

Effect of dexamethasone or synthetic ACTH administration on endogenous ACTH concentrations in healthy dogs

Andrew C. Bugbee, DVM; Jo R. Smith, MA, VetMB, PhD; Cynthia R. Ward, VMD, PhD

Objective—To determine the effects of dexamethasone or synthetic ACTH administration on endogenous ACTH concentrations in healthy dogs.

Animals—10 healthy neutered dogs.

Procedures—Each dog received dexamethasone (0.01 mg/kg), synthetic ACTH (5 µg/kg), or saline (0.9% NaCl) solution (0.5 mL) IV at intervals of ≥ 30 days. Plasma endogenous ACTH concentrations were measured before (baseline; time 0) and 1, 8, 12, and 24 hours after drug administration; serum cortisol concentrations were measured before and 1 hour after synthetic ACTH and saline solution administration and 8 hours after dexamethasone administration.

Results—Analysis of serum cortisol concentrations confirmed effects of drug administration. Dexamethasone significantly decreased the endogenous ACTH concentration from the baseline value at both 8 and 12 hours. Synthetic ACTH administration significantly decreased the endogenous ACTH concentration from the baseline value at 8 hours. Saline solution administration had no significant effect on endogenous ACTH concentration.

Conclusions and Clinical Relevance—Dexamethasone and synthetic ACTH administered IV at doses used routinely during testing for hyperadrenocorticism caused significant but transient reductions of endogenous ACTH concentrations in healthy dogs. Thus, a 2-hour washout period following ACTH stimulation testing before collection of samples for measurement of the endogenous ACTH concentration may be insufficient. Although this effect has not been verified in dogs with hyperadrenocorticism, these data suggested that samples for measurement of endogenous ACTH concentrations should be obtained before or > 8 hours after initiation of an ACTH stimulation test or before or > 12 hours after the start of a low-dose dexamethasone suppression test. (*Am J Vet Res* 2013;74:1415–1420)

Hyperadrenocorticism is a common endocrinopathy in dogs, and it affects mainly small-breed dogs.¹ Various mechanisms have been linked to the development of this syndrome, but all ultimately result in the same pathological abnormality of hypercortisolemia.^{2–4} Spontaneous development of pituitary-dependent or adrenal-dependent hyperadrenocorticism are the most common and clinically relevant forms of the disease in dogs. Different stimuli cause excess cortisol secretion, specifically excess endogenous ACTH secretion from the anterior pituitary gland in pituitary-dependent hyperadrenocorticism and autonomous endogenous ACTH-independent adrenal gland production in adrenal-dependent hyperadrenocorticism.

Diagnostic testing commonly used to confirm abnormal hormone concentrations in patients suspected of hav-

ABBREVIATIONS

CRH	Corticotropin-releasing hormone
CV	Coefficient of variation
HPA	Hypothalamic-pituitary-adrenal
LDDST	Low-dose dexamethasone suppression test
MCR	Melanocortin receptor

ing hyperadrenocorticism includes the ACTH stimulation test and LDDST as well as evaluation of the urine cortisol-to-creatinine concentration ratio.² The ACTH stimulation test and LDDST are reportedly the hyperadrenocorticism screening tests most commonly performed by 204 board-certified veterinary internists and dermatologists.⁵ Both tests rely on manipulation of the HPA axis, with identification of inappropriate serum cortisol responses following administration of a drug. After a patient with clinical signs consistent with hyperadrenocorticism has a positive result for a hyperadrenocorticism screening test, attempts to differentiate between adrenal-dependent and pituitary-dependent forms of the disease are recommended, given that this distinction has both therapeutic and prognostic implications.

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From the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

Supported by the University of Georgia College of Veterinary Medicine Clinical Research and Companion Animal Fund.

Address correspondence to Dr. Ward (crward@uga.edu).

