Serum Inhibin Concentration in Dogs with Adrenal Gland Disease and in Healthy Dogs

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Background: Studies in humans identified the synthesis and secretion of inhibin from adrenocortical tumors, but not pheochromocytoma (PHEO). Inhibin has not been examined in dogs as a serum biomarker for adrenal gland tumors.

Objective: To determine serum inhibin concentration in dogs with adrenal gland disease and in healthy dogs.

Animals: Forty-eight neutered dogs with adrenal disease including pituitary-dependent hyperadrenocorticism (PDH, 17), adrenocortical tumor (18), and PHEO (13), and 41 healthy intact or neutered dogs.

Methods: Prospective observational study. Dogs were diagnosed with PDH, adrenocortical tumor (hyperadrenocorticism or noncortisol secreting), or PHEO based on clinical signs, endocrine function tests, abdominal ultrasound examination, and histopathology. Inhibin concentration was measured by radioimmunoassay in serum before and after ACTH stimulation, and before and after treatment.

Results: In neutered dogs, median inhibin concentration was significantly higher in dogs with adrenocortical tumors (0.82 ng/mL) and PDH (0.16 ng/mL) than in dogs with PHEO and healthy dogs (both undetectable). Median inhibin concentration was significantly higher in dogs with adrenocortical tumors than in those with PDH and decreased after adrenalectomy. Median inhibin concentration was significantly higher in intact than in neutered healthy dogs and was similar in pre- and post-ACTH stimulation. Sensitivity, specificity, and accuracy of serum inhibin concentration for identifying an adrenal tumor as a PHEO were 100, 88.9, and 93.6%, respectively.

Conclusions and Clinical Importance: Adrenocortical tumors and PDH but not PHEOs are associated with increased serum inhibin concentration; undetectable inhibin is highly supportive of PHEO in neutered dogs with adrenal tumors.

Key words: Hyperadrenocorticism; Neoplasm; Pheochromocytoma; Pituitary gland; Tumor.

Introduction

Inhibin is a glycoprotein synthesized predominantly in ovarian granulosa and testicular Sertoli cells.[1,2] The primary physiologic roles of inhibin are suppression of follicle-stimulating hormone release from the pituitary gland and regulation of cellular functions in the gonads.[3,4] Circulating inhibin in healthy dogs and humans mainly originates from the gonads.[5,6] The adrenal glands are known as extragonadal sources of inhibin in humans, but little is known regarding the role of adrenal inhibin.[7,8] An association of inhibin with adrenocortical disease has been reported by the analysis of serum and adrenal tissue in humans. In vivo and in vitro studies identified both synthesis

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Abbreviations:

ATH	adrenocortical tumor-associated hyperadrenocorticism
FI	female intact
FS	female spayed
IR	immunoreactivity
MC	male castrated
MI	male intact
NCS	noncortisol secreting
PDH	pituitary-dependent hyperadrenocorticism
PHEO	pheochromocytoma
RIA	radioimmunoassay

and secretion of inhibin from adrenocortical tumors in humans, with the highest secretion rates in cortical adenomas associated with Cushing's disease.[7,9] Expression of inhibin in tissue also is used for differentiating adrenocortical tumors from pheochromocytoma (PHEO).[10] Immunohistochemical studies in humans identified inhibin alpha expression in adrenocortical hyperplasia, adenomas, and carcinomas, whereas PHEOs were negative.[10–12]

Inhibin concentration in serum or expression in the adrenal glands has not been reported in dogs with adrenal disease, and the value of inhibin as a serum biomarker for adrenal gland tumors has not been investigated. In addition, serum inhibin concentrations have not been evaluated in healthy female dogs. The effect of ACTH on serum inhibin concentration in dogs also is not known. On the basis of findings in humans, we hypothesized that dogs with adrenocortical tumors but not those with PHEOs have increased serum inhibin concentrations compared with healthy dogs. The objectives of the study reported here were to validate

an assay for inhibin immunoreactivity (IR) in the serum of dogs and to determine serum inhibin concentrations in healthy dogs and in dogs with adrenal gland disease to assess the value of inhibin in discriminating adrenocortical tumors from PHEOs.

