Functional hyperthyroidism as a result of thyroid neoplasia in guinea pigs (Cavia porcellus) has recently been documented and appears to be encountered in the clinical setting, but it is a poorly documented condition in these animals. The relative prevalence is 4.6% at 1 pathology facility, making thyroid neoplasia the second most commonly reported malignancy after lymphoma. However, the percentage of these nodules, which are actively secreting hormone or are active metabolically, is currently unknown. Similar to this observation, a German study found that hyperthyroidism is a relatively common clinical condition in guinea pigs. Typical clinical signs include hyperactivity, hyperesthesia, and polyphagia with weight loss. Most guinea pigs with hyperthyroidism have an elevated resting thyroxine concentration and a palpable neck mass. However, some animals have only marginally elevated thyroxine concentrations and a rare few have normal or low resting thyroxine concentrations; not all hyperthyroid guinea pigs have a palpable neck mass. Until very recently, there have been few validated reference values for thyroid hormone concentrations in guinea pigs, making interpreting results of a hormone profile problematic. Occasionally, guinea pigs are examined with typical clinical signs of hyperthyroidism or have no palpable neck tumor but are euthyroid on a hormone profile. These cases, in particular, are difficult to accurately diagnose as hyperthyroidism.

Normal thyroxine and triiodothyronine concentration reference ranges for guinea pigs were published in 1986. In addition, a recent study also established thyroid-stimulating hormone (TSH) reference values for guinea pigs. The goal of this study was to evaluate the effects of administration of recombinant human (rh) TSH for evaluation of thyroid function in euthyroid guinea pigs (Cavia porcellus).

Objective—To evaluate the effects of administration of recombinant human (rh) TSH for evaluation of thyroid function in euthyroid guinea pigs (Cavia porcellus).

Design—Prospective, experimental study.

Animals—10 healthy, sexually intact, pet guinea pigs (approx 1 year of age).

Procedures—Guinea pigs were given rhTSH (100 µg, IM); plasma thyroxine concentrations were determined prior to and 3 and 4 hours after rhTSH injection. The animals were housed in 2 groups on the basis of sex and fed different commercial maintenance diets according to their husbandry.

Results—There was no significant difference in thyroxine concentrations between males and females before or after rhTSH injection. There was also no difference between thyroxine concentrations at 3 versus 4 hours after rhTSH injection. There was a significant difference between thyroxine concentrations before (median, 9.05 nmol/L [0.70 µg/dL]; 10% to 90% range, 7.39 to 16.99 nmol/L [0.57 to 1.32 µg/dL]) and after (mean ± SD, 23.95 ± 4.2 nmol/L) rhTSH injection. Euthyroid guinea pigs had plasma thyroxine concentrations of at least 7.30 nmol/L (0.57 µg/dL) and an increase of at least 2.6 times prestimulation thyroxine concentrations at 3 or 4 hours after stimulation.

Conclusions and Clinical Relevance—The results suggested that rhTSH administered IM can be used for the TSH stimulation testing in guinea pigs. We suggest thyroxine concentration in an euthyroid guinea pig should at least double 3 to 4 hours after rhTSH injection.

Abbreviations

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<th>Description</th>
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<tr>
<td>rhTSH</td>
<td>Recombinant human thyroid-stimulating hormone</td>
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<td>TSH</td>
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roxine concentrations for euthyroid guinea pigs. These concentrations ranged from 14.2 to 66.9 nmol/L (1.1 to 5.2 μg/dL), with a median of 27.0 nmol/L (2.1 μg/dL). Thyroxine concentration determined by radioimmunoassay requires a very small amount of plasma (25 μL). A TSH stimulation test can be an effective test for diagnosing some of these difficult-to-diagnose cases. If thyroxine concentration is evaluated for only a single sample, it is impossible to differentiate true hypothyroidism from euthyroid sick syndrome, in which animals with nonthyroidal illness can have substantial decreases in circulating thyroxine concentrations.

