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Secretion pattern of thyroid-stimulating hormone in dogs during euthyroidism and hypothyroidism

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Abstract

In as many as one third of dogs with primary hypothyroidism a plasma thyrotropin (TSH) concentration within the reference range for euthyroid dogs is found. To determine whether this is due to fluctuations in the release of TSH, the plasma profiles of TSH were analyzed in 7 beagle bitches by collecting blood samples every 10 min for 6 hr, both before and after induction of primary hypothyroidism. After induction of primary hypothyroidism, a 37-fold increase in mean basal plasma TSH concentration and a 34-fold increase in mean area under the curve for TSH were found. Analysis by the Pulsar program demonstrated pulsatile secretion of TSH in the hypothyroid state, characterized by relatively low amplitude pulses (mean [±SEM]) amplitude 41 ± 3% of basal plasma TSH level) and a mean pulse frequency of 2.0 ± 0.5 pulses/6 hr. In the euthyroid state, significant TSH pulses were identified in only 2 dogs. The mean basal plasma TSH level correlated positively ($r = 0.84$) with the mean amplitude of the TSH pulses, and correlated negatively ($r = -0.88$) with the TSH pulse frequency. The results of this study demonstrate pulsatile secretion of TSH in dogs during hypothyroidism and only small fluctuations in plasma TSH concentrations during euthyroidism. The findings also suggest that the low TSH values occasionally found in dogs with spontaneous primary hypothyroidism may in some cases in part be the result of ultradian fluctuations.

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1. Introduction

Primary hypothyroidism is one of the most common endocrine diseases in the dog. In the last two decades, the diagnosis of primary hypothyroidism in dogs has been based upon a low plasma thyroxine concentration that is not responsive to thyrotropin (TSH) stimulation [1]. Because TSH is no longer commercially available for diagnostic purposes in veterinary practice, the diagnosis of canine primary hypothyroidism is now often based upon the combination of a low thyroxine concentration and an elevated TSH concentration within a single plasma sample [2,3]. However, it has been reported that in as many as one-third of dogs with primary hypothyroidism the plasma TSH concentration is within the reference range for euthyroid dogs [3–8]. Possible reasons why dogs with primary hypothyroidism have plasma TSH concentrations within the reference range include concurrent secondary or tertiary hypothyroidism [5], failure of the TSH assay to detect all isoforms of circulating TSH [3], and a decrease in elevated TSH values in hypothyroid dogs with time [9]. In addition, fluctuations in the circulating concentrations of TSH could be a reason for this phenomenon.

In humans and rats, frequent sampling studies have demonstrated that TSH is secreted intermittently. In 1965, Bakke and Lawrence reported circadian TSH rhythmicity in the pituitary gland of euthyroid and hypothyroid rats [10]. With the development of a sensitive radioimmunoassay for TSH, a circadian TSH secretion pattern was also reported in humans [11]. Parker et al. [12] were the first to demonstrate a pulsatile or ultradian TSH secretion pattern in humans. The ultradian TSH release in healthy humans follows a high-frequency, low-amplitude pulsatile pattern superimposed on a low-frequency, high-amplitude circadian TSH rhythm [13–15]. Ultradian pulsatile TSH secretion has also been demonstrated in hypothyroid but not in euthyroid rats [16].

All pituitary hormones studied thus far in the dog are also released in a pulsatile pattern [17–20]. However, there is almost no information on the TSH secretion pattern in this species. In a study by Bruner et al. [7], serum samples were collected at 2-hr intervals and fluctuations in TSH concentrations were detected throughout the day in both euthyroid and hypothyroid dogs. Although these authors were unable to identify any diurnal or pulsatile secretion pattern, the random fluctuations in serum TSH concentration may suggest pulsatile secretion of TSH in the dog.

In this study we analyzed the 6-hr secretion patterns of TSH in 7 beagle bitches, both before and after induction of primary hypothyroidism.

2. Materials and methods

2.1. Dogs

Seven healthy, neutered beagle bitches entered the study at the age of 3 years. The dogs were housed singly in indoor-outdoor runs, fed a standard commercial dry dog food twice daily, and given water ad libitum. The daily food intake of the dogs was approximately 300 grams. According to the manufacturer, the iodine content of the commercial dry dog food

was 250–300 μg per 100 gram. On the days on which blood samples were collected, the dogs were fed their usual commercial dog food between 09:00 and 09:30 a.m.

