Serum 17-α-hydroxyprogesterone and corticosterone concentrations in dogs with nonadrenal neoplasia and dogs with suspected hyperadrenocorticism

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Objective—To assess serum 17-α-hydroxyprogesterone (17OHP) and corticosterone concentrations in dogs with nonadrenal neoplasia and dogs being screened for hyperadrenocorticism.

Design—Prospective study.

Animals—16 clinically normal dogs, 35 dogs with nonadrenal neoplasia, and 127 dogs with suspected hyperadrenocorticism.

Procedure—ACTH stimulation tests were performed in all dogs. Baseline serum cortisol and corticosterone concentrations were measured in the healthy dogs; baseline serum cortisol concentration and ACTH-stimulated cortisol, corticosterone, and 17OHP concentrations were measured in all dogs. Endogenous plasma ACTH concentration was also measured before administration of ACTH in dogs with neoplasia.

Results—In 35 dogs with neoplasia, 31.4% had high serum 17OHP concentration and 22.9% had high serum corticosterone concentration. Of the 127 dogs with suspected hyperadrenocorticism, 59 (46.5%) had high ACTH-stimulated cortisol concentrations; of those, 42 of 59 (71.2%) and 32 of 53 (60.4%) had high serum 17OHP and corticosterone concentrations, respectively. Of dogs with serum cortisol concentration within reference range after ACTH administration, 9 of 68 (13.2%) had high serum 17OHP concentration and 22.9% had high serum corticosterone concentration. Of the 127 dogs with suspected hyperadrenocorticism, 59 (46.5%) had high serum 17OHP concentration and 22.9% had high serum corticosterone concentration. In all 23 dogs, high serum concentrations of 17OHP were induced in response to ACTH administration and it was concluded that those high concentrations served as a marker of HAC. In another study, however, the sensitivity and specificity of post-ACTH serum 17OHP concentration as a diagnostic marker for HAC were low and post-ACTH 17OHP analysis was not recommended as a routine screening test for HAC.

Conclusions and Clinical Relevance—Serum concentrations of 17OHP or corticosterone after administration of ACTH may be high in dogs with nonadrenal neoplasia and no evidence of hyperadrenocorticism. Changes in serum 17OHP or corticosterone concentrations after administration of ACTH are proportionate with changes in cortisol concentration. (J Am Vet Med Assoc 2005; 227:1762–1767)

Hyperadrenocorticism (HAC) is one of the most common endocrinopathies affecting older dogs. Because of the high prevalence and nonspecific nature of the clinical signs associated with the disease, older dogs are commonly screened for HAC. The disease is diagnosed by means of the low-dose dexamethasone suppression test (LDDST) or the ACTH stimulation test (AST) with measurement of pre- and post-ACTH serum cortisol concentrations. Both tests have been associated with false-positive results in dogs with nonadrenal illness and in dogs with lymphoma.

In recent studies, concentrations of sex hormones were measured as a means of assessing adrenal function, and high serum 17-α-hydroxyprogesterone (17OHP) concentrations were suggested as a cause of occult HAC. In 1 study, 23 dogs with clinical and certain laboratory findings suggestive of HAC but with normal results of either the AST, LDDST, or both were assessed. In all 23 dogs, high serum concentrations of 17OHP were induced in response to ACTH administration and it was concluded that those high concentrations served as a marker of HAC. In another study, however, the sensitivity and specificity of post-ACTH serum 17OHP concentration as a diagnostic marker for HAC were low and post-ACTH 17OHP analysis was not recommended as a routine screening test for HAC.

A corticosterone-secreting tumor in a dog that was evaluated because of clinical signs of hypercortisolism has been reported. Because clinical signs of hypercortisolism may be caused by excessive corticosterone, which is a more potent stimulator of glucocorticoid receptors than are sex hormones, occult HAC (ie, clinical signs of HAC develop in response to excess secretion of hormones other than cortisol from the adrenal cortex) may also be caused by excessive synthesis and secretion of corticosterone.

However, before determination of serum concentrations of a specific hormone can be advocated as a marker of excessive adrenal gland activity, the specificity of adrenocortical function tests in patients with chronic illness must be assessed because false-positive test results may lead to inappropriate and potentially harmful treatment recommendations. The objectives of our study were to assess secretion of 17OHP and corticosterone in dogs with chronic illness and to assess whether concentrations of those hormones in sick dogs were similar to concentrations in dogs with HAC.