Materials and Methods

Study Population

The study included 48 neutered dogs with naturally occurring adrenal gland disease and 41 healthy control dogs. Dogs with adrenal gland disease had been examined at the Veterinary Medical Teaching Hospital, University of California, Davis and were enrolled with the informed consent of their owners. The study was performed in compliance with institutional guidelines for research on animals. Dogs with adrenal gland disease included 17 with pituitary-dependent hyperadrenocorticism (PDH), 18 with adrenocortical tumors, and 13 with PHEO. Dogs with adrenocortical tumors included 14 with adrenocortical tumor-associated hyperadrenocorticism (ATH, 13 carcinomas, 1 adenoma) and 4 with noncortisol secreting (NCS) cortical tumors (3 carcinomas, 1 adenoma). Median age (range) was 10.2 years (range, 6.1–14.6 years), 10.4 years (range, 6.1–13.5 years), and 11.8 years (range, 7.1–15.6 years) in dogs with PDH, adrenocortical tumors, and PHEO, respectively. Breed and sex distribution was as follows: PDH, 11 female spayed (FS), 6 male castrated (MC); adrenocortical tumors, 11 FS, 7 MC; PHEO, 4 FS, 9 MC. Dogs were purebred in 8/17, 11/18, and 6/13 cases with PDH, adrenocortical tumors, and PHEO, respectively. Breeds that occurred more than once included Standard Poodle (4; 2 PHEOs, 1 cortical adenoma, 1 PDH), Labrador Retriever (3; cortical carcinomas), Shi Tzu (2; PHEOs), and Dalmatian (2; 1 cortical carcinoma, 1 PDH).

All dogs with hyperadrenocorticism had clinical signs, physical examination findings, and clinicopathologic abnormalities consistent with hyperadrenocorticism and abnormal results of at least 2 of 3 endocrine screening tests (ACTH stimulation test,[a] low-dose dexamethasone suppression test, or urine cortisol[a]-to-creatinine[b] ratio determination). Dogs with PDH also had 2 approximately equal-sized adrenal glands identified by ultrasonography. Dogs with ATH had low or undetectable plasma concentrations of endogenous ACTH, ultrasonographic evidence of an adrenal gland mass, and histologic confirmation of an adrenocortical tumor after adrenalectomy. All dogs with NCS cortical tumors and PHEO were evaluated after an adrenal gland mass had been identified by ultrasonography. These dogs lacked clinical signs and physical examination findings of hyperadrenocorticism and had results of at least 2 of 3 screening tests for hyperadrenocorticism within reference intervals (ACTH stimulation test, low-dose dexamethasone suppression test, urine cortisol-to-creatinine ratio determination). Each of these dogs was suspected of having a PHEO and therefore underwent adrenalectomy without further evaluation for secretory function. Each of these dogs had histologic confirmation of an adrenocortical tumor or PHEO, respectively. Dogs that had been treated for adrenal gland disease (eg, hyperadrenocorticism) and dogs with multiple adrenal gland disorders (eg, PDH and adrenal gland tumor; ATH and PHEO) were not included in the study.

Healthy dogs used as controls were privately owned and consisted of 10 female intact (FI), 10 male intact (MI), 11 FS, and 10 MC purebred or mixed-breed dogs. Median age was 8.8 years (range, 5.0–14.0 years). All control dogs were considered healthy on the basis of lack of clinical signs of disease and unremarkable findings of physical examination. All control dogs had results of serum biochemical analyses,[c] CBC,[d] and urinalyses within reference intervals. Healthy neutered dogs were used

for comparisons with neutered diseased dogs and underwent the following additional tests: serum cortisol[a] concentrations before and 1 h after ACTH stimulation; urine cortisol[a]-to-creatinine[b] ratio; and plasma endogenous ACTH concentration. Results were within reference intervals for all the dogs. Results of ultrasonographic examination of the adrenal glands were normal. Healthy female and male intact dogs were included as positive controls expected to synthesize and secrete inhibin from the gonads. One sexually intact control dog that had been euthanized for reasons not related to this study served as a donor of normal adrenal tissue. This dog also had normal findings of a complete necropsy with histologic examination of all organs including the adrenal and pituitary glands. The only treatments being administered in healthy dogs were heartworm and flea preventive medications.