Euthyroidism was confirmed by the finding of basal and bovine TSH-stimulated plasma thyroxine concentrations within their respective reference ranges, a plasma TSH concentration within the reference range, a normal image of the thyroid glands obtained by scintigraphy using radioactive pertechnetate ($^{99m}TcO_4^-$), and, eventually, by the finding of normal thyroid gland tissue by histologic examination after thyroidectomy.

Primary hypothyroidism was induced by surgical removal of the thyroid glands, leaving the external parathyroid glands in situ. Two months after surgery, scintigraphy with $^{99m}TcO_4^-$ was used to detect remnant thyroid gland tissue. Approximately 1 month later, the dogs were fed a low-iodine diet (meat and white bread) for 3 weeks to increase the iodine uptake by any remnant thyroid tissue, which was then destroyed by administering 5 mCi (=185 MBq) of sodium-^{131}I-iodide orally.

2.2. Sample collection

Blood samples (0.8 mL) for the determination of the plasma concentrations of TSH and total thyroxine were collected at 10-min intervals between 08:00 a.m. and 2:00 p.m. This was done 1.5 months prior to the surgical thyroidectomy and again one month after the administration of sodium-^{131}I-iodide. Blood samples were collected by jugular venipuncture, immediately placed in chilled, heparin-coated tubes, and centrifuged. Plasma was stored at -20°C until assayed.

2.3. Hormone determination

Plasma TSH concentrations were determined by a homologous solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite canine TSH, Diagnostic Products Corporation (DPC), Los Angeles, CA) according to the instructions of the manufacturer. The intraassay coefficients of variation were 5.0%, 4%, and 3.8% at TSH levels of 0.20, 0.50, and 2.6 μg/liter, respectively. The interassay coefficients of variation were 6.3% and 8.2% at TSH levels of 0.16 and 2.8 μg/liter, respectively. The lowest detectable amount of TSH was 0.03 μg/liter. Values of TSH measured with the Immulite canine TSH assay after serial dilution of three canine samples showed good linearity throughout its calibration range. To establish the spiking recovery, a 13 μl aliquot of three spiking solutions (4.3, 8.5, and 18 μg/liter) was spiked into 247 μl aliquots of three different canine samples (0.46, 2.8, and 5.2 μg/liter). The recovery ranged from 94 to 104%. These results confirm that the Immulite canine TSH assay yields an accurate measure of canine TSH throughout its calibration range. Cross-reactivity with FSH and LH was negligible. The upper limit of the reference range for the plasma TSH concentration in euthyroid dogs in our laboratory is 0.6 μg/liter.

Plasma total thyroxine concentrations were determined by a homologous solid-phase, chemiluminescent enzyme immunoassay (Immulite canine Total T4, DPC, Los Angeles, CA) according to the instructions of the manufacturer and validated for the dog by Bruner et al. [7]. The intraassay coefficients of variation were 13.8% and 8.2% at thyroxine levels of 8 and 25 nmol/liter, respectively. The lowest detectable amount of thyroxine was 1.5

nmol/liter. Values of total thyroxine measured with the Immulite canine Total T4 assay after serial dilution of four canine samples showed good linearity throughout its calibration range. To establish the spiking recovery, a 50 μl aliquot of three spiking solutions (219, 412, and 798 nmol/liter) was spiked into 950 μl aliquots of four different canine samples (7, 14, 15, and 23 nmol/liter). The recovery ranged from 96 to 114%. These results confirm that the Immulite canine Total T4 assay yields an accurate measure of canine total thyroxine throughout its calibration range.

2.4. Data analysis

The secretion pattern of TSH was analyzed using the Pulsar program developed by Merriam and Wachter [21]. The program identifies secretory peaks by height and duration from a smoothed baseline, using the assay SD as a scale factor. The cut-off parameters G1-G5 of the Pulsar program were set at 5.78, 2.89, 1.84, 1.27, and 0.89 times the intraassay SD as criteria for accepting peaks 1, 2, 3, 4, and 5 points wide, respectively [22], resulting in a false-positive error rate less than 5%. The smoothing time, a window used to calculate a running mean value, was set at 5 hr. The weight assigned to peaks was 0.05. The A, B, and C values of the Pulsar program, used to calculate the variance of the assay, were set at A = 0.05, B = 3.52, and C = 0.71. The values extracted from the Pulsar analyses included: the overall mean of the smoothed baseline, the area under the curve (AUC), the amplitude of the pulses, and the number of pulses. The AUC was calculated above the zero level.

