Cortisol Acts Through Central Mechanisms to Blunt Counterregulatory Responses to Hypoglycemia in Conscious Rats

Darleen A. Sandoval, Ling Ping, Anthony Ray Neill, Sachiko Morrey, and Stephen N. Davis

Physiological levels of cortisol have been found to blunt neuroendocrine and metabolic responses to subsequent hypoglycemia in humans. The aim of this study was to determine whether cortisol acts directly on the brain to elicit this effect. A total of 41 conscious unrestrained Sprague-Dawley rats were studied during 2-day experiments. Day 1 consisted of two episodes of clamped 2-h hyperinsulinemic (30 pmol · kg^{-1} · min^{-1}) hypoglycemia (2.8 ± 0.1 mmol/l; n = 12; ANTE HYPO), euglycemia (6.2 ± 0.1 mmol/l; n = 12; ANTE EUG), or euglycemia (6.2 ± 0.1 mmol/l) plus simultaneous intracerebroventricular (ICV) infusion of cortisol (25 μg/h; n = 9; ANTE EUG+Cort) or saline (24 μl/h; n = 8; ANTE EUG+Sal). For all groups, day 2 consisted of a 2-h hyperinsulinemic (30 pmol · kg^{-1} · min^{-1}) hypoglycemic (2.9 ± 0.2 mmol/l) clamp. Plasma epinephrine and glucagon incremental area under the curve (ΔAUC) responses were significantly less in ANTE EUG+Cort and ANTE HYPO versus both ANTE EUG and ANTE EUG+Sal (P < 0.05). The ΔAUC responses of plasma norepinephrine were significantly lower in ANTE EUG+Cort versus both ANTE EUG and ANTE EUG+Sal (P < 0.05). Endogenous glucose production was significantly less in ANTE HYPO and ANTE EUG+Cort versus the other groups (P < 0.05). Lastly, the glucose infusion rate to maintain the desired hypoglycemia was significantly greater in ANTE EUG+Cort and ANTE HYPO versus the other two groups (P < 0.05). In summary, ICV infusion of cortisol significantly blunted norepinephrine, epinephrine, glucagon, and endogenous glucose production responses to next-day hypoglycemia. We conclude that cortisol can act directly on the central nervous system to blunt counterregulatory responses to subsequent hypoglycemia in the conscious rat. *Diabetes* 52:2198–2204, 2003

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ACTH, adrenocorticotropic hormone; ΔAUC, incremental area under the curve; ANTE EUG, antecedent euglycemia; ANTE EUG+Cort, antecedent euglycemia plus intracerebroventricular infusions of cortisol; ANTE EUG+Sal, antecedent euglycemia plus intracerebroventricular infusions of saline; ANTE HYPO, antecedent hypoglycemia; EGP, endogenous glucose production; ICV, intracerebroventricular; PVN, paraventricular nucleus; R_a, rate of glucose appearance.

Intensive glucose control in type 1 diabetic patients can slow the progression or prevent complications of diabetes such as retinopathy, nephropathy, or neuropathy (1). Unfortunately, it is also well established that intensive glucose treatment increases the frequency of severe hypoglycemia (2). The mechanism for the increased frequency of hypoglycemia may not be simply due to insulin excess. It has been clearly established (3–5) that repeated exposure to hypoglycemia can reduce neuroendocrine (catecholamine, glucagon, and growth hormone) and metabolic (endogenous glucose production [EGP], lactate, and glycerol) counterregulatory responses to subsequent hypoglycemia by as much as 50% in healthy humans and type 1 diabetic subjects (6,7). Thus, blunted counterregulatory responses make type 1 diabetic patients susceptible to an increased vicious cycle of hypoglycemia.

Cortisol has been proposed to play a role in blunting neuroendocrine and metabolic responses to subsequent hypoglycemia (8,9). Similar to antecedent hypoglycemia, antecedent elevations of cortisol through infusion of cortisol (9) or adrenocorticotropic hormone (ACTH) (10) blunted neuroendocrine and metabolic counterregulatory responses to subsequent day 2 hypoglycemia in nondiabetic subjects. In addition, patients with adrenocortical failure had preserved catecholamine, pancreatic polypeptide, glucagon, and muscle sympathetic nerve activity responses to hypoglycemia after antecedent hypoglycemia (ANTE HYPO) (8).

In animal models, the role glucocorticoids play in modulating responses to repeated stress has provided mixed results. One study, using microdialysis in the brain, found that chronically elevated cortisol blunted norepinephrine synthesis and release to a similar extent as repeated immobilization stress (11). Other studies have demonstrated that cortisol or dexamethasone blunted epinephrine and ACTH responses to insulin-induced hypoglycemia in sheep (12) and dogs (13), respectively. However, three recent studies in rats (14–16) showed no significant blunting of sympathetic responses to subsequent hypoglycemia with antecedent infusions of corticosterone. Although these differences may be attributed to variations in experimental design, the impact of antecedent elevations of glucocorticoids on subsequent responses to hypoglycemia remain controversial.

