20</td>
<td>92 ± 20</td>
<td>50 ± 9</td>
<td>47 ± 8</td>
<td>62 ± 11</td>
<td>62 ± 14</td>
<td>68 ± 12</td>
<td>43 ± 11</td>
<td>46 ± 8</td>
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<td>Lactate, µmol/l</td>
<td>After saline</td>
<td>948 ± 104</td>
<td>828 ± 110</td>
<td>831 ± 111</td>
<td>990 ± 88</td>
<td>1,358 ± 66</td>
<td>1,318 ± 119</td>
<td>1,071 ± 84</td>
<td>1,102 ± 110</td>
<td>1,245 ± 104</td>
<td>1,457 ± 154</td>
<td>1,543 ± 116</td>
<td>1,577 ± 151</td>
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<tr>
<td>After ACTH</td>
<td>992 ± 72</td>
<td>884 ± 83</td>
<td>873 ± 113</td>
<td>1,146 ± 125</td>
<td>1,435 ± 144</td>
<td>1,311 ± 135</td>
<td>1,174 ± 99</td>
<td>1,087 ± 100</td>
<td>1,240 ± 83</td>
<td>1,367 ± 95</td>
<td>1,433 ± 132</td>
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<td>(P &lt; 0.4201)</td>
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<td>Alanine, µmol/l</td>
<td>After saline</td>
<td>350 ± 50</td>
<td>360 ± 55</td>
<td>360 ± 58</td>
<td>306 ± 48</td>
<td>376 ± 51</td>
<td>385 ± 65</td>
<td>294 ± 42</td>
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<td>309 ± 39</td>
<td>294 ± 35</td>
<td>292 ± 34</td>
<td>274 ± 45</td>
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<tr>
<td>After ACTH</td>
<td>456 ± 46</td>
<td>452 ± 54</td>
<td>439 ± 63</td>
<td>424 ± 41</td>
<td>460 ± 59</td>
<td>415 ± 58</td>
<td>355 ± 47</td>
<td>327 ± 38</td>
<td>341 ± 37</td>
<td>356 ± 39</td>
<td>302 ± 33</td>
<td>316 ± 36</td>
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<td>(P &lt; 0.1504)</td>
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Values are means ± SE.
The mechanism(s) by which cortisol elevations shift the glycemic thresholds for autonomic and symptomatic responses to subsequent hypoglycemia to lower plasma glucose concentrations remains to be established. It is likely a direct result of actions of cortisol on key centers in the brain (10). Evidence that antecedent central nervous system infusion of cortisol, but not dexamethasone, reduces autonomic responses to subsequent hypoglycemia in rats has been presented (9). On the other hand, evidence that intracerebroventricular administration of cortisosterone did not reproduce the phenomenon in rats has also been presented (23). These findings are consistent with the suggestion of Davis and colleagues (9–11, 16) that, in people with diabetes, the cortisol response to recent antecedent iatrogenic hypoglycemia mediates HAAF and thus a vicious cycle of recurrent iatrogenic hypoglycemia (3, 4, 6–8, 13, 14, 24, 29). They do not, however, establish that point. The cortisol elevations produced in the present study (~45 μg/dl, ~1,240 nmol/l) and in the cortisol infusion study (~32 μg/dl, ~885 nmol/l) (10) were higher than those that occur normally during hypoglycemia (~25 μg/dl, ~690 nmol/l) (7, 10, 18, 22). Therefore, it remains conceivable that both the exogenous (10) and the endogenous (present data) cortisol elevations demonstrated to reduce autonomic responses, and the latter to reduce neurogenic symptomatic responses, to subsequent hypoglycemia were pharmacological from the perspective of antecedent hypoglycemia. Clearly, additional studies will be required to establish that endogenous cortisol elevations comparable to those that occur during hypoglycemia reduce autonomic and neurogenic symptom responses to subsequent hypoglycemia. Nonetheless, the present documentation that increased endogenous cortisol secretion, albeit to very high plasma cortisol concentrations, reproduces the key features of HAAF, reduced adrenomedullary, sympathetic neural, and neurogenic symptom responses to subsequent hypoglycemia, is a critical prerequisite to the possibility that the cortisol response to antecedent iatrogenic hypoglycemia mediates HAAF in people with diabetes. It is, of course, conceivable that factors in addition to the cortisol response might be involved.

In contrast to the effect to reduce autonomic and sympathetic responses, these marked antecedent cortisol elevations did not reduce the glucagon response to subsequent hypoglycemia. There is agreement that recent antecedent hypoglycemia reduces the glucagon response to subsequent hypoglycemia (10, 11, 18). Galassetti et al. (16) reported that antecedent exercise, which releases cortisol, reduced the glucagon response to subsequent hypoglycemia. However, we found no effect of seemingly similar antecedent exercise on the glucagon response (22) and, in the present study, despite marked antecedent cortisol elevations, we again find no effect on the glucagon response to subsequent hypoglycemia. This finding of an intact glucagon response despite a reduced autonomic (adrenomedullary, sympathetic, and parasympathetic) response indicates that signals in addition to autonomic inputs (31) have key roles in the mechanisms of the pancreatic α-cell glucagon secretory response to hypoglycemia. Those factors might include low α-cell glucose concentrations per se, intraislet hypoinsulinemia, or both (1, 26). It also suggests that factors in addition to the cortisol response mediate the effects of antecedent hypoglycemia to reduce some of the responses, specifically the glucagon response, to subsequent hypoglycemia.

