Luteinizing hormone receptor is immunoexpressed within the canine thyroid

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Abstract

Gonadectomy has been implicated as a risk factor for development of canine hypothyroidism, due to loss of negative feedback to the anterior pituitary, resulting in persistently elevated luteinizing hormone concentrations. Receptors for luteinizing hormone have been identified in various canine gonadal and extragonadal tissues, including bladder and skin. The aim was to investigate if luteinizing hormone receptors were also expressed in the canine thyroid. Formalin-fixed, paraffin-embedded thyroid tissues from eight dogs of various sexes and breeds were subjected to routine immunohistochemical methods. Expression of luteinizing hormone receptors was evident in thyrocytes from all thyroid tissue sections examined; the percentage of positive luteinizing hormone receptors cellular expression was 46.5 ± 23.8% (mean ± standard deviation). Immunoexpression of luteinizing hormone receptors was localized to thyroid follicular epithelial cells, with no immunoreactivity in other thyroid constitutive structures or in any negative control tissue sections. This is apparently the first report to demonstrate luteinizing hormone receptors expression in the canine thyroid gland. Functional studies are needed to determine whether unregulated hypersecretion of luteinizing hormone after gonadectomy affects thyroid function.

Keywords: Dog, hypothyroidism, immunohistochemistry, luteinizing hormone receptor

Introduction

Hypothyroidism is the most common endocrine disorder of dogs, affecting 0.2 - 0.8% of dogs.[1-3] The most common presenting dermatological and metabolic symptoms are alopecia, dry/poor quality coat, seborrhea, lethargy, exercise intolerance, weight gain, skin hyperpigmentation, pyoderma and cold intolerance. These symptoms occur in combination more than 60% of the time.[1,3,4] Hypothyroidism is more common with advancing age;[5] the mean age at diagnosis is 7.2 years (range, 0.5 - 15 years).[3] The risk of developing hypothyroidism is higher in certain breeds, including Doberman Pinschers, Miniature Schnauzers, Golden Retrievers, Cocker Spaniels, Shetland Sheepdogs, Irish Setters and Dachshunds.[3,4,6,7] In these high-risk breeds, young dogs may also develop hypothyroidism.[7] One gene has been implicated in hypothyroidism and lymphocytic thyroiditis in several dog breeds, including the Doberman Pinscher, Rhodesian Ridgeback, English Setter and Giant Schnauzer. This gene is the major histocompatibility complex dog leukocyte antigen haplotype or allele associations.[8,9] This is in contrast to humans, in which there are several genetic risk factors for development of autoimmune thyroid disease,[1] including a polygenic predisposition or autoimmune hypothyroidism resulting from one of seven genes (AIRE, FOXP3, IL2RA, ITCH, LRBA, STAT1, and STAT3).[10,11]

Gonadectomized dogs have a higher risk for developing hypothyroidism, with an overall incidence of 30%, significantly higher than the general dog population.[7] It is noteworthy that hypothyroidism also affects 10 - 15% of postmenopausal women,[12] an endocrine state with many hormonal similarities to gonadectomized dogs. Following gonadectomy or menopause, circulating luteinizing hormone (LH) concentrations increase significantly and remain persistently elevated, due to a lack of negative hormonal feedback. Previous research has demonstrated the presence of LH receptors (LHR) within normal and adenomatous human thyroid glands.[13] However, effects of LHR activation in the human thyroid has not been well described, but may involve intracellular signaling similar to thyroid stimulating hormone (TSH) receptor activation (e.g. adenylate cyclase). To our knowledge, the presence of LHR in the canine thyroid gland has not been reported. We hypothesized that LH receptors are present in the canine thyroid and that LHR localizes to cells with TSH receptors.

