Feedback Inhibition of Adrenocorticotropic Hormone by Physiological Increases in Plasma Corticosteroids in Conscious Dogs

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ABSTRACT We have tested the effect of physiological increases in plasma corticosteroids in conscious dogs on the levels of basal and hypoglycemia-stimulated adrenocorticotropic hormone (ACTH) 2 h later. Increases in plasma corticosteroids, produced by infusion of α-1-24 ACTH or corticosteroids for 40 min, suppressed basal and stimulated ACTH levels. The magnitude of inhibition produced by an increase in plasma corticosteroids induced by the infusion of ACTH was equivalent to the inhibition produced by the same increase in plasma corticosteroids induced by corticosteroid infusion. The infusions did not affect basal plasma glucose concentrations or the decrease in plasma glucose concentrations after administration of 0.1 U insulin/kg. Basal ACTH concentration was less sensitive than hypoglycemia-stimulated ACTH concentration to corticosteroid-induced suppression. Basal and stimulated secretion were significantly inhibited in all dogs after approximately half-maximal increases in plasma corticosteroids; maximum inhibition occurred after maximal increases in plasma corticosteroids. Therefore, physiological increments in plasma corticosteroids, similar to those produced by acute stress, are effective suppressors of subsequent stress-induced ACTH secretion.

INTRODUCTION

Feedback regulation of adrenocorticotropic hormone (ACTH)[1] by glucocorticoids is a prominent control mechanism in the adrenocortical system. Clinically, knowledge of this regulation is used for differential diagnosis of Cushing's syndrome with the dexamethasone suppression test (1). Cortisol infusions have also been used in man to determine the suppressibility of ACTH (2). With a single exception (3), the doses of steroids used for suppression have been large, and probably supraphysiological.

The purpose of this series of experiments was to test the effect of a temporary increase in corticosteroids on basal and stimulus-induced ACTH secretion. We have used ACTH infusions to stimulate adrenocortical secretion; the ACTH infusion rates and the duration of the infusion used were chosen to simulate the changes in ACTH that occur after acute stimuli, such as hypoglycemia. To test whether the inhibitory effect of ACTH infusion was a consequence of the corticosteroids produced, cortisol and corticosterone were infused to increase plasma corticosteroids to levels at which feedback occurred after ACTH infusion. We used insulin-induced hypoglycemia as the stimulus to the hypothalamo-pituitary-adrenal axis since we have found that this is a reproducible stimulus for which the relation between the stimulus intensity (change in plasma glucose) and ACTH response is known (4). We examined the ACTH response to hypoglycemia 2 h after the onset of the ACTH infusion since studies in rats have shown that corticosteroid feedback is effective at this time (5–7).

METHODS

Three male and two female mongrel dogs, weighing 17–28 kg, were studied. Each dog participated in six to eight experiments. In six of these experiments saline or each of four (20, 50, 100, and 200 ng/min) doses of ACTH (α-1-24 ACTH; Cortrosyn, Organon Diagnostics, West Orange, NJ) or cor-

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[1] *Abbreviations used in this paper:* ACTH, adrenocorticotropic hormone; ANOVA, analysis by one- or two-way analysis of variance.

ticosteroids (25–30 μg/min) were infused for 40 min, and 2 h after the start of the infusion 0.10 U insulin/kg body wt was injected. Two of these dogs and two additional dogs were studied in two further experiments in which two doses of ACTH (500 and 1,000 ng/min) were infused and 0.10 U/kg insulin was injected 2 h later. The order in which the experiments were performed was varied from dog to dog.

The dogs were housed in individual cages in a room with a controlled (12 h light, 12 h dark) light-dark cycle. All dogs had been trained to stand in a mesh sling (Alice King Chatham Medical Arts, Inc., Los Angeles, CA) before the day of the first experiment. The dogs were fasted overnight (at least 17 h) before each experiment, but were otherwise allowed free access to food (Purina dog chow, Ralston Purina Co., St. Louis, MO) and water.

On the day of an experiment, a dog was brought to the laboratory, weighed and placed in the sling. A catheter was inserted into a saphenous vein (Angiocath, 18-gauge needle, eight in catheter; Deseret Co., Sandy, UT) and used for the withdrawal of blood samples and the injection of insulin. A second catheter was inserted into the other saphenous vein or a cephalic vein and used for the infusion of saline, ACTH, or corticosteroids.

