Ovine corticotrophin-releasing factor in dogs:
dose-response relationships and effects of dexamethasone

R. J. Kemppainen¹, D. V. Filer¹, J. L. Sartin¹,
and R. B. Reed²

Department of Physiology and Pharmacology¹, School of Veterinary Medicine and Research Data Analysis²,
Auburn University, Auburn, Alabama 36849, USA

Abstract. Dose-response relationships between iv bolus injections (0, 0.1, 1 or 10 μg/kg) of synthetic ovine corticotropin-releasing factor (oCRF) and plasma immunoreactive (i) ACTH and cortisol concentrations were examined in healthy, conscious dogs. All doses of oCRF resulted in elevated plasma iACTH and cortisol levels over those of the controls. Maximum (or Peak) plasma iACTH concentrations were generally observed 20–30 min after oCRF and the magnitude of these peaks was a linear function (P < 0.001) of the logarithm of the oCRF dose. The time of peak cortisol concentrations was more variable but the peak cortisol level was also linearly related (P < 0.001) to the logarithm of the oCRF dose. An estimate for the response areas for both hormones demonstrated a quadratic (P < 0.05) relationship with the logarithm of the oCRF dose. The relationship between oCRF and the iACTH response suggested a progressively greater response at increasing oCRF doses while a maximally effective oCRF dose was predicted in the cortisol response area relationship. Graded (0, 0.01, 0.1 or 1 mg/kg) bolus doses of dexamethasone produced a dose-dependent (P < 0.03) decline in baseline plasma iACTH levels and a non-dose-dependent suppression in baseline plasma cortisol. Pre-treatment with 0.001 mg dexamethasone/kg 4 or 8 h before injection of 1 μg oCRF/kg did not alter the plasma iACTH or cortisol response; however, 0.1 mg dexamethasone/kg administered at these times totally abolished the responses to oCRF. An intermediate dose (0.01 mg/kg) of dexamethasone inhibited the plasma iACTH response by an average of 79% (P < 0.01) when administered 4 h before oCRF, but did not significantly alter this response when given 8 h prior to oCRF. The plasma cortisol response to oCRF was inhibited (P < 0.01) when 0.01 mg dexamethasone/kg was given as a 4 h, but not as a 8 h, pre-treatment. Iv administration of oCRF produces a profound, dose-dependent stimulation of the pituitary-adrenocortical axis of dogs and should prove useful in studies of this system.

Dose-related pituitary-adrenocortical responses to administration of ovine corticotropin-releasing factor (oCRF) have been examined in several species including humans (Orth et al. 1983), sheep (Kalil et al. 1983) and rats (Rivier et al. 1982). Iv injection of oCRF in dogs resulted in a dose-dependent increase in superior mesenteric arterial blood flow with a concomitant decline in mean arterial blood pressure (Brown et al. 1982), but the endocrine responses were not determined. The dog is frequently used in studies involving pituitary-adrenocortical responses to stimuli such as haemorrhage (Wood et al. 1981), hypoxia (Raff et al. 1983) or insulin-induced hypoglycaemia (Keller-Wood et al. 1981). Cushing's syndrome has been frequently documented in dogs, and characteristics of the disease in this species are quite similar to those noted in human patients (Meijer et al. 1978). Because of the potential usefulness of oCRF in studies of pituitary-adrenocortical function, we examined the effects of a relatively wide dosage range of this agent on plasma ACTH and cortisol concentrations in healthy, conscious dogs. Also, the effects of pre-treatment with various doses of dexamethasone on the responses to oCRF were studied.
Materials and Methods

Fifteen adult, healthy mixed breed dogs (7 females, 8 males) weighing 10.0 to 23.5 kg were studied. Normal pituitary-adrenocortical function was determined in each dog on the basis of a suppression in plasma cortisol levels to less than 1.5 µg/100 ml 8 h after iv injection of 0.01 mg dexamethasone/kg. The dogs were housed individually in outdoor covered pens or in cages in an environmentally controlled room (12 h light: 12 h dark). A standard pelleted ration was fed once a day (morning) with water continually available. Some of the dogs were trained over a period of 4 weeks to stand calmly in a nylon sling (Alice Chatham Medical Arts, Inc., Los Angeles, CA).

