Cortisol Elevations Comparable to Those That Occur During Hypoglycemia Do Not Cause Hypoglycemia-Associated Autonomic Failure

Bharathi Raju, Veronica P. McGregor, and Philip E. Cryer

The concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes posits that recent antecedent iatrogenic hypoglycemia causes both defective glucose counterregulation (by reducing the epinephrine response in the setting of an absent glucagon response) and hypoglycemia unawareness (by reducing the autonomic–sympathetic neural and adrenomedullary response and the resulting neurogenic [autonomic] symptom responses) and thus causes a vicious cycle of recurrent hypoglycemia. To assess the suggestion that it is the cortisol response to antecedent hypoglycemia that mediates HAAF, we tested the hypothesis that plasma cortisol elevations during euglycemia that are comparable to those that occur during hypoglycemia reduce sympathoadrenal and neurogenic symptom responses to subsequent hypoglycemia. To do this, 12 healthy subjects were studied with hyperinsulinemic–stepped hypoglycemic clamps the day after saline or cortisol (1.3 ± 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) infusions from 0930 to 1200 and from 1330 to 1600. Compared with saline, antecedent cortisol elevations did not reduce the sympathoadrenal (e.g., final plasma epinephrine levels of 674 ± 84 vs. 606 ± 80 pg/ml and final plasma norepinephrine levels of 332 ± 26 vs. 304 ± 26 pg/ml) or neurogenic symptom (e.g., final scores of 9.3 ± 1.1 vs. 13.2 ± 1.3) responses to subsequent hypoglycemia. Thus, these data do not support the suggestion that cortisol mediates HAAF. *Diabetes* 52:2083–2089, 2003

Iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes (1). It is typically the result of the interplay of relative or absolute insulin excess and compromised physiological and behavioral defenses against developing hypoglycemia in type 1 diabetes (1,2) and advanced type 2 diabetes (1,3). The concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes posits that recent antecedent iatrogenic hypoglycemia causes 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–sympathetic neural as well as adrenomedullary response and the resulting neurogenic [autonomic] symptom responses to a given level of subsequent hypoglycemia) and thus causes a vicious cycle of recurrent iatrogenic hypoglycemia (1–4). The clinical impact of HAAF, including the finding that as little as 2–3 weeks of scrupulous avoidance of hypoglycemia reverses hypoglycemia unawareness and improves the reduced epinephrine component of defective glucose counterregulation in most affected patients is well documented (1,4). However, the mediator(s) and mechanisms(s) of HAAF are unknown (1,4).

Davis and colleagues have suggested that the cortisol response to antecedent hypoglycemia mediates HAAF (5–7). They found that cortisol infusions, which raised mean plasma cortisol concentrations to ~32 μg/dl, reduced autonomic neuroendocrine (among other) responses to hypoglycemia the following day in healthy subjects (5); effects on symptom responses were not reported (5). They also found that deficient cortisol secretion (in patients with primary adrenocortical failure) minimized the effects of antecedent hypoglycemia (6). These investigators presented evidence that intracerebroventricular cortisone (7) or cortisol (8), but not dexamethasone (8), reduced plasma epinephrine and norepinephrine responses to subsequent hypoglycemia. They also found that intravenous dehydroepiandrosterone, a putative glucocorticoid antagonist, blocked the effect of antecedent hypoglycemia to reduce these responses to subsequent hypoglycemia (9) in rats. Furthermore, the effect of subcutaneous 2-deoxyglucose to reduce the feeding and hyperglycemic responses to cellular glucopenia produced by the administration of 2-deoxyglucose 6 h later was not observed in adrenalectomized rats and was mimicked by prior subcutaneous dexamethasone (rather than 2-deoxyglucose) administration (10). However, others reported that intracerebroventricular (11), intravenous (12), or subcutaneous (13) corticosterone did not reduce plasma epinephrine or norepinephrine responses to subsequent hypoglycemia in rats.

In addition, Galassetti et al. (14) reported that two bouts of exercise, which raised mean plasma cortisol levels to ~21 and ~16 μg/dl, respectively, reduced autonomic responses to hypoglycemia the following day in healthy subjects. We found antecedent exercise, which raised mean plasma cortisol levels to ~17 μg/dl after both bouts

From the Division of Endocrinology, Metabolism and Lipid Research, Washington University School of Medicine, St. Louis, Missouri; the General Clinical Research Center, Washington University School of Medicine, St. Louis, Missouri; and the Diabetes Research and Training Center, Washington University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to Philip E. Cryer, Campus Box 8127, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110. E-mail: pcryer@im.wustl.edu.

Received for publication 13 February 2003 and accepted in revised form 22 April 2003.

B.R. and V.P.M. contributed equally to this work.