At least 45 min after the insertion of the catheters, the experiment was started (between 1000–1130 h). Two control blood samples were withdrawn from the venous catheter for plasma glucose, ACTH, and corticosteroid measurements. Then an infusion of saline, ACTH, or corticosteroids was begun.

All infusions were delivered for 40 min at the rate of about 0.8 ml/min using a syringe pump (Harvard Apparatus, Inc., S. Natick, MA). The vehicle for all infusates was sterile, pyrogen-free saline (Travenol Laboratories, Inc., Deerfield, IL) containing 10 U sodium heparin/ml (Organon Inc., West Orange, NJ). The ACTH infusate was prepared by adding a thawed aliquot of frozen α-1-24 ACTH solution to saline. The corticosteroid infusate was prepared by adding an aliquot of a stock solution of cortisol and corticosterone (3:1 cortisol/corticosterone; Sigma Chemical Co., St. Louis, MO) to saline. The ratio of 3:1 cortisol/corticosterone was used because Hechter et al. (8) have reported this to be the average ratio of these steroids in dog adrenal venous blood. Blood samples were withdawn for plasma glucose measurements at 20, 30, and 40 min after the start of the infusion, and for plasma ACTH corticosteroids at 25, 30, 35, 40, 60, 80, 100, and 120 min after the start of the infusion. After the infusion of 500 or 1,000 ng/min ACTH, however, samples were not withdrawn at 60, 80, or 100 min.

2 h after the start of the ACTH or steroid infusion, regular insulin (Eli Lilly & Co., Indianapolis, IN) in 5 ml of sterile saline was injected and immediately flushed with another 5 ml of saline. Venous blood samples were withdrawn 0, 10, 20, 25, 30, 35, 40, and 45 min after the injection of saline. Blood for plasma glucose measurements was placed in tubes containing sodium fluoride and potassium oxalate (Sigma Chemical Co.) on ice. Blood for hormone measurements was placed in heparinized plastic tubes on ice. Blood samples were centrifuged at 3,000 rpm for 20 min, and aliquots of plasma frozen for later hormone analysis. The erythrocytes were resuspended in sterile saline and returned to the dog. At least 96 h elapsed before another experiment was performed on the same dog.

Plasma glucose measurements were performed on the day of the experiment using the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Inc., Fullerton, CA). Plasma ACTH concentrations were measured by radioimmunoassay (9). Plasma corticosteroid levels were measured by competitive protein binding assay using human transcortin as the binding protein (10).

Glucose and ACTH responses over time, and the effect of ACTH infusions on these responses were analyzed by one- and two-way analysis of variance (ANOVA) corrected for repeated measures (11). Differences between time points were analyzed by Duncan's multiple range test (12). The relationships between plasma corticosteroid levels during the infusion and basal ACTH and ACTH responses to hypoglycemia were analyzed by linear regression analysis, and the slopes of the two lines compared by Student's t test (13).

RESULTS

The ACTH infusions produced dose-related increases in plasma ACTH and corticosteroids. ACTH infusions reduced both the basal plasma ACTH concentration measured 2 h after the onset of the infusion and the magnitude of the hypoglycemia-induced increase in plasma ACTH 2 h later. ACTH infusions did not alter plasma glucose concentrations at any time (Fig. 1).

The threshold ACTH infusion rate for subsequent suppression of basal ACTH and the ACTH response to hypoglycemia was 20 ng/min, since the peak ACTH response was reduced (by ~50%) in two of five dogs and the onset of the responses was slower in all dogs. At this ACTH infusion rate ACTH was increased to 109±8 pg/ml and corticosteroids were increased to 4.9±0.5 μg/100 ml. Complete suppression of the ACTH response to hypoglycemia occurred after 500 or 1,000 ng/min ACTH (P = NS by one-way ANOVA). These infusion rates increased plasma ACTH to 981±112 and 2,140±78 pg/ml and plasma corticosteroids to 10.2±0.6 and 11.4±0.6 μg/100 ml, respectively. These infusion rates also completely suppressed basal ACTH concentrations (as compared with the limit of detectability of ACTH in the assay). Following ACTH infusion rates between 20 and 200 ng/min, there were still significant ACTH responses to hypoglycemia ($P < 0.05$ by one-way ANOVA). After these infusion rates, mean ACTH concentrations during hypoglycemia plateaued during the experimental period, but at a lower plasma ACTH concentration (Fig. 1).