Sample Collection and Assay Validation

Serum was harvested from all the dogs and stored at −70°C until analysis. In neutered dogs with adrenal disease and neutered controls, inhibin concentration was measured in serum before and 1 h after ACTH stimulation by IM injection of 0.25 mg cosyntropin[e] per dog. In sexually intact controls, inhibin concentration was measured in serum at baseline. Treatment of adrenal disease consisted of adrenalectomy in all dogs with adrenal gland tumors or medical treatment of PDH with mitotane[f] or trilostane.[g] After treatment of adrenal gland disease, blood samples for the determination of inhibin concentration were available in 8/17 dogs with PDH (mitotane, 4; trilostane, 4), 14/18 dogs with adrenocortical tumors (ATH, 10; NCS, 4), and 8/13 dogs with PHEO. Blood samples 1 h after ACTH stimulation[e] were analyzed in 27 dogs (3 dogs with PHEO were not tested). At the time of blood sample collection after treatment of hyperadrenocorticism, post-ACTH serum cortisol concentration was <5 μg/dL in all the dogs with PDH and in 8/10 dogs with ATH. Post-ACTH serum cortisol concentration was within the reference interval (6–15 μg/dL) in the remaining two dogs with ATH. In dogs with adrenal gland tumors, ACTH stimulation tests usually were performed 1 day after adrenalectomy. The weight of adrenal glands with neoplasms was determined immediately after adrenalectomy.

Inhibin IR was measured in serum by radioimmunoassay (RIA) using an antibody raised against 31 kDa bovine inhibin.[h] The antibody detects all inhibin forms containing the mature region of the alpha subunit. This includes the free alpha subunit and inhibin alpha-beta dimers (inhibin A, inhibin B). The antibody shows crossreactivity with some alpha subunit precursors (pro–alpha-C, pro–alpha-N-alpha C), but does not detect activin (beta-beta dimers).[13] The tracer was I[125]-labeled purified 31 kDa bovine follicular fluid inhibin.[h] This antibody and tracer have been used previously to detect inhibin IR in canine plasma.[14] Purified bovine follicular fluid inhibin was iodinated[i] using chloramine T and purified by column chromatography essentially as previously described.[15] All the samples were analyzed in duplicate. Sensitivity, dilutional parallelism, and precision were determined on canine serum. Sensitivity was determined by identifying the number of raw counts at B_0 (the maximum binding of antibody to tracer in the absence of standard). Two standard deviations were subtracted from the raw count at B_0 and the resulting value estimated on the standard curve, ranging from 0.05 to 0.12 ng/mL (mean sensitivity, 0.09 ng/mL). Inhibin concentrations that were undetectable were assigned the sensitivity value of the assay, which is the lowest concentration measurable with the assay. Parallelism with the standard curve was evaluated and confirmed by serial dilution of canine serum containing high inhibin IR with zero standard (Fig. 1). Intra-assay coefficients of variation were determined by analyzing each of 2 canine sera with known low and high inhibin

IR at least 4 times within the same assay. This was performed in 3 assays. Interassay coefficients of variation were determined by analyzing each of 3 canine sera with known low, intermediate, and high inhibin IR in 3 consecutive assays. Mean coefficients of variation for intra- and interassay precision were 7.1 and 11.7%, respectively.

Immunohistochemistry

Sections from the following tissues were examined for expression of inhibin by immunohistochemistry: adrenocortical tumors (6 carcinomas, 1 adenoma), PHEOs (5), healthy adrenal gland (1), positive controls (3 canine testes, 1 canine ovary), and negative controls for each section (no anti-inhibin alpha antibody added). Immunohistochemistry was performed with an anti-inhibin alpha mouse monoclonal antibody[j] that has been used previously on canine tissues. Positive immunostaining was reported in testicular Sertoli cells,[16] Sertoli cell[17] and Leydig cell tumors,[16] and ovarian granulosa cells and granulosa cell tumors in dogs.[18] After deparaffinization and rehydration, tissue sections were incubated for 30 min in 0.3% H_2O_2 in methanol for quenching of endogenous peroxidase activity, then washed in buffer for 5 min. Heat-induced antigen (epitope) retrieval was performed with antigen unmasking solution.[k] Subsequent steps were performed according to manufacturer's instructions.[l] Briefly, sections were blocked in normal equine serum for 20 min at room temperature to decrease nonspecific staining. Excess blocking solution was blotted from the sections. Sections were incubated with primary antibody (1 : 5 in buffer) for 16 h at 4C. Incubation with normal mouse serum (1 : 5 in buffer) instead of primary antibody was used as a negative control. Sections were washed for 5 min in buffer, then incubated for 30 min at room temperature in kit secondary antibody (1 : 200 in kit blocking serum).[l] Sections were incubated for 5 min in peroxidase substrate solution (diluted in distilled water and in reagents according to the manufacturer's instructions),[m] rinsed with tap water, counterstained with hematoxylin, rinsed with tap water, dehydrated, and mounted. Sections were examined microscopically for the presence or absence of immunohistochemical staining.