HAAF, hypoglycemia-associated autonomic failure.

of exercise, to have a more limited impact (15). Among the responses to subsequent hypoglycemia, only the epinephrine response was reduced, and that by only ~30%, the day after exercise. Symptom responses to hypoglycemia were not reduced in either study (14,15). These findings led us to question the basic phenomenon. However, we found that marked antecedent cortisol elevations, to ~36 and ~45 μg/dl, produced by infusions of a pharmacological dose of α_{1-24}ACTH in healthy subjects, reduced sympathoadrenal (plasma epinephrine and norepinephrine) and neurogenic symptom responses to hypoglycemia the following day (16). These data supported the phenomenon but did not establish that antecedent cortisol elevations comparable to those that occur during hypoglycemia, a mean of ~26 μg/dl (2,6,15–17), reproduce the reduced autonomic neuroendocrine and neurogenic symptom responses to hypoglycemia that characterize HAAF in people with diabetes.

Accordingly, we tested the hypothesis that plasma cortisol elevations during euglycemia that are comparable to those that occur during hypoglycemia reduce the sympathoadrenal responses and the resultant neurogenic symptom responses to subsequent hypoglycemia. These are key features of defective glucose counterregulation and hypoglycemia unawareness, and thus the concept of HAAF in diabetes and, therefore, that the cortisol response to antecedent hypoglycemia mediates, at least in part, HAAF in people with diabetes. To do this, we infused cortisol, in doses lower than those used by Davis et al. (5), or saline intravenously on day 1 and measured the neuroendocrine and symptom responses to hyperinsulinemic–stepped hypoglycemic clamps on day 2 in 12 healthy volunteers.

RESEARCH DESIGN AND METHODS

Subjects. A total of 12 healthy young adults (5 women and 7 men) aged 26 ± 7 years (mean ± SD) and with a BMI of 23.0 ± 2.6 kg/m^2 gave their written consent to participate in this study, which was approved by the Washington University Medical Center Human Studies Committee and was conducted at the Washington University General Clinical Research Center. They were in good health as assessed by medical history and physical examination and had normal fasting plasma glucose concentrations, hematocrits, and electrocardiograms. Aside from estrogen-progestin oral contraceptives, used by two of the women, they took no medications.

Experimental design. Each subject participated in two studies, separated by at least 2 weeks, that involved 2 consecutive study days. On day 1, in random sequence, they received either saline or cortisol (SoluCortef; Pharmacia and Upjohn, Kalamazoo, MI) infusions from 0930 to 1200 and from 1330 to 1600. The cortisol infusion rate (means ± SD) was 1.3 ± 0.2 μg · kg^{-1} · min^{-1}. A dose of 1.4 μg · kg^{-1} · min^{-1} was used in seven subjects, 1.2 μg · kg^{-1} · min^{-1} in three subjects, and 1.0 μg · kg^{-1} · min^{-1} in two subjects. The initial dose used (1.4 μg · kg^{-1} · min^{-1}) was calculated from the dose of 2.0 μg · kg^{-1} · min^{-1} used by Davis et al. (5) in an attempt to produce peak plasma cortisol concentrations of ~26 μg/dl rather than those of ~32 μg/dl produced by Davis et al. (5). Because our target was exceeded in the first seven subjects, lower doses were used in the remaining five subjects.

On day 2 on both occasions, hyperinsulinemic–stepped hypoglycemic clamps (18) were performed after an overnight fast. Insulin was infused in a dose of 2.0 mU · kg^{-1} · min^{-1} for 5 h, and variable infusions of 20% dextrose, based on plasma glucose measurements with a glucose oxidase method (Yellow Springs Analyzer 2; Yellow Springs Instruments, Yellow Springs, OH) every 5 min, were used to clamp plasma glucose concentrations at ~85, 75, 65, 55, and 45 mg/dl in hourly steps. Insulin and glucose were infused through a catheter in an antecubital vein. Arterialized venous samples (for the analytes listed below) were drawn through an indwelling line in a dorsal hand vein, with that hand kept in a ~55°C plexiglass box. Samples were drawn at −30, −15, 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min. At the same time points, the subjects scored (from 0 [none] to 6 [severe]) each of 12 symptoms: 6 neurogenic (autonomic) symptoms (adrenergic: heart pounding, shaky/tremulous, and nervous/anxious; cholinergic: sweaty, hungry, and tingling) and 6 neuroglycopenic symptoms (difficulty thinking/confused, tired/drowsy, weak, warm, faint, and dizzy) based on our published data (19). Heart rates and blood pressures (Propaq Encore; Protocol Systems, Beaverton, OR) were recorded serially, and the electrocardiogram was monitored throughout.

Analytical methods. Plasma insulin (20), C-peptide (20), pancreatic polypeptide (21), glucagon (22), growth hormone (23), and cortisol (24) concentrations were measured with radioimmunoassays, plasma epinephrine, and norepinephrine concentrations with a single isotope–derivative (radioenzymatic) method (25). Serum nonesterified fatty acid (26), blood lactate (27), β-hydroxybutyrate (28), and alanine (29) concentrations were measured with enzymatic methods.

Statistical methods. Data are reported as the means ± SE, except where the SD is specified. The data were analyzed by repeated-measures ANOVA. P values <0.05 were considered to indicate statistically significant differences.