The prior infusion of ACTH delayed the onset, as well as the magnitude, of the ACTH response to subsequent hypoglycemia. After saline infusion plasma ACTH was increased by 140 min (20 min after the injection of insulin); however, after the infusion of 20 ng/min ACTH the first increase in plasma ACTH was at 145 min (25 min after the injection of insulin) and after the infusion of 50 ng/min ACTH the first increase in plasma ACTH occurred at 150–155 min (30–35 min after the injection of insulin).

When corticosteroids were infused at rates chosen to approximate the smallest increase in plasma steroids

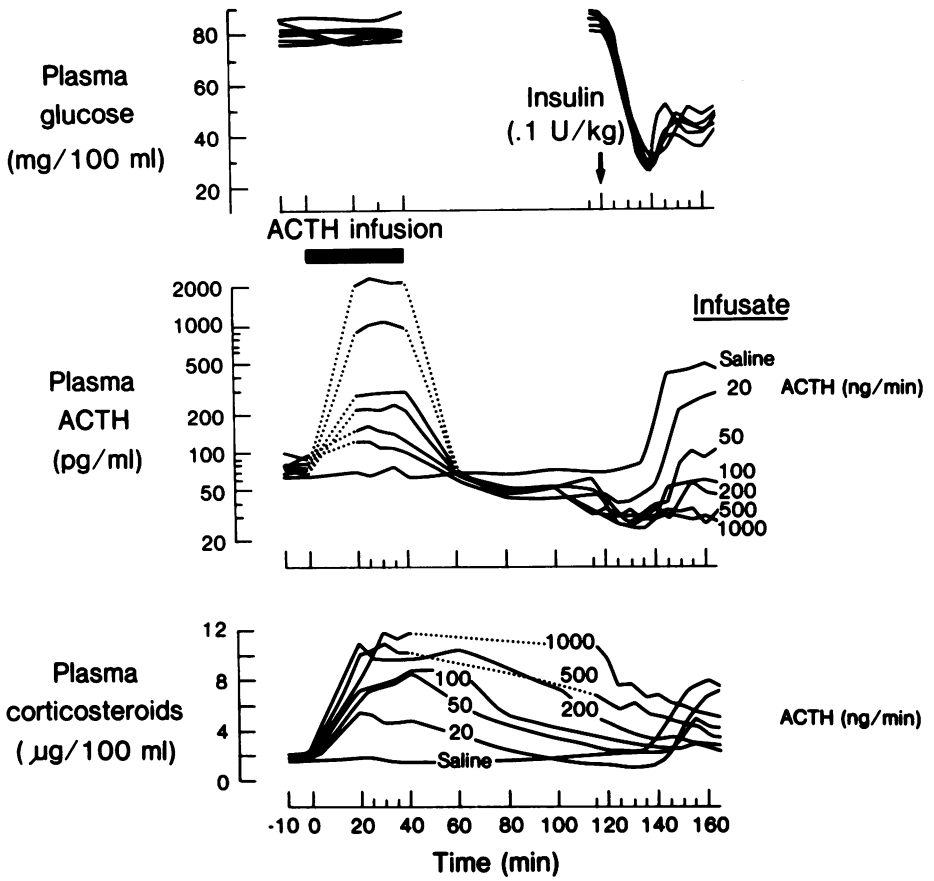

FIGURE 1 The mean plasma glucose, ACTH, and corticosteroid responses to ACTH infusion and insulin injection. α-1-24 ACTH was infused from 0 to 40 min at the rate shown to the right of the corresponding ACTH curve, and on the corticosteroid curve. 0.10 U/kg insulin was injected in all experiments at 120 min.

in each dog that reduced the ACTH response to hypoglycemia, both basal and hypoglycemia-stimulated ACTH concentrations were reduced (Fig. 2). During the period of the corticosteroid infusion, basal plasma ACTH concentrations were suppressed relative to preinfusion concentrations (mean control = 77±5 pg/ml; mean during infusion = 54±4 pg/ml; $P < 0.05$ by Duncan multiple range after two-way ANOVA). It appeared that basal ACTH secretion was also suppressed during the ACTH infusions, since during the infusion of 20 ng/min ACTH, the magnitude of the increase in plasma ACTH tended to fall. Basal ACTH concentrations tended to fall further after the end of the corticosteroid or ACTH infusions and were always lower than the preinfusion controls in samples withdrawn between 60 and 125 min. Maximum inhibition of basal ACTH levels occurred between 120 and 140 min; in most dogs an obvious drop in plasma ACTH concentrations occurred between 115 and 125 min, although the timing of this sharp decrease varied among dogs and experiments.

