Control of canine ACTH by corticosteroids: an integral feedback effect of steroids

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KELLER-WOOD, MAUREEN. Control of canine ACTH by corticosteroids: an integral feedback effect of steroids. Am. J. Physiol. 257 (Regulatory Integrative Comp. Physiol. 26): R427–R430, 1989.—These experiments were designed to test whether the pattern of change in plasma corticosteroids or the total corticosteroid dose is important in determining the degree of inhibition of adrenocorticotropic hormone (ACTH) responses to stress by corticosteroid intermediate-delayed feedback. Five conscious dogs were studied. The ACTH response to induced hypoglycemia was measured after no prior corticosteroid feedback signal or after a corticosteroid feedback signal produced by infusion, two bolus injections, or three bolus injections of cortisol and corticosterone. The total corticosteroid dose (45 µg/kg) and the total interval of steroid treatment (60–30 min before hypoglycemia) were the same in all three cases of corticosteroid treatment. Changes in plasma glucose concentration during induced hypoglycemia were not altered by corticosteroid treatment. The plasma ACTH response to hypoglycemia was inhibited by all three patterns of treatment with corticosteroids. The inhibition of ACTH response was not significantly altered among the patterns of treatment with corticosteroids. The data suggest that the integrated (total) or the mean change in plasma corticosteroid concentration over time determines the degree of inhibition of stimulated ACTH in this time domain.

corticosteroid feedback; cortisol; corticotropin; adrenocorticotropic hormone

CORTICOSTEROIDS REGULATE plasma concentration of adrenocorticotropic hormone (ACTH) by negative-feedback effects on ACTH secretion and synthesis. The mode and magnitude of the corticosteroid feedback effect depends on the dose and timing of administration of the steroids (7). Long-term (≥12 h) treatment with corticosteroids inhibits ACTH synthesis and secretion by a slow-delayed feedback effect. Shorter periods of treatment cause inhibition of secretion, but not synthesis, by an “intermediate”-delayed-feedback effect. Very rapid inhibition of secretion (within minutes) also occurs by a fast-feedback mechanism.

The degree of the suppression of ACTH by the delayed-feedback effect of corticosteroids has been described as proportional to either the steroid dose or plasma level achieved (1, 2). Empirical clinical observations had led to the assertion that the long-term suppressive action of corticosteroids is dependent on the total steroid dose administered over a period of time and can be achieved with large, intermittent doses or smaller, more frequent doses of steroid. However, the doses of steroid used clinically probably all produce plasma corticosteroid concentrations above those necessary to completely suppress ACTH synthesis when administered over days or weeks.

This study was designed to test whether the pattern administration of corticosteroids with short-term treatment alters the degree of suppression of stimulated ACTH, that is, whether the suppressive effect on ACTH secretion depends on the pattern of change of plasma corticosteroids and the maximum plasma corticosteroid concentrations achieved or on the total, or integral, plasma corticosteroid concentration over time.

METHODS

Five mongrel dogs (two females and three males), weighing 18–34 kg, were studied. Each dog participated in four separate experiments. In one experiment, ethanol-saline vehicle [1–2.8% ethanol (0.5 µl·kg⁻¹·min⁻¹) in 0.9% saline] was infused as a control. In each of three experiments, a mixture of cortisol and corticosterone (2:1, cortisol-corticosterone) in ethanol-saline was infused for 30 min or injected at intervals over 30 min, and, at a later time, insulin was injected. Cortisol and corticosterone were infused at the combined rate of 1.5 µg·kg⁻¹·min⁻¹ for 30 min beginning at −60 min relative to the injection of insulin. Cortisol and corticosterone were injected in a dose of 22.5 µg/kg at −60 and −30 min or in a dose of 15 µg/kg at −60, −45, and −30 min relative to the injection of insulin. The total dose of steroid was the same in all three experiments (45 µg/kg). The timing of the infusions or injections was chosen so that both the total duration of the increase in plasma corticosteroids as well as the interval between the change in plasma corticosteroids and the onset of the stimulus were approximately the same in all experiments; duration and interval were found to be important variables in determining the feedback efficacy of corticosteroids in the intermediate-feedback domain (5). The relative concentrations of cortisol and corticosterone in the injectate or infusate were chosen based on previous data on relative rates of cortisol and corticosterone secretion during ACTH infusion in the dog (9). The dose of steroid was chosen to increase combined plasma corticosteroids to approximately half-maximal levels for the dog (9). Increases of plasma steroids of these magnitudes over this time interval were previously shown to reduce ACTH responses to hypoglycemia (5, 7) and hypoxia (11). In all experiments, the dose in insulin injected was 0.50 U/kg.