Material and methods

Formalin-fixed, paraffin-embedded archived canine thyroid tissues from 8 dogs were provided by the Oregon State University Veterinary Diagnostic Laboratory and used for this investigation (Table). In addition, formalin-fixed paraffin-embedded archived skin tissue from another dog was used as a positive control, as canine skin expresses abundant LHR.[14] Serial 6-µm sections were cut from paraffin blocks to determine LHR and TSH receptor expression on adjacent tissue sections. Sections were mounted on poly-l-lysine-coated slides, deparaffinized in xylene, rehydrated in a graded ethanol series (100, 75, and 50%, respectively) and subjected to heat-induced epitope retrieval (#S1700, Dako, Carpinteria, CA). For this, slides were placed in boiling sodium citrate in a Nordicware® tender cooker and boiling continued in a microwave for a total of 10 minutes. Thereafter, slides were left in solution and allowed to cool to room temperature for ~ 20 minutes. Tissue-specific endogenous peroxidase activity was inhibited by incubating slides in 3% hydrogen peroxide and nonspecific binding blocked with 1% horse serum. Goat polyclonal anti-human LH receptor (SC-26341, Santa Cruz Biotechnology, Dallas, TX) or mouse monoclonal IgG1 TSH receptor (SC-53542, Santa Cruz Biotechnology) were applied at dilutions of 1:50 and 1:100, respectively. Primary antibodies were incubated for 1 hour at room temperature. Negative controls from each tissue were similarly treated, but without primary antibody. Slides were then reacted for 30 minutes with biotinylated horse anti-goat IgG (SK-5300 Vector Laboratories, Burlingame, CA) and incubated for 30 minutes with avidin-biotin-peroxidase complex (PK6105, ABC kit, Vector Laboratories) followed by a brief incubation with Nova Red Peroxidase substrate (SK4800, Vector Laboratories,). Slides were counter-stained with hematoxylin, dehydrated and mounted. Cells positively expressing LH and TSH receptors were those with red staining in the cytoplasm. Cells were counted from five randomly selected fields per antibody per dog with a Leica DM4000B microscope using bright field microscopy at 400X magnification. The number of cells evaluated per dog in these fields was 281.3 ± 142.3 (mean ± SD). Representative images from each dog were digitally captured using a QImaging camera (QICAM 12-bit, #QIC-F-M-12-C, QImaging, Surrey, BC) and QCapturePro image capturing software (QImaging). Data were reported as mean ± SD.

Results

Immunostaining for LHR was strong in epidermis and hair bulbs (Figure 1). However, there was no TSH receptor expressed in skin (data not shown). Expression of LHR was evident in all thyroid tissue sections, with a mean ± SD percentage of positive cellular expression of 46.5 ± 23.8%. The pattern of LH receptor immunoexpression was localized to thyroid follicular epithelial cells, with no signs of immunoreactivity in other thyroid constitutive structures (Figure 2). Some cells had cap-like immunostaining. It was noteworthy that LHR immunostaining in the canine thyroid could not be differentiated from that of TSH receptor (Figure 3), whose immunoexpression has been previously reported in cows and rats.[15] Thyroid stimulating hormone receptor expression was 56.0 ± 13.3% (mean ± SD). There was no positive staining evident in any negative control tissue section (Figures 1-3).

Discussion

Immunoexpression of LHR has been described in many canine tissues including the skin,[14,16] lower urinary tract (bladder and urethra),[17,18] and adrenal cortex,[19] lymph node,[20] and musculoskeletal tissues (ligaments, synovia, subchondral bone).[21] However, this was apparently the first report that the canine thyroid gland expressed LHR. Using a cut-off of $\geq$ 25% of cells staining positive for LHR, 16.7% of normal human thyroids were positive for LHR.[13] If a cut-off of $\geq$ 25% of cells staining positive were applied for dogs, 6 out of 8 (75%) thyroids examined in the current study would be positive for LHR.

Molecular mechanisms underlying regulation of LHR expression in gonadal and non-gonadal tissues have been investigated. In chickens, LH increases ovarian LHR gene expression, whereas activin A prevents LH-induced increases in LHR gene expression.[22] Expression of LHR is inhibited post-transcriptionally in rat luteal cells by mevalonate kinase.[23] Estrogen reduced ovine LHR gene expression in several extra-gonadal tissues, including medulla oblongata, hypothalamus, ruminant stomach (except reticulum), intestinal tissues (except colon), pancreas, liver, kidney, and uterus. In contrast, progesterone increased LHR gene expression in various tissues, including the hypophysis, olfactory bulb, rumen, small intestine, kidney, oviduct, and uterus.[24]

Regulation of LHR expression in the thyroid gland needs additional investigation. In prepubertal rats with PTU (6-N-propyl-2-thiouracil) induced hypothyroidism, thyroid LH receptor gene expression was not significantly different from controls.[25] In humans, there was increased thyroid LHR expression in women with high serum LH concentrations.[13] However, thyroid glands from these patients were adenomatous and it is not known if the women were hypothyroid at sample collection. In the current study, serum tetraiodothyronine (T_4) and LH concentrations in these dogs were unknown, due to use of archived tissues.

In women who have undergone gonadectomy, LH concentrations are three times higher after ovary removal, whereas both triiodothyronine and T_4 concentrations are significantly reduced after ovary removal.[26] This observation provides evidence that a causal relationship could exist in dogs as well. That gonadectomized dogs have significantly elevated circulating LH concentrations and are at greater risk for developing hypothyroidism[7] is an impetus to determine effect of reducing LH concentrations on thyroid hormone secretion.

The current study provided evidence that LH receptors may have a role in thyroid hormone secretion. However, more research is needed to determine whether LHR expression is increased in gonadectomized dogs.

