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Comparison of Intravenous versus Intramuscular Administration of Corticotropin-Releasing Hormone in Healthy Cats

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Background: Because of the lack of a current validated assay for feline endogenous adrenocorticotropic hormone (ACTH) in response to administration of currently available ovine corticotropin-releasing hormone (oCRH) preparations, a complete evaluation of the hypothalamic-pituitary-adrenal axis in cats has not been possible.

Objective: This study was undertaken to (1) determine the pituitary (ACTH) and adrenal (cortisol) response to both IV and IM administration of a currently available oCRH product in healthy cats, and (2) validate an endogenous ACTH assay for use in cats.

Animals: Seventeen healthy cats receiving oCRH (n = 11) or placebo (n = 6).

Methods: Prospective, randomized, placebo-controlled study. oCRH at 1 μg/kg or placebo was given either IM or IV. Endogenous cortisol and ACTH concentrations were evaluated after the injection. A comparison of IM versus IV and placebo versus treatment was made.

Results: The DiaSorin immunoradiometric assay (IRMA) assay for ACTH performed well, showing both parallelism and acceptable intra- and interassay coefficients of variation. There was a significant difference between groups ($P = .025$) and a significant difference between times ($P = .025$) when endogenous ACTH concentrations were compared after oCRH IV or IM. No significant differences were observed in cortisol concentrations comparing IV to IM oCRH.

Conclusions: IM administration of oCRH results in significantly greater ACTH concentrations but not cortisol concentrations when compared with IV administration. Samples should be drawn before and at 60 minutes after the injection. The Diasorin IRMA is valid for feline endogenous ACTH measurements.

Key words: ACTH; Adrenal; Cortisol; Pituitary.

Evaluation of the hypothalamic-pituitary-adrenal (HPA) axis can be useful when dysfunction in the axis is suspected.[1–5] Little research has been done to evaluate the HPA axis in healthy cats, cats with hypothalamic or pituitary disease, or cats with nonadrenal illness.[6–13] The paucity of studies may be due, at least in part, to the lack of a current validated feline adrenocorticotropic hormone (ACTH) assay and of reference ranges for the ovine corticotropin-releasing hormone (oCRH) stimulation test using currently available CRH preparations. Three studies since 2004 have utilized several different ACTH assays in healthy cats,[14] cats with suspected PDH[15] and a single case report of a cat with a pituitary macroadenoma, diabetes, hypercortisolism, and neurologic signs.[16] However, ACTH assay validation data were often incomplete.

In the case report,[16] information on the ACTH assay (immunoluminometric; ILMA) was listed as unpublished observations. The Fracassi paper was then cited as the reference for the ACTH assay used in the study evaluating ACTH precursors in cats with PDH.[15] In this study, poor correlation was seen between the ILMA assay and plasma ACTH precursors and the authors remarked that very little is known about ACTH concentrations in cats with PDH. In the paper evaluating renin activity, aldosterone, cortisol, ACTH, and α-Melanocyte Stimulating Hormone (αMSH) in healthy cats[14] an immunoradiometric assay (Nichols Institute; Wijchen, the Netherlands) was used. Information provided on the assay included the manufacturer's references regarding cross-reactivity of ACTH with other pituitary peptides in human serum and a statement regarding serial dilutions of a single feline sample showing parallelism to the standard curve.

This study was undertaken to (1) determine the pituitary (endogenous ACTH) and adrenal (cortisol) response to both IV and IM administration of a currently available oCRH product in healthy cats, and

Abbreviations:

ACTH	adrenocorticotropic hormone
CAP	combined anterior pituitary
CBC	complete blood count
CIRCI	critical illness-related corticosteroid insufficiency
CRH	corticotropin-releasing hormone
EDTA	ethylenediaminetetraacetic acid
GHRH	growth hormone-releasing hormone
GnRH	gonadotropin-releasing hormone
HPA	hypothalamic-pituitary-adrenal
ILMA	immunoluminometric
IRMA	immunoradiometric assay
αMSH	α-melanocyte stimulating hormone
oCRH	ovine corticotropin-releasing hormone
RAI	relative adrenal insufficiency
RIA	radioimmunoassay
TRH	thyrotropin-releasing hormone
TT4	total thyroxine

