

The Effects of Dehydroepiandrosterone Sulfate on Counterregulatory Responses During Repeated Hypoglycemia in Conscious Normal Rats

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We previously determined that both antecedent hypoglycemia and elevated cortisol levels blunt neuroendocrine and metabolic responses to subsequent hypoglycemia in conscious, unrestrained rats. The adrenal steroid dehydroepiandrosterone sulfate (DHEA-S) has been shown in several studies to oppose corticosteroid action. The purpose of this study was to determine if DHEA-S could preserve counterregulatory responses during repeated hypoglycemia. We studied 40 male Sprague-Dawley rats during a series of 2-day protocols. Day 1 consisted of two 2-h episodes of 1) hyperinsulinemic (30 pmol · kg⁻¹ · min⁻¹) euglycemia (6.2 ± 0.2 mmol/l; n = 12; ANTE EUG), 2) hyperinsulinemic euglycemia (6.0 ± 0.1 mmol/l; n = 8) plus simultaneous intravenous infusion of DHEA-S (30 mg/kg; ANTE EUG + DHEA-S), 3) hyperinsulinemic hypoglycemia (2.8 ± 0.1 mmol/l; n = 12; ANTE HYPO), or 4) hyperinsulinemic hypoglycemia (2.8 ± 0.1 mmol/l; n = 8) with simultaneous intravenous infusion of DHEA-S (30 mg/kg; ANTE HYPO + DHEA-S). Day 2 consisted of a single 2-h hyperinsulinemic hypoglycemic (2.8 ± 0.1 mmol/l) clamp. During the final 30 min of day 2, hypoglycemia norepinephrine levels were significantly lower in the ANTE HYPO group versus the ANTE HYPO + DHEA-S group (2.0 ± 0.2 vs. 3.3 ± 0.6 nmol/l; P < 0.05). In addition, epinephrine (8 ± 1 vs. 17 ± 2, 14 ± 3, and 15 ± 3 nmol/l), glucagon (91 ± 8 vs. 273 ± 36, 231 ± 42, and 297 ± 48 ng/l), and corticosterone (1,255 ± 193 vs. 1,915 ± 212, 1,557 ± 112, and 1,668 ± 119 pmol/l) were significantly lower in the ANTE HYPO group versus the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (P < 0.05). Endogenous glucose production was also significantly less in the ANTE HYPO group versus the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (13 ± 5 vs. 32 ± 3, 38 ± 7, and 29 ± 8 μmol/l · kg⁻¹ · min⁻¹; P < 0.05). Consequently, the amount of exogenous glucose needed to maintain the glycemic level during the clamp studies was significantly higher in the ANTE HYPO versus the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (57 ± 8 vs. 22 ± 5, 18 ± 6, and 18 ± 3

μmol/l · kg⁻¹ · min⁻¹; P < 0.05). In summary, day-1 antecedent hypoglycemia blunted neuroendocrine and metabolic responses to next-day hypoglycemia. However, simultaneous DHEA-S infusion during antecedent hypoglycemia preserved neuroendocrine and metabolic counterregulatory responses during subsequent hypoglycemia in conscious rats. *Diabetes* 53:679–686, 2004

The Diabetes Control and Complications Trial established that intensive glucose control in type 1 diabetic patients can slow the progression or significantly reduce the onset of diabetic microvascular complications (e.g., retinopathy, nephropathy, neuropathy) (1). Unfortunately, the study also established that intensive glucose treatment causes an approximate threefold increase in the frequency of severe hypoglycemia (2). This increased frequency of hypoglycemia is at least partially caused by deficient autonomic nervous system (ANS) counterregulatory responses to hypoglycemia (3–5). Repeated exposure to hypoglycemia reduces neuroendocrine, ANS, and metabolic (endogenous glucose production [EGP], lactate, and glycerol) counterregulatory responses to subsequent hypoglycemia by as much as 50% in nondiabetic and type 1 diabetic subjects (6–9). These blunted counterregulatory responses are significant contributing factors to a vicious cycle of hypoglycemia for type 1 diabetic patients (9).

The mechanisms responsible for the blunted counterregulatory responses seen after antecedent hypoglycemia are not fully understood. Controversy exists regarding the role played by corticosteroids in the pathophysiology of blunted ANS responses during hypoglycemia (10–14). Reports in rodents (12) and humans (15) have demonstrated that, similar to antecedent hypoglycemia, antecedent increases of cortisol can blunt neuroendocrine and metabolic counterregulatory responses to next day hypoglycemia. In addition, patients with adrenocortical failure have preserved catecholamine, pancreatic polypeptide, glucagon, growth hormone, and muscle sympathetic nerve activity responses to hypoglycemia after antecedent hypoglycemia (16). Other animal studies have found that corticosteroids can blunt catecholamine responses to a variety of stressors, including insulin-induced hypoglycemia in sheep (17) and immobilization stress in rats (18,19). However, in contrast to the above studies, three other reports have indicated that prior corticosterone administration has little or no effect on ANS responses to subsequent hypoglycemia in the conscious rat (10,13,14).

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Received for publication 30 April 2003 and accepted in revised form 2 December 2003.

ANS, autonomic nervous system; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; EGP, endogenous glucose production; GABA, γ-aminobutyric acid; HPLC, high-performance liquid chromatography; NMDA, N-methyl-D-aspartate.