Diagnostic tests and procedures that reportedly are of benefit when attempting to discriminate between adrenal-dependent and pituitary-dependent hyperadrenocorticism include the LDDST,⁶ measurement of endogenous ACTH concentration,^{7,8} ultrasonography of the adrenal glands,^{7,9-11} high-dose dexamethasone suppression test,⁶ desmopressin stimulation test,¹² determination of extended urine cortisol-to-creatinine concentration ratio,¹³ and advanced imaging techniques such as CT or MRI.¹⁴ Ultimately, the decision regarding the testing options that will be used depends on a variety of factors, including clinician preference, individualized patient care, financial constraints, time constraints, and access to equipment, supplies, or laboratories. The differentiation test preferred by 204 surveyed board-certified veterinary internists and dermatologists is the high-dose dexamethasone suppression test (57 [28%]), followed by measurement of the endogenous ACTH concentration (41 [20%]).⁵

Technical advancements have reduced the interval between obtaining definitive screening results and initiating discriminatory testing in veterinary medical settings. For practitioners in private clinical practice, a commercially available cortisol assay can be used to conveniently provide a rapid in-house diagnosis of hyperadrenocorticism. Furthermore, large referral practices and academic institutions commonly have access to high-capacity, specialized diagnostic laboratories. Thus, same-day screening and differentiation of hyperadrenocorticism have become common in veterinary medicine. Measurement of an endogenous ACTH concentration on the same day as hyperadrenocorticism screening is an attractive method to expedite differentiation of the cause of hyperadrenocorticism, thereby reducing the need for additional clinic visits, tests, and reliance on more expensive or referral-based diagnostic imaging modalities. Sensitivity and specificity for determination of endogenous ACTH concentrations range from 85% to 100% and 95% to 100%, respectively, although reference values differ.⁷

For such testing options, there is concern that manipulation of the HPA axis for purposes of hyperadrenocorticism screening may unintentionally impact results of subsequent adrenal differentiation tests performed in rapid succession. The effect of an ACTH stimulation test or LDDST on the endogenous ACTH concentration obtained within hours after performing these screening tests is unknown. It has been suggested¹⁵ that there be a 2-hour washout period following ACTH stimulation testing prior to obtaining a sample for measurement of endogenous ACTH concentrations, although this recommendation was inferred from a pharmacokinetic study and not actually confirmed. This recommendation contradicts previously accepted concepts of physiologically predicted hormone responses.² Determination of the effect hyperadrenocorticism screening has on measurement of endogenous ACTH concentrations would permit the development of appropriate protocols to ensure accurate results are obtained when attempting to differentiate pituitary-dependent and adrenal-dependent hyperadrenocorticism. We hypothesized that the LDDST and ACTH stimulation tests would affect subsequent plasma endogenous ACTH concentra-

tions in dogs. Therefore, the purpose of the study reported here was to investigate the effect that an ACTH stimulation test or LDDST would have on subsequent endogenous ACTH concentrations in healthy dogs.

Materials and Methods

Animals—Ten healthy neutered dogs owned by staff or veterinary students at the University of Georgia College of Veterinary Medicine were recruited for participation in the study. Inclusion requirements included owner consent, body weight > 4.0 kg, no abnormalities detected during physical examination, and unremarkable clinicopathologic test results (a CBC, serum biochemical analysis, and urinalysis of a sample obtained via cystocentesis). Dogs were excluded from participation if they had a preexisting underlying disease or had received any nonpreventative medications (including orally administered, topically administered, or injected corticosteroids) within the 30 days prior to enrollment. The study protocol was approved by the University of Georgia Veterinary Teaching Hospital Clinical Research Committee.

On test days, dogs were housed in bedded hospital cages or runs and walked outside on a leash every 6 to 8 hours. Dogs had unlimited access to water. After the last blood sample of the test day was obtained, dogs were offered a low-fat prescription food^a and allowed to eat for 20 minutes. A final blood sample was obtained the following morning, and each dog was then discharged to its owner.

Treatments—A randomized, controlled crossover study was performed. Each dog was assigned an identification number by selecting a dog's name from a container and then selecting a number (1 to 10) from another container. The order of drug administration for each dog was determined by selecting the sequence of treatments from a third container. Each dog received each of the 3 treatments: dexamethasone^b (0.01 mg/kg IV), synthetic ACTH^c (5.0 µg/kg, IV), and saline (0.9% NaCl) solution^d (0.5 mL, IV). Treatments were administered at an interval of ≥ 30 days. The dose of synthetic ACTH was rounded to the nearest 50 µg, and 250 µg was the maximum total dose of synthetic ACTH administered to any dog. All treatments were injected into a cephalic or lateral saphenous vein by a study investigator or veterinary technician.