Plasma concentrations of thyroxine and TSH below the detection limit of the assay were considered to be 1.5 nmol/liter and 0.03 μg/liter, respectively. Differences in the AUC and basal concentrations were evaluated by Student's *t*-test for related samples (two-tailed). Correlations between basal levels and pulse characteristics were analyzed using the linear correlation coefficient. Values are expressed as mean $\pm$ SEM or as mean and range. $P < 0.05$ was considered significant.

2.5. Ethics

The experiments in this study were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Utrecht University.

3. Results

Two months after thyroidectomy, scintigraphy with $^{99m}TcO_4^-$ demonstrated that remnant thyroid gland tissue was present in the neck at the normal thyroid location (5 dogs), under the base of the tongue (2 dogs), and at the base of the heart (3 dogs).

The mean plasma thyroxine concentrations was 20.7 $\pm$ 2.6 nmol/liter prior to the thyroidectomy and 2.5 $\pm$ 0.4 nmol/liter 1 month after the administration of ^{131}I. The plasma thyroxine levels in the euthyroid and hypothyroid states did not overlap. Although minor fluctuations were observed in the 6-hr secretion patterns of thyroxine, neither in euthyroidism nor in hypothyroidism was thyroxine secreted in a pulsatile fashion. In both situations the

Table 1
Characteristics of the TSH secretory profiles in euthyroidism and hypothyroidism

	Euthyroid state	Hypothyroid state
Thyroxine basal (nmol/liter)	20.7 ± 2.6	2.5 ± 0.4
TSH basal (μg/liter)	0.10 ± 0.03 (0.03–0.26)	3.7 ± 0.6 (1.1–5.7)
TSH min (% of basal)	—	−16 ± 3 (7–26)
TSH max (% of basal)	—	47 ± 7 (19–74)
TSH pulse amplitude (μg/liter)	—	1.2 ± 0.3 (0.4–4.1)
TSH pulse frequency (pulses/6 hr)	—	2.0 ± 0.5 (0–4)
AUC for TSH (μg/liter·6 hr)	0.68 ± 0.32 (0.18–1.89)	23.3 ± 4.0 (7.0–38.2)

mean basal 6-hr plasma thyroxine concentration and the mean basal 6-hr plasma TSH concentration were not significantly correlated.

After induction of primary hypothyroidism a 37-fold increase in mean basal plasma TSH concentration and a 34-fold increase in mean AUC for TSH were found. The basal plasma TSH concentrations (mean 3.7 ± 0.6 μg/liter) and the AUCs for TSH (23.3 ± 4.0 μg/liter · 6 hr) in the hypothyroid state were significantly higher ($P = 0.001$) than the basal plasma TSH concentrations (mean 0.10 ± 0.03 μg/liter) and the AUCs for TSH (mean 0.68 ± 0.32 μg/liter · 6 hr) before induction of primary hypothyroidism.

Analysis of the TSH secretory profiles in euthyroidism (summarized in Table 1) revealed the presence of significant TSH pulses in only 2 of the 7 dogs, although fluctuations in the plasma TSH concentration were evident in 5 dogs throughout the 6-hr period of 10-min sampling. An example of the TSH pattern in 1 of the euthyroid dogs is presented in Fig. 1. In 2 dogs the plasma TSH concentrations in the euthyroid state were continuously below the detection limit of the TSH assay.

After induction of primary hypothyroidism significant TSH pulses were identified by the Pulsar program in all but 1 of the dogs. Two examples are presented in Fig. 2. The TSH

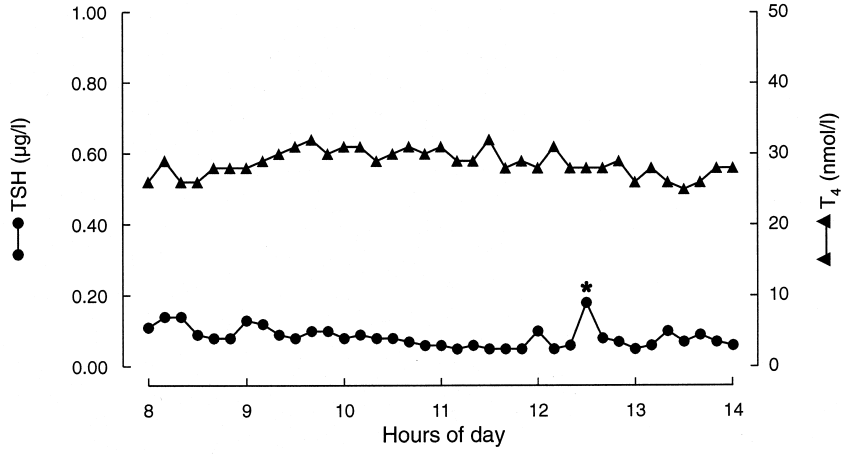

Fig. 1. The 6-hr secretory profiles of TSH (●) and thyroxine (T4) (▲) in a 3-year-old euthyroid beagle bitch. Significant pulses, calculated by the Pulsar program, are indicated by an asterisk.