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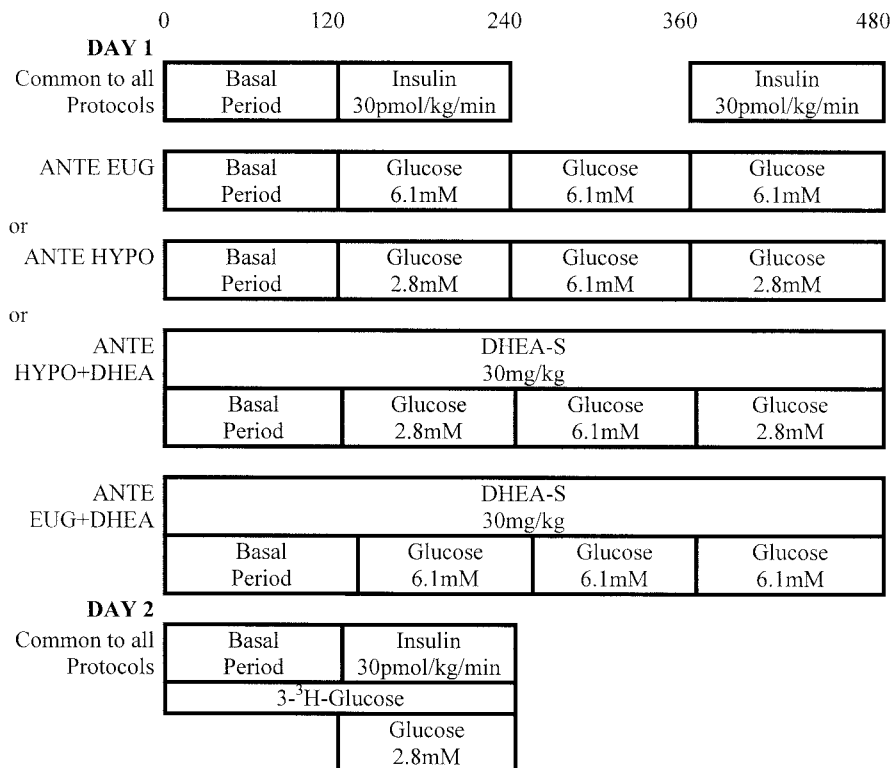


FIG. 1. Protocol for the 2-day studies in four groups of conscious rats.

Dehydroepiandrosterone (DHEA) and its corresponding sulfate ester (dehydroepiandrosterone sulfate; DHEA-S) are steroid hormones secreted from the adrenal gland. After being orally or intravenously administered, the two hormones are interconverted at a high rate and have similar physiological effects (20,21). Little is known about their biological significance, but many studies have shown that they work contrary to corticosteroid actions. For example, in rats, a simultaneous infusion of DHEA-S counteracts the detrimental effects of corticosterone on neuronal function (22) and neurogenesis (23) in the dentate gyrus. In Zucker rats, simultaneous infusion of DHEA and dexamethasone prevents the dexamethasone-induced increase in hepatic tyrosine aminotransferase activity (24). In addition, intracerebroventricular infusion of DHEA has been shown to increase norepinephrine and epinephrine levels in the paraventricular nucleus and ventromedial hypothalamus in obese Zucker rats (25). Although corticosteroids have been found to increase gluconeogenesis (26), proteolysis (27), and net fat deposition in humans (28), DHEA has been found to elicit the opposite effect on these metabolic processes (29–32). Based on the wealth of existing studies, we therefore hypothesized that if DHEA-S can antagonize corticosteroid actions, it may also prevent any corticosteroid-induced inhibition of neuroendocrine and metabolic responses to repeated hypoglycemia. Thus, the aim of this study was to determine if peripheral infusion of DHEA-S during antecedent hypoglycemia could preserve counterregulatory responses during a subsequent bout of hypoglycemia in conscious, unrestrained rats.

RESEARCH DESIGN AND METHODS

We studied 40 male Sprague-Dawley rats (300–350 g) bred and purchased from Harlan (Indianapolis, IN). The rats were housed and individually caged under a 12:12-h light:dark cycle, with room humidity and temperature at 50–60% and 25°C, respectively. All animals had 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.

At 1 week before experiments, catheters were placed in the rats' 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 them with 150 unit/ml of heparin every 3 days. Rats had free access to rat diet on the days preceding surgery and the experiments. All rats used for the 2-day experiments maintained >90% of their presurgery body weight.

Four groups of rats were studied after an overnight fast during a 2-day experimental protocol, as outlined in Fig. 1. Rats were fasted overnight before each day of the 2-day study and remained conscious and unrestrained throughout the 2-day protocols. The morning of each study day, extensions were placed on the exteriorized catheters for ease of access and were removed between day-1 and -2 studies. At $t = 0$ min, rats were moved to an experimental cage and allowed to become acclimated to the surroundings.

Day-1 procedures. Day 1 consisted of two 2-h (120–240 and 360–480 min) hyperinsulinemic-euglycemic clamps (ANTE EUG; $n = 12$), hyperinsulinemic-hypoglycemic clamps (ANTE HYPO; $n = 12$), hyperinsulinemic-euglycemic clamps plus infusion of DHEA-S (30 mg/kg) (ANTE EUG + DHEA-S; $n = 8$) or hyperinsulinemic-hypoglycemic clamps plus DHEA-S infusion (ANTE HYPO + DHEA-S; $n = 8$). The DHEA-S was continuously infused into the jugular vein starting at $t = 0$ min throughout day-1 procedures. DHEA-S rather than DHEA was infused because of the former's water solubility. DHEA-S (30 mg/kg) was dissolved in 1.5 ml of normal saline and infused at a rate of 3 μ l/min. This dosage of DHEA-S was chosen because it has been previously shown to have anticorticosteroid effects on the brain (22). At the conclusion of day-1 procedures, rats were fed 5–8 g of rat diet. To maintain hematocrit, after each blood draw the rats' own erythrocytes plus normal saline were reinfused through the jugular cannula. If a rat's hematocrit was <40% at the beginning of the day-2 studies, that rat was removed from the study. Plasma measurements of glucose were taken every 5 min during the clamp periods and at $t = 240$ and 480 min for insulin levels. 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.

