Control of prolactin secretion in canine hypothyroidism
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Abstract
Hypothyroidism in bitches has been reported to cause elevated serum prolactin concentration and reproductive abnormalities. Dopamine and thyrotropin releasing hormone (TRH) are important factors in normal prolactin homeostasis. The objective of this study was to evaluate the effect of experimentally induced hypothyroidism in bitches on prolactin stimulation and suppression. Eighteen healthy multiparous bitches were used; hypothyroidism was induced (by radioiodine administration) in nine bitches and the remaining nine served as untreated controls. During anestrus, each bitch was treated with TRH (10 µg/kg intravenously), metoclopramide (0.4 mg/kg intravenously), and cabergoline (5 µg/kg subcutaneously) at one week intervals between treatments. Serum prolactin was measured using a commercially available assay. Basal prolactin concentrations were not affected by hypothyroidism. Prolactin was increased in hypothyroid as compared to control bitches after administration of metoclopramide and TRH; and a greater effect was seen after metoclopramide administration as compared to TRH. Suppression of prolactin by cabergoline administration was greater in control than in hypothyroid bitches. Prolactin secretion is increased in hypothyroid bitches during anestrus and may contribute to reproductive failure seen in hypothyroidism.

Keywords: Hypothyroidism, bitch, prolactin, dopamine, TRH

Introduction
Hypothyroidism is reported to be a common cause of reproductive abnormalities in dogs. However, the effects of hypothyroidism on reproduction in the bitch have not been well documented. Reported findings include infertility, prolonged anestrus, early embryonic death or abortion, and stillbirth. Recently, short-term hypothyroidism in bitches has been found to result in lower birth weight and increased periparturient mortality, while hypothyroidism of approximately one year duration resulted in infertility.

Prolactin is the primary luteotrophic factor in the bitch, and inhibition of prolactin secretion shortens anestrus intervals. Prolactin is also responsible for lobuloalveolar development of mammary tissue. Hyperprolactinemia is reported to occur hypothyroidism, and may be associated with inappropriate galactorrhea. The role of prolactin in reproductive failure associated with hypothyroidism is unclear, but hyperprolactinemia may be associated with ovulatory dysfunction and infertility.

Prolactin secretion is controlled primarily by dopaminergic inhibition, and also by TRH and other releasing factors. Elevated TRH in hypothyroidism may be the cause of hypothyroid-associated hyperprolactinemia, and subsequent reproductive failure. In this study, we investigated the effect of short-term hypothyroidism during anestrus in bitches on regulation of prolactin secretion by TRH and dopamine.

Materials and methods
Animals
Eighteen intact adult female mongrels, 2-3.5 years old, 8-13 kg body weight, were obtained from a commercial breeder (Covance, Cumberland, VA, USA). Dogs were housed in indoor runs at 21 °C, with a 12 hour light:dark cycle. All bitches were determined to be clinically normal, based on lack of significant abnormalities on physical examination, complete blood count, serum biochemistry, urinalysis, heartworm antigen test, and zinc sulfate fecal floatation. Serum concentrations of total thyroxine (T4), free T4 by equilibrium dialysis, and endogenous canine thyroid stimulating hormone (TSH) were within respective reference ranges. All bitches had previously produced at least two normal litters. Dogs were fed a commercial maintenance diet (Hill’s Science Diet Adult dry kibble, Topeka, KS) and offered water ad libitum. The study was approved by the Virginia Tech Animal Care and Use Committee.
Induction of hypothyroidism

Following 12-18 weeks of acclimation and data collection, hypothyroidism was induced in nine randomly selected bitches by intravenous administration of 1mCi/kg \(^{131}\)Iodine (Cardinal Health, Charlottesville, VA). Hypothyroidism was confirmed 9 weeks and 38-45 weeks after \(^{131}\)I by measurement of serum T4 concentrations <10 nmol/L before and 4 hours after administration of 50 ug human recombinant TSH (Thyrogen®, Genzyme Corp., Framingham, MA). The remaining nine untreated bitches acted as controls.

