Stimulation of both type I and type II corticosteroid receptors blunts counterregulatory responses to subsequent hypoglycemia in healthy man

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Gustavson SM, Sandoval DA, Ertl AC, Bao S, Raj SR, Davis SN. Stimulation of both type I and type II corticosteroid receptors blunts counterregulatory responses to subsequent hypoglycemia in healthy man. Am J Physiol Endocrinol Metab 294: E506–E512, 2008. First published January 8, 2008; doi:10.1152/ajpendo.00589.2007.—Antecedent increases of corticosteroids can blunt counterregulatory responses to subsequent stress. Our aim was to determine whether prior activation of type I corticosteroid (mineralocorticoid) or type II corticosteroid (glucocorticoid) receptors blunts counterregulatory responses to subsequent hypoglycemia. Healthy volunteers participated in five randomized 2-day protocols. Day 1 involved morning and afternoon 2-h hyperinsulinemic (9 pmol·kg⁻¹·min⁻¹) euglycemic clamps (PE; n = 14), hypoglycemic clamps (PH; n = 14), or euglycemic clamps with oral fludrocortisone (PE + F; type I agonist, 0.2 mg, n = 14), oral dexamethasone (PE + D; type II agonist, 0.75 mg, n = 13), or both (PE + F + D; n = 14). Day 2 was identical in all protocols and consisted of a 2-h hyperinsulinemic hypoglycemic clamp. Day 2 insulin (625 ± 40 pmol/l) and glucose (2.9 ± 0.1 mmol/l) levels were similar among groups. Levels of epinephrine, norepinephrine, glucagon, growth hormone, and MSNA were significantly blunted by prior activation of both type I and type II corticosteroid receptors to PE. Prior activation of both corticosteroid receptors also significantly blunted NEFA during subsequent hypoglycemia. Thus, levels of a wide spectrum of key counterregulatory mechanisms (neuroendocrine, ANS, and metabolic) were blunted by antecedent pharmacological stimulation of either type I or type II corticosteroid receptors in healthy man. These data suggest that activation of type I corticosteroid receptors in man can have acute and profound regulating effects on physiological stress in man. Both type I and type II corticosteroid receptors may be involved in the multiple mechanisms controlling counterregulatory responses to hypoglycemia in healthy man.

human; counterregulation; cortisol

RIGOROUS GLYCEMIC CONTROL can be associated with diminished symptom awareness along with decreased neuroendocrine, metabolic, and autonomic nervous system (ANS) responses to hypoglycemia. Recent work (10) has implicated antecedent hypoglycemia as a major contributor to these blunted counterregulatory responses. In fact, the counterregulatory defects found in intensely treated type 1 DM can all be produced by repeated hypoglycemia in patients with insulinoma and in healthy individuals (11, 34).

The mechanisms responsible for reduced ANS responses during hypoglycemia are not fully elucidated. Several hypotheses have been proposed, including increased alternate cerebral fuel substrate delivery such as lactate or ketone bodies (4, 19), changes in cerebral glucose uptake (19, 42), and activation of the hypothalamo-pituitary axis (15). All of the proposed mechanisms explaining reduced ANS responses during hypoglycemia are somewhat controversial, with data both supporting and contrary to each hypothesis (10, 16, 44). Studies in several species, including sheep (30), dogs (28), and humans (15, 33), have demonstrated that prior elevations of corticosteroids can reduce ANS responses to subsequent hypoglycemia. Additionally, acute elevations of cortisol can blunt sympathetic nervous system tone as measured by muscle sympathetic nerve activity (MSNA) in healthy man (18). Furthermore, antecedent hypoglycemia produced no blunting of ANS and metabolic responses to next-day hypoglycemia in patients with Addison’s disease (who have an absent corticosteroid response to stress) and in healthy individuals whose endogenous cortisol responses were blocked by metyrapone (14, 36). On the other hand, two recent studies incorporating lower antecedent increases in plasma cortisol have not observed reduced ANS responses to subsequent hypoglycemia in healthy man (22, 37).