Statistical Analysis

Statistical software was used for all the analyses.[n] Data distributions were assessed by the Anderson-Darling normality test. Data were not normally distributed; therefore, nonparametric tests were used and data are presented as medians and ranges. For comparisons of data among multiple groups, the Kruskal-Wallis ANOVA was used, and if differences were significant (ie, $P < .050$), that analysis was followed by posthoc Mann-Whitney tests. Wilcoxon signed-rank tests were used for the comparisons of inhibin concentrations within groups (eg, pre- versus post-ACTH stimulation). Comparisons of data between 2 groups were performed with Mann-Whitney tests. Correlations between variables were evaluated by the use of Spearman's rank correlation coefficient. Box plots were generated by SigmaPlot.[o]

Results

In healthy dogs, serum inhibin concentration at baseline was significantly higher in intact than in neutered dogs of both sexes (MI, median, 1.46 ng/mL; range, 0.51–2.50 ng/mL, versus MC, median, 0.11 ng/mL; range, 0.05–0.11 ng/mL, $P < .001$; FI, median, 0.17 ng/mL; range, 0.11–1.47 ng/mL, versus FS, median, 0.11 ng/mL; range, 0.05–0.29 ng/mL, $P = .036$; MI versus FS, $P < .001$; FI versus MC, $P = .014$). Serum inhibin concentration at baseline also was significantly higher in MI than in FI healthy dogs ($P < .001$, Fig 2). In all healthy neutered dogs with 1 exception (FS outlier, Fig 2), baseline serum inhibin concentration was below the sensitivity of the RIA and therefore considered undetectable with this assay.

Serum inhibin concentration at baseline was significantly higher in dogs with adrenocortical tumors (median, 0.82 ng/mL; range, 0.05–20.0 ng/mL, $P < .001$) and in dogs with PDH (median, 0.16 ng/mL; range, 0.05–0.58 ng/mL, $P < .001$) than in healthy neutered dogs (median, 0.11 ng/mL; range, 0.05–0.29 ng/mL). Serum inhibin concentration at baseline was similar in dogs with PHEO (median, 0.11 ng/mL; range, 0.05–0.12 ng/mL) and in healthy neutered dogs ($P = .140$, Fig 3A). Among
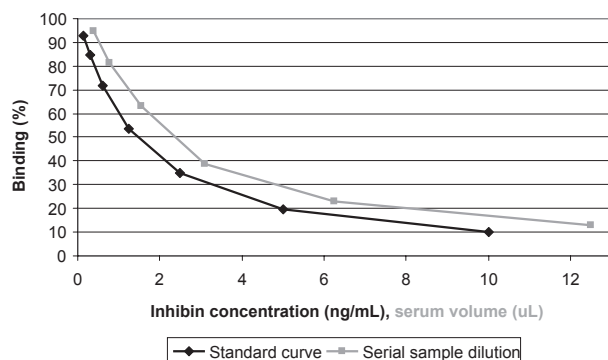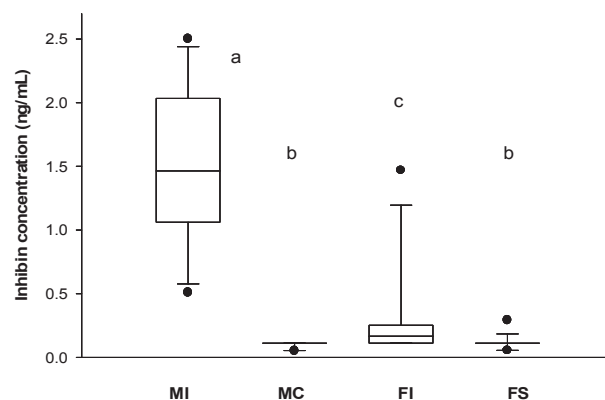
Fig 1. Standard curve of the radioimmunoassay used for the measurement of serum inhibin concentrations in all dogs (black line). Parallelism with the standard curve was confirmed by serial dilution of canine serum containing high inhibin concentration with zero standard (gray line).