Day-2 procedures. At $t = 0$ min, rats were moved to an experimental cage and allowed to become acclimated to the surroundings. The study consisted of a basal period ($t = 90$ –120 min) and an experimental period ($t = 120$ –240 min), during which time a hyperinsulinemic-hypoglycemic clamp (described below) was performed. To measure glucose kinetics during the clamp, a primed (10 μ Ci), constant (0.2 μ Ci/min) infusion of [3 -H] glucose (PerkinElmer, Boston, MA) purified by high-performance liquid chromatogra-

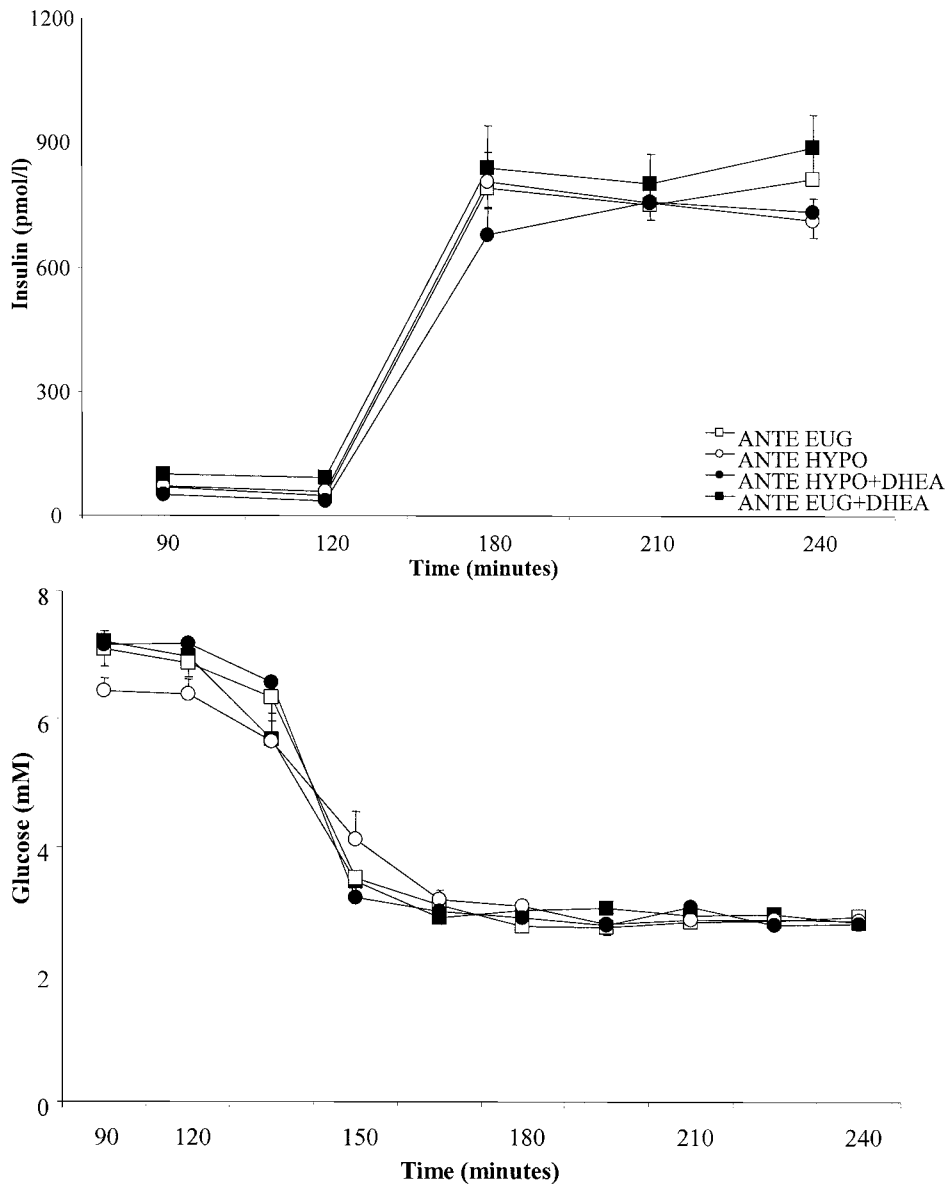


FIG. 2. Plasma glucose and insulin levels during day-2 exposure to hyperinsulinemic hypoglycemia after ANTE EUG, ANTE HYPO, ANTE HYPO + DHEA-S, or ANTE EUG + DHEA-S.

phy (HPLC) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) at $t = 0$ min and continued through $t = 240$ min. During the experimental period, blood was drawn every 5 min for measurement of plasma glucose, every 10 min during the basal period and every 15 min during the experimental period for $[3\text{-}^3\text{H}]\text{glucose}$, and at $t = 90, 120, 180, 210,$ and 240 min for counterregulatory hormones. For clarity of presentation and because counterregulatory hormones were not significantly different between $t = 210$ and 240 min (indicating steady state), these time points were averaged in RESULTS. Rats were killed after the day-2 procedures and the placement of carotid and jugular cannulae was verified.

Glycemic clamping procedures. At $t = 120\text{--}240$ min (days 1 and 2) and $360\text{--}480$ min (day 1 only), a primed ($60 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) continuous ($30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) infusion of insulin (Eli Lilly, Indianapolis, IN) containing 9.7% (vol/vol) rat plasma was administered via a precalibrated infusion pump (Harvard Apparatus). Plasma glucose was measured every 5 min. For the euglycemic clamp, a 50% dextrose infusion was adjusted to maintain glucose at $\sim 6.1 \text{ mmol/l}$. For the hypoglycemic clamp, glucose levels were allowed to fall and a 20% dextrose infusion was adjusted to maintain glucose at $\sim 2.9 \text{ mmol/l}$ for 90 min.

Tracer calculations. The rate of glucose appearance (R_a), EGP, and glucose utilization was calculated according to the methods of Wall et al. (33). EGP was calculated by determining the total R_a , which 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, as underestimates of total R_a and the rate of glucose disposal (R_d) can be obtained. Using 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 proportionally with increases in exogenous glucose infusion.

Analytical methods. Plasma glucose was measured by the glucose oxidase technique on a Beckman glucose analyzer. Catecholamines were determined by HPLC (34) 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 a 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%), insulin interassay (CV 11%) (35), glucagon (Linco Research, St. Louis, MO; interassay CV 15%), and DHEA-S (Diagnostic Systems, Webster, TX; interassay CV 5%) were all measured using radioimmunoassay techniques.