On the day of an experiment, dogs were brought to the laboratory and placed in a loose mesh sling (Alice King Chatham Medical Arts, Los Angeles, CA). Two intrave-
nous catheters (Delmed I-cath, Canton, MA) were placed transcutaneously in saphenous and/or cephalic veins. One catheter was used to inject insulin and to withdraw blood samples, and the other was used to infuse or inject corticosteroids or vehicle. At least 45 min elapsed between the time of catheter placement and the beginning of the experiment. All experiments were begun between 1030 and 1200 h.

Blood samples for plasma corticosteroid measurements were withdrawn before the corticosteroid or vehicle infusion or injection, at 5- to 10-min intervals during the next hour, and at 30-min intervals for 90 min after the injection of insulin. Blood samples for plasma glucose measurements were withdrawn at 5- or 10-min intervals for 90 min after the injection of insulin. Blood samples for plasma ACTH measurements were withdrawn at 10-min intervals for 90 min after the injection of insulin. All blood samples were placed in plastic tubes on ice until centrifugation. The tubes for glucose samples contained potassium oxalate and sodium fluoride (Sigma, St. Louis, MO), and the tubes for hormone samples contained EDTA (1.5 mM, Sigma). A total of 85-100 ml (±6 ml/kg) was withdrawn over the 150-min experimental period.

Plasma glucose concentrations were measured with a Yellow Springs Instruments glucose analyzer (Yellow Springs, OH). Plasma ACTH concentrations were measured by radioimmunoassay (RIA) (8, 14), and combined plasma corticosteroid concentrations were measured by competitive protein-binding assay (10).

The instantaneous changes in plasma corticosteroid concentrations were calculated using estimated volume of distribution determined in a previous study (320 ml/kg; see Ref. 6).

The glucose and hormone data were analyzed by two-way analysis of variance (ANOVA; Ref. 15) to test for the effect of varied pattern of administration of steroid on glucose, corticosteroid, or ACTH responses over time. The steroid data were also calculated as the area under the plasma concentration vs. time curve (ng·ml⁻¹·min) and analyzed by one-way ANOVA to test for differences in the total steroid signal among the patterns of steroid administration. Differences between individual means were analyzed by Duncan’s multiple range test (16). In all analyses, the null hypothesis was rejected if $P < 0.05$.

**RESULTS**

The intravenous administration of the same dose of corticosteroids in three different patterns: infusion, two boluses, or three boluses over 30 min resulted in three distinct patterns of increase of corticosteroids (Fig. 1). During infusion of corticosteroids at a rate of 1.5 μg·kg⁻¹·min⁻¹, plasma corticosteroid concentration increased gradually to a mean of 57.1 ± 5.3 ng/ml at ~30 min. After injection of corticosteroids in two boluses of 22.5 μg/kg each, plasma corticosteroids increased instantly and then were rapidly cleared; plasma corticosteroid concentrations were 52.3 ± 7.6 and 55.4 ± 7.7 ng/ml at 5 min after the successive injections. After injection of three boluses of corticosteroid, of 15 μg/kg each, plasma corticosteroids also rapidly increased; plasma concentrations at 5 min after successive injections were 37.5 ± 5.7, 40.9 ± 4.6, and 51.2 ± 9.1 ng/ml. When the initial volume of distribution estimated from the disappearance curve of cortisol and corticosterone in previous experiments (6) was used, the instantaneous changes in plasma corticosteroid concentrations with each injection

![FIG. 1. Plasma corticosteroids before, during, and after infusion of saline (○), infusion of cortisol and corticosterone (●), or 2 or 3 injections of cortisol and corticosterone (■ and ▲, respectively). Measured plasma corticosteroid concentrations are shown as symbols connected by solid lines. Calculated changes in concentration after corticosteroid injection are shown as dashed lines. Same total dose of combined corticosteroids (45 μg/kg) was administered in the 3 sets of experiments with steroid treatment. Timing of saline or steroid infusions (circles with lines) or steroid injections (squares and triangles) are indicated above each graph. Insulin was injected at 0 min. Corticosteroid dose effect and interaction are significant. Mean square of interaction is 64.95 (95% confidence limits about the mean = 7.17 ng/ml).](http://ajpregu.physiology.org/ by 10.220.33.6 on June 28, 2017)
were calculated to be ~63 and 42 ng/ml after injection of 22.5 and 15 μg/kg of corticosteroids, respectively. These produce corticosteroid concentrations of ~90 and 75 ng/ml immediately after the injection at ~30 min. Thus the estimated maximum levels produced by the injections are appreciably greater than those produced during infusion of the steroids. The changes in plasma corticosteroid concentrations were significantly different (by two-way ANOVA) when the concentrations for samples taken at common time points were compared, reflecting the difference in pattern of change of plasma corticosteroids. However, in the three sets of experiments in which corticosteroids were administered, neither the total corticosteroid concentrations integrated over time (area under the concentration vs. time curve between -60 and 0; infusion: 2,307 ± 191, two boluses: 2,177 ± 210, three boluses: 1,962 ± 262, control: 819 ± 114 ng min/ml) nor the average corticosteroid concentration over time (area divided by time; infusion: 38.5 ± 3.2, two boluses: 36.3 ± 3.5, three boluses: 32.9 ± 4.5, control: 13.6 ± 1.9 ng/ml) were significantly different, indicating that the integrated plasma corticosteroid signal or average corticosteroid signal, as well as the total steroid dose, are not significantly different among experiments.