Fig 2. Box plots of serum inhibin concentration in 41 healthy MI, MC, FI, and FS dogs at baseline. Boxes, generated in SigmaPlot, represent the interquartile ranges from the 25th to the 75th percentiles. Horizontal lines within the boxes indicate median values. Whiskers represent the 10th and 90th percentiles. Observations outside these percentiles are represented by dots, but all observations were included in the statistical analysis. Different letters represent significant differences in inhibin concentrations (a versus b, $P < .001$; a versus c, $P < .001$; b versus c, $P < .050$).

dogs with adrenal gland disease, serum inhibin concentration at baseline was significantly higher in dogs with adrenocortical tumors than in both dogs with PDH ($P < .001$) and with PHEO ($P < .001$) and significantly higher in dogs with PDH than in dogs with PHEO ($P = .016$, Fig 3A). Among dogs with adrenocortical tumors, serum inhibin concentration at baseline was similar in dogs with ATH (median, 0.82 ng/mL; range, 0.11–14.16 ng/mL) and with NCS adrenocortical tumors (median, 0.76 ng/mL; range, 0.05–20.0 ng/mL; $P = .96$; post-ACTH, $P = 0.96$; posttreatment – baseline, $P = .34$; post-ACTH, $P = 0.56$). Inhibin concentrations in 2 dogs with cortical adenomas (0.28 ng/mL, ATH; 0.92 ng/mL, NCS) were within the range of inhibin concentrations in dogs with cortical carcinomas (median, 0.82 ng/mL; range, 0.05–20.0 ng/mL).

Serum inhibin concentrations at baseline were undetectable in 13/13 dogs with PHEO. Inhibin was detected in the serum of 16/18 dogs with adrenocortical tumors

and in 10/17 dogs with PDH. Based on these results, the sensitivity, specificity, and accuracy of an undetectable serum inhibin concentration for identifying an adrenal gland tumor as a PHEO are 100% (95% confidence interval, 75.3–100.0%), 88.9% (95% confidence interval, 65.3–98.6%), and 93.6% (95% confidence interval, 78.6–99.2%), respectively. Serum inhibin concentrations in dogs with PDH may or may not be detectable and, therefore, did not distinguish dogs with PDH from either dogs with adrenocortical tumors or PHEO.

Post-ACTH serum inhibin concentrations were similar to baseline concentrations in healthy dogs (FS, $P = .250$; MC, $P = .250$) and in all groups of diseased dogs before and after treatment (adrenocortical tumors, $P = .860$ and $P = .630$; PDH, $P = .700$ and $P = 1.00$; PHEO, $P = 1.00$ and $P = 1.00$ before and after treatment, respectively; data not shown). Serum inhibin concentration in dogs with adrenocortical tumors decreased after adrenalectomy (pre-ACTH, median, 0.11 ng/mL; range, 0.05–1.92 ng/mL, $P = .032$; post-ACTH, $P < .001$), and significant differences in inhibin concentrations between dogs with adrenocortical tumors and dogs with PHEO (pre-ACTH, median, 0.11 ng/mL; range, 0.05–0.12 ng/mL, $P = .340$, Fig 3B; post-ACTH, $P = .910$) were no longer present. There was no significant association between serum inhibin concentration and tumor weight (adrenocortical tumors; $r = 0.39$, $P = .13$; PHEO; $r = -0.65$, $P = .190$), between serum cortisol concentration and tumor weight (adrenocortical tumors; $r = -0.17$, $P = .530$; PHEO; $r = 0.31$, $P = .570$), or between serum inhibin concentration and cortisol concentration at baseline (adrenocortical tumors; $r = -0.32$, $P = .19$; PHEO; $r = 0.13$, $P = .67$; PDH; $r = -0.02$, $P = .950$).