There is much evidence to suggest that hypoglycemia

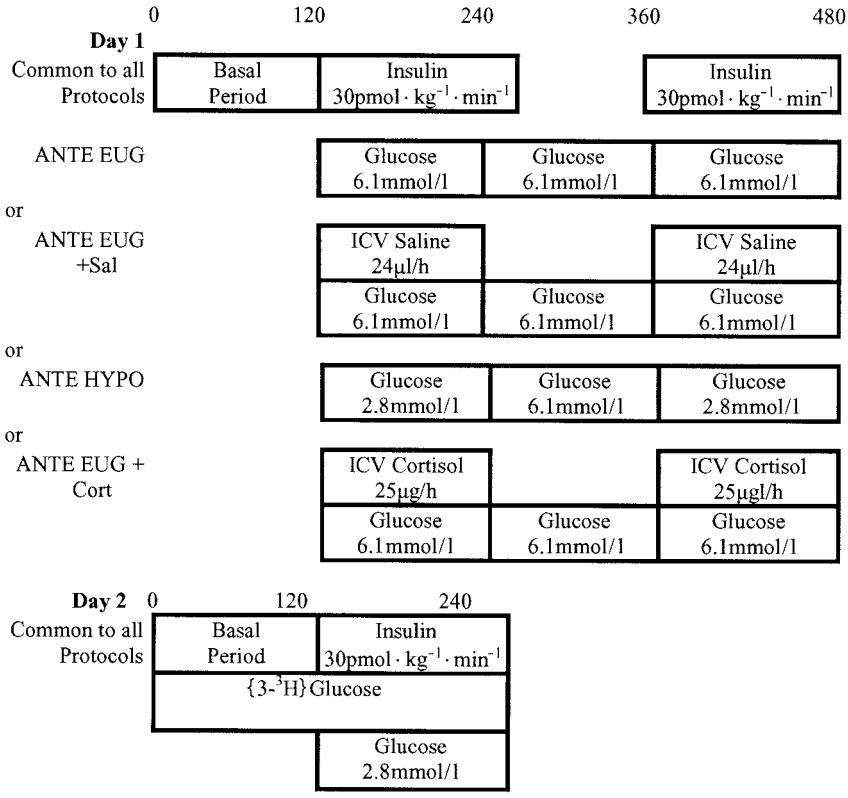

FIG. 1. Protocol for the 2-day studies in four groups of rats (ANTE EUG, ANTE EUG+Sal, ANTE HYPO, or ANTE EUG+Cort).

can be sensed in the hypothalamus (17–20). It is also known that repeated stress activates the paraventricular nucleus (PVN) and ventromedial areas of the hypothalamus. In fact, in rats, inactivation of the PVN by infusion of lidocaine blunted plasma catecholamine responses to hyperinsulinemic hypoglycemia (15). Thus, the brain may be an important site of action for cortisol to blunt responses to subsequent stress. The purpose of this study was to test the hypothesis that acute antecedent intracerebroventricular (ICV) infusions of cortisol can be sensed directly by the brain and blunt sympathetic and metabolic responses to subsequent hypoglycemia.

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats (300–350 g) bred and purchased from Harlan (Indianapolis, IN) were studied. The rats were housed and individually caged under control conditions (12:12 light-dark cycle, 50–60% humidity, 25°C) with free access to water in the Vanderbilt University Animal Care Facility. All procedures for animal use were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Animal preparation. Two weeks before the study, in 17 rats, a 6-mm stainless steel cannula was placed in the lateral ventricle for ICV infusions under a general anesthesia mixture (5 mg/kg acepromazine, 10 mg/kg xylazine, and 50 mg/kg ketamine). Rats were placed on a stereotaxic frame (KOPF Instruments, Tujunga, CA) for placement of the guide cannula at stereotaxis coordinates of −0.9 mm anteroposterior, +1.4 mm mediolateral, and −4.5 dorsoventral from bregma according to the atlas of Paxinos and Watson (21). The intracranial cannulas were held in place with cranioplastic cement to three skull screws. Seven days after surgery in the first group of rats and in 24 additional rats, catheters were then placed in the carotid artery (for blood sampling) and the external jugular vein (for infusions) under a general anesthesia mixture (5 mg/kg acepromazine, 10 mg/kg xylazine, and 50 mg/kg ketamine). Catheter lines were kept patent by flushing with 150 units/ml heparin every 3 days. Rats had free access to rat diet the days before surgery and experiments. Seven days after surgery, only rats with >90% of their presurgery body weight were used for the 2-day experiments.