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(2) validate an endogenous ACTH assay for use in cats. The results of this study may be used as a reference for future experimental and clinical studies investigating feline diseases such as hyperadrenocorticism and to assess the HPA axis in critical illness-related corticosteroid insufficiency (CIRCI), previously known as relative adrenal insufficiency (RAI).

Materials and Methods

Study Cats

The study population consisted of healthy, employee-owned cats (11 domestic short hairs and 1 Bengal, age range 3–13 years, 10 neutered males and 2 spayed females). Cats were determined to be healthy based on history, physical examination findings, complete blood count (CBC), serum chemistry profile (albumin, globulin, alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin, gamma glutamyl transpeptidase, creatinine, phosphorus, glucose, calcium, magnesium, sodium, potassium, chloride, cholesterol, triglyceride, amylase, lipase, creatine phosphokinase), total thyroxine (TT4) concentration, and urinalysis. Cats were not receiving any medications with the exception of flea control and had not been exposed to any medications known to affect the HPA axis in the preceding 3 months, including desoxycorticosterone, glucocorticoids (aerosolized, oral, or parenteral), selective serotonin reuptake inhibitors, progestins, and tricyclic antidepressants.

Blood Collection and Sample Handling

Cats were randomized using a random number generator into 4 groups: placebo (IV and IM) and oCRH (IV and IM) groups. Each cat was sedated with hydromorphone (0.1 mg/kg IM) on day 1 followed by placement of a 16 gauge long catheter[a] in the medial saphenous vein. On day 2, samples were drawn 15 minutes (baseline) before IV administration of 1 μg/kg of oCRH[b] or an equal volume of 0.9% saline. A 2nd sample was drawn at the time of the intravenous injection of oCRH or saline (time 0). Subsequent blood samples were collected at 5, 15, 30, 60, and 120 minutes after the injection. ACTH and cortisol blood samples were collected in plastic tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinin (ACTH) and serum separator tubes (cortisol) and centrifuged; the plasma and serum were stored in plastic tubes at −80°C. On day 3, cats underwent the same protocol with an intramuscular injection of oCRH or saline. All cats in the placebo group always received saline and those in the treatment group received oCRH (IV and IM).

Eleven cats initially received oCRH (IV and IM) with 6 cats receiving placebo (IV and IM). Because of catheter malfunction or cats becoming fractious, the size of the oCRH IM group was 8 cats. Because of a limited number of cats enrolled in the study and concerns that we would not be able to detect differences between the IM and IV treatment groups, more animals were assigned to treatment groups than placebo.

Endocrine Assays

Cortisol Assay. Cortisol concentrations were determined utilizing a radioimmunoassay (RIA)[c] previously validated for cats.[17,18]

Validation of ACTH Assay. In a prior pilot study, plasma ACTH and cortisol concentrations were determined in 6 normal healthy domestic short-hair cats after IV administration of 1 μg/kg oCRH (Table 1). Samples were drawn at 1 minute before injection and 10 minutes after the injection. Plasma immunoreactive ACTH concentrations were determined using a DiaSorin ACTH immunoradiometric assay (IRMA),[d] DiaSorin ACTH RIA,[e] and Scantibodies ACTH assay[f] (Table 1). Parallelism was assessed by serially diluting and assaying 3 feline plasma pools with samples diluted using human plasma (feline plasma not available) depleted of ACTH ("0" standard). Slopes were determined for the displacement lines generated by dilution of each of the cat plasma pools (n = 3) and 4 standard curves. These values were compared using nonpaired *t*-test. Based on parallelism and the intra- and interassay coefficients of variation, the ACTH IRMA assay was used in the current study.