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

RESULTS

Glucose and insulin. Plasma glucose levels were similar during morning and afternoon day-1 euglycemic clamps in

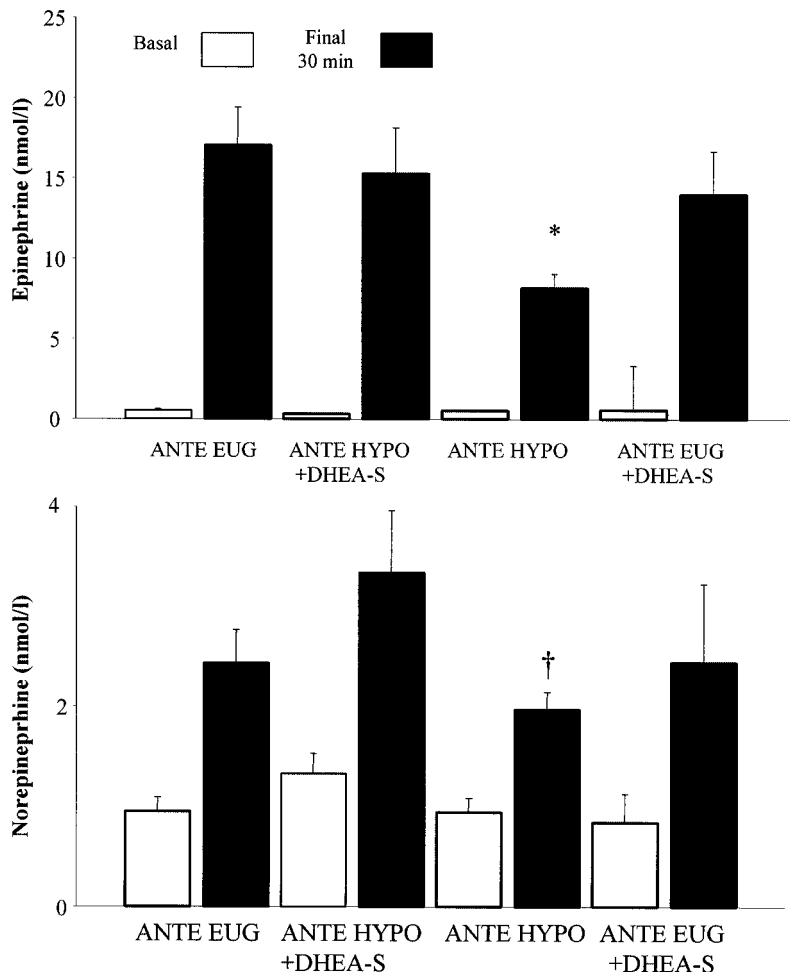


FIG. 3. Norepinephrine and epinephrine levels during basal and final 30-min period of day-2 hyperinsulinemic hypoglycemia after ANTE EUG, ANTE HYPO, ANTE HYPO + DHEA-S, or ANTE EUG + DHEA-S in conscious, unrestrained rats. * $P < 0.05$ vs. ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S; † $P < 0.05$ vs. ANTE HYPO + DHEA-S.

the ANTE EUG and ANTE EUG + DHEA-S groups (6.2 ± 0.2 and 6.1 ± 0.2 nmol/l) and during hypoglycemic clamps in the ANTE HYPO and ANTE HYPO + DHEA-S groups (2.8 ± 0.1 and 2.8 ± 0.1 nmol/l). Glucose levels were also similar during day-2 hypoglycemic clamps in all groups (2.8 ± 0.1 mmol/l) (Fig. 2). In addition, day-1 and -2 insulin levels were similar in all four groups of rats (day 1: 726 ± 38 pmol/l; day 2: 750 ± 28 pmol/l for final 30 min).

Counterregulatory hormones. The increase in plasma norepinephrine during the final 30 min of day-2 hypoglycemia was significantly lower in the ANTE HYPO versus the ANTE HYPO + DHEA-S group (2.0 ± 0.2 vs. 3.3 ± 0.6 nmol/l; $F = 7.31$; $P < 0.05$) (Fig. 3). Also, the increase in epinephrine levels during the final 30 min of day-2 hypoglycemia was significantly lower in the ANTE HYPO group versus the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (8 ± 1 vs. 17 ± 2 , 14 ± 3 , and 15 ± 3 nmol/l; $F = 8.0$; $P < 0.0001$) (Fig. 3). Similarly, glucagon and corticosterone responses during the final 30 min of day-2 hypoglycemia were significantly lower in the ANTE HYPO group versus the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (91 ± 8 vs. 273 ± 36 , 231 ± 42 , and 297 ± 78 ng/l; and $1,255 \pm 93$ vs. $1,915 \pm 212$, $1,557 \pm 112$, and $1,668 \pm 119$ nmol/l, respectively; $F = 4.37$; $P < 0.01$) (Fig. 4).

Glucose kinetics. Specific activity was stable during both the basal and the final 30-min periods for all groups, with a CV of $<5 \pm 1\%$ (Table 1). EGP was significantly lower in

the ANTE HYPO group versus the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (13 ± 5 vs. 32 ± 3 , 38 ± 7 , and 29 ± 8 $\mu\text{mol/l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $F = 7.3$; $P < 0.001$) (Fig. 5). The R_d was similar among groups and did not change during hypoglycemia (Fig. 5). As a consequence of the lower EGP, the exogenous glucose infusion rate needed to maintain the glycemic level during the clamp was significantly greater in the ANTE HYPO group compared with the ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S groups (57 ± 8 vs. 18 ± 3 , 18 ± 6 , and 22 ± 5 $\mu\text{mol/l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $F = 13.43$; $P < 0.0001$) (Fig. 5).