Experimental design. Four groups of rats were studied during a 2-day experimental protocol outlined in Fig. 1. Day 1 consisted of two 2-h clamped hyperinsulinemic euglycemia (ANTE EUG; $n = 12$), hypoglycemia (ANTE HYPO; $n = 12$), or euglycemia plus ICV infusion of saline (24 µl/min) (ANTE EUG+Sal; $n = 8$), or cortisol (25 µg/h; ANTE EUG+Cort; $n = 9$). The total volume of fluid infused into the lateral ventricle was 96 µl for both the ICV saline and cortisol studies. The cortisol infusate was prepared by dissolving hydrocortisone (Sigma, St. Louis, MO) in 0.9% saline. Using saline avoids any potential side effects of the dissolving medium. This dose of cortisol was chosen because our preliminary data using 100 and 50 µg/h of cortisol caused increases in peripheral levels of cortisol that were maintained through the day 2 hypoglycemic clamp. On day 2, all rats were exposed to a 2-h hyperinsulinemic-hypoglycemic clamp. Rats were fasted overnight before each day of the 2-day studies and remained conscious and unrestrained throughout the experimental protocols. To prevent a fall in hematocrit, after each blood draw, erythrocytes plus normal saline were re-infused through the jugular cannula into the rat. The morning of the study, extensions were placed on the exteriorized catheters for ease of access and were removed between day 1 and day 2 studies.

Day 1 procedures. At time 0 min, rats were moved to an experimental cage and allowed to acclimate to the surroundings. At time 120–240 and 360–480 min, the ICV infusions of saline or cortisol was started in the ANTE EUG+Sal and ANTE EUG+Cort groups, respectively. Also during these time periods, a hyperinsulinemic-euglycemic (ANTE EUG, ANTE EUG+Sal, and ANTE EUG+Cort) or hypoglycemic (ANTE HYPO) clamp was performed (clamping procedures described below). Plasma measurement of glucose was taken every 5 min and of insulin at time 240 and 480 min. Between morning and afternoon clamps, plasma glucose was measured every 15–30 min and glucose infusion was adjusted to maintain euglycemia at 6.1 mmol/l. At the conclusion of day 1 procedures, rats were fed 5–8 g of rat diet.

Day 2 procedures. At time 0 min, rats were moved to an experimental cage and allowed to acclimate to the surroundings. The experiment consisted of a basal period (time 90–120) and an experimental period from time 120 to 240 min, during which a hyperinsulinemic-hypoglycemic clamp (described below) was performed. To measure glucose kinetics during the clamp, a primed (10 µCi) constant (0.2 µCi/min) infusion of high-pressure liquid chromatography–purified [3-^{3}H]glucose (Perkin Elmer Life Sciences, Boston, MA) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) at time 0 min and continued through 240 min. During the experimental period, blood was drawn every 5 min for measurements of plasma glucose, every 10 min during the basal period, every 15 min during the experimental periods for [3-^{3}H]glucose, and at time 90, 120, 180, 210, and 240 min for counterregulatory hormones. Rats were killed after day 2 procedures, and placement of ICV (by infusion of cresyl violet staining), carotid, and jugular cannulas was verified.

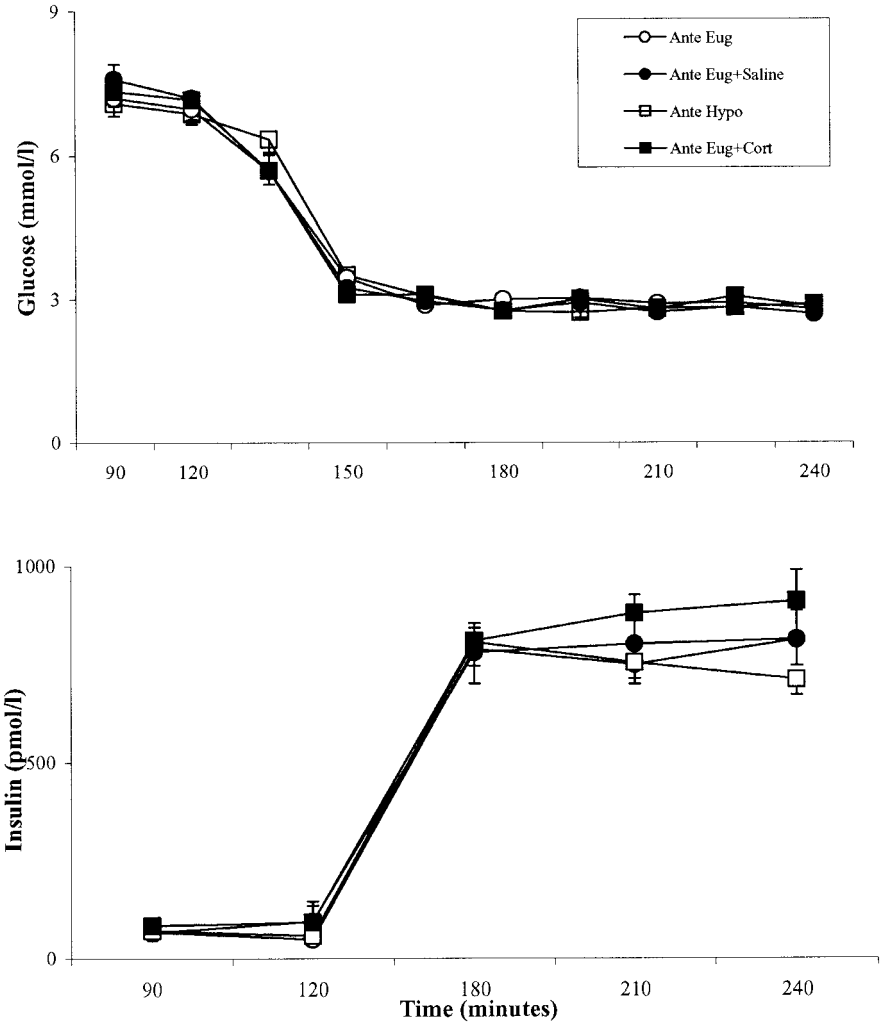

FIG. 2. Plasma glucose and insulin levels during day 2 exposure to hyperinsulinemic hypoglycemia after either ANTE EUG, ANTE EUG+Sal, ANTE HYPO, or ANTE EUG+Cort.