Table 1. Post-oCRH endogenous cortisol and ACTH compared by route and compared with placebo in 8 cats. There was no significant difference noted in the endogenous ACTH concentrations when placebo and oCRH IM or IV were compared. The difference in endogenous cortisol between the placebo and oCRH IV or IM approached significance.

	Endogenous Cortisol	Endogenous ACTH
oCRH IV versus oCRH IM	$P = .58$ No significant difference	$P = .025$
oCRH IV versus placebo IV	$P = .07$	$P = .14$ No significant difference
oCRH IM versus placebo IM	$P = .06$	$P = .18$ No significant difference

Statistical Analysis

Longitudinal analyses of group (route of administration oCRH IV versus IM) and time effects were evaluated with repeated measure analysis of variance (RMANOVA). A two-repeated factor RMANOVA[g] was performed when comparing ACTH and cortisol concentrations after IV or IM administration of oCRH. ACTH and cortisol concentrations were also compared at times −15 and 0 (baseline, ie, before administration) in the oCRH IV and IM groups. A one-grouping factor (route) and one-repeated factor (time) RMANOVA was used to compare ACTH and cortisol response to oCRH IM versus IM placebo and oCRH IV versus IV placebo. In addition to examining the main effects of route and time, the interaction between these 2 factors (indicating that the effect of one factor is dependent on the level of the other factor) was also evaluated. When main or interaction effects were significant ($P < .05$), posthoc analyses were performed using a Bonferroni–Holm multiple comparison adjustment to preserve the nominal Type I error rate.

Results

Validation of ACTH Assay

Six individual normal healthy domestic short-haired cats given 1 μg/kg oCRH IV showed the expected rapid increase in circulating concentrations of ACTH and cortisol. Plasma ACTH concentrations were measured with 3 different assays. ACTH samples were obtained before and 10 minutes after IV oCRH. Several of the after oCRH IV values were below the

standard curve. The ACTH IRMA assay performed well based on the expected rise in ACTH after oCRH administration. When evaluating eACTH with the Scantibodies assay, values for 5 of the 6 cats were below the assay detection limit for both before and after oCRH administration. All dilutions yielded lines with slopes similar to the standard ($P > .5$). Intra- and interassay coefficients of variation were calculated in 4 assay runs including 3 feline plasma pools that showed relative low, medium, or high concentrations of plasma ACTH (low 12 ± 1.9 pg/mL, medium 65 ± 6.9 pg/mL, high 118 ± 10.9 pg/mL). Intra-assay CVs were for the low pool, 3.1%; medium pool, 4.4%; and high pool, 3.3%. The interassay coefficient of variation was for the low pool, 8.1%; medium pool, 8.3%; and high pool; 3%. The detection limit for the purposes of this study was the lowest standard curve concentration that provided counts/minute significantly ($P < .05$) different from that measured in the 0 standard.

ACTH and Cortisol Responses to oCRH. There was a significant main effect of route of administration of oCRH on endogenous ACTH concentration ($P = .025$), a significant main effect of time on ACTH concentration ($P = .025$) across times (−15 to 120 minutes), and a significant change in ACTH concentration over time ($P = .025$) for both administration routes (Figs 1 and 2). There were significant concentrations of ACTH relative to baseline at times 5, 15, and 30 minutes. No significant interaction was detected between route and time ($P = .31$). The mean difference between the IM and IV groups across times was 7.7 pg/mL. Posthoc comparisons showed significant increases in ACTH concentrations relative to baseline (Time 0) at times 5 and 15 minutes.

There was no significant difference detected in endogenous cortisol concentration between administration routes ($P = .58$), but there was a significant change in concentration across time ($P = .0031$) (Figs 3 and 4). However, post hoc comparisons between time = 0 and later times were not significant. There was no significant interaction between route and time ($P = .12$).