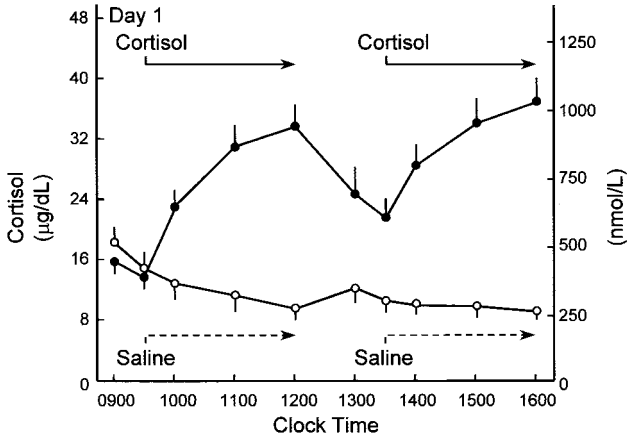

FIG. 1. Plasma cortisol concentrations (means ± SE) during two 2.5-h cortisol (●) or saline (○) infusions on day 1 of each study occasion.

RESULTS

Day 1, cortisol or saline infusions. On day 1, 2.5-h cortisol infusions raised plasma cortisol concentrations to 33.8 ± 3.0 μg/dl at 1200 and 37.0 ± 3.6 μg/dl at 1600 compared with corresponding levels of 9.6 ± 1.6 and 9.1 ± 1.1 μg/dl, respectively, during saline infusions (Fig. 1). When the data from the two subjects taking oral contraceptives were excluded, the peak plasma cortisol concentrations were 30.9 ± 2.2 μg/dl at 1200 and 33.7 ± 3.2 μg/dl at 1600 on the cortisol-infusion days.

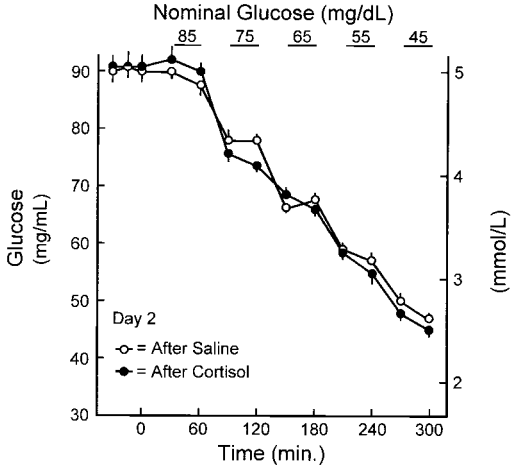

FIG. 2. Plasma glucose concentrations (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

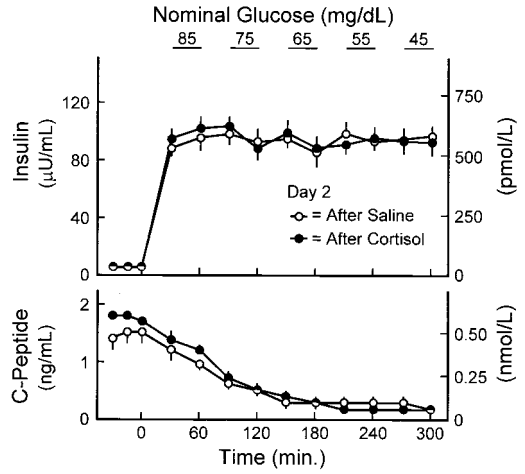

FIG. 3. Plasma insulin and C-peptide concentrations (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

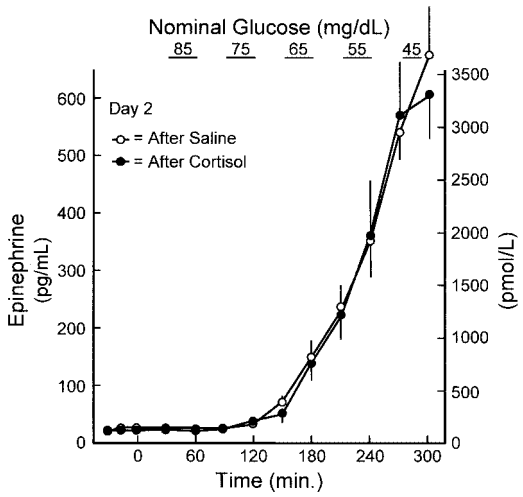

FIG. 5. Plasma epinephrine concentrations (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

Day 2, hyperinsulinemic–stepped hypoglycemic clamps. Plasma glucose targets were approximated and glucose concentrations were similar on the day after saline infusion and the day after cortisol infusion (Fig. 2). Plasma insulin and C-peptide concentrations (Fig. 3) and the glucose infusion rates required to maintain the glucose steps (Fig. 4) were similar on both occasions.

Plasma epinephrine (Fig. 5) and norepinephrine (Fig. 6) responses to hypoglycemia were not reduced on the day after cortisol infusion. Neurogenic (Fig. 7) and neuroglycopenic (Fig. 8) symptom scores were also not reduced on the day after cortisol infusion; indeed, they were increased ($P = 0.0015$ and 0.0104, respectively). Plasma pancreatic polypeptide, glucagon, growth hormone, and cortisol responses to hypoglycemia were unaltered on the day after cortisol infusion (Table 1). Serum nonesterified fatty acid, blood lactate, β-hydroxybutyrate, and alanine concentrations were similar on both occasions (Table 2). Similarly, heart rates, systolic blood pressures, and diastolic blood pressures did not differ (Table 3).