Glycemic clamping procedures. From time 120 to 240 min (day 1 and day 2) and from time 360 to 480 (day 1 only), a primed (60 pmol · kg^{-1} · min^{-1}) continuous (30 pmol · kg^{-1} · min^{-1}) infusion of insulin (Eli Lilly, Indianapolis, IN) containing 9.7% (vol/vol) of rat plasma was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA). Plasma glucose was measured every 5 min. For the euglycemic clamp, a 50% dextrose infusion was adjusted to maintain glucose at ~6.1 mmol/l. For the hypoglycemic clamp, glucose levels were allowed to fall (reaching nadir in ~30 min), and a 20% dextrose infusion was adjusted to maintain glucose at ~2.9 mmol/l for 90 min.

Tracer calculations. Rates of glucose appearance (R_a), EGP, and glucose utilization were calculated according to the methods of Wall et al. (22). EGP was calculated by determining the total R_a (this comprises both EGP and any exogenous glucose infused to maintain the desired hypoglycemia) and subtracting from it the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, because underestimates of total R_a and rate of glucose disposal can be obtained. The use of a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity) in the presence of low glucose flux eliminates most, if not all, of the problems. In addition, to maintain a constant specific activity, isotope delivery was increased commensurate with increases of exogenous glucose infusion.

Analytical methods. Plasma glucose was measured in duplicate by the glucose oxidase technique on a Beckman Glucose analyzer. Catecholamines were determined by high-pressure liquid chromatography (23) with an interassay coefficient of variation (CV) of 12% for both epinephrine and norepinephrine. We made two modifications to the procedure for catecholamine determination: *1*) we used a five-point rather than one-point standard calibration curve, and *2*) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant catecholamine peaks could be made. Corticosterone (ICN Biomedicals, Irvine, CA; interassay CV 7%), cortisol (Clinical Assays Gamma Coat Radioimmunoassay Kit; interassay CV 6%), insulin (24) (interassay CV 11%), and glucagon (Linco Research, St. Louis, MO; interassay CV 15%) were all measured using radioimmunoassay techniques.

Statistical analysis. Data are expressed as means ± SE and were analyzed using standard parametric one-way ANOVA, with repeated measures where appropriate. A Tukey's post hoc analysis was used to delineate statistical significance. A P value ≤0.05 was accepted as statistically significant.

RESULTS

Glucose and insulin. Plasma glucose levels were similar during all morning and afternoon day 1 euglycemic clamps (6.2 ± 0.1 and 6.3 ± 0.1 mmol/l, respectively; Fig. 2) in ANTE EUG, ANTE EUG+Sal, and ANTE EUG+Cort. Plasma glucose levels were also equivalent during day 1 morning and afternoon hypoglycemic clamps (2.8 ± 0.1 in the morning and 2.8 ± 0.2 in the evening) in ANTE HYPO. Glucose levels were also similar during day 2 hypoglycemic clamps between all groups (2.9 ± 0.2 mmol/l). In addition, day 1 and day 2 insulin levels were similar between all four groups of rats (day 1: 744 ± 32 pmol/l for final 30 min; day 2: 74 ± 10 and 800 ± 26 pmol/l for basal and final 30 min).

Counterregulatory hormones. Cortisol levels at the end of day 1 euglycemic clamps were below the lower limit of detection of our assay in ANTE EUG+Cort (<27 nmol/l). Thus, there was little or no spillover of cortisol from the brain into the peripheral circulation.