DISCUSSION

In the current study, we compared the counterregulatory responses of four groups of rats during 2 h of hyperinsulinemic hypoglycemia on day 2 after different interventions on day 1. The day-1 interventions included antecedent euglycemia, antecedent euglycemia plus DHEA-S infusion, antecedent hypoglycemia, and antecedent hypoglycemia plus DHEA-S infusion. Similar to previous findings from human studies, we found in these conscious, unrestrained rats that two 2-h episodes of hypoglycemia blunted neuroendocrine and metabolic responses to a subsequent episode of hypoglycemia occurring 1 day later. Peripheral infusion of DHEA-S during hypoglycemia on day 1, however, prevented counterregulatory failure and preserved

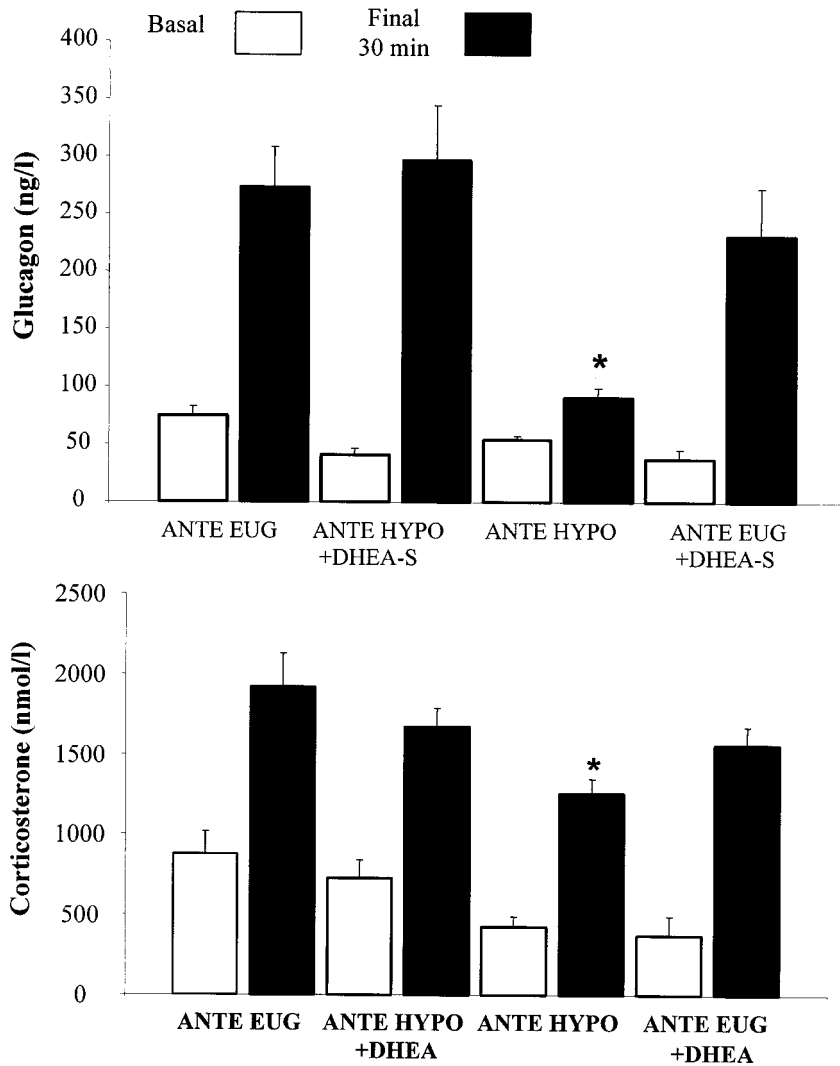


FIG. 4. Glucagon and corticosterone responses to day-2 hyperinsulinemic hypoglycemia after ANTE EUG, ANTE HYPO, ANTE HYPO + DHEA-S, or ANTE EUG + DHEA-S in conscious, unrestrained rats. * $P < 0.05$ vs. ANTE EUG, ANTE EUG + DHEA-S, and ANTE HYPO + DHEA-S.

catecholamine, glucagon, corticosterone, and EGP responses to next day hypoglycemia.

In this study, DHEA-S rather than DHEA was infused because the former is water soluble. Using DHEA-S avoids the potentially confounding variable of using a substance (e.g., alcohol or DMSO) that has independent effects on the central nervous system as a vehicle for administering the DHEA-S. Although the structures of DHEA-S and DHEA differ slightly, studies have shown similar physiological effects of DHEA-S and DHEA (20,21) and in humans the hormones are interconverted at a high rate.

The two euglycemic control groups, ANTE EUG + DHEA-S and ANTE EUG, produced similar neuroendocrine and metabolic counterregulatory responses during

day-2 hypoglycemia. This indicated that DHEA-S did not have any direct action on modulating counterregulatory responses during day-2 hypoglycemia. However, DHEA-S infused during day-1 hypoglycemia preserved counterregulatory function during day-2 hypoglycemia. The mechanism for this effect is unknown, as a specific DHEA-S receptor has not been identified. It is interesting that many studies have shown that DHEA and its sulfated ester can antagonize corticosteroid actions (22,24,25,36). The role of glucocorticoids in blunting physiological responses to stress are somewhat controversial, with a number of studies both supporting (11,17,19,37) and contradicting (14) this finding (10,13). Several studies, in addition to our own, have shown that corticosteroids can reduce ANS

TABLE 1

Glucose specific activity in conscious rats at baseline and during the final 30-min period of day-2 hyperinsulinemic hypoglycemia

Group	Time (min)					
	100	110	120	210	225	240
ANTE EUG	334 ± 61	319 ± 53	325 ± 50	424 ± 77	410 ± 62	438 ± 84
ANTE HYPO + DHEA-S	397 ± 47	404 ± 48	370 ± 43	509 ± 51	548 ± 44	570 ± 63
ANTE HYPO	503 ± 40	508 ± 38	538 ± 53	852 ± 103	916 ± 142	938 ± 138
ANTE EUG + DHEA-S	403 ± 53	407 ± 46	390 ± 57	612 ± 41	629 ± 45	626 ± 79

Data are means ± SD. Glucose specific activity given as dpm/mmol. Hyperinsulinemic hypoglycemia is defined as 2.8 ± 0.1 mmol/l.