Immunohistochemistry results of adrenal tumor sections demonstrated positive immunostaining in adrenocortical tumors with high serum inhibin concentrations and minimal immunostaining in adrenocortical tumors with lower serum inhibin concentrations. Immunostaining was not detectable in 4 of 5 PHEOs with undetectable serum inhibin concentrations; with faint positive immunostaining was observed in the remaining PHEO (Fig 4). The section of healthy adrenal gland with corresponding low serum inhibin concentration also lacked positive immunostaining. All positive controls (testes, ovary) and negative controls showed positive and negative immunostaining, respectively.

Fig 3. Box plots of serum inhibin concentration in 44 neutered dogs with adrenal gland disease and in 21 neutered control dogs at baseline before treatment (A; 4 cortical carcinomas with extreme values ranging from 4.1–20.0 ng/mL data not shown) and after treatment (B, 30 dogs). Different letters indicate significant differences in inhibin concentrations (3A; a versus b, $P < .001$; a versus c, $P < .001$; b versus c, $P < .050$; 3B; a versus b, $P < .050$). Inhibin concentrations before treatment of PDH (A) were similar in dogs subsequently treated with mitotane or trilostane (pre-ACTH, $P = .200$; post-ACTH, $P = .11$). Tm – tumor (ATH and noncortisol secreting [NCS]); T – trilostane; M – mitotane. See Figure 1 for remainder of key.

Discussion

Results of this study indicate that serum inhibin concentrations are higher in neutered dogs with hyperadrenocorticism (ATH, PDH) and NCS adrenocortical tumors than in those with PHEO and healthy dogs. In neutered dogs with adrenocortical tumors, median serum inhibin concentration at baseline was >7.5 times higher than in neutered controls, suggesting that adrenocortical tumors synthesize and secrete inhibin into the circulation. The relatively small number of dogs with NCS adrenocortical tumors is a limitation of this study. In contrast to dogs with adrenocortical tumors or PDH, serum inhibin concentration in
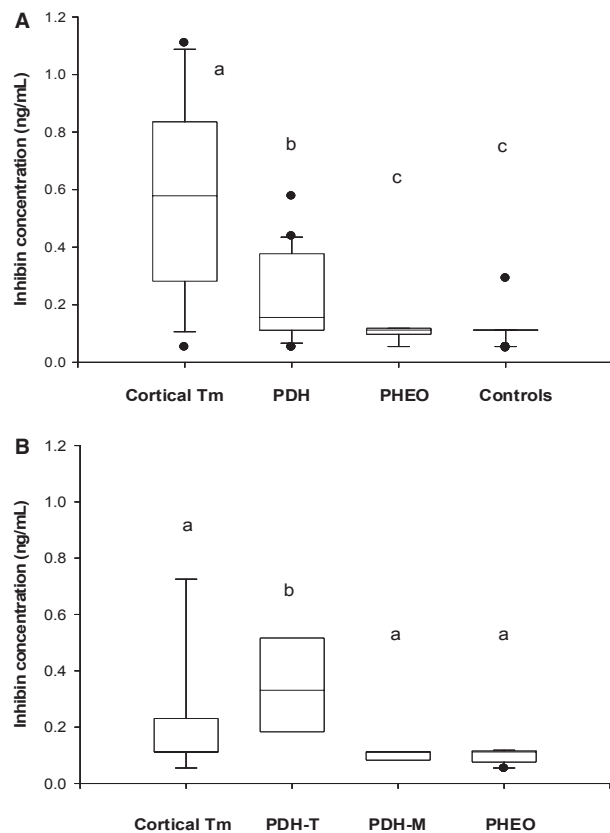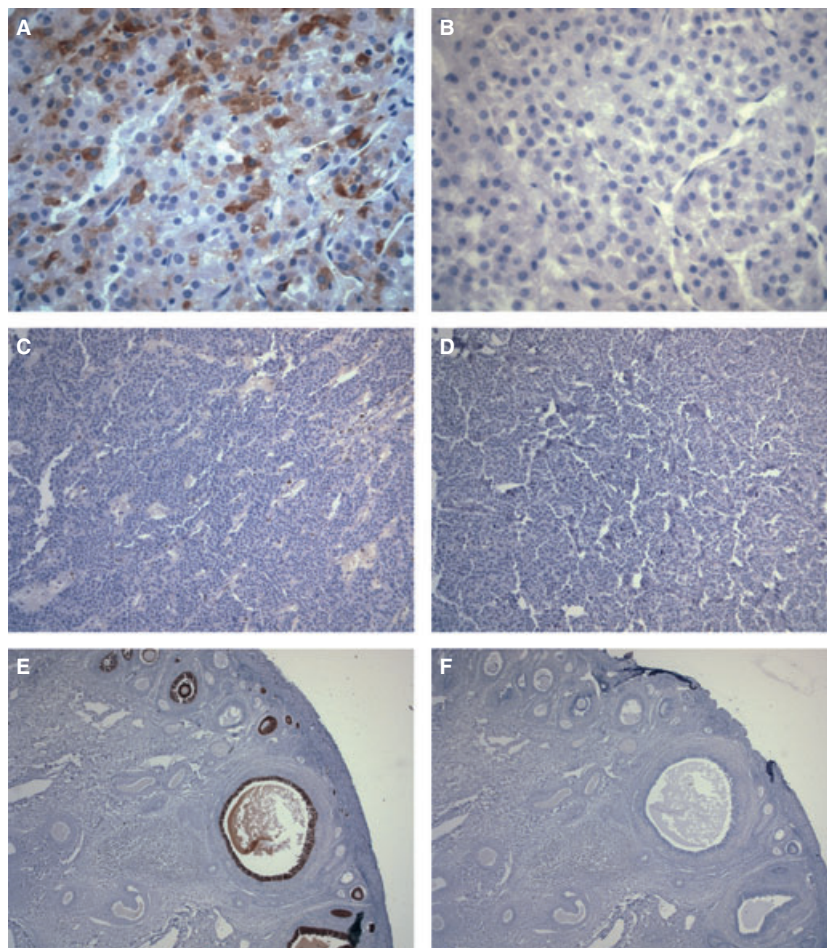
Fig 4. Immunohistochemical labeling using an anti-inhibin alpha antibody. Strong, diffuse immunoreactivity is observed in the adreno-cortical carcinoma (A), without immunoreactivity in the adrenocortical carcinoma negative control (B). Immunostaining was not detectable in the PHEO (C) or the corresponding PHEO negative control (D). Intense immunoreactivity is present in ovarian follicular walls (eg, granulosa cells, E), without immunoreactivity in the negative control of the ovary (F).