Endogenous corticosteroids can initiate their actions by binding to mineralocorticoid receptors (MR; type I corticosteroid receptors) and/or glucocorticoid receptors (GR; type II) or via nongenomic mechanisms. It is classically believed that type I corticosteroid receptors are nearly continuously occupied (~90%) and exert no acute regulatory action. This is in contrast to the type II receptors that acutely modulate responses to stress. Previous studies (17) have shown that activation of both receptors can acutely downregulate hippocampal-hypothalamic-autonomic function. However, no data are available regarding whether activation of type I receptors can acutely influence neuroendocrine or ANS responses to hypoglycemia. Furthermore, it is unknown whether cortisol exerts its action to downregulate subsequent ANS homeostatic mechanisms via specific effects on type I and/or type II corticosteroid receptors. Therefore, the specific aim of this study was to determine, using pharmacological probes, which corticosteroid receptor(s) is responsible for blunting ANS, neuroendocrine, and metabolic responses to subsequent hypoglycemia in healthy humans. To test this hypothesis, fludrocortisone (a type I-specific corticosteroid agonist), dexamethasone (a type II-specific agonist), or both were administered on day 1 during systemic hyperinsulinemic euglycemia, and responses to subsequent clamped hypoglycemia were studied the following day.
RESEARCH DESIGN AND METHODS

Subjects. We studied 17 (8 male/9 female) healthy volunteers (age 28 ± 2 yr, body mass index 25 ± 1 kg/m², Hb A₁c, 4.8 ± 0.1). The subjects were nonsmokers, had no family history of diabetes, and were not taking any medications, and all subjects had normal liver, renal, and hematological parameters. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board, and all subjects gave informed written and verbal consent. Some of the data from the two control groups in this paper came from individuals studied previously (13).

Experimental design. The volunteers participated in five separate 2-day experiments, with differing day 1 protocols, separated by ≥2 mo. The experiments were randomized and performed in a single-blind fashion. Women were studied at the same point in their menstrual cycle for each arm of the study. All subjects were instructed to avoid intense exercise and alcohol and to consume their usual weight-maintaining diet for 3 days before each study. Each subject was admitted to the Vanderbilt University Clinical Research Center the evening before an experiment. The next morning, after an overnight 10-h fast, subjects had an intravenous cannula placed into each arm under local 1% lidocaine anesthesia. One cannula was placed in a retrograde fashion into a vein in the back of the hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained (2). The other cannula was placed in a large vein in the contralateral arm for infusions of dextrose, insulin, and potassium chloride.

Day 1 consisted of different antecedent challenges (euglycemia alone, hypoglycemia alone, or euglycemia with the administration of a steroid hormone), the effects of which were measured during standardized hypoglycemia on day 2. On day 1 in all studies, following an overnight 10-h fast, there was a baseline period (0–120 min) and a 2-h hyperinsulinemic experimental “clamp” period (120–240 min). An insulin infusion solution was prepared with normal saline containing 3% (vol/vol) of the subject’s own plasma. At the onset of the experimental period a primed, continuous infusion of regular human insulin (ElI Lilly, Indianapolis, IN) was administered at a rate of 9 pmol·kg⁻¹·min⁻¹ for 120 min (Medfusion 3010; Medex-A Furon Healthcare, Duluth, GA). Potassium chloride (5 mmol/h, Iomed pump; Imed, San Diego, CA) was also infused during the clamp period to reduce insulin-induced hypokalemia. During the clamp periods, plasma glucose levels were measured every 5 min, and a variable infusion of 20% dextrose (variable-rate volumetric infusion pump; Imed) was adjusted so that plasma glucose levels were held at either euglycemia (5.2 mmol/l) or hypoglycemia (2.9 mmol/l) (16). After completion of the initial 2-h test period, plasma glucose was either restored to or maintained at euglycemia for an additional 2 h. At that point, insulin was restarted, and a second euglycemic (PE) or hypoglycemic clamp (PH), identical to the morning’s study, was performed. At completion of the second glucose clamp, subjects consumed a large, standardized meal and a bedtime snack prior to 10 PM and remained in the Clinical Research Center.