Sample collection—Prior to each treatment, food was withheld from each dog by its owner for a minimum of 12 hours. Sample collection was initiated between 7:30 AM and 8:00 AM on each test day.

Blood samples (2 to 3 mL/sample) for measurement of plasma concentrations of endogenous ACTH were collected by jugular venipuncture before (baseline; time 0) and 1, 8, 12, and 24 hours after drug administration. Blood samples were placed in prechilled plastic tubes containing EDTA, mixed by inversion, and immediately centrifuged. Serum cortisol concentrations were used to confirm proper drug administration and absorption. Blood samples (2 to 3 mL/sample) for cortisol analysis were concurrently obtained with samples used for measurement of endogenous ACTH con-

centrations at 1 hour after administration of synthetic ACTH and saline solution or 8 hours after administration of dexamethasone. The samples were placed in additive-free plastic serum blood tubes and allowed to clot at room temperature (approx 22°C) for 10 minutes before centrifugation.

All blood samples were centrifuged (5,000 × g at 4°C for 10 minutes); plasma or serum was then decanted into polypropylene tubes, which were stored in a -80°C freezer for up to 150 days. Samples were shipped on dry ice for overnight delivery to a veterinary diagnostic laboratory^c for analysis.

Analysis of plasma endogenous ACTH and serum cortisol concentrations—All samples were assayed in duplicate, and the mean of the 2 values was reported. Samples with a CV > 20% were reassayed to confirm the values obtained. A series of 6 control samples were assayed each day; results for these samples were required to be within established laboratory quality-control ranges.

Analysis of cortisol concentrations was performed with a validated solid-phase chemiluminescent immunoassay.^{16,f} Interassay precision was 0.29 nmol/L (0.01 µg/dL) for a sample containing 29.0 nmol of cortisol/L (1.05 µg/dL). The assay limit of detection was < 0.2 µg/dL; results below the limit of detection were assigned a value of 0.19 µg/dL. The criterion used to define effective dexamethasone administration was posttreatment suppression of the cortisol concentration by ≥ 50% from the baseline value or a decrease to an absolute value < 1.4 µg/dL.^{2,6} The criterion used to define effective synthetic ACTH administration was a posttreatment increase in the cortisol concentration to an absolute value ≥ 6 µg/dL.²

Endogenous ACTH analysis was performed with a validated chemiluminescent enzyme immunometric assay.^{17,g} The intra-assay precision (CV) at low, medium, and high concentrations was 8.2%, 4.1%, and 4.3%, respectively; and the interassay CV for low, medium, and high concentrations was 6.0%, 4.6%, and 14.8%, respectively. The assay limit of detection was < 10 pg/mL; results below the limit of detection were assigned a value of 9.9 pg/mL.

Statistical analysis—Data analysis was performed with statistical software.^h A repeated-measures model for multiple observations from the same dog was used to test for differences in endogenous ACTH concentrations following synthetic ACTH or dexamethasone administration, compared with results after saline solution administration, at 1, 8, 12, and 24 hours. Plasma endogenous ACTH concentration at 1, 8, 12, and 24 hours was compared with the baseline value for each treatment. The full model included factors of group, time, and a group-by-time interaction. Adjustments for multiple comparisons were made with a Bonferroni correction. An unstructured covariance structure was used in all repeated-measures models. All hypothesis tests were 2-sided, and values of α equal to 0.05 divided by the number of simultaneous tests performed were used to determine significant differences. The repeated-measures analysis was performed with a mixed procedures function.ⁱ

Results

Dogs—Six spayed females and 4 neutered males (mean ± SD age, 6.9 ± 3.7 years; mean body weight, 16.36 ± 10.56 kg) were enrolled in the study. Two dogs were < 4 years old and weighed > 15 kg, 6 dogs were < 10 years old and weighed < 15 kg, and 2 dogs were > 10 years old and weighed > 15 kg. Dogs comprised 5 mixed-breed dogs, 1 Beagle, 1 Border Collie, 1 German Wirehaired Pointer, 1 Irish Terrier, and 1 Jack Russell Terrier.