The degree of inhibition of basal and hypoglycemia-stimulated ACTH concentrations was exponentially related to the plasma corticosteroid levels achieved during the ACTH or corticosteroid infusions (Fig. 3). The ACTH infusions produced a similar degree of inhibition as corticosteroid infusions that achieved the same plasma corticosteroid levels, indicating that the increase in plasma corticosteroids caused the inhibition after ACTH infusion. Basal ACTH was significantly less sensitive to inhibition by corticosteroids than was the hypoglycemia-stimulated increase in ACTH, as indicated by the lower slope for the relation between corticosteroids and the logarithm of the basal ACTH concentrations as compared with the logarithm of the stimulated ACTH concentration ($t_{slopes} = 6.62$; $P < 0.01$).

We have previously found that the total ACTH re-

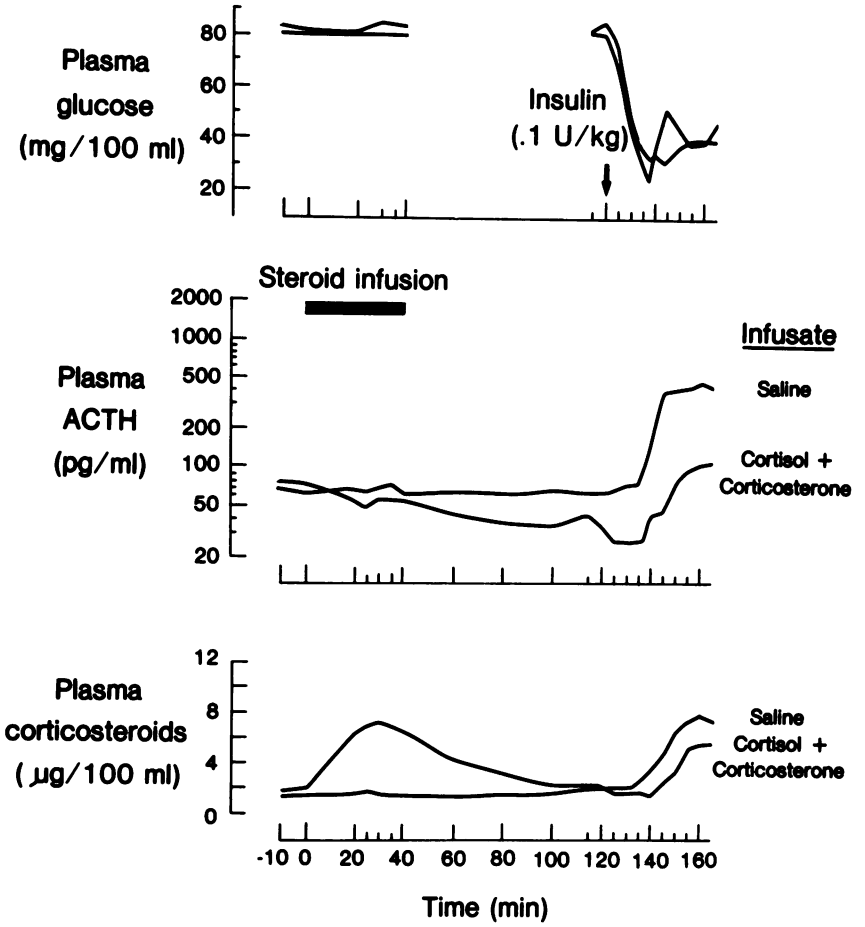

FIGURE 2 The mean plasma glucose, ACTH, and corticosteroid responses to corticosteroid infusion and insulin injection. A combination of cortisol and corticosterone or saline was infused from 0 to 40 min and 0.10 U/kg insulin was injected in all dogs at 120 min. The infusate is indicated to the right of the corresponding line.