Hypothyroidism in animals can be congenital, acquired, or iatrogenic (as a result of treatment of hyperthyroidism). For dogs and cats, it has been customary to use bovine TSH for the TSH stimulation test; however, bovine TSH is no longer available as a pharmaceutical preparation. The hormone has been replaced with the notably more expensive rhTSH. Because of this development, new protocols for the TSH stimulation test in companion animals have been established for the use of rhTSH. Protocols have been described for dogs and cats, but we are not aware of a similar protocol for guinea pigs.

The purpose of the study reported here was to evaluate the effects of rhTSH on plasma thyroxine concentrations in euthyroid guinea pigs to develop an antemortem method for evaluating thyroid function in guinea pigs. The specific hypotheses were that rhTSH can be safely and effectively used for TSH stimulation testing of guinea pigs, an increase in thyroxine concentration would be noted in animals given rhTSH, and plasma from the suspected hyperthyroid guinea pig was 98.0%, 134.0%, 93.0%, 111.0% and 97.0%, respectively. When pooled plasma was serially diluted, results were 97.0%, 101.0%, 116.0%, and 164.0% of the expected values. Results of serially diluted plasma from suspected hyperthyroid guinea pigs were 98.0%, 134.0%, 93.0%, and 62.0% of the expected values. Analytic sensitivity of the assay was 2.80 nmol/L (0.22 μg/dL) on the basis of information provided by the manufacturer.

Results

Median thyroxine concentration prior to TSH injection was 9.05 nmol/L (0.70 μg/dL) to 10% to 90% range; 7.39 to 16.99 nmol/L [0.57 to 1.33 μg/dL]; range, 7.30 to 17.10 nmol/L [0.56 to 1.33 μg/dL]). The mean thyroxine after TSH injection was 23.95 ± 4.2 nmol/L.

Materials and Methods

Animals—The effects of TSH on plasma concentrations of thyroxine were determined in euthyroid guinea pigs. Ten sexually intact guinea pigs (6 females and 4 males) that were approximately 1 year of age were used for this study. The guinea pigs were healthy pet animals recruited for the study, and informed owner consent was acquired. The animals were housed in 2 groups on the basis of sex and fed different commercial maintenance diets according to their maintenance husbandry protocol. Physical examinations, CBCs, plasma biochemical analyses, and determination of baseline thyroxine concentrations were performed on the 10 study animals to ensure that they were healthy prior to the start of the study. Blood samples were taken from the jugular vein. All guinea pigs were allowed to acclimate to their surroundings and to handling procedures for 1 week prior to testing.

Measurement of thyroxine concentrations—Plasma thyroxine concentrations were measured by use of a commercially available 125I solid-phase competitive radioimmunoassay kit. Plasma thyroxine concentrations were determined prior to and 3 (females only) and 4 hours (males only) after rhTSH injection. Performance characteristics for the thyroxine assay were determined with pooled plasma from 5 healthy male guinea pigs and plasma from 1 suspected hyperthyroid guinea pig. Intra-assay coefficients of variation for the pooled plasma from healthy guinea pigs and plasma from the suspected hyperthyroid guinea pig were 9.6% and 11.8%, respectively. Inter-assay coefficients of variation for the pooled plasma from healthy guinea pigs and plasma from the suspected hyperthyroid guinea pig were 18.0% and 12.6%, respectively. Mean recovery of known amounts of thyroxine standards added to the pooled plasma from healthy guinea pigs and plasma from the suspected hyperthyroid guinea pig was 111.0% and 97.0%, respectively. When pooled guinea pig plasma was serially diluted, results were 97.0%, 101.0%, 116.0%, and 164.0% of the expected values. Results of serially diluted plasma from suspected hyperthyroid guinea pigs were 98.0%, 134.0%, 93.0%, and 62.0% of the expected values. Analytic sensitivity of the assay was 2.80 nmol/L (0.22 μg/dL) on the basis of information provided by the manufacturer.

Protocol—Each animal was given 100 μg of rhTSH IM. The dose was established by a small pilot study performed prior to this study. The rhTSH was divided into 100-μg aliquots and kept frozen at –20°C until use. Blood samples were collected into plasma separator tubes immediately before the rhTSH injection (time 0) and 3 and 4 hours after injection to measure thyroxine concentrations. The guinea pigs were monitored for 3 to 4 hours for any adverse effects, such as anaphylaxis, that may have occurred as a result of the IM administration of the foreign antigen.

