Serum concentrations of cortisol, sex hormones of adrenal origin, and adrenocortical steroid intermediates in healthy dogs following stimulation with two doses of cosyntropin

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Objective—To compare the effects of 2 doses of cosyntropin (5 µg/kg vs 250 µg, IV) on serum concentrations of cortisol, sex hormones of adrenal origin, and adrenocortical steroid intermediates and determine the optimal sample collection time after adrenal stimulation with cosyntropin.

Animals—10 healthy, privately owned, neutered dogs.

Procedure—Dogs were randomly assigned to initially receive cosyntropin at 5 µg/kg or as a total dose of 250 µg, IV. Dogs received the alternate dose 1 to 2 weeks later. Serum was obtained from blood samples collected before (0 minutes) and 30, 60, 90, and 120 minutes after cosyntropin administration.

Results—Maximum stimulation of cortisol, androstenedione, progesterone, and 17-hydroxyprogesterone production was achieved at 60 minutes following IV administration of cosyntropin at 5 µg/kg or as a total dose of 250 µg. Serum estradiol concentration did not increase in response to either cosyntropin dose. For all hormones, no significant difference in serum hormone concentrations was found among sample collection times of 0, 30, 60, and 90 minutes when comparing the 2 doses of cosyntropin.

Conclusions and Clinical Relevance—Cosyntropin, when administered at 5 µg/kg, IV, effectively stimulated maximum production of cortisol, sex hormones of adrenal origin, and adrenocortical steroid intermediates at 1 hour after administration. (*Am J Vet Res* 2004;65:1631–1633)

The **adrenocorticotropic hormone (ACTH)** stimulation test is commonly used to screen dogs suspected of having hypo- or hyperadrenocorticism.[1] In addition to measuring cortisol, sex hormones of adrenal origin (estradiol, progesterone, and testosterone) and adrenocortical steroid intermediates (adrenocortical steroid precursors such as **17-hydroxyprogesterone [17-OHP]** and androstenedione) may be measured in dogs suspected of having atypical hyperadrenocorticism, whereby dogs have clinical signs of hyperadrenocorticism but do not have hypercortisolemia.[2,3] Instead, dogs have an increase in plasma concentrations of precursor hormones to cortisol such as 17-OHP.[2,3] Although becoming more controversial, measuring adrenocortical steroid concentrations in addition to cortisol is also common in dogs with nonendocrine alopecia.[4,5]

Currently, serum is assayed for cortisol and adrenocortical steroids before and 1 hour after IV administration of cosyntropin (5 µg/kg).[4,6] This dose of synthetic ACTH has been shown to maximally stimulate cortisol production by the adrenal glands.[7,8] It is not known, however, whether the production of other adrenocortical steroids is also maximized by stimulation with cosyntropin at this dose (ie, 5 µg/kg) and time interval (1 hour). The purpose of the study reported here was to compare the stimulation effects of 2 doses of cosyntropin on adrenocortical steroid production and determine the optimal sample collection time after stimulation.

Materials and Methods

Animals—Ten healthy, privately owned, neutered dogs (5 males and 5 females) were entered into the study. All dogs were enrolled with the informed consent of their owners. The institutional animal care and use committee approved the study. All dogs weighed between 6.58 and 20.87 kg (mean 12.36 kg). Smaller dogs were selected to maximize the difference between doses. Dogs were excluded from the study if they had received any corticosteroid preparation (systemic or topical) within 6 months before the start of the study. Dogs did not receive any adrenocortical steroid preparation while participating in the study.

Experimental protocol—Dogs were randomly assigned to initially receive cosyntropin[a] at 5 µg/kg or as a total dose of 250 µg, IV. Dogs were returned in 1 to 2 weeks, at which time the doses were reversed. Serum samples were obtained from blood samples collected before and 30, 60, 90, and 120 minutes after cosyntropin administration. Blood was left to clot at room temperature (approx 21°C) prior to centrifugation. Serum samples were stored in a −70°C freezer until assayed for cortisol and adrenocortical steroid concentrations at the completion of the study. Serum samples from all dogs were evaluated for cortisol, progesterone, 17-OHP, androstenedione, testosterone, and estradiol.