DISCUSSION

These data do not support the suggestion that the cortisol response to antecedent iatrogenic hypoglycemia mediates HAAF in diabetes (5–10). Compared with intravenous infusions of saline, intravenous infusions of cortisol that raised plasma cortisol concentrations during euglycemia to (and indeed above) levels that occur during hypoglycemia did not reduce the sympathoadrenal (plasma epinephrine and norepinephrine) or neurogenic (autonomic) symptom responses to hypoglycemia the following day. Thus, these physiological cortisol elevations did not reproduce the key features of HAAF in diabetes (1–4).

Using a generically similar experimental design—interventions on day 1 and quantitation of the responses during hyperinsulinemic-hypoglycemic clamps on day 2—antecedent hypoglycemia (rather than cortisol elevations in the absence of hypoglycemia) has consistently been found to reduce autonomic neuroendocrine and neurogenic symptom responses to a given level of subsequent hypoglycemia in healthy subjects (17) and patients with type 1

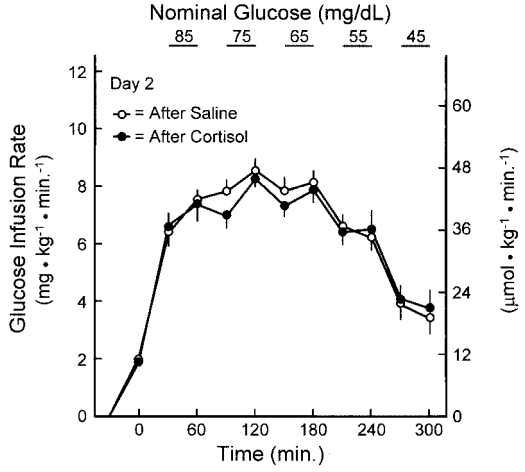

FIG. 4. Glucose infusion rates (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

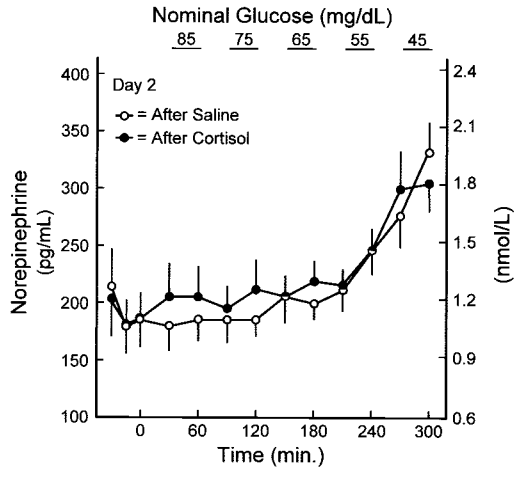

FIG. 6. Plasma norepinephrine concentrations (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

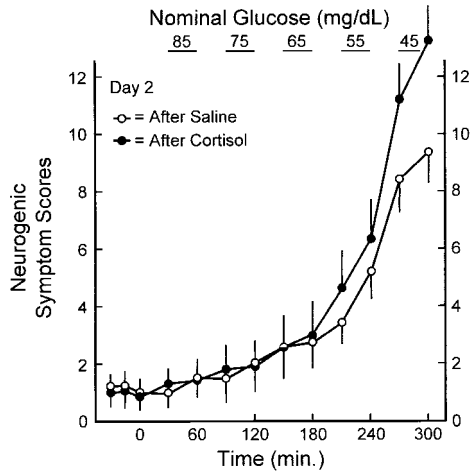

FIG. 7. Neurogenic (autonomic) symptom scores (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

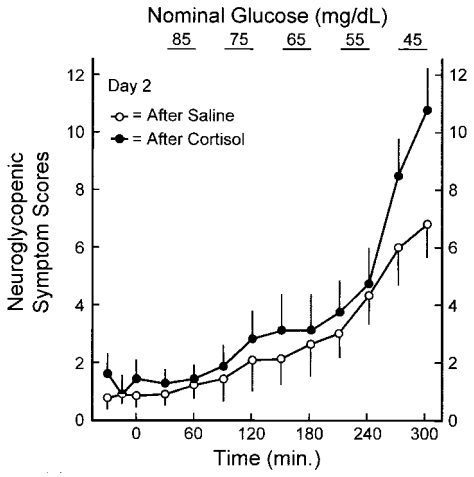

FIG. 8. Neuroglycopenic symptom scores (means ± SE) during hyperinsulinemic–stepped hypoglycemic clamps on day 2 after saline infusions on day 1 (○) and on day 2 after cortisol infusions on day 1 (●).

diabetes (2) as well as those with advanced type 2 diabetes (3). In patients with type 1 diabetes, recent antecedent hypoglycemia has been shown to shift glycemic thresholds for autonomic, neurogenic symptom, and cognitive dysfunction responses to subsequent hypoglycemia to lower plasma glucose concentrations (2,30), impair glycemic defense against hyperinsulinemia (2), and reduce detection of hypoglycemia in the clinical setting (31). Given the present findings, one cannot attribute these effects of antecedent hypoglycemia to the cortisol response to that hypoglycemia.