Materials and Methods

Sixteen clinically normal student-owned dogs were used for determination of the reference range for post-ACTH serum cortisol.
aliquots of serum from a canine serum pool and these were in various known concentrations (up to 1,000 nmol/L) to testosterone. Reported cross-reactivity for cortisol was 4.1% for 17OH pregnenolone and 1.3% for progesterone. Important cross-reactivities (coefficients of variation were 8% and 10%, respectively).

Cross-reactivity for cortisol and 17OHP was 0.05% cross-reactivity for corticosterone and 0.94% cross-reactivity for corticosterone and < 0.02% cross-reactivity for progesterone. Plasma eACTH concentration was measured by use of a validated radioimmunoassay kit. The sensitivity (ie, the concentration of hormone that resulted in a significant [P < 0.05] difference in percentage binding, compared with the zero standard) of the corticosterone assay was 6 ng/mL. Cross-reactivity for cortisol and 17OHP was < 1%. To further evaluate possible cross-reactivity between analytes, cortisol was added in various concentrations (up to 1,000 nmol/L) to aliquots of serum from a canine serum pool and those specimens were assayed by use of the corticosterone and cortisol assays. Although the cortisol assay accurately detected the amounts of cortisol added, addition of the corticosteroid had no effect on reported concentrations of corticosterone.

Results

The 20 dogs in the lymphosarcoma group had a median age of 9 years (range, 2 to 13 years), and the 15 dogs in the NHN group also had a median age of 9 years (range, 6 to 13 years). The control group consisted of 16 dogs with a median age of 4 years in 14 of the dogs (range, 2 to 12 years); the exact age of 2 dogs was unknown, but they were known to be ≥ 2 years old. The clinically normal dogs included 1 sexually intact male, 3 castrated males, and 12 spayed females. Dogs in the lymphosarcoma group included 1 sexually intact male, 6 castrated males, and 13 spayed females. In the NHN group, there were 1 sexually intact male, 5 castrated males, 1 sexually intact female, and 8 spayed females. Lymphosarcoma was diagnosed by means of cytologic (n = 18) or histologic (2) evaluation of peripheral lymph nodes. A diagnosis of NHN was made via histologic evaluation of biopsy specimens in all dogs; all specimens were reviewed by a single pathologist (EMW).

Dogs with lymphosarcoma were classified according to the modified World Health Organization staging system, and distribution was as follows: stage Va (n = 6 dogs), stage Vb (3), stage Iva (6), stage Ivb (2), stage IIa (1), stage IIb (1), and stage IIib (1). Tumors diagnosed in the NHN group included transitional cell carcinoma (n = 4 dogs), hepatocellular adenoma (1), hepatocellular carcinoma (1), metastatic adenocarcinoma (1; origin likely a perianal tumor), metastatic mammary carcinoma (1), metastatic jejunal adenocarcinoma (1), rectal adenocarcinoma (1), anal sac apocrine gland adenocarcinoma (1), insulinoma (1), pheochromocytoma (1), hemangiosarcoma (1), and osteosarcoma (1).

In the dogs with neoplasia, median post-ACTH serum cortisol concentration was 357 nmol/L (range, 53 to 933 nmol/L; reference range, 220 to 560 nmol/L). Of 20 dogs (4 with lymphosarcoma and 2 with NHN) had a decreased post-ACTH serum cortisol concentration, and 3 (1 with lymphosarcoma and 2 with NHN) had an increased post-ACTH serum cortisol concentration. In addition, median eACTH concentration in dogs with nonadrenal neoplasia was 17 pg/mL (range, 5 to 91 pg/mL; reference range, 10 to 80 pg/mL). Of 20 dogs with lymphosarcoma, 1 had high plasma eACTH concentration. Of 15 dogs with nonhematopoietic neoplasia, no dogs had high eACTH concentration. Plasma eACTH concentrations were lower than reference range in 2 dogs with lymphosarcoma and in 1 with NHN.

The reference range for post-ACTH serum 17OHP concentration in healthy dogs was 0.4 to 2.8 ng/mL (1.2 to 8.5 nmol/L), calculated as the mean value obtained in the healthy dogs ± 2 SDs. There was no difference.
between stimulated serum 17OHP concentrations for dogs with lymphosarcoma (2.0 ng/mL; range, 0.5 to 6.1 ng/mL) and dogs with NHN (2.6 ng/mL; range, 0.7 to 4.5 ng/mL), so the dogs with neoplasia were combined into a single group for further evaluation. Serum 17OHP concentrations after ACTH administration were significantly ($P = 0.014$) higher in dogs with neoplasia than in clinically normal dogs (Figure 1). Eleven dogs with neoplasia (31.4%) had high post-ACTH serum 17OHP concentrations, compared with reference range values for healthy dogs. In the 35 dogs with neoplastic disease, the reference range for post-ACTH serum 17OHP concentration, calculated as the mean value obtained ± 2 SDs, was 0.05 to 5.0 ng/mL (0.15 to 15.2 nmol/L).