In the three corticosteroid experimental protocols, volunteers underwent two hyperinsulinemic euglycemic clamps as described above. In addition, at times 0 and 245 min (~8 AM and 12 PM), volunteers received oral doses of either fludrocortisone (0.2 mg at each time) and placebo (PE + F; n = 14, 7 males, 7 females), dexamethasone (0.75 mg at each time) and placebo (PE + D; n = 13, 6 males, 7 females), or both drugs (fludrocortisone, 0.2 mg, and dexamethasone, 0.75 mg; PE + F + D; n = 14, 7 males, 7 females) in a randomized single-blind fashion.

Day 2 was identical for all five protocols and was started after an overnight 10-h fast. Each study consisted of a basal period (0–120 min) and a 2-h experimental period (120–240 min). At the onset of the experimental period a primed, constant (9 pmol·kg⁻¹·min⁻¹) infusion of insulin was started and continued for the next 2 h. The rate of fall of glucose was controlled (<0.08 mmol/min), and the hypoglycemic nadir (2.9 mmol/l) was achieved and then held constant for the remainder of the study. Potassium chloride (5 mmol/h) was infused during the clamp as on day 1.

Direct measurement of MSNA via microneurography. MSNA was measured in the peroneal nerve at the level of the fibular head or popliteal fossa (12). A recording of MSNA was considered adequate when there was 1) spontaneous appearance of pulse-linked bursts, 2) increased nerve activity during phase II (hypotensive phase) and suppressed activity during phase IV (blood pressure overshoot) of the Valsalva maneuver, 3) increased nerve activity in response to held expiration (apnea), 4) insensitivity to emotional stimuli (loud yell or clap), and/or 5) proprioceptiveafferent signals in response to stretching the tendons in the foot or tapping the muscle belly but not cutaneous stimulation by stroking the skin.

Sympathetic nerve activity was expressed as bursts per minute. Measurements of MSNA were made from original tracings or online recordings (DI-220; Dataq Instruments, Akron, OH) by an operator blinded to the sequence of experiments.

Analytical methods. The collection and processing of blood samples have been described previously (7). Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Blood for hormones and intermediary metabolites was drawn twice during the control period and every 15 min during the experimental period. Glucagon was measured according to the method of Aguilar-Parada et al. (3), with an interassay coefficient of variation (CV) of 15%. Insulin was measured as previously described (45) with an interassay CV of 11%. Catecholamines were determined by high-pressure liquid chromatography (5), with an interassay 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. Growth hormone (interassay CV = 8%) (26), cortisol (interassay CV = 6%; Clinical Assays Gamma Coattoimmunoassay kit), pancreatic polypeptide (interassay CV = 8%) (23), and glucagon (interassay CV = 15%; Linco Research, St. Louis, MO) were measured using radioimmunooassay techniques. Lactate, glycerol, alanine, and β-hydroxybutyrate were measured on deproteinized whole blood using the method of Lloyd et al. (32). Nonesterified fatty acids (NEFA) were measured using the Wako kit adopted for use on a centrifugal analyzer (24).

Cardiovascular parameters (heart rate, systolic, diastolic, and mean arterial blood pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min throughout each 2-h insulin clamp. Symptoms of hypoglycemia were assessed every 15 min during the hypoglycemic clamps using a previously validated semiquantitative questionnaire (9).

Statistical analysis. Data are expressed as means ± SE and were analyzed using standard, parametric one- and two-way analysis of variance and with repeated measures where appropriate (SigmaStat; SPSS Science, Chicago, IL). Tukey’s post hoc analysis was used to delineate statistical significance across time within each group and for each group compared with the PE control group. A P value of <0.05 was accepted as statistically significant. Baseline period data represent an average of two time points (110 and 120 min), and final 30-min data represent an average of three measurements taken during this time (210, 225, and 240 min).

RESULTS

Glucose and insulin levels from day 1. Plasma glucose levels reached steady state by 30 min and were similar in the morning and afternoon during the prior hypoglycemic study (PH = 3.0 ± 0.1 and 3.1 ± 0.1 mmol/l, respectively). Likewise, glucose levels were similar during morning and afternoon.
euglycemic studies (5.1–5.3 ± 0.1 mmol/l). Plasma insulin levels were also similar among groups during the morning and afternoon glucose clamps (528 ± 66 to 690 ± 54 pmol/l).

