Use of recombinant human thyroid-stimulating hormone for thyrotropin stimulation testing in euthyroid ferrets

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Objective—To evaluate the effects of IM administration of recombinant human thyroid-stimulating hormone (rhTSH) on plasma total thyroxine (T\textsubscript{4}) concentrations in euthyroid ferrets.

Design—Evaluation study.

Animals—25 healthy neutered ferrets (14 female and 11 male) of various ages from 2 populations (laboratory ferrets from Georgia and pet ferrets from Pennsylvania).

Procedures—Each ferret underwent a physical examination and standard hematologic testing to ensure it was healthy and had clinically normal thyroid function. Once determined to be euthyroid, ferrets received a single IM injection of 100 µg of rhTSH. Blood samples were collected into plasma-separator tubes immediately before the rhTSH injection (time 0) and 4 hours after injection to measure T\textsubscript{4} concentrations.

Results—Males did not differ from females in regard to prestimulation or poststimulation plasma T\textsubscript{4} concentrations; however, prestimulation and poststimulation T\textsubscript{4} concentrations were significantly different between the 2 groups of ferrets. A significant difference was also identified between prestimulation T\textsubscript{4} concentration (mean ± SD: 213 ± 6.1 nmol/L) and poststimulation T\textsubscript{4} concentration (29.9 ± 8.2 nmol/L). All 25 ferrets had high poststimulation T\textsubscript{4} concentrations (median difference, 7.5 nmol/L; 10% to 90% interval, 3.26 to 17.70 nmol/L [0.25 to 1.38 µg/dL]; range, 2.50 to 20.70 nmol/L [0.19 to 1.61 µg/dL]). This represented a median increase in T\textsubscript{4} concentration of 35% (10% to 90% interval, 18% to 81%; range, 8% to 126%).

Conclusions and Clinical Relevance—Results suggested that rhTSH can be used for thyrotropin stimulation testing in ferrets when administered IM. According to the findings, a euthyroid ferret should have an increase of approximately 30% in plasma T\textsubscript{4} concentration 4 hours after rhTSH injection. (J Am Vet Med Assoc 2013;243:1432–1435)

Analysis of serum or plasma total T\textsubscript{4} concentration is considered a first-line test when an animal is suspected of having hypothyroidism or hyperthyroidism.1–3 Although these endocrinopathies are not common in ferrets (Mustela putorius furo), the authors have encountered ferrets in which thyroid disease was highly suspected. Case reports1–4 of ferrets with disease of the thyroid gland have been published in the peer-reviewed literature; however, the antemortem diagnosis of thyroid disease in ferrets is challenging because of limited diagnostic options.

The radioimmunoassay method is often used for measurement of plasma total T\textsubscript{4} concentration in companion animals.7 A benefit of the radioimmunoassay is that it can be performed with a small volume (25 µL) of plasma, which is particularly useful for small exotic animal species in which blood volume is limited. Although the diagnostic approach for domestic small animals in which hypothyroid disease is suspected includes measurement of plasma total T\textsubscript{4} concentration, TSH concentration, autoantibody titer, and free T\textsubscript{4} concentration by equilibrium dialysis and possibly TSH stimulation testing, many of these tests have not been evaluated for use in exotic pets.

For dogs and cats suspected to have thyroid disease, it has been customary to use bovine TSH for TSH stimulation testing; however, bovine TSH is no longer available as a pharmaceutical preparation and has been replaced with the considerably more expensive rhTSH. Because of this development, new protocols for TSH stimulation testing in companion animals have been established with the use of rhTSH instead of bovine TSH. Protocols have been described for dogs and cats,8,9 but we are not aware of a similar protocol for ferrets.

ABBREVIATIONS

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>rhTSH</td>
<td>Recombinant human thyroid-stimulating hormone</td>
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<td>T\textsubscript{4}</td>
<td>Thyroxine</td>
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<td>TSH</td>
<td>Thyroid-stimulating hormone</td>
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The purpose of the study reported here was to evaluate the effects of IM injection of rhTSH on plasma T4 concentration in euthyroid ferrets with the aim of developing an antemortem method for evaluation of thyroid gland function in ferrets. The specific hypotheses were that rhTSH could be safely and effectively used for TSH stimulation testing of ferrets, an increase in plasma T4 concentration would occur in ferrets given rhTSH, and no significant difference between the sexes or between ferret populations would be identified in plasma T4 concentrations before or after rhTSH administration.