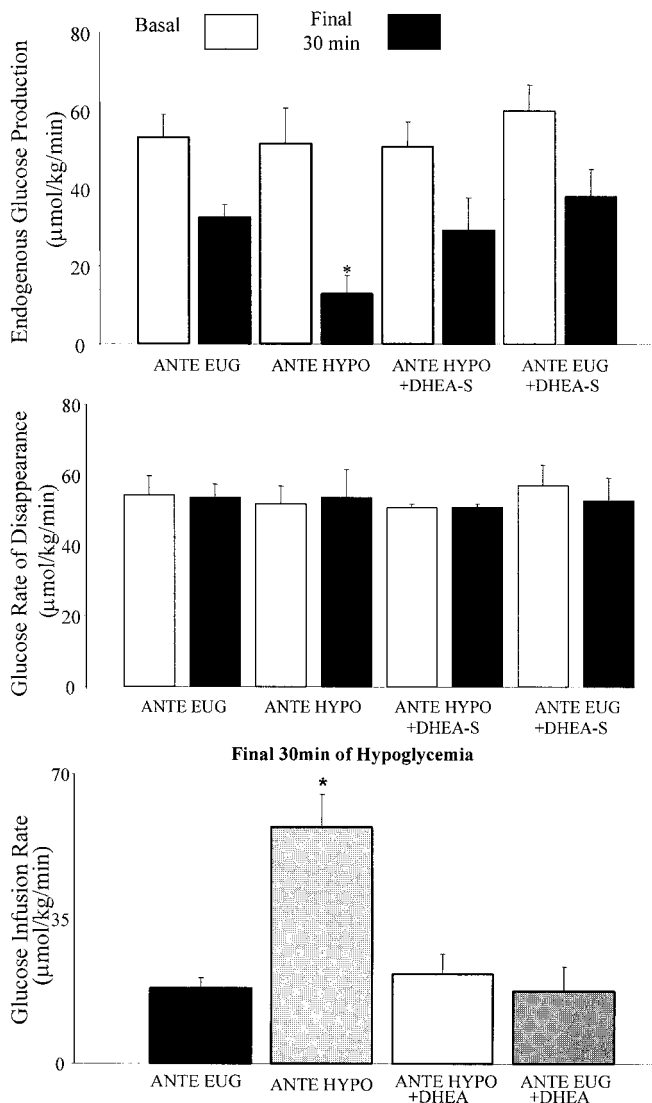


FIG. 5. EGP, R_{a1} , and R_{d1} during the final 30 min of day-2 hyperinsulinemic hypoglycemia after ANTE EUG, ANTE HYPO, ANTE HYPO + DHEA-S, or ANTE EUG + DHEA-S in conscious, unrestrained rats. * $P < 0.05$ vs. ANTE HYPO + DHEA-S, ANTE EUG + DHEA-S, and ANTE EUG.

responses to differing forms of stress in a variety of species, such as rats (37), sheep (17), and dogs (38). However, DHEA-S does not antagonize corticosteroid actions by competitively binding to corticosteroid receptors, at least in hepatocytes (39). In addition, chronically administered DHEA has been shown to prevent stress-induced increases in glucocorticoid receptor number (40), which minimizes the biological effects of the elevated corticosteroids. Brain *N*-methyl-D-aspartate (NMDA) and γ -aminobutyric acid (GABA) receptors are known to have excitatory and inhibitory effects on neuronal norepinephrine release, and thus ANS function, respectively. Although DHEA-S has been shown to have stimulatory effects on NMDA activity (21) and receptor number (41) and inhibitory effects on GABA receptor activity (42), corticosterone has been found to increase the affinity of GABA receptors (43). Therefore, DHEA-S could be stimulatory to the ANS (stimulatory to NMDA and inhibitory to GABA receptors), whereas cortisol may be inhibitory (i.e., may increase activity of GABA receptors). Future studies should be

aimed at determining the mechanism for the protective effect of DHEA-S against repeated hypoglycemia in rats.

A number of the effects observed in the present study with antecedent DHEA-S infusion appear to have been mediated via the ANS. Stimulation of the ANS increases plasma catecholamines, plasma glucagon, and, subsequently, EGP. ANS activation also results in reduced glucose uptake during hypoglycemia. Alterations in glucose flux are critical end points in the defense against hypoglycemia. During acute periods of hypoglycemia, increases in EGP provide the primary defense (44). However, during more prolonged hypoglycemia, both reduced glucose uptake and increased EGP become important (44). It is therefore relevant that during the present study DHEA-S infusion prevented the blunting of EGP during subsequent hypoglycemia. Regulation of glucagon responses during hypoglycemia is complex, with data demonstrating that direct α -cell sensing (45), prevailing insulinemia (46), epinephrine, and the ANS (47) may all modulate levels of the hormone during stress. The finding that DHEA-S infusion preserved glucagon responses during our studies may also indicate an action of the compound on preventing ANS dysfunction during repeated episodes of hypoglycemia. The site of a DHEA-S action that protects the ANS cannot be determined from our study. However, DHEA-S readily crosses the blood-brain barrier and peripheral infusions have been found to affect various sites in the brain (20,23,41). We would therefore speculate that DHEA-S can influence ANS responses via direct brain sensing, but future studies will be needed to determine whether this is in fact the case.

DHEA-S also preserved the response of corticosterone during repeated hypoglycemia, thereby suggesting additional non-ANS-mediated effects. Although DHEA-S may antagonize corticosteroid actions, some (48) but not all (49,50) studies have found that DHEA-S can also decrease corticosteroid levels. If this is correct, it suggests that DHEA-S may have the ability to antagonize multiple levels of glucocorticoid negative feedback loops (i.e., on both the ANS and the hypothalamopituitary adrenal axis).