Norepinephrine incremental area under the curve

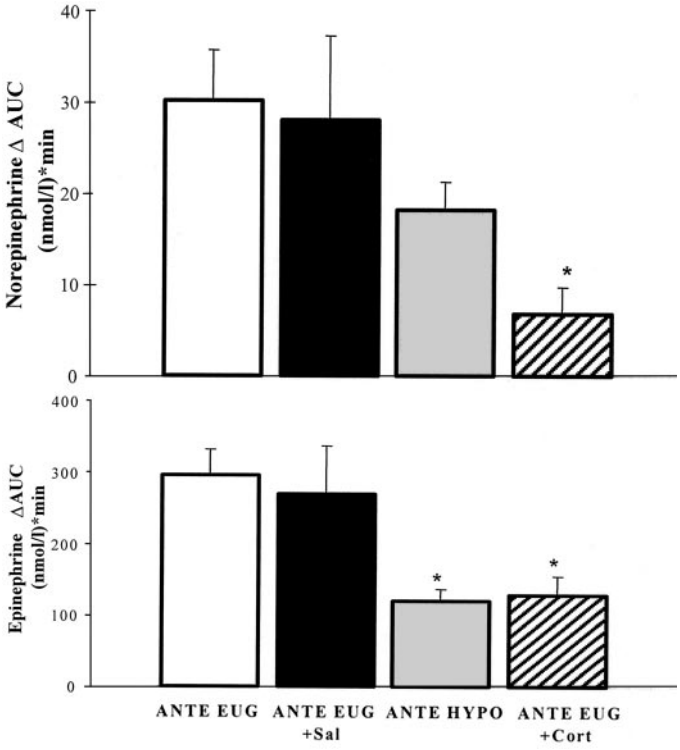

FIG. 3. Norepinephrine and epinephrine ΔAUC responses to day 2 hyperinsulinemic hypoglycemia after either ANTE EUG, ANTE EUG+Sal, ANTE HYPO, or ANTE EUG+Cort in conscious unrestrained rats. *P < 0.05 vs. both ANTE EUG and ANTE EUG+Sal.

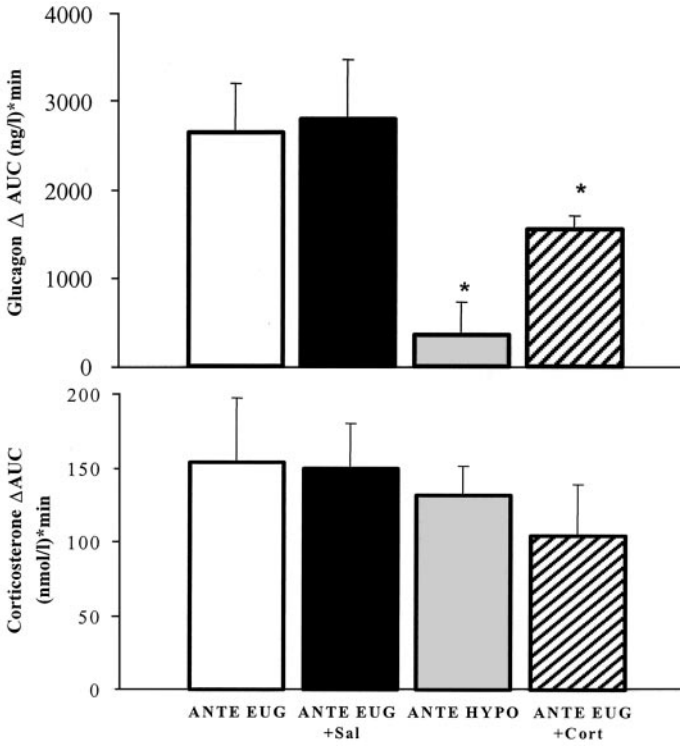

FIG. 4. Glucagon and corticosterone ΔAUC responses to day 2 hyperinsulinemic hypoglycemia after either ANTE EUG, ANTE EUG+Sal, ANTE HYPO, or ANTE EUG+Cort in conscious unrestrained rats. *P < 0.05 vs. both ANTE EUG and ANTE EUG+Sal.

(ΔAUC) responses to day 2 hypoglycemia were significantly lower in ANTE EUG+Cort versus ANTE EUG and ANTE EUG+Sal (7 ± 6 vs. 28 ± 9 and 30 ± 6 nmol · l^{-1} · min, respectively; $P < 0.05$) but not compared with ANTE HYPO (18 ± 3 nmol · l^{-1} · min; Fig. 3). Day 2 epinephrine ΔAUC responses to hypoglycemia were significantly less in both ANTE HYPO and ANTE EUG+Cort compared with ANTE EUG and ANTE EUG+Sal (120 ± 17 and 129 ± 26 vs. 295 ± 37 and 270 ± 66 pmol · l^{-1} · min; Fig. 3; $P < 0.05$). Glucagon ΔAUC responses to hypoglycemia were significantly lower in ANTE EUG+Cort and ANTE HYPO versus ANTE EUG and ANTE EUG+Sal (1,559 ± 360 and 359 ± 144 vs. 2,658 ± 541 and 2,800 ± 680 ng · l^{-1} · min; Fig. 4; $P < 0.05$). Corticosterone levels significantly increased with day 2 hypoglycemia similarly among the groups (Fig. 4). Basal and final 30-min levels of hormones are listed in Table 1.