Statistical analysis—The distribution of the data was evaluated by means of the Shapiro-Wilk test. Normally distributed data are reported as mean, SD, and range. Nonnormally distributed data are reported as median, 10% to 90% range, and minimum to maximum range. A Levene test was used to evaluate for homogeneity of variance in the data. On the basis of distribution of the data, a Mann-Whitney test was used to compare thyroxine concentrations before TSH injection between the sexes and a Student t test was used to compare thyroxine concentrations 3 (females only) and 4 (males only) hours after TSH injection. A related-samples Wilcoxon signed rank test was used to compare thyroxine concentrations before and after TSH injection for all guinea pigs. A Student t test was also used to compare the overall difference in thyroxine concentration between the sexes before and after TSH injection. Values of P < 0.05 were considered significant, and a Bonferroni correction was applied to minimize the potential for introducing familywise error due to multiple comparisons for thyroxine concentrations before and after TSH injection (P < 0.025). A power analysis was done for any values of P < 0.1. A commercial software package was used to perform the analyses.
(range, 18.2 to 31.1 nmol/L). There was no significant difference in the thyroxine concentration prior to TSH injection by sex ($P = 0.09$; power = 0.2) or between the thyroxine concentrations before and after TSH injection by sex ($P = 0.4$). There was also no significant ($P = 0.5$) difference between thyroxine concentrations 3 and 4 hours after TSH injection. However, there was a significant ($P = 0.003$) difference in thyroxine concentrations before and after TSH injection, with a 2.6 times increase in the thyroxine concentration after TSH injection. Because there was no difference between the thyroxine concentrations before and after TSH injection by sex or time, the data were combined. In addition, because the thyroxine concentrations after TSH injection were normally distributed, a specific reference range (mean ± 2 SD) was calculated: 15.55 to 32.35 nmol/L (1.21 to 2.51 µg/dL).

**Discussion**

The results of the present study suggest that rhTSH administered IM can be used for the TSH stimulation test in guinea pigs. We suggest that thyroxine concentrations in a euthyroid guinea pig should at least double 3 to 4 hours after TSH injection. Thyrotropin or TSH is considered the most important regulator of thyroid 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. Thyroid-stimulating 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 several assays, they share the same biological activity, which explains why canine thyroid glands respond 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 risk of hypersensitivity reactions if repeated administrations occur. It has been shown that highly purified rhTSH was effective in stimulating CAMP production in rat cells. It has also been demonstrated that rhTSH binds to the thyroid TSH receptor in mice and rats.

The results of the present study suggest that the administration of rhTSH had the expected biological effect on the normal thyroid gland of guinea pigs. This is hypothesized to result from rhTSH binding to the guinea pig thyroid TSH receptor and stimulating cAMP production. On the basis of data obtained from our study and the lack of other comparable data in the scientific literature, the administration of 100 µg was found to be an adequate and sufficient IM dose of rhTSH to cause stimulation in guinea pigs; however, we realize that the cost of rhTSH is a limiting factor in a clinical setting. Unfortunately, there is no report on the biological half-life of TSH in guinea pigs, nor in dogs. In humans, the half-life of TSH is reported to be 1 hour; this may be used as a reference. In human medicine, the use of rhTSH appears safe. Only a few minor adverse effects, such as headache, fatigue, vomiting, and dizzi- ness, have been reported. No rhTSH antibodies were detected in the plasma of any human patients. In a study of dogs, repeated administration of rhTSH did not induce adverse effects. Although antibody production against rhTSH was not evaluated, none of the dogs in that study had any anaphylactic reactions or obvious resistance to rhTSH.