Experimental protocol

Dogs were determined to be in anestrous based on at least 90 days beyond onset date of the most recent estrus, noncornified vaginal cytology, and serum progesterone less than 1 ng/ml (Target®, Biometals, Inc., Princeton, NJ). Testing began at a mean of 39 weeks (range 33-45) from induction of hypothyroidism. Each dog was subjected to testing with one of three substances (thyrotropin releasing hormone, metoclopramide, and cabergoline) in a randomized design with a one-week interval between treatments. Sampling occurred at 0, 10, 20, 30, 45, 60, 90 minutes after intravenous administration of TRH (10 ug/kg; Sigma Chemical, St. Louis, MO) and metoclopramide (0.4 mg/kg; Reglan®, Baxter Healthcare, Deer Park, IL), and at 0, 4, 8, 12, 24, 36, 48, 60 hours after subcutaneous administration of cabergoline (5 ug/kg; Galastop®, CEVA VETEM, Milano, Italy). Time 0 samples were obtained immediately prior to treatment. Blood samples (8 ml) were collected by jugular venipuncture and allowed to clot at room temperature for 30 minutes prior to centrifugation at 1200 x g for 20 minutes. Serum was then divided into aliquots and stored at -70 ºC until assayed.

Sample analysis

Serum prolactin concentrations were measured using a previously validated\(^{15}\) commercial homologous canine prolactin enzyme immunoassay (ALPCO Diagnostics, Windham, NH), as described by the manufacturer. Interassay coefficient of variation was 12% and intrassay coefficient of variation was 9%. Prolactin assays were performed at the Small Animal Reproduction Clinic Endocrinology Laboratory, University of Florida College of Veterinary Medicine.

Statistical analysis

Area under the curve (AUC) of prolactin concentration during each test was calculated using the trapezoidal rule. Results of baseline concentrations were excluded from calculation of AUC in the TRH and metoclopramide response tests. Values of prolactin >160 ng/ml were assigned a value of 160 ng/ml and those <0.3 ng/ml were assigned a value of 0.3 ng/ml for purposes of analysis. Testing for Gaussian distribution was accomplished using the Shapiro-Wilk test. Comparisons between groups were accomplished by ANOVA using a general linear model (SAS Enterprise, SAS Institute Inc., Cary, NC). The Wilcoxon sign-rank test was used for data that was not normally distributed. Serum prolactin concentrations were compared on basal samples, and the difference between basal and subsequent prolactin concentrations after administration of test substances. Comparisons of absolute and proportional change in prolactin from time 0 in response to stimulation and suppression were made between the control and hypothyroid groups. The proportional change between hypothyroid and control groups for AUC of prolactin in response to TRH and metoclopramide was compared using ANOVA. The prolactin response to TRH and metoclopramide were also compared. Level of significance was set at P<0.05. Values are expressed as mean +/- SD unless otherwise stated.

Results

At the time of testing, all hypothyroid dogs showed clinical signs of hypothyroidism including weight gain, thin hair coat or alopecia, and lethargy. Galactorrhea was not noted in any dog. Serum T4 concentrations before and after TSH administration were <5 nmol/L in all hypothyroid dogs at both 9 and 38-45 weeks after \(^{131}\)I administration. In control dogs, all post-TSH serum T4 concentrations were >35 nmol/L. The mean +/- SD serum T4 concentrations before and after TSH in control dogs was 25 +/- 11 nmol/L and 59 +/- 14 nmol/L at 9 weeks, and 25 +/- 7 and 59 +/- 17 at 38-45 weeks, respectively. Emesis occurred in 9 of 18 dogs within 4 hours following cabergoline administration. No significant side effects were seen with administration of TRH or metoclopramide. With the exception of differences between AUC of TRH and metoclopramide tests, data were found to be normally distributed. Prolactin concentration in response to TRH was log-transformed prior to analysis. There was no difference in baseline prolactin concentration between the hypothyroid and control groups. The mean serum prolactin concentration increased above baseline after TRH and metoclopramide administration in both hypothyroid (P=0.0001, P<0.0001, respectively) and control (P=0.02, P=0.02, respectively) groups (Fig. 1). The mean serum prolactin concentration 90 minutes after TRH administration was higher in hypothyroid than control dogs (P=0.02).
Following metoclopramide administration, the mean serum prolactin concentration was higher in hypothyroid than control dogs at all sample times (P<0.001), and remained significantly above the baseline concentration throughout the 90 minute sampling period (Fig. 2). Serum prolactin concentrations were decreased from basal concentrations in both control and hypothyroid groups at 4 to 36 (both P<0.05) hours after cabergoline administration (Fig. 3). The proportional change in prolactin concentrations from baseline was greater in control as compared to hypothyroid dogs at 24 (P=0.029), 36 (P=0.0087), and 60 (P=0.021) hours after cabergoline administration. No significant difference was found between hypothyroid and control dogs in AUC analysis of prolactin concentrations after TRH administration (P=0.09, Table). AUC was different between hypothyroid and control dogs after administration of metoclopramide (P<0.0001) and cabergoline (P<0.0001). When the relative difference of the AUC of prolactin in hypothyroid and control dogs was compared for metoclopramide (6.7 +/- 2.6 times) and TRH (2.1 +/- 2.0 times), the difference was significant (P<0.005).