There was no significant difference in ACTH concentration between the IV and IM routes ($P = .58$) at time = −15 and time = 0 (baseline) but there was a close-to-significant change over these 2 times ($P = .054$). There was no significant interaction between route and these 2 times ($P = 0.54$). With IV administration, the mean difference between time = −15 and time = 0 was −11.6 pg/mL, with the ACTH concentrations being higher at time = 0. With the IM route, the mean difference between time = −15 and time = 0 was −5.3 pg/mL, with the ACTH concentrations being higher at time = 0.

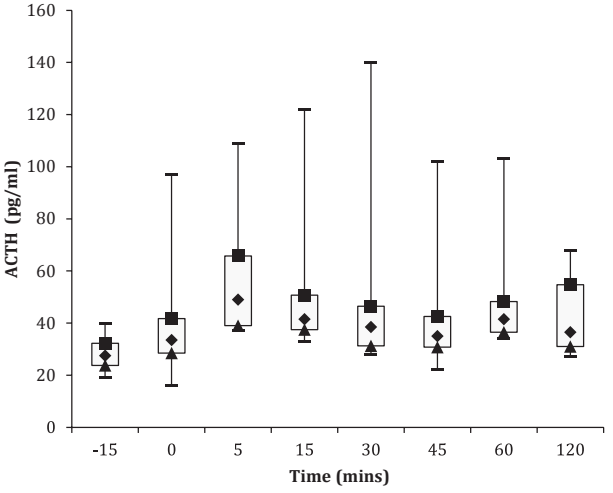

Fig 1. Box and whisker plot depicting the ACTH response following the IV administration of oCRH. There was a significant difference in endogenous ACTH concentration between IV and IM oCRH administration ($P = .025$) across times (−15 to 120 minutes), and a significant change in ACTH concentration over time ($P = .025$) for both administration routes.

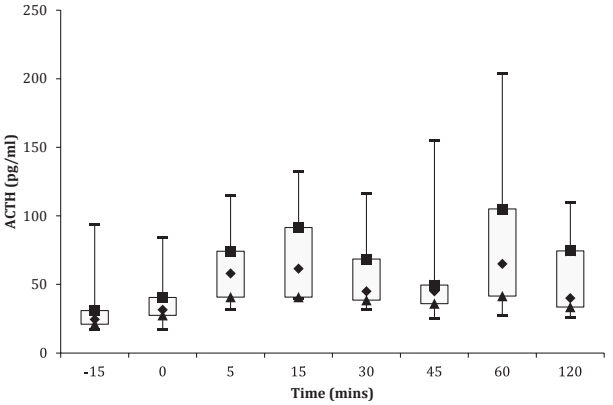

Fig 2. Box and whisker plot depicting the ACTH response following the IM administration of oCRH. Refer to legend for Figure 1 for explanation of plot. There was a significant difference in endogenous ACTH concentration between IV and IM oCRH administration ($P = .025$) across times (−15 to 120 minutes), and a significant change in ACTH concentration over time ($P = .025$) for both administration routes.

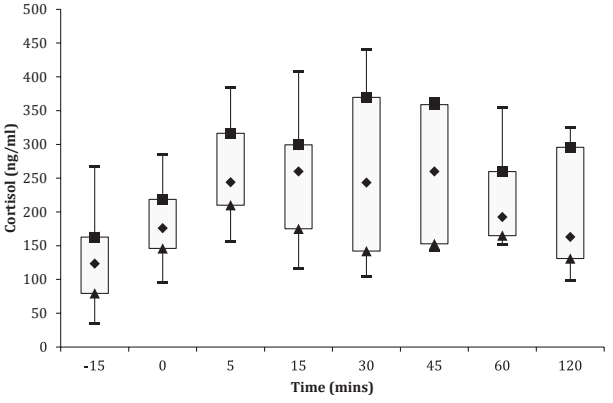

Fig 3. Box and whisker plot depicting the cortisol response following the IV administration of oCRH. There was no significant difference detected in endogenous cortisol concentration between administration routes ($P = .58$) or across times (−15 to 120 minutes), but there was a significant change in concentration across times ($P = .0031$) for both routes.