Glucose, insulin, and counterregulatory hormones from day 2. Basal levels of insulin were similar in the PE (49 ± 6 pmol/l), PH (48 ± 9 pmol/l), and PE + F (44 ± 6 pmol/l) groups. However, basal insulin levels were increased in the PE + D (77 ± 11 pmol/l) and PE + F + D (66 ± 11 pmol/l) groups (P < 0.05). Insulin infusions resulted in steady-state glucose and insulin levels that were similar among groups (Fig. 1).

Plasma epinephrine, plasma norepinephrine, and MSNA increased from basal in all treatment groups during hypoglycemia (Fig. 2). Plasma epinephrine and MSNA during hypoglycemia were significantly blunted (P < 0.05) after prior hypoglycemia (2,222 ± 288 pmol/l, 33 ± 4 bursts/min) compared with prior euglycemia (4,193 ± 392 pmol/l, 45 ± 3 bursts/min). All three steroid treatments resulted in reduced epinephrine (PE + F = 3,109 ± 275, PE + D = 3,212 ± 427, PE + F + D = 2,643 ± 307 pmol/l), norepinephrine (PE = 1.9 ± 0.2, PE + F = 1.4 ± 0.1, PE + D = 1.4 ± 0.1, PE + F + D = 1.49 ± 0.2 nmol/l), and MSNA (PE + F = 36 ± 4, PE + D = 32 ± 4, PE + F + D = 30 ± 5 bursts/min) during hypoglycemia (all P < 0.05). Prior administration of the steroid hormones also resulted in a significant reduction (P < 0.05) in baseline norepinephrine and MSNA (PE + F = 20 ± 4, PE + D = 16 ± 2, PE + F + D = 17 ± 3 bursts/min) compared with PE (32 ± 5 bursts/min).

Pancreatic polypeptide and glucagon levels during hypoglycemia (Fig. 3) were also significantly blunted (P < 0.05) after prior hypoglycemia (197 ± 27 pmol/l, 132 ± 18 ng/l) compared with prior euglycemia (263 ± 33 pmol/l, 241 ± 34 ng/l). Prior administration of any of the three steroids also resulted in a blunting of pancreatic polypeptide (PE + F = 184 ± 28, PE + D = 180 ± 49, PE + F + D = 172 ± 25 pmol/l), glucagon (PE + F = 111 ± 12, PE + D = 139 ± 47, PE + F + D = 129 ± 12 pg/ml), and growth hormone (PE = 32 ± 4, PE + F = 51 ± 5, PE + D = 17 ± 3, PE + F + D = 18 ± 4, PE + F + D = 19 ± 4 µg/l) during hypoglycemia (all P < 0.05). Basal plasma cortisol levels were significantly reduced (P < 0.05) following day 1 administration of dexamethasone. Cortisol levels increased similarly during hypoglycemia in all groups (Table 1).

Glucose kinetics from day 2. The exogenous glucose infusion rate required to clamp glucose levels at 2.9 mmol/l on day 2 was significantly elevated (P < 0.05) after prior hypoglycemia compared with prior euglycemia (Table 2). Prior administration of fludrocortisone and both steroids in combination also resulted in an increased (P < 0.05) exogenous glucose requirement compared with prior euglycemia.

Intermediary metabolism from day 2. Glycerol levels fell significantly (P < 0.05) during hypoglycemia in all treatment groups except PE, implying a reduction in lipolysis (Table 3). Hypoglycemia resulted in significant reductions (P < 0.05) in plasma NEFA levels in all groups. NEFA levels during base-
line and hypoglycemia were reduced in the steroid treatment groups compared with prior euglycemia. Plasma lactate, alanine, and blood β-hydroxybutyrate responses during hypoglycemia were not different among groups.