Serum adrenocortical steroid concentrations were determined in a laboratory[b] via commercially available radioimmunoassay kits.[c-h] Samples were run in duplicate, and each hormone was run in a single assay. Sensitivity as determined by the manufacturer for cortisol, progesterone, 17-OHP, androstenedione, testosterone, and estradiol radioimmunoassay was 0.2 µg/dL, 0.02 ng/mL, 0.08 ng/mL, 0.05 ng/mL, 0.04 ng/mL, and 7.2 pg/mL, respectively. Intra- and interassay coefficients of variation for the radioim-

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munoassay were as follows: cortisol, 5.4% and 5.9%; progesterone, 7.3% and 13.0%; 17-OHP, 10.7% and 9.8%; androstenedione, 7.7% and 7.1%; testosterone, 9.3% and 7.9%; and estradiol, 7.3% and 7.3%, respectively.[6] No noteworthy cross-reactivity exists with other adrenocortical steroids for each assay, with the exception of prednisolone in the cortisol assay.[c-h]

Hormones tested for cross-reactivity include the following: the cortisol assay was tested for cross-reactivity with aldosterone, betamethasone, danazol, 11-deoxycorticosterone, 11-deoxycortisol, dexamethasone, estriol, estrone, flumethasone, methotrexate, methylprednisolone, prednisolone, prednisone, pregnenolone, progesterone, tetrahydrocortisol, and triamcinolone; the progesterone assay was tested for cross-reactivity with corticosterone, cortisol, danazol, 11-deoxycorticosterone, 11-deoxycortisol, dihydroepiandrosterone sulfate, 20α-dihydroprogesterone, estradiol, 17-OHP, medroxyprogesterone, pregnenolone, and testosterone; the 17-OHP assay was tested for cross-reactivity with 11-descoxycortisol, 17α-hydroxypregnenolone, and progesterone; the androstenedione assay was tested for cross-reactivity with dihydroepiandrosterone sulfate, dihydroepiandrosterone, estrone, testosterone, progesterone, 17β-estradiol, aldosterone, cholesterol, corticosterone, cortisol, dihydrotestosterone, desoxycorticosterone, 11-desoxycortisol, estriol, 17-OHP, pregnenolone, pregnenolone sulfate, and 17α-hydroxypregnenolone; the testosterone assay was tested for cross-reactivity with aldosterone, androstenedione, androsterone, corticosterone, cortisol, cortisone, danazol, 11-deoxycortisol, dexamethasone, dihydroepiandrosterone, dihydroepiandrosterone sulfate, 5α-dihydrotestosterone, estradiol, estrone, methyltestosterone, prednisone, progesterone, and triamcinolone; and the estradiol-17β assay was tested for cross-reactivity with estrone, estriol, estradiol-17α, ethinyl estradiol, androstenedione, dihydroepiandrosterone, 5α-dihydrotestosterone, 20α-dihydroprogesterone, progesterone, testosterone, pregnenolone, 17-hydroxypregnenolone, dihydroepiandrosterone sulfate, aldosterone, cortisol, 11-desoxycortisol, 17-OHP, and cholesterol.

Statistical analysis—Data was analyzed by use of a software package.[i] For each sample collection time, the individual hormone concentrations after stimulation with the 2 doses of cosyntropin were compared by use of the paired Student *t* test. When normality failed, the Wilcoxon signed rank test was used. A repeated-measures ANOVA was used to compare the hormone concentrations among the sample collection times. Friedman repeated-measures ANOVA on ranks was used when normality failed. Values of $P < 0.05$ were considered significant.