Discussion

Results of the present study show that prolactin secretion is increased in female hypothyroid dogs during anestrus. The increased prolactin secretion in response to TRH administration is similar to that previously found in dogs with experimental and spontaneous hypothyroidism.9,10,15 Hypothyroidism is associated with decreased negative feedback of thyroid hormones to the hypothalamus, resulting in an increase in TRH secretion into the hypothalamo-hypophyseal portal system. Prolactin secretion is increased at least in part as a result of the stimulatory effect of TRH on pituitary lactotrophs. Diaz-Espineira, et al. reported that the increase in prolactin secretion in response to TRH peaked six months after induction of hypothyroidism before declining thereafter. Because the present study evaluated prolactin response to TRH at a single time and used intact bitches, while dogs in the longer duration study were ovariectomized, it is not possible to make direct comparisons of results.

Basal prolactin concentration was not affected by hypothyroidism in the present study, while previous studies in dogs have reported increased, unchanged, or decreased basal prolactin. The differences between the studies may be due to the influence of the duration of hypothyroidism, gender, and stage of estrus.9,10,15 In a previous study of experimental hypothyroidism, basal prolactin was unchanged for the first six months of hypothyroidism, then decreased significantly thereafter for the next 2.5 years.9 Because the present study was carried out nine months after induction of hypothyroidism and comprised one time point, a similar trend cannot be determined. Patients with spontaneous hypothyroidism will likely not be examined until they are exhibiting classic signs of disease, as long as one to two years after actual onset of clinical signs of hypothyroidism in one study.15 Therefore, the effect of more prolonged hypothyroidism on prolactin secretion may differ from the results reported here. Serum prolactin concentration varies depending on the stage of estrus in normal bitches and has been suggested to affect prolactin levels and responsiveness in previous studies of hypothyroid dogs.15,16 The present study attempted to control for this effect by testing bitches only during anestrus, and further investigation will be necessary to determine the effects of hypothyroidism on prolactin secretion in other stages of estrus.

In addition to basal prolactin and response to TRH, the present study evaluated the influence of dopamine as a regulator of prolactin secretion. Prolactin secretion in mammals is primarily controlled by the inhibitory effects of dopamine, through tuberoinfundibular dopamine (TIDA) neurons, on the otherwise unrestrained secretion by lactotrophs in the anterior pituitary gland. Using metoclopramide and cabergoline as dopamine-2 receptor antagonist and agonist, respectively, prolactin secretion was altered. Control of prolactin secretion in hypothyroid dogs appears particularly sensitive to dopaminergic control. Hypothyroidism induced an increase in prolactin secretion in response to metoclopramide that was 3.2 times greater than that caused by TRH when results were normalized by that of control dogs. This may indicate increased sensitivity to dopamine or merely hyperplasia of lactotrophs resulting in increased secretory capacity that is revealed when the restraint of dopamine on secretion is removed. The role of altered TIDA neuron activity as a cause of increased prolactin secretion in hypothyroidism is unclear, but it was recently shown to not be altered in hypothyroid rats.17