Cardiovascular responses from day 2. Heart rate increased significantly (P < 0.05) and similarly in all treatment groups (ranging from +8 ± 2 to +13 ± 4 beats/min) (Table 4). There were greater reductions (P < 0.05) in mean arterial pressure during day 2 hypoglycemia following day 1 corticosteroid administration.

Hypoglycemic symptoms from day 2. After prior euglycemia, autonomic symptom scores increased by 10 ± 4 during hypoglycemia. Prior hypoglycemia significantly blunted (P < 0.05) the increase in autonomic symptoms (4 ± 1). However, prior

Table 2. Glucose infusion rate during the baseline and final 30 min of hyperinsulinemic hypoglycemia in overnight-fasted healthy volunteers exposed to PE, PH, PE + F, PE + D, or PE + F + D

<table>
<thead>
<tr>
<th>Glucose Infusion Rate</th>
<th>Baseline period, min</th>
<th>Final 30 min of hypoglycemia, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>PE</td>
<td>0</td>
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</tr>
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<tr>
<td>PE + F + D</td>
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</tbody>
</table>

Data are means ± SE for each group and in μmol·kg⁻¹·min⁻¹. *Significant difference vs. PE (P < 0.05); †significant change (P < 0.05) from basal within a group.

Table 3. Intermediary metabolite levels during the baseline and final 30 min of hyperinsulinemic hypoglycemia in overnight-fasted healthy volunteers exposed to PE, PH, PE + F, PE + D, or PE + F + D

<table>
<thead>
<tr>
<th>Blood lactate, mmol/l</th>
<th>Baseline Period</th>
<th>Final 30 min of Hypoglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>1.0±0.1</td>
<td>1.6±0.1†</td>
</tr>
<tr>
<td>PH</td>
<td>0.9±0.1</td>
<td>1.4±0.1†</td>
</tr>
<tr>
<td>PE + F</td>
<td>1.3±0.1</td>
<td>2.0±0.2†</td>
</tr>
<tr>
<td>PE + D</td>
<td>1.2±0.1</td>
<td>1.7±0.1†</td>
</tr>
<tr>
<td>PE + F + D</td>
<td>1.2±0.1</td>
<td>1.6±0.2†</td>
</tr>
</tbody>
</table>

Blood alanine, μmol/l

| PE                    | 388±31           | 322±20†                     |
| PH                    | 394±27           | 327±19†                     |
| PE + F                | 437±40           | 354±36†                     |
| PE + D                | 454±33           | 387±22†                     |
| PE + F + D            | 484±38           | 382±30†                     |

Blood glycerol, μmol/l

| PE                    | 44±5             | 41±7                         |
| PH                    | 43±5             | 33±5†                        |
| PE + F                | 85±15*           | 70±13†                       |
| PE + D                | 66±8             | 47±3†                        |
| PE + F + D            | 70±12            | 47±5†                        |

Plasma NEFA, μmol/l

| PE                    | 359±53           | 160±28†                      |
| PH                    | 452±42           | 160±15†                      |
| PE + F                | 229±24*          | 76±12†                       |
| PE + D                | 269±22*          | 75±12†                       |
| PE + F + D            | 238±29*          | 60±9†                        |

Blood β-OHB, μmol/l

| PE                    | 34±7             | 14±2†                        |
| PH                    | 32±6             | 8±1†                         |
| PE + F                | 37±4             | 24±3†                        |
| PE + D                | 36±5             | 20±3†                        |
| PE + F + D            | 42±7             | 18±2†                        |

Data are means ± SE for each group. NEFA, nonesterified fatty acids; β-OHB, β-hydroxybutyrate. *Significant difference (P < 0.05) vs. PE within a time period (either basal or final 30 min); †significant change from basal (P < 0.05) within a group.
administration of fludrocortisone, dexamethasone, and both steroids together did not blunt autonomic symptoms (13 ± 2, 10 ± 2, and 9 ± 1, respectively).

After prior euglycemia, neuroglycopenic symptom scores increased by 10 ± 3 during hypoglycemia. Prior hypoglycemia significantly blunted the increase in neuroglycopenic symptoms (4 ± 1). Prior administration of fludrocortisone, dexamethasone, and both steroids together also reduced the increase in neuroglycopenic symptoms (5 ± 2, 6 ± 2, and 6 ± 2, respectively).