Acknowledgement

The authors thank Kay Fischer and the Oregon State University Histology Laboratory for technical assistance.

Authors' contributions

Zwida contributed to the design of the study, performed all of the immunohistochemistry experiments, and image acquisitions. Kutzler conceived and contributed to the design of the study and oversaw immunohistochemistry and image acquisition. Both authors drafted the manuscript, critically revised it, gave final approval and agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors thank the Ministry of Scientific Research and Higher Education in Libya for graduate school financial assistance and Dr. Howard Meyer of Chippewa Kennels for funding research supplies.

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Table. Signalment of the subjects used to determine LH receptor immunoexpression in canine thyroid tissue. The expression of thyrocytes varied considerably among individuals.

Breed	Reproductive Status	Age	Thyroid cells expressing LH receptors
Labrador Retriever	Spayed Female	2 months	5%
American Pit Bull Terrier	Intact Male	5 months	51%
Labrador Retriever	Spayed Female	6 months	19%
Maltese	Spayed Female	2 years	62%
Rottweiler	Intact Male	6 years	66%
Irish Setter	Castrated Male	10 years	38%
Cairn Terrier	Spayed Female	12 years	60%

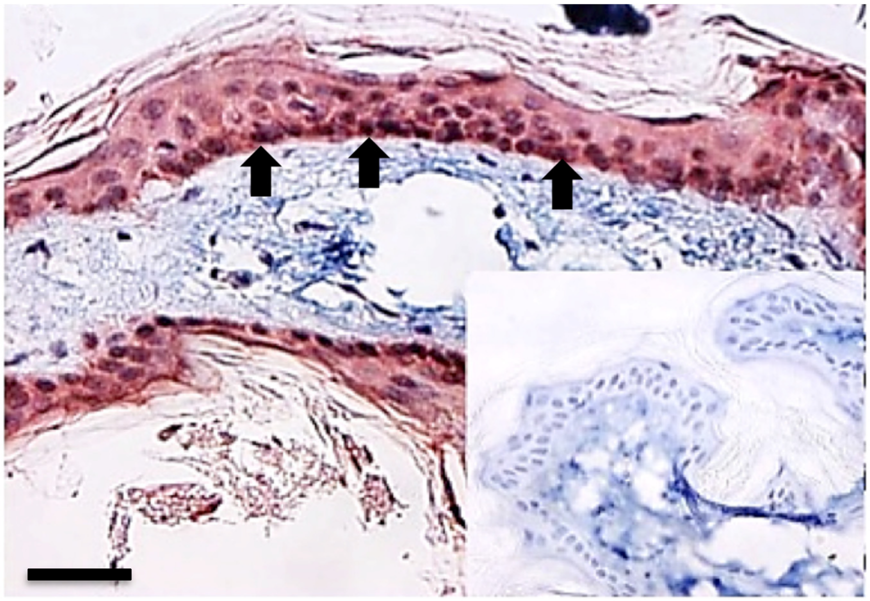

Figure 1. Representative image of immunohistochemical localization; arrows indicate immunoexpression for LH receptor in the canine epidermis (scale bar = 10 μm). Inset image is negative control for LH receptor immunostaining.

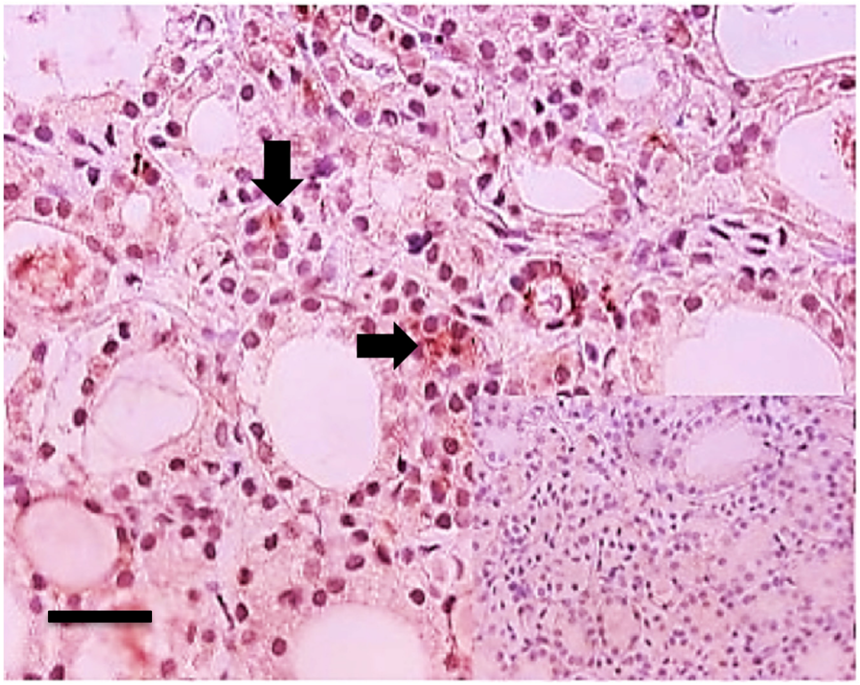

Figure 2. Representative image of immunohistochemical localization; arrows indicate a low level of immunoexpression for LH receptor in the canine thyroid (scale bar = 10 μm). Inset image is negative control for LH receptor immunostaining.