Serum cortisol concentrations—Analysis of serum cortisol concentrations confirmed proper drug administration in all dogs (Figure 1). At 1 hour after administration of synthetic ACTH, cortisol concentrations increased from a mean ± SD baseline value of 2.6 ± 2.1 µg/dL to 13.4 ± 5.8 µg/dL. One dog had a cortisol concentration of 26.1 µg/dL and another had a concentration of 21.1 µg/dL at 1 hour after synthetic ACTH administration. The values in these 2 dogs may have been suggestive of hyperadrenocorticism; however, both dogs lacked clinical or clinicopathologic evidence of hyperadrenocorticism, and both had an anticipated suppression of cortisol concentrations following dexamethasone administration. At 8 hours after dexamethasone administration, all dogs had cortisol concentrations < 1.4 µg/dL (range, 0.19 to 0.5 µg/dL), which was consistent with suppression. Saline solution administration caused no significant change in serum cortisol concentrations at 1 hour after injection (mean change from baseline value, 0.0 ± 1.0 µg/dL).

Plasma endogenous ACTH concentrations—At 8 hours after administration of synthetic ACTH, there was a significant ($P = 0.001$) reduction in the endogenous ACTH concentration, compared with the base-

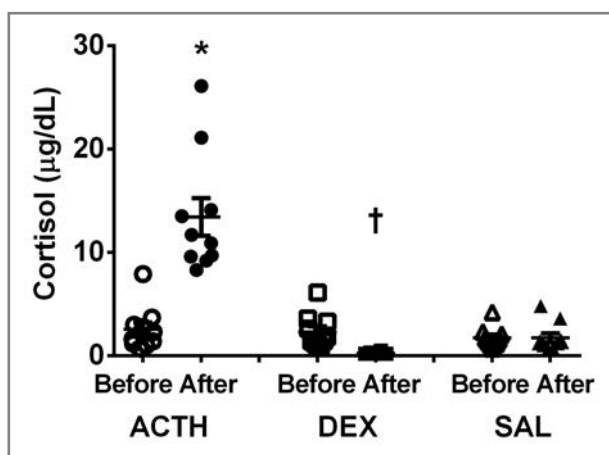


Figure 1—Serum cortisol concentrations in samples obtained before (baseline; time 0) and after IV administration of synthetic ACTH (5 µg/kg), dexamethasone (0.01 mg/kg [DEX]), or saline (0.9% NaCl) solution (0.5 mL [SAL]) to 10 healthy neutered dogs; there was an interval of ≥ 30 days between successive treatments. Samples were obtained 1 hour after administration of synthetic ACTH or saline solution and 8 hours after administration of dexamethasone. Each symbol represents results for 1 dog. For each treatment, the horizontal line represents the mean, and the error bar represents the SD. *Concentration is significantly ($P < 0.05$) increased from the baseline concentration. †Concentration is significantly ($P < 0.05$) decreased from the baseline concentration.

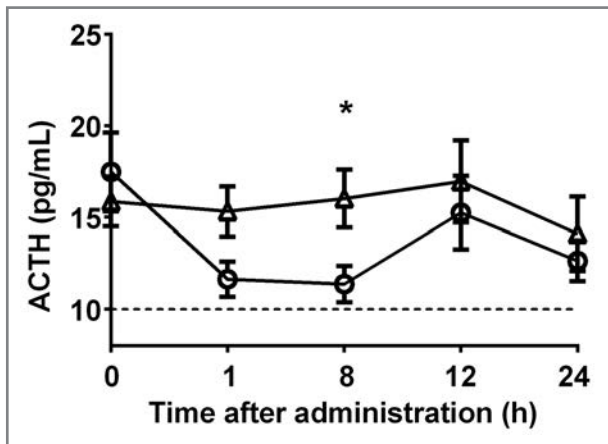


Figure 2—Mean \pm SD plasma endogenous ACTH concentrations after IV administration of saline solution (triangles) and synthetic ACTH (circles) to the same 10 healthy dogs in Figure 1. Results at 1 hour after synthetic ACTH administration represent values for only 9 dogs (the value for 1 dog was excluded because of discordant results [CV > 20% for duplicate analyses]). The dashed horizontal line represents the assay limit of detection; results below the limit of detection were assigned a value of 9.9 pg/mL.*Within a time point, concentrations differ significantly ($P < 0.05$) between treatments.