We infused lower doses of cortisol on day 1 than Davis et al. (5) because we intended to produce mean cortisol elevations comparable to those that occur during hypoglycemia (~26 μg/dl) (2,6,8,10). They infused cortisol intravenously in a dose of 2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and produced mean peak plasma cortisol concentrations of ~32 μg/dl (5). Based on those data, we started with a cortisol infusion dose of 1.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($n = 7$) and then doses of 1.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($n = 3$) and 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($n = 2$); the cortisol dose (mean ± SD) was 1.3 ± 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Despite these lower cortisol doses, peak cortisol concentrations (mean ± SE) were 33.8 ± 3.0 μg/dl at the end of the first infusion and 37.0 ± 3.6 μg/dl at the end of the second infusion. If the data from two women using estrogen-containing oral contraceptives (which raise corticosteroid binding globulin and thus total plasma cortisol concentrations) were excluded, the corresponding mean peak cortisol concentrations were 30.9 ± 2.2 and 33.7 ± 3.2 μg/dl, respectively. Despite these somewhat supraphysiological (with respect to hypoglycemia) cortisol elevations on day 1, sympathoadrenal and neurogenic symptom responses to hypoglycemia were not reduced on day 2.

We used hyperinsulinemic–stepped hypoglycemic clamps, with hourly steps of ~85, 75, 65, 55, and 45 μg/dl, to assess autonomic neuroendocrine and neurogenic symptom responses to hypoglycemia on day 2. Davis et al. (5) used hyperinsulinemic–single-step (to ~50 μg/dl for 2 h) hypoglycemic clamps and generally analyzed re-

TABLE 1
Plasma pancreatic polypeptide, glucagon, growth hormone, and cortisol concentrations during hyperinsulinemic–stepped hypoglycemic (85, 75, 65, 55, and 45 mg/dl) clamps on day 2 after saline or cortisol infusions on day 1

Time (min)	Pancreatic polypeptide (pg/ml)		Glucagon (pg/ml)		Growth hormone (ng/ml)		Cortisol (μg/dl)	
	After saline	After cortisol	After saline	After cortisol	After saline	After cortisol	After saline	After cortisol
−30	81 ± 12	71 ± 10	77 ± 5	75 ± 9	3.6 ± 1.6	3.2 ± 1.3	16.5 ± 2.0	11.3 ± 1.5
−15	80 ± 11	78 ± 16	76 ± 5	76 ± 6	3.9 ± 1.8	2.7 ± 1.1	14.8 ± 1.8	9.3 ± 1.2
0	62 ± 7	68 ± 13	74 ± 5	70 ± 6	3.4 ± 1.5	2.5 ± 0.8	14.0 ± 2.1	8.8 ± 1.1
30	60 ± 8	63 ± 8	66 ± 5	64 ± 6	4.7 ± 1.6	1.1 ± 0.3	13.0 ± 2.0	8.3 ± 1.0
60	58 ± 7	54 ± 6	61 ± 5	58 ± 6	2.9 ± 0.9	2.0 ± 1.3	12.7 ± 2.1	8.5 ± 0.9
90	67 ± 13	59 ± 10	62 ± 4	61 ± 6	1.0 ± 0.3	1.8 ± 1.2	11.4 ± 2.0	8.0 ± 1.0
120	58 ± 9	54 ± 7	62 ± 4	62 ± 8	1.0 ± 0.4	1.2 ± 0.5	10.7 ± 1.4	7.2 ± 0.9
150	80 ± 14	56 ± 7	69 ± 6	72 ± 8	1.4 ± 0.6	1.0 ± 0.3	10.9 ± 1.9	7.8 ± 1.1
180	217 ± 70	104 ± 21	77 ± 10	81 ± 12	6.0 ± 0.4	4.0 ± 1.7	11.3 ± 1.1	9.6 ± 1.4
210	240 ± 59	253 ± 95	97 ± 11	92 ± 13	12.1 ± 3.3	6.5 ± 1.9	14.8 ± 1.4	10.3 ± 1.3
240	398 ± 79	267 ± 50	103 ± 11	112 ± 18	17.8 ± 3.8	11.2 ± 2.1	17.8 ± 1.0	14.7 ± 1.9
270	518 ± 63	392 ± 68	112 ± 13	98 ± 10	19.8 ± 4.5	16.2 ± 3.5	21.4 ± 1.5	17.2 ± 1.8
300	467 ± 56	398 ± 53	103 ± 13	121 ± 22	20.1 ± 3.2	18.8 ± 4.0	25.7 ± 2.0	21.2 ± 2.0

Data are means ± SE. *To convert pancreatic polypeptide to pmol/l multiply by 0.239, glucagon to pmol/l multiply by 0.287, growth hormone to pmol/l multiply by 44.15, and cortisol to nmol/l multiply by 27.59.