Glucose kinetics. Specific activity, listed in Table 2, was stable during the basal and final 30 min of the hyperinsulinemic-hypoglycemic clamp for all groups with an average CV of 5 ± 1% for both periods. During the final 30 min of day 2 hypoglycemia, EGP in the ANTE HYPO group was significantly less than that in the ANTE EUG and ANTE EUG+Sal groups (10 ± 5 vs. 28 ± 3 and 38 ± 4 μmol · kg^{-1} · min^{-1}; $P < 0.05$; Fig. 5). In addition, EGP was significantly less in the ANTE EUG+Cort versus the ANTE EUG+Sal group (21 ± 5 vs. 38 ± 5 μmol · kg^{-1} · min^{-1}; $P < 0.05$). Glucose rate of disappearance during the final 30 min of hypoglycemia was significantly greater in ANTE EUG+Cort and ANTE HYPO versus ANTE EUG (63 ± 7 and 64 ± 9 vs. 45 ± 3 μmol · kg^{-1} · min^{-1}; $P < 0.05$; Fig. 5). Consequently, glucose infusion rates were significantly greater in both ANTE HYPO and ANTE EUG+Cort compared with both ANTE EUG and ANTE EUG+Sal (57 ± 8

TABLE 1
Hormonal measurements during baseline and the final 30 min of day 2 hyperinsulinemic hypoglycemia (2.9 ± 0.1 mmol) in concious rats

	Norepinephrine		Epinephrine		Glucagon		Corticosterone	
	Basal (nmol/l)	Final 30 min (nmol/l)	Basal (nmol/l)	Final 30 min (nmol/l)	Basal (ng/l)	Final 30 min (ng/l)	Basal (nmol/ml)	Final 30 min (nmol/ml)
ANTE EUG	1 ± 0.1	2.6 ± 0.3	0.5 ± 0.1	17 ± 2	67 ± 2	258 ± 39	8 ± 2	19 ± 2
ANTE EUG+Sal	1 ± 0.3	2.1 ± 0.1	0.5 ± 0.2	15 ± 3	45 ± 4	246 ± 45	5 ± 2	15 ± 2
ANTE HYPO	1 ± 0.2	2.1 ± 0.2	0.5 ± 0.1	8 ± 1†	54 ± 4	91 ± 8†	5 ± 1	13 ± 1
ANTE EUG+Cort	1 ± 0.2	1.5 ± 0.2*	0.7 ± 0.2	8 ± 1†	48 ± 5	169 ± 24†	8 ± 1	15 ± 1

Data are means ± SE. *$P < 0.05$ vs. ANTE EUG; †$P < 0.05$ vs. ANTE EUG and ANTE EUG+Sal.

TABLE 2
Specific activity (dpm/mmol) during baseline and the final 30 min of day 2 hyperinsulinemic hypoglycemia (2.9 ± 0.1 mmol) in concious rats

	Time (min)					
	100	110	120	210	225	240
ANTE EUG	334 ± 61	319 ± 53	325 ± 50	424 ± 77	410 ± 62	438 ± 84
ANTE EUG+Sal	380 ± 46	388 ± 46	423 ± 36	720 ± 118	738 ± 127	781 ± 142
ANTE HYPO	503 ± 40	508 ± 38	538 ± 53	862 ± 103	932 ± 142	938 ± 138
ANTE EUG+Cort	390 ± 40	391 ± 40	381 ± 43	634 ± 92	717 ± 90	

Data are means ± SE.

and 40 ± 7 vs. 17 ± 5 and 18 ± 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $P < 0.05$; Fig. 5).

DISCUSSION

We have previously found that antecedent peripheral infusions of cortisol blunted neuroendocrine and metabolic responses to subsequent hypoglycemia in humans. To test the hypothesis that cortisol acts through central mechanisms (i.e., the brain) to induce the blunted neuroendocrine and consequently metabolic responses to repeated hypoglycemia, we infused cortisol directly into the brain of conscious nonrestrained rats during morning and afternoon hyperinsulinemic-euglycemic clamps on day 1 and measured neuroendocrine and metabolic responses to subsequent day 2 hypoglycemia. Two experimental control groups (ANTE EUG and ANTE EUG+Sal) were used to determine whether ICV infusion itself would cause antecedent stress. Importantly, there were no significant differences between ANTE EUG compared with ANTE EUG+Sal in any variable. Our results demonstrate that antecedent elevations of cortisol or antecedent hypoglycemia blunt norepinephrine, epinephrine, glucagon, and EGP responses and increase the need for exogenous glucose during subsequent hypoglycemic clamps in a similar fashion. Thus, the results support the hypothesis that antecedent elevations of cortisol are sensed by the brain and can blunt neuroendocrine and metabolic responses to subsequent hypoglycemia.

Previous studies in a number of species (rats, dogs, sheep, and humans) have provided a rationale for the role played by cortisol in blunting counterregulatory responses to subsequent hypoglycemia (8–10,13). Davis et al. (9) infused cortisol in healthy humans and achieved levels in the high physiological range (~32 μg/dl) on day 1 and examined responses to a subsequent single-step hyperinsulinemic-hypoglycemic clamp on day 2. Similar to our current study in rats, plasma epinephrine, norepinephrine, glucagon, and EGP were all significantly lower after antecedent cortisol and hypoglycemia as compared with day 1 euglycemia. McGregor et al. (10) found similar results in humans with antecedent ACTH infusion that increased endogenous cortisol levels to ~40 μg/dl. Interestingly, in Addison's patients who lack cortisol, day 2 hypoglycemic responses were preserved between day 1 and day 2 hypoglycemia (8), again supporting a regulatory role for cortisol in mediating the blunted counterregulatory responses that occur with repeated hypoglycemia.