Although clinicians have a notable problem in diagnosing thyroid pathological changes in exotic animal species, it is important to note that the diagnosis of thyroid pathological changes in domesticated species (mainly dogs and cats) is still controversial. To date, actual recommendations to assess thyroid function in domestic species include a large variety of assays, ranging from radioactive imaging, thyroid-stimulating hormone tests, and home thyroid hormone suppression tests. A recent study in cats concluded that the additional expense and limited availability of free thyroxine analysis did not appear to add useful information to the test protocol; therefore, the authors recommend measurement of only the thyroxine response for the TSH stimulation test. However, many clinicians feel that measuring thyroid-stimulating hormone via equilibrium dialysis is very helpful in diagnosing feline hyperthyroidism. The TSH-stimulation test in cats has been documented both with bovine TSH and rhTSH. Clinicians in the United Kingdom have described measurement of TSH in cats, but this practice has not yet been adopted by most clinicians. One newer way to diagnose feline hyperthyroidism is by means of a triiodothyronine suppression test. The triiodothyronine suppression test relies on the ability of administered liothyronine, through negative feedback, to decrease thyroxine 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 thyroxine production. We suggest that, as for dogs and cats, differences of opinion between clinicians may exist regarding the appropriate diagnostic tests for guinea pigs with thyroid disease. Other tests may need to be evaluated in guinea pigs together with or independent of evaluation of rhTSH responses on thyroxine (or free thyroxine) concentrations before firm statements can be made on thyroid testing in this species.

The primary limitations of the present study were the small sample size and the sampling strategy used for determining thyroxine concentrations after TSH injection. The sample size selected for this study was small because this was considered a pilot study. The primary objective of this study was to determine whether thyroxine concentrations would increase in euthyroid guinea pigs after rhTSH stimulation. The findings of the study confirm that this is possible. Further, we attempted to control for a potential type I error for this comparison with a Bonferroni correction. This was also used to control for familywise error when looking at potential differences by the sexes. We did not expect there to be a difference. Whereas it is common to perform a power analysis in cases where the $P$ value approaches the determined level of significance, which we did in this case, we were more concerned about a type I error. Additional study to determine whether sex has
an effect on thyroxine concentrations could be done to further assess this question.

It is interesting to note that the median baseline thyroxine concentration of our population was lower than the published data of Müller et al.\(^3\) Müller et al\(^3\) reported a median thyroxine concentration of 27 nmol/L (2.1 µg/dL), whereas the median concentration for the present study was 0.95 nmol/L (0.7 µg/dL). The discrepancy in baseline thyroxine concentrations between the 2 studies may be explained, in part, by the difference in number of animals used in each study and by the methodologies used for analysis of thyroxine concentration. The number of animals was higher in the study by Müller et al\(^3\) with 40 animals being used, compared with 10 animals in the present study. Furthermore, Müller et al\(^3\) used a chemiluminescence test,\(^6\) whereas we relied on a commercially available \(^5\) solid-phase competitive radioimmunoassay kit. It has been shown that differences exist in measurement of steroids between the 2 methodologies, and the same may be true for thyroid hormone.\(^6\)\(^8\)\(^10\) In addition, Müller et al\(^3\) also acknowledged that the thyroxine concentrations in their study (14.2 to 66.9 nmol/L [1.1 to 5.2 µg/dL]) were considerably higher than those previously reported for guinea pigs by Ewringmann and Glockner\(^4\) (6.4 to 15.4 nmol/L [0.5 to 1.2 µg/dL]). Ewringmann and Glockner\(^4\) used 30 healthy animals as their study population, and the analytic method in their trial was chemiluminescence. Because our data were obtained with a different method (radioimmunoassay) versus that used in the previous study\(^3\) and the results of our study are very comparable with their results, we feel that these values do reflect the normal thyroxine concentration in the pet guinea pig population. However, the difference in baseline thyroxine concentration between the different studies highlights the need to perform an actual stimulation test when thyroid disease is suspected because the difference between concentrations before and after TSH stimulation can be an important aid in diagnosing true functional pathological changes of the thyroid gland.

Considering our results, it seems that rhTSH administered IM can be used for the TSH stimulation test in guinea pigs. Preliminary testing indicates that 3 hours after TSH stimulation testing is as sensitive as 4 hours after TSH in determining normal thyroid function; thus, we suggest that a euthyroid guinea pig should at least double its thyroxine concentration 3 to 4 hours after TSH injection. Although a relatively small number of guinea pigs were used in this study, we believe the results were quite consistent, and the data gathered confirmed our hypothesis that rhTSH could be used safely in guinea pigs. However, further studies are required to evaluate the optimal dose of rhTSH in hypothyroid guinea pigs and in guinea pigs with non-thyroidal illnesses.

References