Results

Maximum stimulation of serum hormone concentrations following cosyntropin administration at either 5 µg/kg or as a total dose of 250 µg was achieved at 60 minutes for cortisol, androstenedione, progesterone, and 17-OHP (**Table 1**). Serum concentrations of cortisol, progesterone, 17-OHP, and androstenedione were significantly increased above baseline values at 30, 60, and 90 minutes following cosyntropin administration at 5 µg/kg. The serum concentration of 17-OHP was also significantly increased at 120 minutes after stimulation with cosyntropin at 5 µg/kg. Serum concentrations of cortisol, progesterone, and androstenedione were significantly increased above baseline values at 30, 60, 90, and 120 minutes following administration of 250 µg of cosyntropin. The 17-OHP concentrations were only significantly increased above baseline values at 60 and 90 minutes after administration of 250 µg of cosyntropin. Serum androstenedione concentration at 90 minutes following cosyntropin administration at 5 µg/kg was significantly lower than serum androstenedione concentration at 60 minutes following stimulation. No other significant differences were found between serum hormone concentrations at these 2 time points. Serum concentration of estradiol did not increase in response to stimulation with either cosyntropin dose. All serum concentrations of testosterone were below the sensitivity of the assay and therefore are not reported.

No significant differences in serum concentrations of hormones were found between sample collection times 0, 30, 60, and 90 minutes after stimulation when comparing the 2 doses of cosyntropin. Administration of 250 µg of cosyntropin resulted in significantly

Table 1—Mean (± SD) serum adrenocortical steroids* concentrations before and after stimulation with cosyntropin in 10 clinically normal neutered dogs.

		Time (min)				
Hormone	**Cosyntropin dose**	**0**	**30**	**60**	**90**	**120**
Cortisol (µg/dL)	5 µg/kg	3.87 ± 3.91	10.22 ± 3.38†	12.37 ± 2.60†	11.06 ± 3.99†	7.36 ± 2.42‡
	250 µg	1.88 ± 1.34	9.54 ± 2.50†	12.32 ± 3.04†	12.34 ± 3.87†	9.28 ± 3.63†
Progesterone (ng/mL)	5 µg/kg	0.35 ± 0.51	1.37 ± 0.50†	1.59 ± 0.50†	1.20 ± 0.46†	0.57 ± 0.21‡
	250 µg	0.16 ± 0.12	1.33 ± 0.56†	1.68 ± 0.64†	1.46 ± 0.72†	0.79 ± 0.38†
17-OHP (ng/mL)	5 µg/kg	0.40 ± 0.38	1.87 ± 0.78†	2.17 ± 0.88†	1.84 ± 0.97†	1.04 ± 0.50†
	250 µg	0.22 ± 0.14	1.71 ± 0.62	2.21 ± 0.92†	2.22 ± 1.12†	1.42 ± 0.86
Androstenedione (ng/mL)	5 µg/kg	7.1 ± 10.7	27.4 ± 15.1†	31.4 ± 18.0†	19.7 ±16.0†§	9.9 ± 8.1‡
	250 µg	2.1 ± 1.5	25.9 ± 13.0†	30.7 ± 20.0†	26.7 ± 23.6†	16.1 ± 17.2†
Estradiol (pg/mL)	5 µg/kg	56.2 ± 10.1	53.4 ± 12.8	55.5 ± 10.1	56.2 ± 11.9	52.7 ± 12.1
	250 µg	55.9 ± 15.0	54.0 ± 11.7	55.9 ± 10.1	54.3 ± 15.9	55.5 ± 17.4

*Testosterone concentrations were below the sensitivity of the assay and therefore not reported. †Significantly different ($P < 0.05$) from 0-minute values. ‡Significantly different ($P < 0.05$) from the value following administration of 250 µg of cosyntropin. §Significant difference ($P < 0.05$) between 60- and 90-minute values.

17-OHP = 17-hydroxyprogesternone.

Conversion to SI units are as follows: cortisol, µg/dL × 27.59 = nmol/L; progesterone, ng/mL × 3.18 = nmol/L; 17-OHP, ng/mL × 3.03 = nmol/L; androstenedione, ng/mL × 3.49 = nmol/L; and estradiol, pg/mL × 3.67 = pmol/L.

greater serum concentrations of androstenedione, progesterone, and cortisol at 120 minutes after stimulation, compared with cosyntropin administration at 5 µg/kg.