TABLE 2
Blood lactate, serum nonesterified fatty acid, blood β-hydroxybutyrate, and blood alanine concentrations during hyperinsulinemic–stepped hypoglycemic (85, 75, 65, 55, and 45 mg/dl) clamps on day 2 after saline or cortisol infusions on day 1

Time (min)	Lactate (μmol/l)		Nonesterified fatty acids (μmol/l)		β-Hydroxybutyrate (μmol/l)		Alanine (μmol/l)	
	After saline	After cortisol	After saline	After cortisol	After saline	After cortisol	After saline	After cortisol
−30	888 ± 103	1,040 ± 119	412 ± 45	362 ± 42	126 ± 15	141 ± 20	390 ± 62	440 ± 39
−15	778 ± 100	850 ± 122	415 ± 39	390 ± 30	119 ± 18	128 ± 23	310 ± 43	413 ± 35
0	800 ± 102	805 ± 116	416 ± 41	387 ± 32	138 ± 21	125 ± 15	367 ± 50	403 ± 31
30	1,044 ± 87	1,250 ± 178	144 ± 17	116 ± 12	127 ± 43	96 ± 17	337 ± 48	430 ± 46
60	1,298 ± 75	1,453 ± 183	78 ± 9	71 ± 9	87 ± 13	81 ± 14	366 ± 50	404 ± 31
90	1,215 ± 91	1,290 ± 161	74 ± 11	68 ± 9	79 ± 16	93 ± 14	349 ± 49	370 ± 34
120	1,136 ± 67	1,197 ± 140	59 ± 11	72 ± 9	85 ± 16	123 ± 30	316 ± 28	346 ± 28
150	1,167 ± 47	1,037 ± 113	63 ± 12	70 ± 13	75 ± 15	88 ± 10	322 ± 36	318 ± 15
180	1,213 ± 62	1,040 ± 187	59 ± 10	79 ± 17	64 ± 11	72 ± 13	298 ± 22	283 ± 19
210	1,248 ± 98	1,228 ± 149	62 ± 11	63 ± 10	70 ± 10	86 ± 14	272 ± 25	305 ± 20
240	1,477 ± 130	1,654 ± 187	66 ± 12	49 ± 7	75 ± 16	96 ± 16	300 ± 34	305 ± 20
270	1,808 ± 234	1,618 ± 163	72 ± 9	74 ± 15	131 ± 36	128 ± 31	320 ± 53	293 ± 21
300	2,046 ± 199	2,009 ± 214	86 ± 12	83 ± 23	86 ± 18	103 ± 18	288 ± 27	286 ± 17

Data are means ± SE.

sponses during the final 30 min. Thus, the duration of plasma glucose levels below the glycemic thresholds for glucose counterregulatory (including autonomic neuroendocrine) responses (rev. in 30) was nearly 3 h in the present study and nearly 2 h in the study of Davis et al. (5). Therefore, the differences in the findings cannot be attributed to a shorter duration, or lesser magnitude, of hypoglycemia in the present study. Furthermore, the differences cannot be explained by the fact that we analyzed absolute plasma epinephrine and norepinephrine and neurogenic symptom score values rather than increments from baseline, because there were no differences in the baseline values for these parameters under the two study conditions. Cortisol was infused during ambient euglycemia, rather than during hyperinsulinemic clamped euglycemia (5), on day 1 in the present study. Thus, there was greater antecedent hyperinsulinemia in the earlier study, but that was the same on both study occasions. Despite the apparent similarity of the peak cortisol levels produced on day 1 in both studies, we suspect that the critical issue is the antecedent plasma cortisol dose. We found that marked α_{1-24}ACTH–induced cortisol elevations (to ~45 μg/dl) reduced sympathoadrenal and neurogenic symptom responses to hypoglycemia the following day (16). Here, using the same experimental design and methods on day 2, we find that less-marked antecedent cortisol elevations did not do so.

Additional data are seemingly inconsistent with the suggestion that cortisol mediates hypoglycemia-associated autonomic failure in diabetes. Cortisol (among other) responses to nocturnal hypoglycemia are virtually absent during polysomnography-documented sleep in patients with type 1 diabetes (32,33). Nocturnal hypoglycemia, with the patients ostensibly asleep, has been shown to reduce sympathoadrenal and neurogenic symptom responses to hypoglycemia the following morning (30). To the extent that the latter patients were asleep during the nocturnal hypoglycemia, their reduced sympathoadrenal and neurogenic symptom responses to hypoglycemia—the key features of hypoglycemia-associated autonomic failure (1–4)—the following morning cannot, therefore, be attributed

TABLE 3
Heart rate, systolic blood pressure, and diastolic blood pressure during hyperinsulinemic–stepped hypoglycemic (85, 75, 65, 55, and 45 mg/dl) clamps on day 2 after saline or cortisol infusions on day 1