sponse above control levels integrated over the experimental period (change in concentration × time) is linearly related to the magnitude of the glucose change integrated over the same period (4); that is, the hormonal response is proportional to the stimulus intensity. Although prior ACTH or steroid infusions did not alter the stimulus intensity, as measured by the glucose response (P = NS by ANOVA), the greater the prior ACTH infusion rate, and therefore the higher the prior plasma levels of corticosteroids, the smaller the ACTH response to the same degree of hypoglycemia (Fig. 4). There was no significant integrated ACTH response to hypoglycemia after 100–1,000 ng/min ACTH. However, after either the infusion of 100 or 200 ng/min ACTH, ACTH concentrations during hypoglycemia rose relative to control concentrations, and this increase over time was significant ($P < 0.05$ by ANOVA). The absence of a significant integrated ACTH response after 100 or 200 ng/min ACTH infusions resulted from the decrease in basal ACTH concentrations between the time of the control sample (120 min) and the time of the onset of the ACTH response to hypoglycemia.

DISCUSSION

We have shown that physiological concentrations of circulating plasma corticosteroids act to inhibit basal and stimulus-induced ACTH secretion in the dog. We used ACTH infusions to stimulate adrenal corticosteroid secretion and thereby increase endogenous corticosteroid levels. The increase in ACTH and the corresponding levels of plasma corticosteroids that were effective in suppressing subsequent ACTH secretion

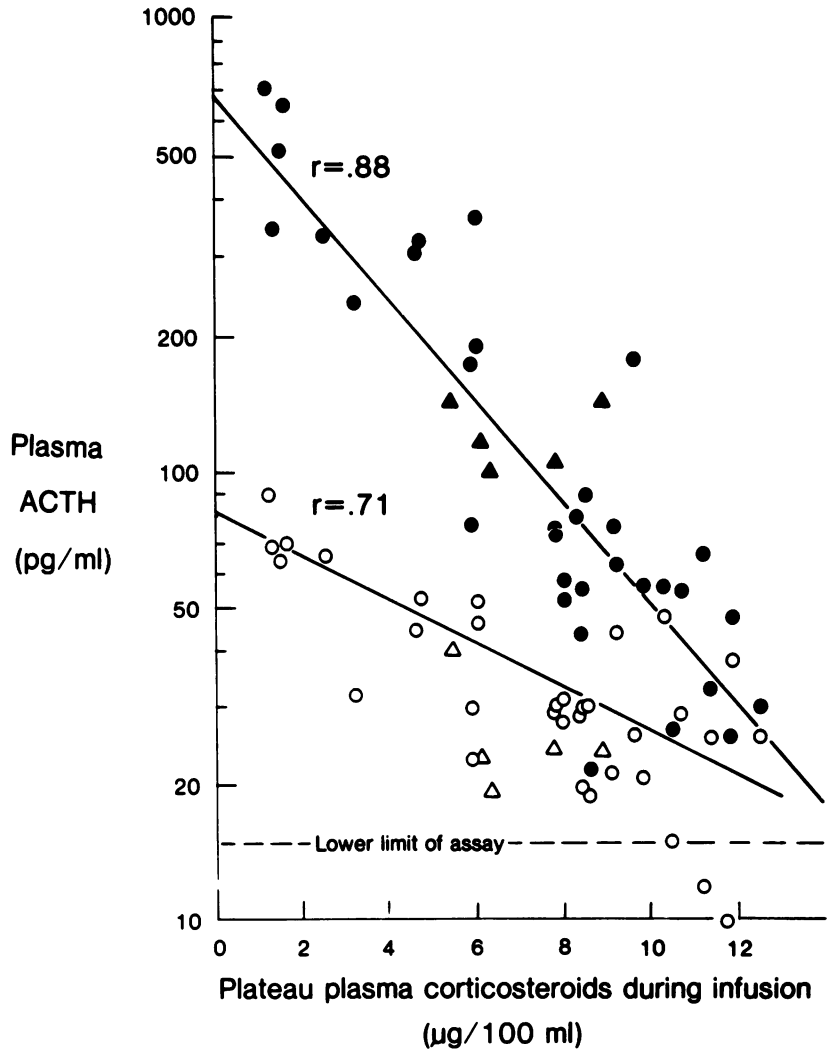

FIGURE 3 Relationship between the plateau plasma corticosteroid concentrations during ACTH or corticosteroid infusions and basal or hypoglycemia-stimulated plasma ACTH concentrations. Triangles (△, ▲) indicate the relationship when corticosteroids were infused (n = 5); circles (○, ●) indicate the relationship when ACTH was infused (n = 33). Open circles and triangles (○, △) and the line indicate the relationship between plasma corticosteroids and basal ACTH at 125 min (r = 0.71, $P < 0.01$); filled circles and triangles (●, ▲) and the line indicate the relationship between plasma corticosteroids and the maximum hypoglycemia-stimulated plasma ACTH concentration (r = 0.88, $P < 0.01$).