Of all 35 dogs, 21 had mild disease (12 with lymphosarcoma and 9 with NHN), 6 had moderate disease (3 with lymphosarcoma and 3 with NHN), and 8 had severe disease (5 with lymphosarcoma and 3 with NHN). No significant difference existed among groups with respect to ACTH-stimulated serum 17OHP concentration (Figure 2).

The reference range for post-ACTH serum corticosterone concentration in healthy dogs was 6.4 to 35.4 ng/mL (18.4 to 102.0 nmol/L), calculated as the mean value in the clinically normal dogs ± 2 SDs. All pre-ACTH serum corticosterone concentrations in clinically normal dogs were less than the sensitivity of the assay, indicating that ACTH administration elicits corticosterone secretion. There was no difference in stimulated serum corticosterone concentrations between dogs with lymphosarcoma (19.0 ng/mL; range, 6.0 to 47.0 ng/mL) and dogs with NHN (26.0 ng/mL; range, 6.0 to 46.0 ng/mL), so the dogs with neoplasia were combined into a single group for further evaluation. Serum post-ACTH corticosterone concentrations were not significantly different between tumor-bearing and clinically normal dogs (Figure 3).

Eight (22.9%) dogs with neoplasia had high post-ACTH serum corticosterone concentrations, compared with reference range values. In the 35 dogs with neoplastic disease, the reference range for post-ACTH serum corticosterone concentration was calculated as the mean value obtained ± 2 SDs and determined to be 6 to 58.2 ng/mL (17.3 to 167.6 nmol/L). In comparisons among dogs with mild, moderate, and severe disease, there was no difference among groups in post-ACTH serum corticosterone concentrations (for dogs with mild disease, 24.0 ng/mL; range, 6.0 to 46.0 ng/mL; for dogs with moderate disease, 19.5 ng/mL; range, 11.0 to 42.0 ng/mL; and for dogs with severe disease, 17.5 ng/mL; range, 6.0 to 47.0 ng/mL).

All 3 dogs with neoplasia that had high post-ACTH serum cortisol concentrations also had high post-ACTH 17OHP and serum corticosterone concentrations. Of the 6 dogs with neoplasia that had low post-ACTH serum cortisol concentrations, serum 17OHP concentration was within the reference range (0.5 to 2.4 ng/mL) for healthy dogs in 5 dogs and high (2.9 ng/mL) in 1 dog. In addition, for those 6 dogs, post-ACTH serum corticosterone concentrations were in the lower 33% (11 to 15 ng/mL) of the reference range.
range in 4 dogs and were lower than reference range values in 2 dogs (6 ng/mL in both).

Of the 127 dogs with suspected HAC, 59 (46.5%) had high serum cortisol concentration after ACTH stimulation (> 560 nmol/L [> 20.3 μg/dL]). Serum 17OHP concentration after ACTH administration was significantly (P < 0.001) lower in dogs with post-ACTH serum cortisol concentration within reference range than in dogs with high post-ACTH serum cortisol concentration (Figure 4). In addition, serum corticosterone concentration post-ACTH was significantly (P < 0.001) lower in dogs with post-ACTH serum cortisol concentrations (22.8 ng/mL; range, 6.0 to 58.5 ng/mL) within reference range than in dogs with increased post-ACTH serum cortisol concentrations (39.8 ng/mL; range, 17.3 to 333.0 ng/mL). Of the dogs with a high post-ACTH cortisol concentration, 42 of 59 (71.2%) and 32 of 53 (60.4%) had high serum 17OHP and corticosterone concentrations after ACTH injection, respectively, compared with reference range values in clinically normal dogs, whereas 20 of 59 (33.9%) and 7 of 53 (13.2%) had high serum 17OHP and corticosterone concentrations after administration of ACTH, respectively, compared with reference range values derived from dogs with chronic illness. Of the 68 dogs with suspected HAC that had serum cortisol concentration within reference range after ACTH stimulation, 10 of 68 (14.7%) and 3 of 67 (4.5%) had high post-ACTH serum 17OHP and corticosterone concentrations, respectively, if the reference range for clinically normal dogs was used, whereas 3 of 68 (4.4%) and 1 of 67 (1.5%) had high post-ACTH serum 17OHP and corticosterone concentrations, respectively, if the reference range from dogs with chronic illness was used.