neutered dogs with PHEO was undetectable and did not differ from inhibin concentration in serum of neutered healthy dogs. Results indicate that the inhibin concentration in serum may be useful for differentiating adrenocortical tumors from PHEOs. If a neutered dog with an adrenal gland tumor has a detectable inhibin concentration in serum, then the tumor is likely adrenocortical based on the results of this study. An undetectable serum inhibin concentration in a dog with an adrenal gland tumor suggests that the tumor is a PHEO. Determination of serum inhibin concentration requires a single blood sample, no special sample handling, and no injections for additional pituitary-adrenal function testing.

Surgical removal of adrenocortical tumors was associated with a decrease in serum inhibin concentration, eliminating the difference in inhibin concentration in these dogs compared with neutered controls. The findings from dogs undergoing adrenalectomy for adrenocortical tumors suggest that serum inhibin measured before treatment in these neutered dogs was of adrenal origin. Serum inhibin concentration has not been reported in dogs with adrenal gland disease, but has been

evaluated in humans. Cortisol-producing adenomas secreted inhibin at a higher rate than aldosterone-producing adenomas, clinically nonfunctioning adenomas, and normal adrenal glands.[7] Similarly, tissue inhibin content and secretion rate of inhibin from cultured cells were the highest in people with Cushing's syndrome caused by an adrenal adenoma.[7] Serum inhibin concentration in this study was lower in dogs with PDH treated with mitotane than in those treated with trilostane. Mitotane is cytotoxic to adrenocortical cells, more so for cells within the zona fasciculata and zona reticularis, whereas trilostane inhibits the steroidogenic enzyme 3β-hydroxysteroid dehydrogenase and only rarely is associated with other effects on the adrenal gland in dogs (eg, necrosis). Cytotoxicity to adrenocortical cells in mitotane-treated dogs and surgical removal of the tumor and the associated adrenal gland in dogs with adrenocortical tumors are likely the reasons for the lower inhibin concentrations in these dogs after treatment. However, the number of dogs with PDH treated with mitotane or trilostane was small, dogs with adrenocortical tumors that had been treated with mitotane or trilostane were not included in this study, and a comparison of different

drug treatments is beyond the objectives of this study. Although this test may be useful in dogs treated with trilostane, we do not recommend interpreting serum inhibin concentrations in dogs that have been treated with mitotane or trilostane until a larger dataset is available.