are similar to the levels achieved in dogs during acute hypoglycemia (100–700 pg/ml ACTH, 3–11 μg/100 ml corticosteroids) (4), hemorrhage (20–80 pg/ml, 2–6 μg/100 ml) (14) or hypoxia (100–400 pg/ml, 2–12 μg/100 ml) (15), or after endotoxin injection (1,500 pg/ml, 12 μg/100 ml) (unpublished observation), or during hypoglycemia (100–300 pg/ml, 20 μg/100 ml) (16) in man.

The suppression of the adrenocortical system response to hypoglycemia probably resulted from the increase in plasma corticosteroid concentrations that occurred during the ACTH infusion. Cortisol and corticosterone were infused in a ratio (3:1 cortisol/corticosterone) and at rates chosen to simulate the increase in plasma corticosteroids that occurred during the minimum effective ACTH infusion rate (20 or 50 ng/min ACTH) in each dog; these infusions produced the same suppression of basal and hypoglycemia-stimulated ACTH concentrations for a given plateau of plasma corticosteroid level as the ACTH infusions did

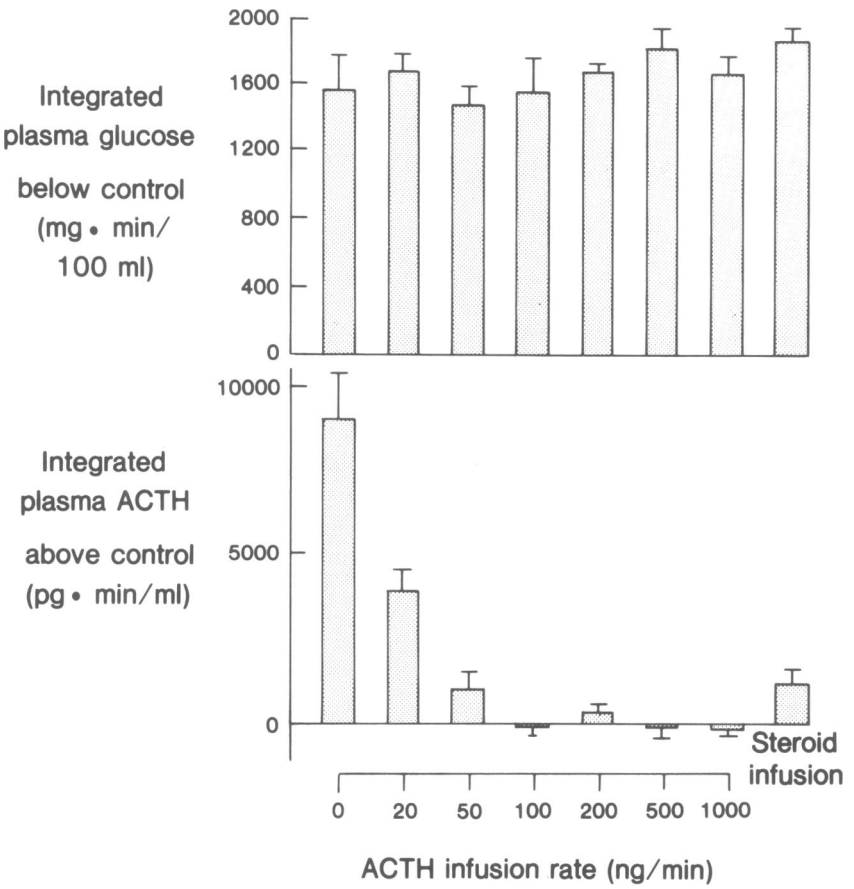

FIGURE 4 Integrated glucose and ACTH responses to the injection of 0.1 U/kg insulin after ACTH and corticosteroid infusions. Integrated glucose responses were calculated as the area (from 120 to 165 min) below the control concentration at 120 min, and integrated ACTH responses were calculated as the area (from 120 to 165 min) above the control concentration at 120 min. The prior infusion is indicated below each column.