In the 16 clinically normal dogs, post-ACTH serum cortisol concentration was not significantly correlated with post-ACTH serum 17OHP concentration (P = 0.13) but was correlated (R² = 0.404; P = 0.008) with post-ACTH serum corticosterone concentration. Post-ACTH serum 17OHP and corticosterone concentrations were also significantly correlated (R² = 0.323; P = 0.02). In the 35 dogs with neoplasia, post-ACTH serum cortisol concentrations were significantly corre-

Discussion

In this study, we validated a new assay for measurement of serum 17OHP concentration in dogs. The reference range for post-ACTH serum 17OHP concentration in clinically normal dogs was 0.4 to 2.8 ng/mL (1.2 to 8.5 nmol/L). These values are similar to previously reported ranges of 0.33 to 1.82 ng/mL (1.0 to 6.0 nmol/L) and 0.32 to 2.76 ng/mL (1.0 to 8.4 nmol/L). The effect of sex on serum 17OHP concentration is unclear. In one study, investigators determined that neither sex nor neutering affected serum 17OHP concentrations, whereas in another study, it was determined that concentrations are higher in neutered males, compared with sexually intact males, and in sexually intact females, compared with neutered females. Our population of clinically normal dogs was too small to detect differences on the basis of sex, and only 3 of the dogs with neoplasia were sexually intact.

Serum concentration of 17OHP was measured after ACTH administration in dogs with neoplasia as a model of chronic illness. On the basis of historical information, clinical signs, and laboratory data, none of those dogs was suspected of having HAC. Overall, the stimulated serum 17OHP concentrations were significantly higher in dogs with neoplasia. Of the 35 dogs with neoplastic disease, 11 (31.4%) had high post-ACTH serum 17OHP concentrations, compared with clinically normal dogs. Because tests of adrenal gland function in dogs may be more likely to yield a false-positive result as the severity of nonadrenal gland illness increases, the post-ACTH serum 17OHP concentration was compared among dogs with neoplasia with mild, moderate, or severe disease. No significant differences existed among groups. Interestingly, dogs with severe disease had the lowest median post-ACTH serum 17OHP concentrations. However, these findings should be interpreted cautiously because of the small group size.

Determination of serum 17OHP concentration before and after ACTH administration has recently been advocated as a means of diagnosing classic or occult HAC. Of 23 dogs suspected of having HAC, all had high serum 17OHP concentrations after ACTH stimulation, although 17 dogs had a post-ACTH serum cortisol concentration within reference range (ie, normal results of an AST), normal LDDST results, or both. However, depending on the serum 17OHP concentration used to distinguish normal from abnormal, the specificity of post-ACTH serum 17OHP concentration as a diagnostic test for HAC may be as low as 71%. We also found that dogs with chronic illness may have a
high serum 17OHP concentration after ACTH administration. Interestingly, in our study, only 3 (8.6%) dogs with neoplasia had high post-ACTH cortisol concentration, whereas 11 (31.4%) had a high post-ACTH serum 17OHP concentration, suggesting that the specificity of serum 17OHP concentration for diagnosis of HAC may be less than that of serum cortisol concentration.

To our knowledge, serum corticosterone concentrations have not been evaluated as a marker of canine adrenal function in a multiple-case series. A dog with clinical signs of HAC associated with corticosterone secretion by an adrenal tumor has been reported. In the present study, post-ACTH serum corticosterone concentration was not significantly different between clinically normal dogs and dogs with neoplasia nor among dogs with mild, moderate, or severe disease. However, the post-ACTH serum corticosterone concentration was high in 8 (22.9%) dogs with neoplastic disease, compared with concentrations in clinically normal dogs. Modification of the assay may allow accurate measurement of the low concentrations of corticosterone in some samples (ie, nondetectable concentrations in baseline serum samples).