Materials and Methods

Animals—Twenty-five healthy neutered adult ferrets were used for this study. The ferrets were obtained from 2 sources: laboratory ferrets housed at TRS Labs Inc, Athens, Georgia, (n = 14; 9 females and 5 males) and pet ferrets obtained from a Pittsburgh ferret rescue organization and housed in a room at the owner's house (11; 5 females and 6 males). The ferrets were brought to a veterinary clinic for the study and returned to the owner the same day. All of the laboratory ferrets were 12 months old at the time of the study and had been neutered between 6 and 8 weeks of age. The pet ferrets were approximately 0.5 to 3 years of age and had been originally obtained from the same supplier as the laboratory ferrets.

The pet ferrets were housed in mixed groups of 2 to 3 ferrets/group. They were fed a commercial food to meet their maintenance requirements, and a whole mouse was given twice a week. All ferrets underwent a physical examination, CBC, plasma biochemical analyses, and determination of baseline total T4 concentration to ensure that they were healthy prior to the start of the study. Owner consent was obtained for the pet ferrets to participate in the study. The study protocol was approved by the institutional animal care and use committee of TRS Labs Inc, Athens, Ga.

Protocol—With each ferret manually restrained, blood samples were collected from the cranial vena cava into plasma-separator tubes immediately before IM injection of rhTSH (time 0) and 4 hours after injection. The ferrets were monitored for 3 to 4 hours after the rhTSH injection for any adverse effects (eg, anaphylaxis). Thyroxine concentrations were measured in plasma harvested from the blood samples by use of a commercially available 125I solid-phase competitive radioimmunoassay kit.

Statistical analysis—The distribution of plasma T4 data was evaluated by means of the Shapiro-Wilk test, q-q plots, and evaluations of kurtosis and skewness. Normally distributed data are reported as mean ± SD, and nonnormally distributed data are reported as median, 10% to 90% interval, and range. The Fisher exact test was used to determine whether a significant difference existed between proportions of males and females among all ferrets. The Levene test was used to evaluate equality of variance. The Student t test was used to determine whether ferret origin (laboratory or rescue organization) or sex had an effect on prestimulation or poststimulation T4 values, and the Mann-Whitney test was used to determine whether ferret origin or sex had an effect on the difference between prestimulation and poststimulation values. A repeated-measures ANOVA was used to determine whether there was a difference between the prestimulation and poststimulation T4 concentrations, controlling for ferret origin and sex. When the homogeneity of covariance was violated, the Greenhouse-Geisser method was used to interpret the results. Statistical software was used to analyze the data. A value of P ≤ 0.05 was used to determine significance. For comparisons in which values of P were between 0.06 and 0.10, a post hoc power analysis was performed.

Results

Animals—No significant difference was evident in proportions of male and female ferrets used in the study (P = 0.69). The median age and weight of all 25 ferrets, regardless of whether they were pet or laboratory ferrets, were 1.0 years (range, 0.5 to 3.5 years) and 1.0 kg (2.2 lb; range, 0.8 to 1.8 kg [1.76 to 3.96 lb]), respectively.

rhTSH stimulation testing—Plasma T4 concentrations did not differ significantly between male and female ferrets for blood samples collected before rhTSH administration (P = 0.51) or those collected 4 hours after rhTSH administration (P = 0.91). However, a significant difference was identified between the 2 origins of ferrets (laboratory ferrets or pets) in prestimulation (P = 0.001) and poststimulation (P < 0.001) plasma T4 concentrations. The 14 laboratory ferrets had a mean ± SD prestimulation plasma T4 concentration of 18.0 ± 3.6 nmol/L (1.40 ± 0.28 µg/dL) and a mean poststimulation value of 23.2 ± 6.6 nmol/L (1.96 ± 0.51 µg/dL), whereas the 11 pet ferrets had a mean prestimulation plasma T4 concentration of 25.5 ± 6.1 nmol/L (1.98 ± 0.47 µg/dL) and a mean poststimulation value of 33.9 ± 5.7 nmol/L (2.79 ± 0.44 µg/dL).