It is interesting that rats do not secrete DHEA-S. This renders the rat a good animal model for testing the hypothesis that DHEA-S could preserve counterregulatory responses during repeated hypoglycemia because any effects of DHEA-S can be isolated. The fact that DHEA-S was infused before and during hypoglycemia on day 1 may be relevant. However, the goal of the present study was to determine if DHEA-S infused during hypoglycemia on day 1 could prevent hypoglycemia-associated autonomic failure 1 day later. The levels of DHEA-S that we infused peripherally reached 0.14 $\mu\text{mol/l}$, which is ~ 5 times the basal levels found in humans. These levels are also similar with those seen in other studies that demonstrated an anticorticosteroid effect on the brain in rats (23) and with levels found with 100 mg of oral DHEA replacement in elderly (51) and young adult subjects (52). Thus, the finding that DHEA-S preserves counterregulatory responses during repeated hypoglycemia in conscious, unrestrained rats could have implications for future human studies.

In conclusion, the present study demonstrated that in the conscious rat, simultaneous DHEA-S administration

during antecedent hypoglycemia preserves neuroendocrine (norepinephrine, epinephrine, glucagon, and corticosterone) and metabolic (EGP) responses to next day hypoglycemia. This preservation of counterregulatory function appears to involve the antagonism of mechanisms responsible for causing blunted counterregulatory responses during repeated hypoglycemia rather than the hormone's directly stimulating neuroendocrine responses per se.

ACKNOWLEDGMENTS

This work was supported by research grants from the Juvenile Diabetes Research Foundation International and the National Institutes of Health (RO1-DK-45369) and a Diabetes Research and Training Grant (5P60-AM-20593).

We thank Eric Allen, Angelina Penalzoza, Pam Venson, and Wanda Snead for their expert technical assistance.

REFERENCES

1. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. The Diabetes Control and Complications Trial Research Group: Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 46:271–286, 1997
3. Davis MR, Shamoon H: Counterregulatory adaptation to recurrent hypoglycemia in normal humans. *J Clin Endocrinol Metab* 73:995–1001, 1991
4. Davis MR, Mellman MJ, Shamoon H: Further defects in counterregulatory responses induced by recurrent hypoglycemia in IDDM. *Diabetes* 41:1335–1340, 1992
5. 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
6. Davis SN, Mann S, Galassetti P, Neill RA, Tate D, Ertl AC, Costa F: Effects of differing durations of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia in normal humans. *Diabetes* 49:1897–1903, 2000
7. Davis SN, Tate D: Effects of morning hypoglycemia on neuroendocrine and metabolic responses to subsequent afternoon hypoglycemia in normal man. *J Clin Endocrinol Metab* 86:2043–2050, 2001
8. Davis SN, Shavers C, Mosqueda-Garcia R, Costa F: Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. *Diabetes* 46:1328–1335, 1997
9. Cryer PE: Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM. *Diabetes* 41:255–260, 1992
10. 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
11. 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
12. Sandoval D, Ping L, Neill AR, Morrey S, Davis SN: Cortisol acts through central mechanisms to blunt counterregulatory responses to hypoglycemia in conscious rats. *Diabetes* 52:2198–2204, 2003
13. 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
14. Evans SB, Wilkinson CW, Bentson K, Gronbeck P, Zavosh A, Foglewicz DP: PVN activation is suppressed by repeated hypoglycemia but not antecedent corticosterone in the rat. *Am J Physiol* 281:R1426–R1436, 2001
15. Davis SN, Shavers C, Costa F, Mosqueda-Garcia R: Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal man. *J Clin Invest* 98:680–691, 1996
16. Davis SN, Shavers C, Costa F: Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. *J Clin Invest* 100:429–438, 1997
17. Komesaroff PA, Funder JW: Differential glucocorticoid effects on catecholamine responses to stress. *Am J Physiol* 266:E118–E128, 1994
18. Pacak K, Kvetnansky R, Palkovits M, Fukuhara K, Yadid G, Kopin IJ, Goldstein D: Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. *Endocrinology* 133:1404–1410, 1993
19. Pacak K, Palkovits M, Kvetnansky R, Matern P, Hart C, Kopin IJ, Goldstein DS: Catecholaminergic inhibition by hypercortisolemia in the paraventricular nucleus of conscious rats. *Endocrinology* 136:4814–4819, 1995
20. Kimonides VG, Khatibi NH, Svendsen CN, Sofroniew MV, Herbert J: Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. *Proc Natl Acad Sci U S A* 95:1852–1857, 1998
21. Compagnone NA, Mellon SH: Dehydroepiandrosterone: a potential signaling molecule for neocortical organization during development. *Proc Natl Acad Sci U S A* 95:4678–4683, 1998
22. Kaminska M, Harris J, Gijbsers K, Dubrovsky B: Dehydroepiandrosterone sulfate (DHEAS) counteracts decremental effects of corticosterone on dentate gyrus LTP: implications for depression. *Brain Res Bull* 52:229–234, 2000
23. Karishma KK, Herbert J: Dehydroepiandrosterone (DHEA) stimulates neurogenesis in the hippocampus of the rat, promotes survival of newly formed neurons and prevents corticosterone-induced suppression. *Eur J Neurosci* 16:445–453, 2002
24. Wright BE, Porter JR, Browne ES, Svec F: Antiglucocorticoid action of dehydroepiandrosterone in young obese Zucker rats. *Int J Obes Relat Metab Disord* 16:579–583, 1992
25. Wright BE, Svec F, Porter JR: Central effects of dehydroepiandrosterone in Zucker rats. *Int J Obes Relat Metab Disord* 19:887–892, 1995
26. Goldstein RE, Rossetti L, Palmer BA, Liu R, Massillon D, Scott M, Neal D, Williams P, Peeler B, Cherrington AD: Effects of fasting and glucocorticoids on hepatic gluconeogenesis assessed using two independent methods in vivo. *Am J Physiol* 283:E946–E957, 2002
27. Lofberg E, Gutierrez A, Wernerman J, Anderstam B, Mitch WE, Price SR, Bergstrom J, Alvestrand A: Effects of high doses of glucocorticoids on free amino acids, ribosomes and protein turnover in human muscle. *Eur J Clin Invest* 32:345–353, 2002
28. Samra JS, Clark ML, Humphreys SM, Macdonald IA, Bannister PA, Frayn KN: Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue. *J Clin Endocrinol Metab* 83:626–631, 1998
29. Kneer N, Lardy H: Thyroid hormone and dehydroepiandrosterone permit gluconeogenic hormone responses in hepatocytes. *Arch Biochem Biophys* 375:145–153, 2000
30. McIntosh MK, Berdanier CD: Antiobesity effects of dehydroepiandrosterone are mediated by futile substrate cycling in hepatocytes of BHE/cdb rats. *J Nutr* 121:2037–2043, 1991
31. Shepherd A, Cleary MP: Metabolic alterations after dehydroepiandrosterone treatment in Zucker rats. *Am J Physiol* 246:E123–E128, 1984
32. Tagliaferro AR, Ronan AM, Payne J, Meeker LD, Tse S: Increased lipolysis to beta-adrenergic stimulation after dehydroepiandrosterone treatment in rats. *Am J Physiol* 268:R1374–R1380, 1995
33. Wall JS, Steele R, DeBodo RD, Altszuler N: Effect of insulin on utilization and production of circulating glucose. *Am J Physiol* 189:43–50, 1957
34. Causon R, Caruthers M, Rodnight R: Assay of plasma catecholamines by liquid chromatography with electrical detection. *Anal Biochem* 116:223–226, 1982
35. Wide L, Porath J: Radioimmunoassay of proteins with the use of sephadex-coupled antibodies. *Biochim Biophys Acta* 130:257–260, 1966
36. McIntosh MK, Pan JS, Berdanier CD: In vitro studies on the effects of dehydroepiandrosterone and corticosterone on hepatic steroid receptor binding and mitochondrial respiration. *Comp Biochem Physiol Comp Physiol* 104:147–153, 1993
37. Kvetnansky R, Fukuhara K, Pacak K, Goldstein D, Kopin IJ: Endogenous glucocorticoids restrain catecholamine synthesis and release at rest and during immobilization stress in rats. *Endocrinology* 133:1411–1419, 1993
38. Keller-Wood M, Leeman E, Shinsako J, Dallman MF: Steroid inhibition of canine ACTH: in vivo evidence for feedback at the corticotrope. *Am J Physiol* 255:E241–E246, 1988
39. Mohan PF, Ihnen JS, Levin BE, Cleary MP: Effects of dehydroepiandrosterone treatment in rats with diet-induced obesity. *J Nutr* 120:1103–1114, 1990
40. Hu Y, Arturo C, Erdal G, Anderson P, Kalimi M: Anti-stress effects of dehydroepiandrosterone. *Biochem Pharmacol* 59:753–762, 2000
41. Wen S, Dong K, Onolfo JP, Vincens M: Treatment with dehydroepiandrosterone sulfate increases NMDA receptors in hippocampus and cortex. *Eur J Pharmacol* 430:373–374, 2001
42. Yoo A, Harris J, Dubrovsky B: Dose-response study of dehydroepiandrosterone sulfate on dentate gyrus long-term potentiation. *Exp Neurol* 137:151–156, 1996