Post-ACTH serum 17OHP and corticosterone concentrations were also measured in the 127 dogs with suspected HAC. Of dogs with high post-ACTH serum cortisol concentration consistent with a diagnosis of HAC, 71.2% and 60.4% also had high post-ACTH serum 17OHP and corticosterone concentrations, respectively, compared with clinically normal dogs. Because the positive predictive value of a post-ACTH cortisol concentration for diagnosing HAC is high in dogs with suggestive clinical signs, these results differed from those of an earlier study in which it was determined that all dogs with HAC had a high post-ACTH serum 17OHP concentration. However, in another study, the sensitivity of post-ACTH serum 17OHP concentration for detecting HAC was reported to be 40% to 71%, depending on the cutoff value used to differentiate reference range from abnormal concentrations. In addition, in 42 dogs with HAC, only 10 (24%) had high serum 17OHP concentrations after ACTH stimulation, whereas in another 10, concentrations were near reference range, resulting in a sensitivity of high post-ACTH serum 17OHP concentrations for diagnosis of HAC in the range of 24% to 48%.

Of the dogs with suspected HAC that had a post-ACTH serum cortisol concentration within reference range, 14.7% and 7.5% had high post-ACTH serum 17OHP and corticosterone concentrations, respectively, with the reference range for clinically normal dogs. Because most dogs tested for HAC have clinical signs of disease (and if they do not have HAC, the clinical signs are likely caused by chronic illness), the more appropriate population to use for determination of reference range values may be dogs with nonadrenal illness (eg, neoplastic disease). Furthermore, linear regression analysis revealed a significant correlation between post-ACTH hormone concentrations in dogs with neoplasia and in those suspected of having HAC, suggesting that as adrenal gland hormone output increased as a direct result of adrenal disease or increased in a nonspecific manner as a result of nonadrenal disease, serum concentrations of all hormones increased proportionately. Accordingly, of the 68 dogs with suspected HAC that had serum cortisol concentrations within reference range after ACTH stimulation, only 2 of 68 (2.9%) and 2 of 67 (3.0%) had high post-ACTH serum 17OHP and corticosterone concentrations, respectively; if the reference range for 17OHP concentration derived from dogs with chronic illness was used. Thus, the specificity of post-ACTH serum 17OHP or corticosterone concentrations for diagnosing HAC may be much higher if dogs with illness are used as the reference population. Whether these dogs had a false-positive test for HAC, had subclinical HAC, or would have benefited from treatment for HAC (eg, received mitotane) could not be determined.

Limitations of our study included the fact that group sizes were relatively small; therefore, the absence of significance as indicated by certain tests should be interpreted cautiously. Second, artifact may have been introduced by a failure to identify all dogs that had received exogenous glucocorticoids at the time of enrollment into the study, despite intensive questioning of owners regarding topically or systemically administered glucocorticoids. In many instances, referring veterinarians were also contacted to confirm dogs’ medication histories. Third, limiting the exclusion criteria for exogenous glucocorticoid use to the previous 4 weeks may not have allowed sufficient time for adrenal gland recovery for all dogs. Fourth, it is possible that some of the dogs with neoplastic disease also had HAC, although this is unlikely given the absence of clinical signs or other indicators of concurrent HAC. Lastly, it is also possible that some of the dogs screened for HAC that had positive results of an AST may not have actually had HAC. However, inclusion criteria for the study specified that accompanying historical information be consistent with a diagnosis of HAC, and the specificity of the AST for diagnosing HAC in dogs with suggestive clinical signs is approximately 86%. Alternatively stated, the chance of a false-positive test was relatively low (14%). Also, in dogs with clinical signs of HAC, positive results of an AST have a high positive predictive value for the disease. Similarly, some of the dogs screened for HAC that had a negative AST may have had HAC. Again, given that the sensitivity of the AST for detecting HAC is approximately 80%, few dogs would be affected.

Our results suggest that dogs with chronic illness may have high post-ACTH serum concentrations of 17OHP and corticosterone. As a result, diagnosing HAC in dogs with increased post-ACTH serum 17OHP concentrations but without hypercortisolemia may lead to misdiagnosis and inappropriate treatment. However, although measurement of these hormones may not be a sensitive marker of HAC, if ill dogs are used as a reference population, the values may be highly specific. Further investigation is warranted, including use of ill dogs as a reference population for post-ACTH serum cortisol concentrations. Although it is unclear whether dogs in which HAC was suspected and that had concentrations of post-ACTH serum cortisol within reference range but high post-ACTH serum 17OHP or corticosterone concentrations would have
benefited from treatment with mitotane, the data suggest that post-ACTH serum 17OHP and corticosterone concentrations may not necessarily be correlated with abnormal adrenal gland function.

References