Dose-response of oCRF

The responses to 5 iv bolus doses of oCRF (0.1, 1 or 10 µg/kg) or oCRF vehicle (controls) were determined using 10 dogs. The synthetic oCRF (Peninsula Laboratories, Inc., Belmont, CA) was solubilized as previously described (Orth et al. 1983) except that dog serum albumin was used in place of bovine serum albumin. Individual aliquots of the drug were stored at -80°C until use, unused portions were discarded. Treatments were administered in a random order with at least 72 h allowed between experiments on an individual dog. No dog received the same treatment twice. The dogs were fasted 12 h before use, treatments were administered at different times of the day (08.00–14.00 h), but the sequence of doses was random. Previous studies from our laboratory have failed to document the existence of a circadian rhythm in pituitary-adrenocortical activity in dogs (Kemppainen & Sartin 1984). Treatments were given through an indwelling jugular catheter (Delmed I-Cath, Delmed, Inc., Canton, MA) which was inserted to the level of the right atrium under general anesthesia (thiamylal sodium) at least 72 h before an experimental period. Following catheter placement, the dogs were housed individual in cages in the animal facility. On the day before an experiment, a dog was brought to the experimental room and housed overnight in a cage. On the following day, the dog was placed in the sling for a 30 min acclimatization period, and then 3 baseline blood samples were collected through the catheter. Following injection of the test substance, blood samples were collected at multiple times up to 120 min post-treatment. Blood samples (2 ml) were placed into chilled polypropylene tubes containing 3 mg EDTA and 1000 Kallikrein Inactivator Units (KIU) aprotinin (trasyrol, Mobay Chemical Corp., New York, NY) and immediately centrifuged. Plasma was stored at -20°C until time of assay.

Dose-response of dexamethasone on baseline plasma ACTH and cortisol

The responses of baseline plasma ACTH and cortisol concentrations to single iv bolus doses of dexamethasone (Azium, Schering Corp., Kenilworth, NJ) were examined in 10 dogs 3 weeks after conclusion of the first experiment. Blood samples were collected via jugular venipuncture before, and at intervals up to 8 h after the injection of 0, 0.01, 0.1 or 1 mg dexamethasone/kg. The dogs were maintained in the individual outdoor pens for this study. The doses were administered in a random order allowing at least 96 h between treatments on an individual dog.

Effect of dexamethasone pre-treatment on the response to oCRF

This experiment was conducted in a manner similar to that of the first study. Jugular catheters were inserted under general anaesthesia at least 72 h before an experiment on an individual dog. Instead of placing the dogs in the sling, blood samples were collected from the dogs in cages in the experimental room. To facilitate sample collection with minimal restraint, an intermittent injection plug (Abbott Laboratories, North Chicago, IL) was attached to the sampling port of the catheter. Dogs were given 0.001, 0.01 or 0.1 mg dexamethasone/kg iv 4 or 8 h prior to the iv administration of 1 µg oCRF/kg. One group of dogs was given the dexamethasone vehicle 4 h before the oCRF dose, and served as controls. In each dog, the oCRF was injected at approximately 13.00 h. Blood samples were collected at the same time intervals as in the first experiment.

Hormone analyses

Plasma concentrations of immunoreactive (i) ACTH (Kemppainen & Sartin 1984; Nicholson et al. 1984) and cortisol (Kemppainen et al. 1983) were determined using RIA. The RIA for ACTH was modified in that porcine ACTH (IgG Corporation, Nashville, TN) was iodinated following previously described procedures (Nicholson et al. 1984) and the [125I]ACTH was repurified immediately before use on a 0.9 x 57 cm column of Sephadex G-50 fine resin (Pharmacia Fine Chemicals, Piscataway, NJ). A similar column was used to characterize the pattern of iACTH in plasma (0.6 ml) obtained 20 min after injection of 10 µg oCRF/kg in 1 dog. The sensitivity of the iACTH assay was 5 pg/ml; for the cortisol assay, 0.2 µg/100 ml. Intra- and inter-assay coefficients of variation were approximately 10 and 15% for the iACTH assay and 5 and 10% for the cortisol assay. The cortisol antiserum showed approximately 2% cross-reaction with corticosterone, the other major endogenous canine glucocorticoid.