**DISCUSSION**

The present study examined the effects of antecedent pharmacological stimulation of MR (type I), GR (type II), or both corticosteroid receptors on counterregulatory drive during subsequent clamped hypoglycemia. Previous-day administration of fludrocortisone (a type I agonist) or dexamethasone (a type II agonist), similarly to prior hypoglycemia, resulted in significant and extensive blunting of a wide spectrum of neuroenocrine and ANS counterregulatory mechanisms during the subsequent hypoglycemia. These results indicate that both type I and type II corticosteroid receptors can acutely regulate physiological responses to subsequent stress. Our data also demonstrate that prior activation of MR can acutely induce a profound reduction in ANS drive during subsequent hypoglycemia.

Traditionally, GR have been implicated as the primary protectors of the body from the effects of repeated stress, especially since GR are mostly unoccupied at basal cortisol levels. Thus, 2-day dexamethasone treatment (2 mg/day) inhibited insulin-induced increases in MSNA during a euglycemic clamp in humans (41). Dexamethasone, similarly to prior glucoprivation induced by 2-deoxyglucose, blunted food intake during subsequent glucoprivation in rats (38). Furthermore, intracerebroventricular injection of dexamethasone attenuated the stress-induced release of catecholamines (21). However, a role for MR during stress is emerging. Kalman and Spencer (27) have recently shown that previous studies may have significantly overestimated the proportion of type I receptors occupied by basal cortisol levels, implying that MR can participate in acute stress responses. Furthermore, MR are abnormally expressed in the hippocampus of obese Zucker rats and diabetic rats under both basal conditions and hypoglycemia (6). Additionally, blockade of MR prevented the inhibitory effects of corticosterone on repeated restraint stress in rats (8). Thus, taken together, these above studies suggest that both MR and GR are involved in mediating stress responses. However, this present study is the first to show that an MR agonist can affect counterregulatory activity during subsequent hypoglycemic stress.

Antecedent administration of both steroid agonists, similarly to antecedent hypoglycemia and cortisol (15), significantly reduced ANS activity during hypoglycemia. Both levels of sympathetic (as evidenced by epinephrine, norepinephrine, and MSNA) and parasympathetic (as evidenced by the partially parasympathetically mediated pancreatic polypeptide) nervous system drive were reduced during hypoglycemia following steroid treatment. Corticosteroid agonists reduced sympathetic nervous system activity during subsequent hypoglycemia by differing mechanisms. Epinephrine levels were reduced by limiting the incremental response of the hormone during day 2 hypoglycemia. On the other hand, levels of both MSNA and norepinephrine were reduced by effects of corticosteroid agonists to blunt basal activity rather than limiting incremental responses during hypoglycemia. In contrast, autonomic hypoglycemic symptoms were not affected by prior steroids, although they were blunted after prior hypoglycemia and prior cortisol (15). Neuroglycopenic symptoms, however, were reduced 40–50% following prior administration of type I and type II corticosteroid receptor agonists. The mechanism for the lack of an effect of the corticosteroid agonists on autonomic symptoms is not apparent but may be related to the fact that incremental increases in sympathetic neural responses were also unaffected by prior steroids. However, the marked reduction of neuroglycopenic symptoms clearly indicates that these agonists have an effect on reducing hypoglycemic symptoms.

Activation of both type I and type II corticosteroid receptors reduced a wide spectrum of pituitary and pancreatic neuroendocrine counterregulatory responses. The blunted growth hormone levels following steroid treatment could be due to corticosteroid effects to decrease ghrelin receptors, decrease responsiveness to growth hormone-releasing hormone, and/or increase somatostatin (25). The blunted glucagon response in the steroid groups could be the result of decreased ANS input to the pancreas, decreased glucose sensing of the α-cells, or a direct decrease in glucagon expression (1, 31). Finally, basal cortisol levels tended to be reduced following administration of the GR, but not the MR, agonist. This is consistent with previous work, as glucocorticoids (29), but not mineralocorticoids (35), have been shown to suppress basal hypothalamic-pituitary-adrenal (HPA) activity. The failure of either agonist to reduce hypoglycemia-stimulated cortisol release is consistent with a previous study (43) that showed no effect of prior
corticosteroid administration on the corticosterone response to hypoglycemia.