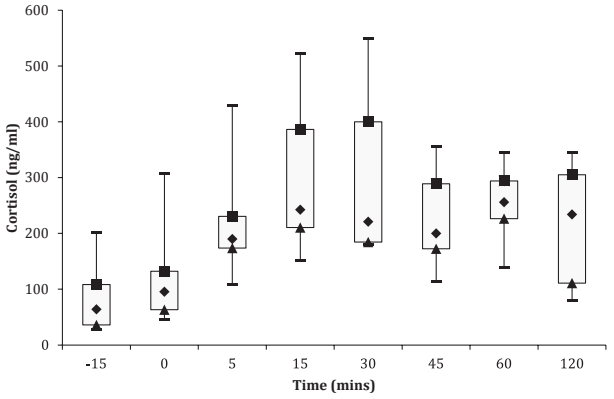

Fig 4. Box and whisker plot depicting the cortisol response following the IM administration of oCRH. There was no significant difference detected in endogenous cortisol concentration between administration routes ($P = .58$) or across times (−15 to 120 minutes), but there was a significant change in concentration across times ($P = .0031$) for both routes.

A similar comparison was made for cortisol concentrations at time = −15 and time = 0 (baseline) when oCRH was administered IV or IM. Significant differences were found between route ($P = .0072$) and the 2 times ($P = .015$). No significant interaction between route and time ($P = .52$) was noted. With the IV route, the mean difference between time = −15 and time = 0 was −51.6 ng/mL, indicating that cortisol values were higher at time = 0 than time = −15. In the IM route, the mean difference between time = −15 and time = 0 was −28.6 ng/mL, again indicating that cortisol values were higher at time = 0 than time = −15. The mean difference at time = −15 between the IV and IM routes was 49.1 ng/mL, indicating that cortisol values were higher when the oCRH was given IV than IM. The mean difference at time = 0 between the IV and IM routes was 66.1 ng/mL, indicating that cortisol values were higher when the oCRH was given IV than IM.

Endogenous ACTH concentrations after IV administration of oCRH and placebo were compared. There were no significant differences in the main effects of treatment (IV versus placebo; $P = .23$), time ($P = .14$), or the interaction between treatment and time ($P = .16$). There were also no significant differences between treatments in endogenous ACTH concentrations after administration of either IM oCRH or IM placebo ($P = .42$), time ($P = .18$), or in the interaction between treatment and time ($P = .51$).

Endogenous cortisol concentrations after IV administration of oCRH and placebo were compared. The difference between treatments approached significance ($P = .070$), but there was no significant change over time ($P = .35$) nor was there a significant treatment and time interaction ($P = .28$). At 5 minutes, the cortisol concentrations in the oCRH IV group were higher than the placebo IV group.

A comparison of cortisol concentrations after IM administration of oCRH and placebo was made. The difference approached significance between treatments ($P = .066$) because the cortisol values in the oCRH IM group were higher than in the placebo IM group. A significant change over time ($P = .0071$) was found but no differences between time = 0 and later times were significant. There was no significant group and time interaction ($P = .31$).

Table 1 summarizes cortisol and ACTH concentrations after the IV or IM administration of oCRH and placebo.

Discussion

There were 2 major findings in this study. Currently, the Scantibodies ACTH assay is commonly used in the clinical setting. Based on our data this assay performed poorly. Several of the after oCRH IV values were below the standard curve. Although both the ACTH IRMA and RIA assays showed the expected rise in endogenous ACTH post oCRH IV, the ACTH IRMA assay demonstrated parallelism and excellent intra- and interassay coefficient of variation; thus, we chose to use the ACTH IRMA assay. The ACTH IRMA assay performed well based on the expected rise in ACTH post oCRH administration. This assay showed both parallelism and acceptable intra- (<5%) and inter- (<10%) assay coefficients of variation using 3 cat plasma pools. The Diasorin IRMA assay is valid for use in cats and should be recommended to laboratories performing endocrine testing.