In addition to the control of prolactin secretion by TRH and dopamine, other factors have been demonstrated to stimulate its release. Vasoactive intestinal peptide (VIP) increases prolactin secretion by lactotrophs at concentrations present in the anterior pituitary.18 Hypothyroidism causes an increase in VIP in the anterior pituitary gland of hypothyroid rats with increased prolactin secretion in the absence of altered TIDA activity.17,19 The significance of other prolactin-releasing substances, including serotonin, vasoactive intestinal peptide, endothelin, prolactin releasing peptide, oxytocin, and endogenous opioids, is unknown in the pathogenesis of increased prolactin secretion in hypothyroidism.

Pulsatile release results in great variability in basal serum prolactin levels. Levels may also vary within a group of dogs and between laboratories.20 Handling stress is also shown to transiently affect prolactin release in some studies. This study accounted for these influences on serum prolactin by using AUC and proportional change
for comparison as well as comparisons with a control group treated in an identical manner. In order to reduce stress, all dogs in this study had been extensively acclimated to handling and sampling procedures. Similar testing with saline administration (unpublished data) showed no significant change from baseline due to pulsatile release or handling stress in this group of dogs.

Galactorrhea was not seen in any bitches in this study. The inconsistent signs related to hyperprolactinemia in hypothyroid bitches and the occurrence of hyperprolactinemia in only a portion of untreated hypothyroid women is evidence for a dominant effect of dopamine over TRH for control of prolactin secretion.\(^{11,12,21}\) Failure to identify elevated basal prolactin concentrations in hypothyroid dogs in the present study may account for the lack of galactorrhea. Based on current understanding of the functions of prolactin, enhanced prolactin secretion in hypothyroid bitches has the potential to cause inappropriate galactorrhea, prolonged anestrus, and early embryonic death, abortion, or stillbirth. While early studies (19 weeks after induction of hypothyroidism) in the dogs used in the present study documented only lower birth weight, and increased periparturient mortality, more prolonged hypothyroidism (one year duration) resulted in infertility in addition to the abnormalities noted earlier.\(^{4,5}\)

In conclusion, basal prolactin concentrations were unchanged in hypothyroid dogs, while the response to TRH and sensitivity to dopaminergic control were increased. The role that altered prolactin secretion has on reproduction is unknown, but may include infertility and decreased puppy viability. Further study is necessary to fully characterize the effects of hypothyroidism on prolactin regulation and to understand the possible clinical implications.

Acknowledgement

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References

Figure 1. Serum prolactin response to TRH.
Mean +/- SD serum prolactin concentration in 9 euthyroid control (dashed line) and 9 hypothyroid (solid line) bitches in response to intravenous administration of TRH (10 µg/kg). Asterisk indicates a significant difference (P=0.02) between groups.

Figure 2. Serum prolactin response to metoclopramide.
Mean +/- SD serum prolactin concentration in 9 euthyroid control (dashed line) and 9 hypothyroid (solid line) bitches in response to intravenous administration of metoclopramide (0.4mg/kg). Asterisks indicate a significant difference (P<0.0001) between groups.
Figure 3. Serum prolactin response to cabergoline.
Mean +/- SD serum prolactin concentration in 9 euthyroid control (dashed line) and 9 hypothyroid (solid line) bitches in response to subcutaneous administration of cabergoline (5 µg/kg). Asterisks indicate a significant difference (P<0.05) between groups.

Table 1. Area under the curve (AUC) results for prolactin response tests.
Mean +/- SD prolactin concentration (ng/ml). Results within a row with different superscripts are significantly different (P<0.05).

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<tr>
<th></th>
<th>Hypothyroid</th>
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<tr>
<td>TRH</td>
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<td>1293 ± 517ᵃ</td>
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<td>1783 ± 923ᵇ</td>
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<tr>
<td>Cabergoline</td>
<td>210 ± 83ᵇ</td>
<td>74 ± 52ᵇ</td>
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