(Fig. 3). Since ACTH infusions did not produce greater suppression of basal or stimulated ACTH concentrations, there is no evidence for a direct inhibitory effect of ACTH. The data suggest that an indirect inhibitory effect of the ACTH infusion resulted from the actions of corticosteroids. A lack of a direct ACTH feedback inhibition on subsequent ACTH secretion was also suggested by previous studies in rats (17).

Both basal and stimulus-induced ACTH secretion are markedly affected by prior increases in corticosteroids. Increasing plasma corticosteroid concentrations to only half-maximal levels for only 40 min was effective in reducing the magnitude of the peak ACTH response to hypoglycemia in two of five of the dogs, and reduced the total integrated ACTH response to this stimulus as well as the basal ACTH concentration, in all of the dogs. Complete suppression of stimulated and basal ACTH secretion was produced by increases in plasma corticosteroids similar to those caused by acute, severe hypoxia (15) or hypoglycemia (4) or by surgery. The results also suggest that corticosteroid feedback both delays the onset of the ACTH response to stress and inhibits the magnitude of the increase in ACTH during stress; the total ACTH secretory response to a stimulus is more sensitive to steroid feedback because of these combined effects.

In this study both basal and hypoglycemia-stimulated ACTH concentrations were exponentially reduced by increases in plasma corticosteroid 2 h earlier. Inhibition of stress-induced adrenocortical responses has been previously shown to depend on the dose of exogenous corticosteroids administered and to follow the increase in corticosteroids by ~2 h ("delayed feedback," 5). The adrenocortical responses to hemorrhage

in dogs (7) and to scald and low doses of histamine (5) and to cold (18) in rats are all inhibited in a steroid dose-dependent manner by the administration of dexamethasone 2–7 h earlier. However, it is clear that there is a difference in penetration and sites of entry of dexamethasone and naturally occurring steroids into the brain and a difference in location of receptors binding synthetic and physiologic steroids (19).

Basal and hypoglycemia-stimulated ACTH secretion are differentially sensitive to corticosteroid feedback, as indicated by the significantly different slopes of the two lines in Fig. 3. The difference between the two relationships cannot be ascribed purely to a difference in the intensity of the drive to the corticotrope since a change in stimulus intensity alone would shift the intercept of the line, but not the slope. The fact that basal and stimulated ACTH secretion have differential sensitivities to corticosteroid inhibition suggests the possibility that at least one site of corticosteroid feedback may be proximal to the corticotrope. Both basal and hypoglycemia-induced ACTH secretion appear to involve only steroid-sensitive pathways. However, the mechanism responsible for basal ACTH secretion is apparently less sensitive to corticosteroid inhibition than is the mechanism responsible for the ACTH response to hypoglycemia.

The time course of corticosteroid feedback on basal ACTH concentrations appears to differ from the time course of feedback on stimulus-induced adrenocortical responses. In rats a fast, rate-sensitive corticosteroid inhibition of stress-induced ACTH secretion is observed within seconds of the rise in corticosteroids, and disappears within 30 min. A slower, steroid dose-sensitive feedback is observed beginning at 2 h after the rise in corticosteroids; this effect lasts for hours. Between these two periods, no inhibition of adrenocortical responses to stress is observed in the rat (5). However, in dogs it appears that after the initial rise in plasma corticosteroids there is progressive inhibition of basal ACTH secretion over the following 2 h, without a "silent" period. A similar pattern of inhibition of "basal" ACTH levels has been shown in patients with Addison's disease (2) and in healthy subjects (3) after cortisol infusions, and is suggested by the reduction in corticosterone content (20) and in vitro corticoid production (21) of rat adrenals 30, 60, and 120 min after corticosteroid injection. Thus, inhibition of basal and stimulated ACTH concentrations may have different time domains, as well as differential sensitivity to feedback, which also indicates a different mechanism or site of steroid inhibition of basal and stimulated ACTH.

This study has shown that both basal and hypoglycemia-stimulated ACTH concentrations are sensitive to physiological increments in circulating plasma corticosteroids. It is possible, therefore, that increases in plasma corticosteroids stimulated by stresses such as hypoglycemia, hypotension, hypoxia, or surgery, may alter an individual's ACTH response to subsequent stresses, particularly when the stresses are temporally separated by only a few hours. We are presently investigating this possibility.

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