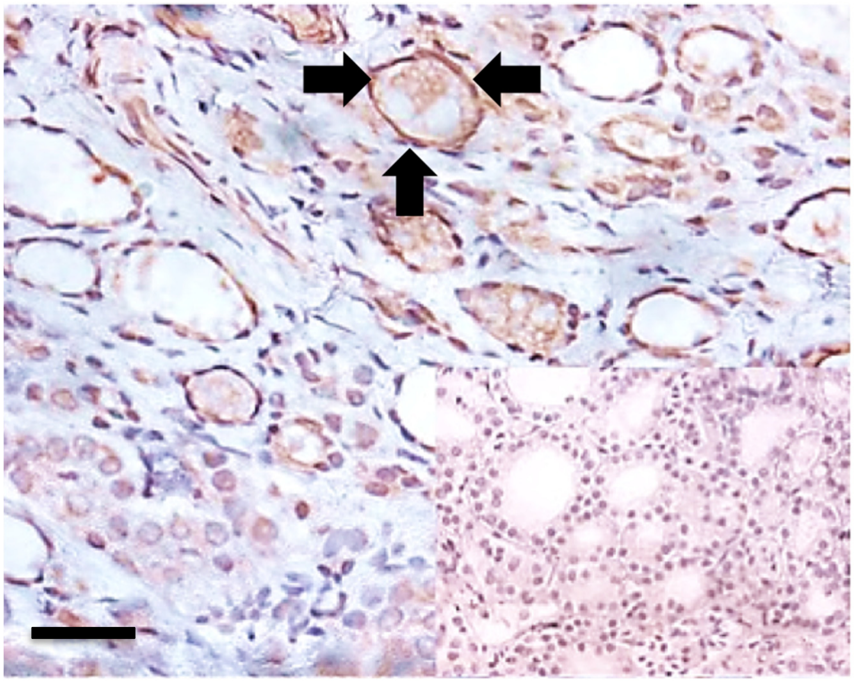

Figure 3. Representative image of immunohistochemical localization; arrows indicate a high level of immunoexpression for LH receptor in the canine thyroid (scale bar = 10 µm). Inset image is negative control for LH receptor immunostaining.

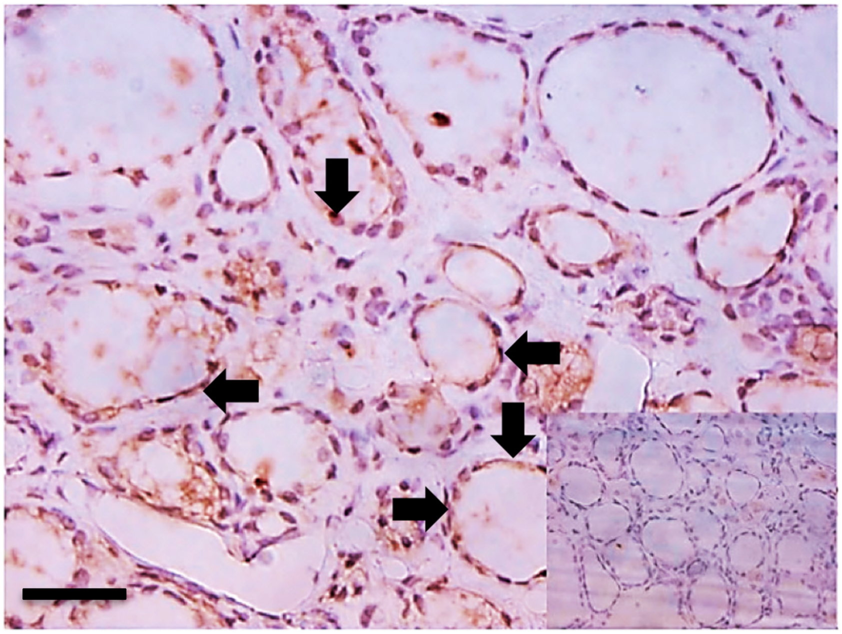

Figure 4. Representative image of immunohistochemical localization; arrows indicate a low level of immunoexpression for TSH receptor in the canine thyroid (scale bar = 10 µm). Inset image is negative control for TSH receptor immunostaining.