The present results are similar to previous studies in rats where a variety of differing antecedent stresses (25–27) have been found to blunt counterregulatory responses to subsequent stress. Kvetnansky et al. (25) demonstrated that adrenalectomy increased norepinephrine responses to immobilization stress in rats and that peripheral infusion of cortisol reversed this effect. Another study in sheep found that cortisol and dexamethasone blunted epinephrine responses to hypoglycemia but not to audiovisual stress (12). Three studies in rodents showed somewhat conflicting results after antecedent corticosterone infusions. None of the three studies found blunted hormonal (catecholamines and glucagon) responses to either antecedent infusions of corticosterone (36 μg/infusion) into the PVN (15) or to repeated (3 and 4 days) peripheral corticosterone injections (2 × 500 μg injections and 4.05 μg/min for 2 h per day) (14,16). However, in contrast to Flanagan et al. (16), glucose flux (EGP) responses during hyperinsulinemic hypoglycemia were blunted after antecedent corticosterone administration in the study by Shum et al. (14). It should be noted that the present study differed from the above three studies (14–16) in a number of important methodological details. First, unlike the present study, corticosterone was administered in the absence of hyperinsulinemic-euglycemic conditions. The lack of insulin during the antecedent challenges may have relevance bearing in mind that Shum et al. reported that antecedent hyperinsulinemic euglycemia, per se, blunted epinephrine, and EGP responses to subsequent hypoglycemia. Although these effects of insulin reported by Shum et al. are in contrast to results in humans (28), they do point to a potentially important role for hyperinsulinemia in models of hypoglycemia-associated counterregulatory failure in rodents.

Second, along similar lines, the above studies used insulin infusion rates that were 4 (16), 8 (15), and 10 (14) times greater (~20, 252, and 300 vs. 30 $pmol \cdot kg^{-1} \cdot min^{-1}$) and achieved insulin levels that were up to 25 times greater (~3,500 and 20,000 vs. ~800 pmol/l) than in our current study. An insulin level of 800 pmol/l is at the high end of the physiological range and can be seen postprandially in rats. Insulin values above the physiological range have been shown to increase plasma catecholamines up to twofold in dogs (29) and humans (30) during hypoglycemia. High insulin in the presence of euglycemia has also been shown to increase cortisol levels (31,32). Thus, the potential effect of insulin to increase catecholamine and cortisol levels could certainly confound the interpretation of the effects of antecedent corticosterone on autonomic nervous system responses during subsequent hypoglycemia. Of course other differences in experimental design may also contribute to the divergent results (i.e., three to four separate single daily administrations of glucocorti-

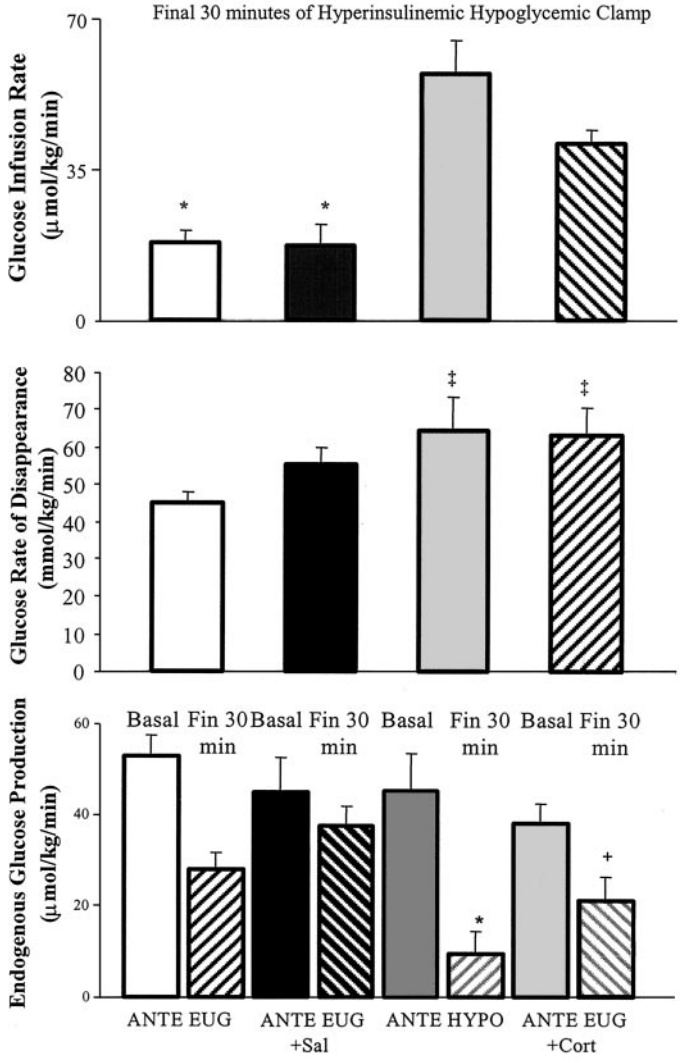

FIG. 5. Glucose infusion rate, glucose rate of disappearance, and EGP during the final 30 min of day 2 hyperinsulinemic hypoglycemia after either ANTE EUG, ANTE EUG+Sal, ANTE HYPO, or ANTE EUG+Cort in conscious unrestrained rats. *$P < 0.05$ vs. ANTE EUG+Cort, ANTE EUG, and ANTE EUG+Sal. +$P < 0.05$ vs. ANTE EUG+Sal; ‡$P < 0.05$ vs. ANTE EUG.

coids versus two same day bouts of glucocorticoid infusions).