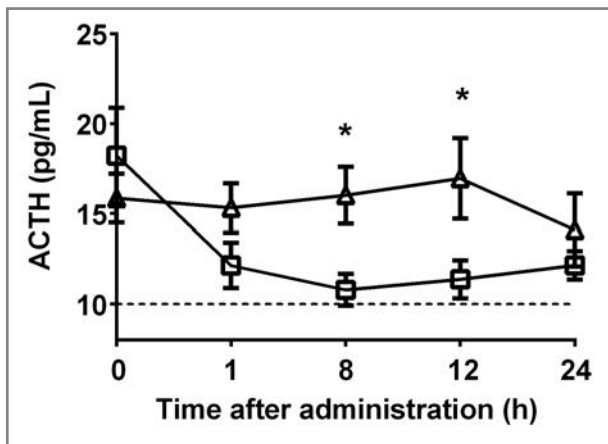


Figure 3—Mean \pm SD plasma endogenous ACTH concentrations after IV administration of saline solution (triangles) and dexamethasone (squares) to the same 10 healthy dogs in Figure 1. See Figure 2 for remainder of key.

line value (Figure 2). Seven of 10 dogs had decreases in the endogenous ACTH concentration (mean maximal decrease, 44.1%) after synthetic ACTH administration; 4 dogs had decreases at all time points, and 3 dogs had decreases that lasted up to 8 hours. Two dogs had baseline endogenous ACTH concentrations below the assay limit of detection; 1 of these dogs had endogenous ACTH concentrations < 10 pg/mL at all time points. One dog failed to have decreases in endogenous ACTH concentrations at any time point. This dog had discordant results (CV > 20%) in the sample obtained 1 hour after synthetic ACTH administration, and there was an insufficient amount of sample for repeated analysis. Therefore, results for that dog at this time point were excluded from analysis.

A significant decrease in the endogenous ACTH concentration, compared with the baseline value, was detected at both 8 ($P = 0.001$) and 12 ($P = 0.002$) hours

after dexamethasone administration (Figure 3). Eight of 10 dogs had decreases in the endogenous ACTH concentration (mean maximal decrease, 42.3%) after dexamethasone administration; 5 dogs had decreases at all time points, 2 had decreases that lasted up to 12 hours, and 1 had decreases that lasted up to 8 hours. One dog had a baseline endogenous ACTH concentration below the assay limit of detection, and the remaining dog had concentrations at 1 and 8 hours after dexamethasone administration that exceeded the baseline value. Administration of saline solution did not result in a significant change in endogenous ACTH concentrations from the baseline value at any time point.

Discussion

Analysis of the results of the study reported here indicated significant and transient decreases in endogenous ACTH concentrations following IV administration of dexamethasone or synthetic ACTH to healthy dogs. Although healthy dogs may have episodic or pulsatile secretion of ACTH and cortisol, management of the dogs and sampling procedures were standardized to account for this variable.¹⁸ Theoretically, it is possible that the variations in endogenous ACTH concentration and the relationship to the cortisol concentration may have reflected physiologic fluctuations and were not direct responses to drug administration. Until these data can be verified in dogs with a confirmed diagnosis of hyperadrenocorticism, results of the present study suggested that sample collection for measurement of endogenous ACTH concentrations should be delayed for up to 8 hours after initiating an ACTH stimulation test and up to 12 hours after the start of an LDDST to minimize the effect of these drugs on subsequent endogenous ACTH concentrations.