A significant (P ≤ 0.001) difference was also identified between prestimulation (21.3 ± 6.1 nmol/L [1.66 µg/dL]) and poststimulation (29.9 ± 8.2 nmol/L [2.32 µg/dL]; range, 16.5 to 42.5 nmol/L [1.28 to 3.3 µg/dL]) plasma T4 concentrations for all ferrets combined. However, there was no significant interaction between prestimulation and poststimulation values and ferret origin (P = 0.11) or sex (P = 0.17). All 25 ferrets had an increase in plasma T4 concentration after rhTSH administration (median difference, 7.5 nmol/L [0.58 µg/dL]; 10% to 90% interval, 3.3 to 17.7 nmol/L [0.25 to 1.38 µg/dL]; range, 2.5 to 20.7 nmol/L [0.19 to 1.61 µg/dL]); this represented a median increase of 35% (10% to 90% interval, 18% to 81%; range, 8% to 126%).

Discussion

Thyrotropin, or TSH, is considered the most important regulator of thyroid gland activity. Thyroid-stimulating hormone is synthesized and secreted by thyrotropic cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid gland. The hormone acts through the initiation of cAMP formation and the phosphorylation of protein kinases.
The TSH molecule is composed of 2 subunits: the α subunit, which is not species specific, and the β subunit, which is species specific. Even though TSHs from different species have variable immunologic responses on different assays, they share the same biological activity and thus can respond to different endocrine stimuli from different species or genera of animals (eg, canine thyroid gland responding to bovine TSH). However, differences in the amino acid sequence make the β subunit potentially immunogenic when used in a nonhomologous species. This might translate into a hypersensitivity reaction with repeated administration of hormones that are not species specific. It has been shown that IM administration of highly purified rhTSH is effective in stimulating cAMP production in rat cells. Evidence has also been obtained that rhTSH binds to the thyroid TSH receptor in mice and rats.

Serum total T₄ concentrations were measured in adult female (sexually intact and neutered) ferrets, juvenile ferrets (< 1 year old) of either sex, and castrated male ferrets in a 1988 study, with results similar to those in cats: 15.44 to 48.91 nmol/L (1.20 to 3.80 µg/dL). Interestingly, sexually intact adult male ferrets had higher serum T₄ concentrations than did adult female ferrets, juvenile ferrets (< 1 year old) of either sex, and castrated male ferrets. The sexually intact adult male ferrets had serum T₄ concentrations that were more comparable with those of dogs: 19.56 to 46.33 nmol/L (1.52 to 3.60 µg/dL). In another study, the effect of IV TSH administration (1 U/ferret) on plasma T₄ concentrations was evaluated in adult male ferrets, revealing an increase in concentration as early as 2 hours after injection and concentrations that remained elevated for 6 hours after injection. Sexually intact male ferrets were used, and pre-stimulation T₄ concentrations were reported to be 32.56 ± 11.58 nmol/L (2.53 ± 0.9 µg/dL). These reported values are in agreement with those in the other study, in which sera from 44 ferrets were used, including 31 males (27 sexually intact and 4 castrated) and 13 females (10 sexually intact and 3 spayed).

In those studies and the present study, the serum or plasma T₄ concentrations were determined with the same analytic method (radioimmunoassay), which makes comparison of the values meaningful. The differences apparent in prestimulation values between our 2 groups of ferrets was an interesting observation and difficult to explain. However, because a significant difference has been reported to exist between age groups in T₄ concentrations, age might have contributed to our findings, given that the laboratory ferrets were younger than the pet ferrets. This observation gives rise to an interesting point about the importance of establishing reference values for an individual patient to facilitate accurate interpretation of an endocrine assay. The reason the 2 populations of ferrets in our study had significant differences in prestimulation plasma T₄ concentrations is unclear. We believe that care needs to be taken when diagnosing endocrine disease on the basis of reference limits established from a uniform population that may not be representative of subjects from other environmental or other husbandry settings. We recently noticed a similar variation in baseline plasma T₄ concentrations in guinea pigs in that values differed between guinea pigs used in a laboratory and those kept as pets.

The results of the present study suggested that IM administration of rhTSH has a biological effect on euthyroid ferrets. We surmise that this effect results from rhTSH binding to TSH receptors in the thyroid gland of ferrets, stimulating cAMP production. Intramuscular administration of 100 µg of rhTSH appeared adequate to induce an increase in plasma T₄ concentration suggestive of a healthy thyroid response; however, we realize that the cost of rhTSH is a limiting factor in a clinical setting.