Statistical analyses

Data were analyzed using an analysis of variance for repeated measures with regression (General Linear Models Procedure, SAS Institute, Cary, NC).
Results

Dose-response of oCRF

Injection of oCRF had no visible effect in any of the dogs. Concentrations of plasma iACTH or cortisol did not differ ($P > 0.3$) between treatments prior to injection of oCRF or vehicle (Fig. 1). Mean baseline plasma iACTH concentrations for the dogs in this experiment were $32 \pm 3$ (± SE) pg/ml with a range of 10 to 70 pg/ml; mean cortisol concentrations at these times were $2.6 \pm 0.2 \mu g/100$ ml with a range of 0.9 to 5.2 $\mu g/100$ ml. Injection of oCRF resulted in a significant increase in plasma iACTH and cortisol concentrations over time ($P < 0.001$), which differed ($P < 0.001$) between treatments (Fig. 1). Peak concentrations of plasma iACTH were generally measured 20–30 min after oCRF administration. The magnitude of the observed peak plasma iACTH level was a linear function of the logarithm of the oCRF dose ($P < 0.001$). Peak plasma cortisol concentrations, whose time of appearance varied with oCRF dose (Fig. 1), were also linearly related to the logarithm of the dose. An index of the total response of plasma iACTH and cortisol to oCRF dose was determined by summing the differences between hormone levels at the various times after injection and the mean concentration for the

![Graph 1](image1)

![Graph 2](image2)

Plasma immunoreactive (i) ACTH and cortisol concentrations determined in dogs before and after iv injection of oCRF or oCRF-vehicle (control). The arrow indicates the time of treatment. Each point is the mean ± SE of 5 dogs.
hormones for times prior to injection. For both iACTH and cortisol, the relationship between the index of the total response and the logarithm of the oCRF dose was quadratic \( (P < 0.05) \). The nature of the relationship between the logarithm of oCRF dose and the iACTH response was such that as the dose of oCRF increased, the iACTH response increased to a greater degree; in contrast, the equation predicted a peak in the cortisol response at an oCRF dose of 7.5 µg/kg.

Plasma obtained 20 min after injection of 10 µg/kg was chromatographed using Sephadex G-50 fine to characterize potential heterogeneity in iACTH associated with the profoundly increased peripheral levels measured at this time. Virtually all of the iACTH in the plasma coeluted with \(^{[125]I}\)ACTH indicating an absence of secreted precursor or metabolic products.

**Dose-response of dexamethasone on baseline plasma iACTH and cortisol**

The patterns of plasma iACTH and cortisol produced in response to various doses of dexamethasone are shown in Fig. 2. Each dose of dexamethasone resulted in a significant \( (P < 0.001) \) suppression in plasma iACTH and cortisol over time. The decline in plasma iACTH was dose-dependent \( (P < 0.03) \), with concentrations in dogs given 1 mg/kg near the assay sensitivity by 2 h post-treatment, while concentrations in dogs given 0.1 mg/kg reached a nadir by 4 h. The decline in plasma iACTH in dogs given the lowest dexamethasone dose was more variable; 1 dog showed an increase in plasma iACTH at 1 h post-treatment, while 2 other dogs had an apparent early escape from suppression. In these latter individuals, plasma iACTH increased to a small degree \( (5–10 \, \text{pg/ml}) \) from 6 to 8 h coincident with a rise in plasma cortisol (Fig. 2). The suppressions in plasma cortisol concentrations were more consistent across doses with no interaction \( (P > 0.3) \) between time and dose. Plasma cortisol had declined to approximately 0.2 µg/100 ml in dogs given either 0.1 or 1 mg dexamethasone/kg by 4 h post-treatment, and these low concentrations were maintained at least to 8 h.