43. Majewska MD, Bissler JC, Eskay RL: Glucocorticoids are modulators of GABAA receptors in brain. *Brain Res* 339:178–182, 1985
44. Cherrington AD, Stevenson RW, Steiner KE, Davis MA, Myers SR, Adkins BA, Abumrad NN, Williams PE: Insulin, glucagon and glucose as regulators of hepatic glucose uptake and production in vivo. *Diabetes Metab Rev* 3:307–332, 1987
45. Gerich JE, Charles MA, Grodsky GM: Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. *J Clin Invest* 54:833–841, 1974
46. Unger RH: Berson Memorial Lecture: insulin-glucagon relationships in the defense against hypoglycemia. *Diabetes* 32:575–583, 1983
47. Havel PJ, Parry SJ, Stern JS, Akpan JO, Gingerich RL, Taborsky GJ Jr, Curry DL: Redundant parasympathetic and sympathoadrenal mediation of increased glucagon secretion during insulin-induced hypoglycemia in conscious rats. *Metabolism* 43:860–866, 1994
48. Kroboth PD, Amico JA, Stone RA, Folan M, Frye RF, Kroboth FJ, Bigos KL, Fabian TJ, Linares AM, Pollock BG, Hakala C: Influence of DHEA administration on 24-hour cortisol concentrations. *J Clin Psychopharmacol* 23:96–99, 2003
49. Meno-Tetang GM, Blum RA, Schwartz KE, Jusko WJ: Effects of oral prasterone (dehydroepiandrosterone) on single-dose pharmacokinetics of oral prednisone and cortisol suppression in normal women. *J Clin Pharmacol* 41:1195–1205, 2001
50. Ceresini G, Morganti S, Rebecchi I, Freddi M, Ceda GP, Banchini A, Solerte SB, Ferrari E, Ablondi F, Valenti G: Evaluation of the circadian profiles of serum dehydroepiandrosterone (DHEA), cortisol, and cortisol/DHEA molar ratio after a single oral administration of DHEA in elderly subjects. *Metabolism* 49:548–551, 2000
51. Arlt W, Haas J, Callies F, Reincke M, Hubler D, Oettel M, Ernst M, Schulte HM, Allolio B: Biotransformation of oral dehydroepiandrosterone in elderly men: significant increase in circulating estrogens. *J Clin Endocrinol Metab* 84:2170–2176, 1999
52. Arlt W, Justl HG, Callies F, Reincke M, Hubler D, Oettel M, Ernst M, Schulte HM, Allolio B: Oral dehydroepiandrosterone for adrenal androgen replacement: pharmacokinetics and peripheral conversion to androgens and estrogens in young healthy females after dexamethasone suppression. *J Clin Endocrinol Metab* 83:1928–1934, 1998