Assessment of the cortisol concentration was performed to verify drug administration and absorption. Administration of synthetic ACTH stimulated a predictable increase in the serum cortisol concentration at the 1-hour time point, and dexamethasone caused suppression of serum cortisol concentrations at the 8-hour time point in all dogs. The basis for these expected responses is the physiologic processes of the HPA axis. Hypothalamic paraventricular neuron-derived CRH induces pro-opiomelanocortin gene transcription and processing within the corticotrophs of the anterior pituitary gland, which results in the generation of various peptides, including ACTH. Endogenous ACTH then binds to MCR2 in the zona fasciculata of the adrenal gland to stimulate the production and immediate release of glucocorticoids.¹⁹ A synthetic derivative of ACTH^c was used for the ACTH stimulation test in the present study to mimic the adrenocortical-stimulating effect of endogenous ACTH.²⁰ Although the synthetic ACTH only contains the first 24 of the 39 amino acids comprising endogenous ACTH, its biological function is retained.^{19,21,22}

Negative long-loop feedback in the HPA axis involves cortisol-mediated activation of glucocorticoid receptors in the hypothalamus and anterior pituitary gland, which induce suppression of pro-opiomelanocortin gene transcription and CRH receptor-1 expression.^{23–25} There are also multiple potential glucocorti-

coid-independent interactions identified at all levels of the HPA axis. In humans, MCR2 mRNA is expressed in the anterior pituitary gland, which thus enables autocrine downregulation of ACTH synthesis and release. Loss of this ultrashort-loop feedback is potentially involved in functional tumorigenesis because approximately 70% of ACTH-secreting pituitary gland tumors fail to express MCR2 mRNA.²⁰ Additionally, although binding to MCR2 requires a specific ACTH epitope (Lys15-Lys16-Arg17-Arg18), all other MCR subtypes (1, 3, 4, and 5) have no binding requirement beyond the first 13 amino acids.^{21,27}

Melanocortin receptor subtype 3, which is expressed within the hypothalamus, has greater affinity for synthetic ACTH (amino acids 1 to 24) than for intact ACTH (amino acids 1 to 39).^{27,28} Therefore, administration of synthetic ACTH (amino acids 1 to 24) may indirectly have negative feedback on ACTH production through activation of hypothalamic MCR3 and subsequent downregulation of CRH. Thus, the transient decrease of ACTH concentrations in the present study may have reflected synthetic ACTH feedback on MCR2 in pituitary corticotrophs (ultrashort loop) or hypothalamic MCR3 (short loop) independent of expected cortisol-mediated responses.

In a previous pharmacokinetic study¹⁵ of synthetic ACTH administration to dogs, it was suggested that a 2-hour delay after starting an ACTH stimulation test was sufficient before subsequent collection of samples for measurement of ACTH concentrations to differentiate between pituitary-dependent and adrenal-dependent hyperadrenocorticism. On the basis of the results of the present study, we recommend that this delay be extended to 8 hours to minimize the possibility that synthetic ACTH or increased cortisol concentrations would cause negative feedback and affect intrinsic endogenous ACTH secretion. The disparity in these results may be attributable to a difference in the form of synthetic ACTH injected or the assay used for ACTH detection. Investigators in that previous study¹⁵ used an unspecified synthetic form (amino acids 1 to 39) of ACTH, which potentially has interactions with the HPA axis that differ from the interactions of the peptide form (amino acids 1 to 24) used in the present study. Also, use of synthetic ACTH (amino acids 1 to 39) would preclude both the radioimmunoassay and immunoradiometric assay used in the previous study¹⁵ from definitively differentiating between synthetic and endogenous forms of ACTH. All ACTH detected after the 2-hour time point in that previous study¹⁵ was considered to be endogenous ACTH because of fluctuation patterns, when, in fact, endogenous ACTH concentrations may have been suppressed and the assay was detecting residual synthetic ACTH (amino acids 1 to 39). In the study reported here, the synthetic ACTH (amino acids 1 to 24) analogue^c could be differentiated from endogenous ACTH (amino acids 1 to 39).¹⁷