Time (min)	Heartrate (beats/min)		Systolic BP (mmHg)		Diastolic BP (mmHg)	
	After saline	After cortisol	After saline	After cortisol	After saline	After cortisol
−30	72 ± 4	69 ± 3	115 ± 3	116 ± 2	65 ± 1	66 ± 2
−15	70 ± 4	68 ± 3	117 ± 2	114 ± 3	66 ± 2	65 ± 2
0	71 ± 4	71 ± 3	116 ± 3	115 ± 3	65 ± 2	68 ± 2
30	71 ± 4	71 ± 3	118 ± 3	117 ± 3	64 ± 2	65 ± 3
60	73 ± 4	70 ± 3	117 ± 3	114 ± 2	63 ± 2	63 ± 2
90	75 ± 4	72 ± 3	118 ± 3	118 ± 3	64 ± 2	62 ± 2
120	79 ± 4	74 ± 4	120 ± 3	118 ± 3	63 ± 2	66 ± 3
150	79 ± 4	74 ± 4	119 ± 3	115 ± 3	62 ± 2	61 ± 3
180	83 ± 4	74 ± 3	116 ± 3	116 ± 3	56 ± 2	58 ± 2
210	83 ± 5	78 ± 4	114 ± 3	116 ± 3	54 ± 2	57 ± 1
240	85 ± 5	76 ± 4	112 ± 3	117 ± 2	52 ± 1	60 ± 4
270	84 ± 4	80 ± 3	114 ± 2	115 ± 2	52 ± 2	54 ± 2
300	84 ± 5	84 ± 4	119 ± 3	114 ± 3	53 ± 2	54 ± 2

Data are means ± SE. BP, blood pressure.

to a cortisol response to the antecedent nocturnal hypoglycemia.

To this point we have focused on the sympathoadrenal and neurogenic symptom responses to hypoglycemia because those are directly relevant to hypoglycemia-associated autonomic failure in diabetes (1–4). Among the other end points measured, plasma glucagon, growth hormone, and cortisol responses to hypoglycemia on day 2 were also not reduced after cortisol infusion on day 1. Growth hormone, but not glucagon or cortisol, responses to hypoglycemia were reduced after pharmacological (ACTH-induced) elevations of cortisol in our earlier study (16). In that study the plasma pancreatic polypeptide response to hypoglycemia, a marker of the parasympathetic response, was reduced significantly. It appeared to be reduced slightly in the present study. However, parasympathetic (in contrast to sympathetic cholinergic) responses are not known to be involved in the glucose counterregulatory or symptomatic responses to hypoglycemia (34). Finally, there were no differences in the glucose infusion rates required to maintain the hypoglycemic clamps on day 2 after saline or cortisol infusions on day 1 (biological support for the absence of differences in the measured glucagon and epinephrine responses) and no differences in the metabolic (lactate, nonesterified fatty acid, β-hydroxybutyrate, and alanine) or hemodynamic (heart rate and systolic and diastolic blood pressure) responses.

In summary, plasma cortisol elevations during euglycemia that are comparable to (and indeed above) levels that occur during hypoglycemia did not reduce sympathoadrenal or neurogenic symptom responses to subsequent hypoglycemia. Therefore, these data do not support the suggestion that the cortisol response to antecedent iatrogenic hypoglycemia mediates hypoglycemia-associated autonomic failure in diabetes.

ACKNOWLEDGMENTS

This work was supported, in part, by U.S. Public Health Service/National Institutes of Health Grants R37 DK27085, M01 RR00036, P60 DK20579, and T32 DK07120 and a fellowship award from the American Diabetes Association.

The authors gratefully acknowledge the analytical assistance of Krishan Jethi, Cornell Blake, Joy Brothers, Zina Lubovich, and Michael Morris; the assistance of the nursing staff of the Washington University General Clinical Research Center in the performance of this study; and the assistance of Karen Muehlhauser and Janet Dedeke in the preparation of this manuscript.