Similarly to prior hypoglycemia, the day 2 exogenous glucose infusion rates were increased following day 1 steroid agonist administration. This finding is consistent with and reflects the reduced ANS and neuroendocrine responses occurring following day 1 steroid agonists. The increased glucose infusion rates during day 2 hypoglycemia are even more notable, as the steroid agonists would have been expected to produce increased insulin resistance. Supporting this, day 2 basal insulin levels in the two dexamethasone groups were in fact higher than those in the control groups, thus indicating an increase in insulin resistance.

Interestingly, each corticosteroid agonist and both steroids combined produced similar effects in most, but not all, counterregulatory responses during subsequent hypoglycemia. The lack of an additive effect of the two corticosteroid agonists is intriguing. Potential explanations include that the strong similarity of the DNA-binding domains of the corticosteroid receptors could lead to activation and repression of the same genes. On the other hand, although many of the physiological responses following the two corticosteroids were similar, there were some differences between fludrocortisone and dexamethasone. For example, cortisol levels and glucose infusion rates were lower, but insulin levels were higher following dexamethasone compared with fludrocortisone. Thus, it is possible that the two corticosteroid agonists may have been working through independent pathways that produced separate and not combined effects. Another explanation for the lack of additive effect of the steroids is that the neuroendocrine and ANS counterregulatory activity to hypoglycemia were already maximally blunted with either agonist. In fact, it is worth noting that each corticosteroid blunted overall counterregulatory activity to a similar extent as prior hypoglycemia.

Although our current results and other literature support a role for the HPA axis in hypoglycemia-associated autonomic failure, the relationship may be complex. For example, Flanagan et al. (20) found that corticotropin-releasing hormone administration, but not ACTH or corticosterone, blunted counterregulatory responses to hypoglycemia in rats. Third-ventricle (19) and peripheral (43) infusion of corticosterone and lateral ventricle infusion of dexamethasone (39) in rats did not cause blunting of the counterregulatory responses to repeated hypoglycemia. This was in contrast to studies in rats that showed blunting of hypoglycemic responses after central cortisol (40), peripheral, and fourth-ventricle dexamethasone (39) and other studies where peripheral cortisol and dexamethasone caused blunting of sympathetic responses to stress (30, 38).

The discrepancies in animal studies may be due to route of administration, discussed in detail elsewhere (39). In humans, McGregor et al. (33) demonstrated that pharmacological doses of ACTH, which increased endogenous cortisol to supraphysiological levels, reduced autonomic, neuroendocrine, and symptom responses to subsequent hypoglycemia. On the other hand, lower levels of exogenous cortisol infusion did not result in diminished ANS responses during subsequent hypoglycemia (22, 37). Therefore, it appears that the differences in the above studies may be plausibly related to dosing issues, with an effect seen only at pharmacological cortisol levels. Although a dose-response curve remains to be performed, we chose doses roughly twice that of normal replacement doses to simulate the approximately threefold rise of cortisol that usually occurs during hypoglycemia in healthy volunteers. Note that, although there is a great deal of evidence for a role of the HPA axis in the pathogenesis of hypoglycemia-associated autonomic failure, other factors such as altered brain glycogen stores and altered brain glucose sensing and uptake may also be important in the dysfunction.

In summary, these studies demonstrate that, in healthy humans, prior pharmacological stimulation of type I and type II corticosteroid receptors with fludrocortisone or dexamethasone can blunt autonomic, neuroendocrine, and metabolic counterregulatory activity during subsequent clamped hypoglycemia. We thus conclude that both type I and type II corticosteroid receptors can acutely regulate stress responses and may be involved in the multiple mechanisms controlling counterregulatory drive during hypoglycemia in healthy man.

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CORTICOSTEROID RECEPTORS AND HYPOGLYCEMIA