After synthetic ACTH was administered, 2 dogs with baseline concentrations within the reference range had persistent decreases in endogenous ACTH concentrations for up to 24 hours. Sequential measurements of cortisol concentrations were not obtained to further define possible hormone interactions because the study

objective was to assess drug effects only on endogenous ACTH concentrations. One dog had no measurable decrease in the endogenous ACTH concentration, despite concurrent increases in serum cortisol concentrations. This response could correspond with reports¹⁵ of endogenous ACTH concentrations returning to the reference range (20 to 80 pg/mL) within 2 hours after stimulation with synthetic ACTH, although it does not adhere to the accepted physiologic concept of cortisol-mediated suppression of endogenous ACTH secretion. Dissociation of ACTH and cortisol secretion has been reported in various species, including dogs^{29,30}; however, this has typically been described only after experimentally induced stress. Results for a few dogs in the present study could have represented uncommon physiologic responses, alternate feedback mechanisms, or intrinsic drug-hormone interactions; however, insufficient data were obtained to enable determination of a definitive explanation. Until further investigations are performed, clinicians should use caution when measuring and interpreting endogenous ACTH concentrations in samples obtained within 8 hours after synthetic ACTH administration.

In clinically normal dogs, dexamethasone administered during an LDDST suppresses ACTH secretion by the pituitary gland, which results in an expected decrease in serum cortisol concentration.² A single dose of dexamethasone administered IV has a serum half-life of 1.98 to 2.27 hours and causes the cortisol concentration to decrease to < 1.4 µg/dL within 3 hours after administration.^{2,6} In contrast to the rapid blood clearance rate, the biological half-life of dexamethasone can be prolonged, with cortisol suppression lasting for 24 to 48 hours.³¹ Similar results were obtained in the present study because 9 of 10 dogs had cortisol suppression and corresponding decreases in endogenous ACTH concentrations at 8 hours after dexamethasone administration. However, several dogs had results that conflicted with expected physiologic responses. Four dogs had undetectable endogenous ACTH concentrations at 12 and 24 hours after dexamethasone administration. One of these dogs had concentrations below the limit of detection at all time points. Concurrent measurement of cortisol concentrations was not performed for the other 3 dogs; thus, it cannot be determined whether the endogenous ACTH concentrations at 12 and 24 hours after dexamethasone administration represented persistent dexamethasone suppression of endogenous ACTH and cortisol concentrations, preferential suppression of the endogenous ACTH concentration with cortisol recovery (dissociation), or typical physiologic variation. Another dog had cortisol suppression at 8 hours with no previous or concurrent decrease in the endogenous ACTH concentration. This observation suggested that there were other mechanisms involved in inhibiting cortisol secretion or altering suppression of ACTH in this dog. This likely represented an actual finding, considering that inappropriate collection or storage of a sample would have been expected to decrease, rather than increase, the endogenous ACTH concentration. Analysis of these results indicated that dexamethasone administered to healthy dogs at doses used for the LDDST may cause a decrease in endogenous ACTH concentrations for at least 12 hours following administration.

Pharmacological manipulation of the HPA axis for the purposes of hyperadrenocorticism testing may alter the accuracy of ACTH concentrations in samples obtained subsequently. Although the number of dogs assessed in the present study was small, analysis of the results suggested that healthy dogs can have significant decreases in endogenous ACTH concentrations for at least 12 hours after IV administration of dexamethasone (0.01 mg/kg) and for at least 8 hours after IV administration of synthetic ACTH (5.0 µg/kg). Investigation of these findings in patients with confirmed hyperadrenocorticism is warranted to avoid incorrect diagnosis of adrenal-dependent hyperadrenocorticism or the performance of unnecessary additional testing.

- a. Gastrointestinal Low Fat LF, Royal Canin USA Inc, St Charles, Mo.
- b. Dexium injection, 2 mg (dexamethasone solution, 2 mg/mL), Bimeda Inc, Chicago, Ill.
- c. Cortrosyn injection, 0.25 mg cosyntropin sterile lyophilized powder, Amphastar Pharmaceuticals, Rancho Cucamonga, Calif.
- d. Hospira Inc, Lake Forest, Ill.
- e. University of Minnesota Veterinary Diagnostic Laboratory, Saint Paul, Minn.
- f. Siemens Immulite cortisol assay, catalogue No. LKC01, Siemens Healthcare Diagnostics, Deerfield, Ill.
- g. Siemens Immulite ACTH assay, catalogue No. LKAC1, Deerfield, Ill.
- h. SAS, version 9.2, SAS Institute Inc, Cary, NC.
- i. PROC MIXED, SAS, version 9.2, SAS Institute Inc, Cary, NC.

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