Discussion

In our study, serum concentrations of cortisol, sex hormones of adrenal origin, and adrenocortical steroid intermediates were measured in response to cosyntropin stimulation to assess increased production of these hormones by the adrenal glands. Use of the cosyntropin-stimulation test for assessing cortisol production in dogs with hyperadrenocorticism is well documented.[1,9-11] Our results are in agreement with those of previous studies[7,8] in which 2 doses of ACTH were compared, indicating that the cosyntropin dose of 5 µg/kg maximally stimulates cortisol production and peak cortisol production occurs at 60 to 90 minutes after stimulation, with no significant difference in serum hormone concentrations between the 2 sample collection times.

Serum concentrations of sex hormones of adrenal origin and adrenocortical steroid intermediates in response to ACTH have been assessed in dogs with atypical hyperadrenocorticism and in dogs with nonendocrine alopecia,[2-4] although the usefulness of this test for the latter condition is now being questioned.[5] The optimal dose of ACTH and sample collection times following stimulation have not been previously evaluated in dogs when assessing these hormones. Cosyntropin, when administered at 5 µg/kg, IV, effectively stimulated production of sex hormones of adrenal origin and adrenocortical steroid intermediates at 60 minutes after administration. No significant difference was found between serum hormone concentrations when comparing the 2 doses of cosyntropin at each time point, with the exception of the 120-minute samples. The higher dose of cosyntropin resulted in a more sustained response, as shown by the significantly greater serum concentration of androstenedione, progesterone, and cortisol at 120 minutes after stimulation.

With the great increase in cost of cosyntropin, the ability to use a low amount of drug without affecting the diagnostic capability of the test increases the affordability and therefore the frequency of the use of the drug. Results of previous work[12] indicate that the bioactivity of cosyntropin is maintained when stored at -20°C in plastic syringes for 6 months.

As has been previously reported,[6] the concentration of estradiol did not increase in response to either dose of cosyntropin in our study. Whereas estradiol may originate in the adrenal glands by conversion of adrenocortical steroid intermediates (eg, androstenedione) via aromatase enzyme action, estradiol is also produced from aromatization in extra-adrenal tissues such as adipose, hair follicles, or liver.[13] In light of the lack of postcosyntropin stimulation increase of estradiol from the adrenal glands in dogs, this extra-adrenal production of estradiol may be more important than the actual amount produced by the adrenal glands. More work is needed to determine the importance of an increase in serum estradiol concentrations and its source in neutered dogs.

In summary, results of our study indicate that IV administration of cosyntropin at either 5 µg/kg or as a total dose of 250 µg results in maximum stimulation of cortisol and adrenocortical steroid production from the adrenal glands; therefore, either dose is appropriate to use to evaluate adrenal hormone production in dogs. The preferred sample collection time after administration is at 60 minutes, in agreement with the sample collection time currently recommended for postcosyntropin stimulation cortisol assessment. Although little difference in serum hormone concentrations was found between the 60- and 90-minute sample collection times, maximum stimulation was consistently observed at 60 minutes following stimulation with either dose of cosyntropin.

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[a]Cortrosyn, Oreganon Inc, West Orange, NJ.
[b]Clinical Endocrinology Service, College of Veterinary Medicine, University of Tennessee, Knoxville, Tenn.
[c]Cortisol [I^{125}] RIA, Diagnostic Products Corp, Los Angeles, Calif.
[d]Progesterone [I^{125}] RIA, Diagnostic Products Corp, Los Angeles, Calif.
[e]17-OHP [I^{125}] RIA, MP Biomedicals (formerly ICN Pharmaceuticals Inc), Costa Mesa, Calif.
[f]Androstenedione [I^{125}] RIA, MP Biomedicals (formerly ICN Pharmaceuticals Inc), Costa Mesa, Calif.
[g]Testosterone [I^{125}] RIA, Diagnostic Products Corp, Los Angeles, Calif.
[h]Estradiol 17-beta [I^{125}] RIA, MP Biomedicals (formerly ICN Pharmaceuticals Inc), Costa Mesa, Calif.
[i]SigmaStat 3.0 for Windows, SPSS Inc, Chicago, Ill.

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