**Fig. 2.**

Plasma immunoreactive (i) ACTH and cortisol concentrations determined in dogs before and after iv injection of dexamethasone or dexamethasone-vehicle (control). The arrow indicates the time of treatment. The interrupted line indicates the approximate assay sensitivity. Each point is the mean ± SE of 4 dogs.
Plasma immunoreactive (i) ACTH and cortisol concentrations determined in dogs pre-treated with dexamethasone (DEX) or dexamethasone-vehicle (control) and later given 1 µg oCRF/kg iv. Left, plasma iACTH and cortisol concentrations in dogs given dexamethasone 4 h before oCRF. Right, plasma iACTH and cortisol concentrations in dogs given dexamethasone 8 h before oCRF. Controls were given the vehicle 4 h before oCRF and their responses are shown in both columns. The arrow indicates the time of treatment with oCRF. The interrupted line indicates the approximate assay sensitivity. Each point is the mean ± SE of 5 dogs.

Effect of dexamethasone pre-treatment on the response to oCRF

The effects of various doses of dexamethasone administered 4 or 8 h prior to injection of 1 µg oCRF/kg are shown in Fig. 3. Treatment with 0.001 mg dexamethasone/kg had no effect on the response of plasma iACTH or cortisol when administered either 4 or 8 h before oCRF. However, treatment with 0.01 mg dexamethasone/kg inhibited (P < 0.01) the response of plasma iACTH to oCRF given 4 h later. The mean plasma iACTH response area estimate for this group was 21% of that of the control group (79% inhibition). Although this dose of dexamethasone given 8 h before oCRF appeared to inhibit the plasma iACTH response (37% inhibition compared to controls; Fig. 3), this difference was not significant (P < 0.2). While 0.01 mg dexamethasone/kg administered 4 h prior to oCRF inhibited (P < 0.01) the plasma cortisol response (Fig. 3, lower left), this response determined 8 h after dexamethasone treatment was slightly but not significantly reduced (Fig. 3, lower right). The plasma iACTH and cortisol responses to oCRF were totally abolished by treatment with 0.1 mg dexamethasone/kg given either 4 or 8 h prior to the oCRF injection (Fig. 3).
Discussion

Similar to data obtained in humans (Orth et al. 1983), sheep (Kalin et al. 1983) or rats (Rivier et al. 1982), peripheral iv administration of oCRF resulted in a dose-dependent increase in plasma iACTH concentrations in conscious dogs. Peak concentrations of plasma iACTH were linearly related to the logarithm of the oCRF dose, similar to stimulus-response relationships between insulin-induced hypoglycaemia (Keller-Wood et al. 1981) or hypoxia (Raff et al. 1983) and peak plasma ACTH concentrations in dogs. Following insulin-induced hypoglycaemia, peak plasma ACTH concentrations in excess of those measured in the present study were reported by others (Keller-Wood et al. 1981) and determined in dogs in our laboratory (unpublished). It is therefore unlikely that the highest dose of oCRF (10 µg/kg) maximally stimulated iACTH output. In dogs, oCRF in doses of \( \sim 10^{-9} \) mol/kg (4.6 µg/kg) or greater caused a dose-dependent fall in mean arterial blood pressure (Brown et al. 1982). Hypotension, in addition to stimulating the release of ACTH (Wood et al. 1981), is also associated with increased levels of arginine vasopressin, angiotensin II and catecholamines (Oberg 1976). These substances have been shown to have activity in vivo and in vitro to enhance CRF-induced ACTH release (Rivier & Vale 1983; Vale et al. 1983a,b). It is therefore possible that a dose-dependent release of these substances contributed to the proportionately greater integrated iACTH response observed at increasing oCRF doses in the dogs in the present study. Also, the duration of ACTH release from pituitary fragments in vitro was shown to be increased as the concentration of added CRF was increased (Widmaier & Dallman 1984).

Although peak plasma cortisol concentrations were linearly related to the logarithm of the oCRF dose, the relationship between oCRF and the estimate of the cortisol response area suggested a plateau. This likely related to the fact that the canine adrenal cortex exhibits maximal secretion of cortisol at plasma ACTH concentrations well within the physiological range (Keller-Wood et al. 1981, 1983a). Higher ACTH levels act to prolong this stimulation (Keller-Wood et al. 1983a; Kempainen et al. 1983), although 2 of the 5 dogs given 10 µg oCRF/kg showed a gradual but continued elevation in plasma cortisol up to 120 min follow-

ing treatment. The plasma cortisol response to the largest dose of oCRF was reminiscent to that observed in dogs infused for 40 min with 1000 ng/min ACTH (Keller-Wood et al. 1983a). The prolonged pattern of plasma corticosteroid concentrations observed in that study was associated with a continued elevation in adrenocortical secretory rates rather than alterations in corticosteroid clearance. Longer sampling periods will be required to fully characterize the pattern of plasma cortisol response to large doses of oCRF.