The 2nd finding of this study was that an intramuscular injection of oCRH resulted in an increase of greater magnitude in plasma ACTH than did the IV injection, and there was a significant difference between ACTH concentrations after both routes of administration across all time points. The difference may be because of a slower release of oCRH from the muscle versus a more short-term presence in the intravascular space with IV injection. Maximal median concentrations of ACTH and cortisol were seen at 60 minutes in the oCRH IM versus IV group (Figs 2 and 4). Thus, when performing an oCRH stimulation test in cats, samples for ACTH and cortisol should be drawn before and 60 minutes post IM injection of 1 μg/kg oCRH to assess maximal response.

There was a significant difference in maximal concentrations between the IM versus IV routes for ACTH but not for cortisol. The inability to find a significant difference in the post-oCRH cortisol concentrations may have been because of insufficient sample size. In addition, baseline cortisol concentrations were higher in cats administered oCRH IV versus IM. We hypothesize that this may have been because of stress associated with testing and handling as the oCRH IM study was performed the day after oCRH IV. The effects of stress in cats that have been physically restrained have been previously documented.[19] Thus, the lack of a significant difference in the cortisol response to IV versus IM administration, might have been the result of endogenous release of ACTH in response to the stress, blunting the response to exogenous oCRH.

There was no significant difference noted in the endogenous ACTH concentrations when placebo and

oCRH IM or IV were compared. The difference in endogenous cortisol between the placebo and oCRH IV or IM approached significance (Table 1). Given the rise in endogenous ACTH post oCRH IV that was noted when validating the ACTH IRMA assay, the lack of overall difference between the placebo and post-oCRH results is likely because of a small sample size rather than a lack of effect of the oCRH.

Limitations of this study include a small sample size, which may have made it difficult to find significant differences between placebo and oCRH IV and IM when measuring overall ACTH and cortisol response. However, when significant differences were present, the cortisol and ACTH concentrations in the oCRH IV and IM groups were higher than the placebo groups. An additional limitation is the lack of data in clinically ill animals. Although an oCRH stimulation test has the potential to be clinically useful, it is important to note that the use of this test is limited until further studies in disease states are performed.

This study and others have only assessed a limited number of dosages of oCRH when evaluating the HPA axis. Evaluating additional dosages of oCRH may allow for further assessment of the HPA axis in both normal and disease states and provide researchers and clinicians with a valuable tool for use in the study of the HPA axis in cats with suspected hypothalamic pituitary disease as well as CIRCI.

Cats were not randomized as to the order in which they received the oCRH as the short half-life of oCRH, ranging from 30 to 42 minutes in humans and sheep[20] would not be expected to affect the subsequent test results. In healthy dogs undergoing combined anterior pituitary testing, endogenous ACTH and cortisol concentrations following an injection of CRH appeared to peak and then rapidly decline with no delayed effect noted after the administration of CRH in these dogs.[3]

Using oCRH to stimulate the release of a physiologic amount of endogenous ACTH may result in a more accurate assessment of adrenal responsiveness in critically ill patients. We speculate that using oCRH could more closely approximate physiologic ACTH secretion and, thus cortisol, in critically ill cats. Further studies can now be carried out to evaluate the presence of CIRCI in ill cats using both ACTH and oCRH stimulation testing.

Footnotes

[a] Venocath-16, Hospira, Lake Forrest, IL
[b] Ovine corticotropin-releasing hormone, Acthrel, Ben Venue Laboratories, Bedford, OH
[c] Coat-a-Count Cortisol RIA, Siemens Healthcare Diagnostics, Los Angeles, CA
[d] ACTH IRMA, DiaSorin, Stillwater, MN
[e] ACTH RIA, DiaSorin
[f] Scantibodies ACTH assay, Scantibodies Laboratory, Inc, Santee, CA
[g] Stata/IC 12.1 for Windows, StataCorp LP, College Station, TX

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

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