Third, in the current study, ICV infusion of cortisol rather than corticosterone was chosen because it would allow us to specifically probe the effects of cortisol action on the brain (because cortisol is not synthesized in the rat). It is interesting to note that the studies infusing cortisol in rats (our current study and the study by Kvetnansky et al. [25]) but not corticosterone (14–16) blunted subsequent counterregulatory responses. The reason for this intriguing difference is not clear. In dogs, both antecedent cortisol (the naturally occurring glucocorticoid) and corticosterone infusion have been found to have similar inhibitory effects on ACTH response to subsequent hyperinsulinemic hypoglycemia (13). Previous work has demonstrated that rat brain corticosteroid receptors have up to fivefold less affinity for cortisol compared with corticosterone (33). Thus, although the dose of cortisol we infused was higher than in reference (15), because of the lower receptor affinity, we cannot be certain that the biologic effective doses of cortisol and corticosterone would be similar in the two studies. Furthermore, because corticosterone is the naturally occurring glucocorticoid, differences in the effects of cortisol versus corticosterone in the rat brain cannot be ruled out. It is important to mention that Flanagan et al. (16) did find that repeated antecedent infusions of corticotropin-releasing hormone, but not ACTH, or corticosterone blunted subsequent responses to hypoglycemia. The finding that ACTH did not blunt counterregulatory responses to hypoglycemia also contrasts results in humans (10). From the above information, it can be seen that currently there is considerable debate regarding the mechanisms responsible for hypoglycemia-associated autonomic failure. Nevertheless, there appears to be a consensus building that one or more aspects of the hypothalamo-pituitary-adrenal axis may be involved in this syndrome.

The negligible day 1 cortisol levels in the ANTE EUG+Cort group demonstrated little or no spillover from the ICV infusion into the peripheral circulation. This clearly partitions cortisol effects to central rather than peripheral actions. Infusion of cortisol into the lateral ventricle could allow cortisol to reach several areas within the brain, including the PVN, the ventromedial hypothalamus, and the hindbrain—all areas that are sensitive to changes in glycemic levels (17–20). In fact, lesions in the ventromedial hypothalamus (34) and inactivation of the PVN with infusion of lidocaine (15) both caused blunting of plasma epinephrine and norepinephrine responses to hypoglycemia. In addition, peripheral cortisol infusions have been found to inhibit norepinephrine release and synthesis within the PVN during 2 h of immobilization stress (11). Similarly, immediate early gene expression in the PVN has been found to be suppressed by both repeated stress and chronic elevation of glucocorticoid levels (35). Both minerocorticoid and glucocorticoid receptors are located throughout the brain (pituitary, PVN, hippocampus, and hindbrain). Thus, it is possible that cortisol binds to one or both of its receptors within the brain to inhibit autonomic responses to repeated hypoglycemia.

Although our results strongly support a role for cortisol in causing blunting of the sympathetic and metabolic responses to subsequent stress, there were some differences between the ANTE EUG+Cort and ANTE HYPO groups. First, glucagon responses were blunted to a lesser degree by prior cortisol compared with prior hypoglycemia. These findings are similar to results in humans (9). Control of glucagon responses to hypoglycemia is complex but includes both autonomic control (sympathetic and parasympathetic limbs) and direct sensing by the pancreatic α-cell (36). Thus, it may be possible that antecedent hypoglycemia blunts glucagon secretion through a combination of direct inhibition of pancreatic α-cell sensing of hypoglycemia and a reduced autonomic nervous system drive. However, cortisol may only blunt glucagon release during subsequent hypoglycemia via effects on the autonomic nervous system but does not modulate α-cell sensing of hypoglycemia. Second, EGP was greater in the ANTE EUG+Cort group than in the ANTE HYPO group. The differential levels of glucagon may explain these results. Because both epinephrine and glucagon are important regulators of EGP, the slightly greater

glucagon levels may provide enough stimuli to raise EGP in the ANTE EUG+Cort group during day 2 hypoglycemia.

In conclusion, our results in conscious rats provide additional support for the hypothesis that cortisol can regulate counterregulatory responses to repeated hypoglycemia. Furthermore, they suggest that cortisol acts through central mechanisms to elicit this effect. A physiological function of antecedent elevations of cortisol may be to restrain autonomic nervous system responses during subsequent stress. Unfortunately, for type 1 diabetic patients who lack glucagon responses to hypoglycemia, restraint of the autonomic nervous system may be maladaptive.

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