The changes in plasma glucose concentration after injection of 0.5 U/kg insulin were not significantly different after administration of steroid compared with changes after administration of vehicle (by two-way ANOVA). This was true regardless of the pattern of administration of steroid. For example, mean plasma glucose concentrations at 20 min after insulin were 26 ± 3, 29 ± 2, and 29 ± 3 mg/100 ml, after administration of vehicle, an infusion of steroid, two boluses of steroid, and three boluses of steroid, respectively. Thus neither infusion nor injection of corticosteroids changed the stimulus to ACTH in these experiments.

The ACTH response to hypoglycemia was significantly inhibited after all three patterns of administration of corticosteroids (Fig. 2). In all three groups of experiments, the inhibition of plasma ACTH concentrations was significant between 30 and 90 min after injection of insulin. The pattern of administration of corticosteroids also did not significantly alter the magnitude of inhibition of the ACTH response; the plasma ACTH concentration after insulin was not significantly different among the three sets of experiments in which steroid was administered (by Duncan’s test), although the inhibition was less than maximal (mean peak ACTH concentration after vehicle: 617 ± 85, corticosteroid infusion: 324 ± 40, two boluses: 324 ± 21, three boluses: 248 ± 30 pg/ml).

DISCUSSION

Despite the dramatically different patterns of change in plasma corticosteroids over time, the three different steroid treatment protocols caused similar timing and magnitude of inhibition of plasma ACTH responses. The similarity in the degree of inhibition of ACTH suggests that the integrated corticosteroid signal, or the average corticosteroid concentrations, determine the magnitude of inhibition of ACTH by intermediate-delayed feedback. Thus the relevant signal at the corticosteroid feedback elements would be the time-averaged level of corticosteroids, rather than the minute-to-minute or maximum change or rate of change in level of corticosteroids.

The timing of steroid administration in these experi-
ments was chosen based on the results of previous studies in the dog. Suppression of hypoglycemia-induced ACTH secretion occurs when steroids were infused from 60 to -30 or -30 to 0 min before the administration of insulin (5). Therefore, the steroid concentrations over the period of steroid treatment in the present study are controllers of the ACTH secretion in response to hypoglycemia.

The maximum corticosteroid concentrations measured in these experiments were very similar after the three steroid treatments, however, the first blood sample after injection of steroids missed the maximum. The estimated maximum corticosteroid concentrations are very different after the three patterns of corticosteroid treatment, reflecting the different dynamics of plasma corticosteroids after injection or infusion.

Previous studies by other investigators led to the conclusion that although the rapid (≤10 min) feedback effect is rate sensitive, rather than level sensitive (1-4, 7), the more delayed-feedback effects of corticosteroids are level sensitive or dose sensitive (2, 7). The results reported here support the hypothesis that the more delayed-feedback effects are not dependent on the rate of change of plasma corticosteroids, as similar inhibition of ACTH was observed whether the increase in steroids was produced slowly (by infusion) or rapidly (by injection). It has been previously suggested that the much slower delayed-feedback effect of cortisol to cause inhibition of corticotropin-releasing factor (CRF) and ACTH synthesis is related to the total dose of steroid administered. Clinical observations support the idea that the integrated, or level × time, signal of plasma corticosteroids determine the degree of inhibition by slow-delayed feedback. However, because the effectiveness of suppression of ACTH synthesis also depends on the duration of the feedback signal (12, 13), it has been difficult to separate the effect of varying the duration and intervals between treatments and varying the total feedback signal in designs in which the effectiveness of various patterns of intermittent steroid treatment are compared over many hours or days. Also, because this slow inhibition affects a decrease in ACTH and CRF synthesis, as well as secretion, the results of studies with longer duration steroid treatment cannot be generalized to the case of shorter treatments, which would affect secretion but have little or no effect on synthesis of ACTH and CRF. The study reported here examines the relation between pattern of steroid administration and inhibition of ACTH over a shorter period and separates the effect of prolonged duration of steroid treatment from the effect of differences in level achieved, by administering the same total dose over the same total time interval. In this study, the suppression of ACTH was the same when the same total dose of steroid was administered in different patterns over the same time interval, indicating that the intermediate-delayed-feedback effect depends on the total dose of steroid administered. The results suggest that this integral or time-averaged feedback effect operates over relatively short time courses (30–60 min) associated with intermediate feedback in the dog. The results also reconfirm that small increments in corticosteroids, well within the physiological range, can reduce ACTH responses to subsequent stress.

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