No report exists of the biological half-life of TSH in ferrets, nor in dogs. In humans, the half-life of TSH is 1 hour. Administration of rhTSH appears to be safe in humans, with only a few minor adverse effects reported (eg, headache, fatigue, vomiting, and dizziness). In addition, rhTSH antibodies have not been reported to develop in human patients after treatment. In a study in dogs, repeated administration of rhTSH yielded no adverse effects. The dogs did not develop anaphylactic reactions or obvious resistance to rhTSH, although whether antibody production against rhTSH occurred after rhTSH administration was not evaluated. There are no equivalent data for exotic species. In the present study, only 1 rhTSH injection was given, so it was not possible to evaluate the effects of multiple injections; however, all ferrets were clinically normal following injection and remained clinically normal in the days after the injections were given.

To date, recommendations for assessment of thyroid function in domestic species have differed considerably among sources. Generally speaking, guidelines for testing usually suggest inclusion of the following: T₄ concentration screening with additional testing if clinical signs and other clinicopathologic changes are present to include determination of free T₄ concentration as measured by equilibrium dialysis, TSH concentration, and autoantibody titer and, potentially, provocative thyroid function testing such as TSH stimulation. However, despite the many diagnostic tools or options to evaluate thyroid gland function, studies have yielded inconclusive or even conflicting results regarding the usefulness of specific tests. A study in cats led to the conclusion that, given the additional expense and limited availability, measuring free T₄ concentration did not appear to add useful information to the testing protocol for our intended use; therefore, we followed the recommendations of these investigators, who recommended measuring only T₄ concentration for the rhTSH stimulation test. However, some clinicians believe that measuring serum or plasma T₄ and free T₄ concentrations via equilibrium dialysis is helpful in the diagnosis of hyperthyroidism in cats. The TSH stimulation test can be performed with bovine TSH or rhTSH in cats. Measurement of serum or plasma TSH concentration in cats has been described, but this practice has not yet been adopted by most clinicians. One of the newer methods used to diagnose hyperthyroidism in cats is the triiodothyronine suppression test, which relies on the ability of administered liothryronine, through negative feedback, to decrease T₄ production by the thyroid gland. In hyperthyroidism, because excess circulating thyroid...
hormone concentrations have already suppressed TSH production and secretion, additional triiodothyronine has minimal effect on T₄ production.³

Variations in thyroid metabolism due to local environmental influences need to be considered when looking at differences in thyroid hormone concentrations between populations. One of the main differences between our 2 groups of ferrets was the setting in which they were housed, as 1 group was used in a laboratory and the other served as pets. Differences in nutrition might have also contributed. In addition, the pet ferrets were exposed to many environmental stimuli and potential toxins that the laboratory ferrets would not be exposed to, which could affect thyroid metabolism.

In the United States, the incidence of thyroid disease in humans is increasing substantially, particularly the incidences of thyroid cancer and thyroid autoimmune disease.²¹ Focus has been placed on identifying environmental agents known to interfere with thyroid function at multiple physiologic points, including thyroid hormone synthesis, thyroid hormone metabolism and excretion, and thyroid hormone action.²² Evidence exists to link polychlorinated biphenyls and specific organochlorines to thyroid disruption in wild animals. In Canada, research has found that herring gulls in the Great Lakes area can develop serious thyroid abnormalities and other endocrine diseases.²³ Other research has shown that every top predator fish examined in the Great Lakes had enlarged thyroid glands; most dramatically, the thyroid glands of evaluated fish from Lake Erie had ruptured as a result of severe enlargement.²⁴ Information on thyroid disease in ferrets is scarce. Therefore, other tests may need to be evaluated in concert with or independently of the T₄ response to rhTSH administration before conclusions can be made on thyroid testing in ferrets. The present study yielded evidence that IM administration of rhTSH can be used safely for TSH stimulation testing in ferrets. Although a small number of ferrets were used, the results were consistent (ie, small SDs were obtained, compared with values in other ferret studies). However, additional studies are required to evaluate the suggested rhTSH dose of 100 µg in suspected hypothyroid ferrets and in ferrets with nonthyroidal illnesses.

References