The effect of antecedent cortisol elevations was not, however, limited to the autonomic and sympathetic responses. For example, the growth hormone response to subsequent hypoglycemia were also reduced. This is consistent with most (10, 11), but not all (18), earlier studies of the effect of antecedent hypoglycemia and earlier studies of the effect of antecedent exercise (16, 22). Interestingly, marked antecedent cortisol elevations did not reduce the cortisol response to subsequent hypoglycemia. Cortisol responses to hypoglycemia have been reported to be reduced (11, 18) or unaltered (10) after hypoglycemia and unaltered after exercise (16, 22). The mechanism of the dissociation of

### Table 2. Heart rate and systolic and diastolic blood pressures during hyperinsulinemic stepped hypoglycemic clamps on day 2 after α-(1–24)-ACTH or saline on day 1

|                     | Time, min | -30 | -15 | 0   | 30  | 60  | 90  | 120 | 150 | 180 | 210 | 240 | 270 | 300 |
|---------------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **Heart rate, beats/min** |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| After saline        |           | 68 ± 2 | 71 ± 2 | 67 ± 3 | 68 ± 2 | 68 ± 2 | 71 ± 2 | 75 ± 3 | 76 ± 2 | 79 ± 3 | 79 ± 3 | 82 ± 3 | 85 ± 3 | 81 ± 3 |
| After ACTH          | (P=0.2984)| 67 ± 3 | 66 ± 2 | 66 ± 1 | 70 ± 3 | 71 ± 2 | 72 ± 1 | 71 ± 2 | 73 ± 3 | 72 ± 2 | 78 ± 3 | 80 ± 4 | 77 ± 2 | 81 ± 5 |
| **Systolic blood pressure, mmHg** |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| After saline        |           | 122 ± 3 | 120 ± 2 | 118 ± 2 | 120 ± 3 | 120 ± 3 | 122 ± 4 | 120 ± 3 | 124 ± 3 | 120 ± 3 | 123 ± 3 | 118 ± 3 | 116 ± 3 | 122 ± 4 |
| After ACTH          | (P=0.3606)| 122 ± 2 | 122 ± 3 | 121 ± 3 | 125 ± 3 | 124 ± 3 | 123 ± 3 | 119 ± 3 | 122 ± 2 | 121 ± 3 | 120 ± 3 | 117 ± 5 | 118 ± 4 | 121 ± 5 |
| **Diastolic blood pressure, mmHg** |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| After saline        |           | 66 ± 2 | 65 ± 2 | 66 ± 2 | 62 ± 2 | 63 ± 1 | 62 ± 2 | 63 ± 2 | 60 ± 2 | 56 ± 1 | 57 ± 2 | 55 ± 1 | 53 ± 2 | 57 ± 2 |
| After ACTH          | (P=0.1986)| 66 ± 2 | 66 ± 3 | 68 ± 3 | 66 ± 2 | 63 ± 1 | 63 ± 2 | 59 ± 3 | 65 ± 2 | 61 ± 3 | 57 ± 2 | 56 ± 2 | 56 ± 3 | 60 ± 4 |

Values are means ± SE.
the effects of cortisol elevation on the growth hormone and the cortisol responses to subsequent hypoglycemia found in the present study is unknown.

Aside from a slightly reduced blood β-hydroxybutyrate level at the end of the hyperinsulinemic stepped hypoglycemic clamps, we found no significant effects of marked antecedent cortisol elevations on the levels of the intermediary metabolites measured during subsequent hypoglycemia. However, serum nonesterified fatty acid and blood β-hydroxybutyrate concentrations were suppressed markedly under the hyperinsulinemic conditions of the present study. Blood lactate responses to hypoglycemia have been reported to be decreased after hypoglycemia (10, 11, 18) and increased (16) or unchanged (22) after exercise. They were unaltered by antecedent cortisol elevations in the present study.

In summary, the present data indicate that markedly elevated plasma cortisol levels produced by stimulation of endogenous cortisol secretion, like substantial cortisol elevations produced by infusion of cortisol (10), reduce autonomic neuroendocrine responses to subsequent hypoglycemia in healthy humans. Endogenous cortisol elevations also reduced neurogenic symptom responses to subsequent hypoglycemia. These findings are potentially relevant to the media tors of hypoglycemia-associated autonomic failure, and thus a vicious cycle of recurrent iatrogenic hypoglycemia, in people with diabetes. Further studies will, however, be required to establish the latter role of cortisol unequivocally.

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