REFERENCES

1. Cryer PE: Hypoglycemia: the limiting factor in the glycaemic management of type I and type II diabetes. *Diabetologia* 45:937–948, 2002
2. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin dependent diabetes mellitus. *J Clin Invest* 91:819–828, 1993
3. Segel SA, Paramore DS, Cryer PE: Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. *Diabetes* 51:724–733, 2002
4. Cryer PE: Hypoglycemia-associated autonomic failure in diabetes. *Am J Physiol* 281:E1115–E1121, 2001
5. Davis SN, Shavers C, Costa F, Mosqueda-Garcia R: Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. *J Clin Invest* 98:680–691, 1996
6. Davis SN, Shavers C, Davis B, Costa F: Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. *J Clin Invest* 100:429–438, 1997
7. Ping L, Neill RA, Davis SN: CNS administration of cortisone blunts autonomic nervous system responses to subsequent hypoglycemia (Abstract). *Diabetes* 49:A66, 2000
8. Davis SN, Neill RA, Ping L: Activation of brain type II corticosteroid receptor is not responsible for blunting of autonomic nervous system responses to subsequent hypoglycemia (Abstract). *Diabetologia* 50:A53, 2001
9. Ping L, Neill RA, Cardin S, Survant ME, Davis SN: Dehydroepiandrosterone preserves counterregulatory responses during repeated hypoglycemia in conscious normal rats (Abstract). *Diabetes* 49:A65, 2002
10. Sanders NM, Ritter S: Acute 2DG-induced glucoprivation or dexamethasone abolishes 2DG-induced glucoregulatory responses to subsequent glucoprivation. *Diabetes* 50:2831–2836, 2001.
11. Evans SB, Wilkinson CW, Bentson K, Gronbeck P, Zavosh A, Figlewicz DP: PVN activation is suppressed by repeated hypoglycemia but not antecedent corticosterone in the rat. *Am J Physiol* 281:R1426–R1436, 2001
12. Shum K, Inouye K, Chan O, Mathoo J, Bilinski D, Matthews SG, Vranic M: Effects of antecedent hypoglycemia, hyperinsulinemia, and excess corticosterone on hypoglycemic counterregulation. *Am J Physiol* 281:E455–E465, 2001
13. Flanagan DE, Keshavarz T, Evans ML, Flanagan S, Fan X, Jacob RJ, Sherwin RS: Role of corticotrophin-releasing hormone in the impairment of counterregulatory responses to hypoglycemia. *Diabetes* 52:605–613, 2003.
14. Galassetti P, Mann S, Tate D, Neill RA, Costa F, Wasserman DH, Davis SN: Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. *Am J Physiol* 280:E908–E917, 2001
15. McGregor VP, Greiwe JS, Banarer S, Cryer PE: Limited impact of vigorous exercise on defenses against hypoglycemia: relevance to hypoglycemia-associated autonomic failure. *Diabetes* 51:1485–1492, 2002
16. McGregor VP, Banarer S, Cryer PE: Elevated endogenous cortisol reduces autonomic neuroendocrine and symptom responses to subsequent hypoglycemia. *Am J Physiol* 282:E770–E777, 2002
17. Heller SR, Cryer PE: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after one episode of hypoglycemia in nondiabetic humans. *Diabetes* 40:223–226, 1991
18. Schwartz NS, Clutter WE, Shah SD, Cryer PE: The glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. *J Clin Invest* 79:777–781, 1987
19. Towler DA, Havlin CE, Craft S, Cryer P: Mechanism of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes* 42:1791–1798, 1993
20. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH: Determination of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes* 26:22–29, 1977
21. Gingerich RL, Lacy PE, Chance RE, Johnson MG: Regional pancreatic concentration and in vitro secretion of canine pancreatic polypeptide, insulin, and glucagon. *Diabetes* 27:96–101, 1978
22. Ensinck J: Immunoassays for glucagon. In *Handbook of Experimental Pharmacology*. Vol. 66. Lefebvre P, Ed. Springer Verlag, New York, 1983, p. 203–221
23. Schalch D, Parker M: A sensitive double antibody radioimmunoassay for growth hormone in plasma. *Nature* (*Lond*) 703:1141–1142, 1964
24. Farmer RW, Pierce CE: Plasma cortisol determination: radioimmunoassay and competitive protein binding compared. *Clin Chem* 20:411–414, 1974
25. Shah SD, Clutter WE, Cryer PE: External and internal standards in the single-isotope derivative (radioenzymatic) measurement of plasma norepinephrine and epinephrine. *J Lab Clin Med* 106:624–629, 1985
26. Hosaka K, Kikuchi T, Mitsuhida N, Kawaguchi A: A new colorimetric method for the determination of free fatty acids with acyl-CoA synthetase and acyl-CoA oxidase. *J Biochem* (*Tokyo*) 89:1799–1803, 1981
27. Lowry O, Passoneau J, Hasselberger F, Schultz D: Effect of ischemia on known substrates and co-factors of the glycolytic pathway of the brain. *J Biol Chem* 239:18–30, 1964
28. Pinter J, Hayaski J, Watson J: Enzymatic assay of glycerol, dihydroxacetone and glyceraldehyde. *Arch Biochem Biophys* 121:404–414, 1967
29. Cahill GF Jr, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA Jr, Kipnis DM: Hormone-fuel interrelationships during fasting. *J Clin Invest* 45:1751–1769, 1966
30. Fanelli CG, Paramore DS, Hershey T, Terkamp C, Ovalle F, Craft S, Cryer PE: Impact of nocturnal hypoglycemia on hypoglycemic cognitive dysfunction in type 1 diabetes. *Diabetes* 47:1920–1927, 1998
31. Ovalle F, Fanelli CG, Paramore DS, Hershey T, Craft S, Cryer PE: Brief

twice-weekly episodes of hypoglycemia reduce detection of clinical hypoglycemia in type 1 diabetes mellitus. *Diabetes* 47:1472–1479, 1998
32. Jones TW, Porter P, Sherwin RS, Davis EA, O'Leary P, Frazer F, Byrne G, Stick S, Tamborlane WV: Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med* 338:1657–1662, 1998
33. Banarer S, Cryer PE: Sleep-related hypoglycemia-associated autonomic failure in type 1 diabetes: reduced awakening from sleep during hypoglycemia. *Diabetes* 52:1195–1203, 2003
34. Cryer PE: The prevention and correction of hypoglycemia. In *Handbook of Physiology. Section 7. The Endocrine System. Volume II. The Endocrine Pancreas and Regulation of Metabolism*. Jefferson LS, Cherrington AD, Eds. New York, Oxford University Press, 2001, p. 1057–1092