Single iv injections of dexamethasone caused a rapid and dose-dependent decline in baseline plasma iACTH concentrations, while the depression in plasma cortisol was more consistent across doses. With the exception of the response of 1 dog to the lowest dose of dexamethasone, plasma iACTH and cortisol fell concurrently in all dogs given this glucocorticoid. These data are in contrast to findings in a recent study in humans where dexamethasone-induced suppressions in plasma ACTH and cortisol were dissociated in certain individuals (Sherman et al. 1984). Species or methodological differences may account in part for these contrasting results. In a previous study in dogs, two 4 mg doses of dexamethasone reduced baseline plasma ACTH and corticosteroid concentrations to levels comparable with our results (Wood et al. 1982). Infusions of corticosteroids or ACTH resulting in physiological elevations in plasma corticosteroid concentrations inhibited baseline plasma ACTH levels 2 h later in a dose-related manner (Keller-Wood et al. 1983b). Our findings agree with those of Keller-Wood et al. (1983b) that glucocorticoid inhibition of baseline ACTH in dogs is a continuous phenomenon, with a duration and magnitude likely related to dose and potency of the glucocorticoid. This pattern of feedback inhibition differs from glucocorticoid effects on stress-induced ACTH secretion, where a silent or inactive period is preceded by a rate-sensitive phase and followed by a dose-sensitive period of negative feedback (Keller-Wood & Dallman 1984).

The results of administration of varying doses of dexamethasone prior to injection of oCRF show that the magnitude of the glucocorticoid-induced inhibition of the response to oCRF is dependent on the glucocorticoid dose and the time interval from glucocorticoid to oCRF administration. Whereas 0.001 mg dexamethasone/kg had no effect when administered 4 or 8 h prior to
oCRF, a 100-fold greater dose of dexamethasone totally abolished responses when given at these pre-treatment intervals. An intermediate (0.01 mg/kg) dose of dexamethasone caused a partial inhibition of the plasma iACTH response, which was more profound when dexamethasone was given 4 h before oCRF. These results concur with observations on the time course and dose-dependency of glucocorticoid inhibition of stress-induced pituitary ACTH release (Keller-Wood & Dallman 1984; Takebe et al. 1971). The present results also agree with in vivo (Gonzalez-Lugue et al. 1970; Rivier et al. 1982) and in vitro (Vale et al. 1983b; Widmaier & Dallman 1984) studies which indicated a direct inhibitory effect of dexamethasone at the pituitary level. This synthetic glucocorticoid has been shown to preferentially concentrate in pituitary tissue of rats (De Kloet 1975).

Certain stimuli causing the release of ACTH and adrenal corticosteroids can be inhibited by prior treatment with glucocorticoids while other stimuli are considered to be steroid non-suppressible (Keller-Wood & Dallman 1984). This difference may be explained on the basis of variations in afferent pathways leading to ACTH stimulation (Keller-Wood & Dallman 1984). Dexamethasone pre-treatment did not block the increase in adrenal corticosteroid secretion in dogs following a large haemorrhage or injection of endotoxin while the response to ether anaesthesia was inhibited (Egdahl 1964). However, in a more recent study, dexamethasone administration prevented any change in plasma levels of either ACTH or corticosteroids after a moderate (15 ml/kg) haemorrhage in dogs (Wood et al. 1982). Since pre-treatment with 0.1 mg dexamethasone/kg abolished the pituitary response to oCRF injection in the present study, it is likely that stimuli capable of activating ACTH secretion after moderate or large doses of dexamethasone may override pituitary inhibition either by releasing large quantities of CRF or by activating non-CRF ACTH secretagogues. Further studies of the interactions between glucocorticoid dose, type and pre-treatment time intervals on stress-induced and CRF-induced ACTH secretion would help clarify the understanding of systems regulating glucocorticoid negative feedback.

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References


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