Normal human adrenal glands produce and secrete inhibin into the peripheral circulation.[7,9] Interestingly, serum inhibin concentration was undetectable in healthy neutered dogs in our study, suggesting that if the healthy canine adrenal gland secretes inhibin, it secretes amounts that are not detectable in blood by RIA. Serum or plasma concentrations of inhibin have been reported in male but not in female dogs. A single study evaluated plasma inhibin concentrations in male castrated dogs. Inhibin could not be detected by RIA, which is in agreement with our results.[14] Sexually intact healthy dogs in our study had higher serum inhibin concentrations than neutered dogs, as is expected because the gonads are the primary source of circulating inhibin. Inhibin concentrations in dogs were 10–15 times higher in spermatic venous than in peripheral blood.[6] Inhibin concentrations in male intact dogs in our study were within the range of reported inhibin concentrations in male intact dogs (approximately 0.1[19]–2.2[20] ng/mL).

One objective of this study was to evaluate if the ACTH stimulation test affects serum inhibin concentration in neutered dogs. In dogs with adrenal gland disease and in controls, serum inhibin concentration was similar at baseline and after ACTH stimulation, suggesting that the ACTH stimulation test does not affect circulating inhibin concentration. Baseline data are sufficient to evaluate serum inhibin concentration in dogs, and an ACTH stimulation test does not offer additional information. The effect of ACTH on circulating inhibin has not been reported in dogs. However, inhibin secretion from adrenocortical cells[8,19,20] and serum inhibin concentration increased in response to ACTH in humans with functioning adrenal glands,[9] suggesting that this response may differ among species.

On histologic examination, it may be difficult to distinguish adrenocortical hyperplasia, cortical adenoma, cortical carcinoma, PHEO, and metastatic carcinoma in dogs as well as in humans.[11,12,21,22] Therefore, the value of immunohistochemistry using an antibody to inhibin alpha to distinguish these disorders has been assessed in humans. Positive immunostaining with anti-inhibin alpha was identified in all or almost all cortical adenomas and carcinomas.[10,11,23,24] This finding is consistent with our findings in dogs with positive immunostaining in some, but not all adrenocortical tumors. The lack of immunostaining in 4 of 5 PHEOs in dogs of this study corresponds to the findings in humans where immunopositivity to inhibin alpha was detected in 0 to 14% of PHEOs.[10–12,21,25,26] Possible reasons for positive immunostaining in PHEOs include staining artifacts[10] or tumors with both adrenocortical and medullary differentiation,[26] although this was not observed on histologic examination in this dog. The anti-inhibin alpha antibody is occasionally used by veterinary pathologists to identify tumor tissues of adrenocortical origin. However, this has not been systematically evaluated in dogs, and such an evaluation is beyond the objectives of this study.

Based on the results of this study, determination of inhibin concentration in serum appears to be valuable for the differentiation of adrenocortical tumors from PHEOs in neutered dogs. This test is not applicable in sexually intact dogs because gonadal inhibin in serum cannot be differentiated from inhibin secreted by adrenocortical tumors. In addition, circulating inhibin concentrations are higher in dogs with testicular Leydig cell[6,27] or Sertoli cell tumors (including those in cryptorchids) than in normal dogs,[6,14,28] which should be included in the differential diagnosis of dogs with high serum inhibin concentrations.

In summary, serum inhibin concentration is higher in neutered dogs with adrenocortical tumors and PDH than in dogs with PHEO and healthy dogs. Inhibin concentration in serum appears useful for the differentiation of adrenocortical tumors from PHEOs. If inhibin is detectable in serum, the adrenal gland tumor is most likely to be cortical on the basis of the results of this study. Undetectable inhibin in serum of a dog with an adrenal gland tumor is highly supportive of a PHEO, especially if associated with the appropriate clinical signs. Therefore, serum inhibin concentration can be used as an indirect biomarker for PHEOs in neutered dogs.

Footnotes

Acknowledgments

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