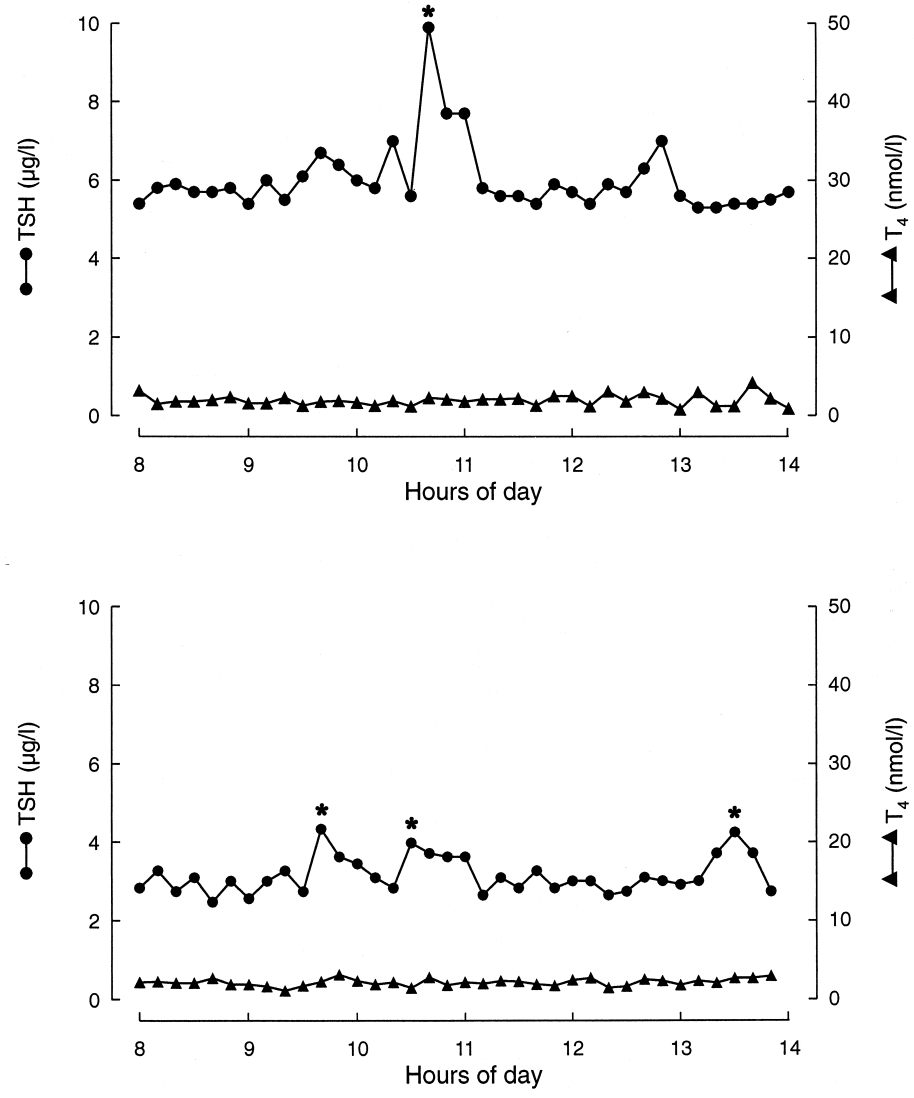

Fig. 2. The 6-hr secretory profiles of TSH (●) and thyroxine (T4) (▲) in two 3-year-old beagle bitches after induction of primary hypothyroidism. Significant pulses, calculated by the Pulsar program, are indicated by an asterisk.

secretory patterns in individual dogs varied considerably in pulse frequency and pulse amplitude, and in all dogs fluctuations around the mean baseline were observed (Table 1, Fig. 2). The mean TSH pulse frequency in hypothyroidism was approximately 2 pulses per 6 hr. The TSH pulses were relatively low, having a mean amplitude of 41 ± 3% of the basal plasma TSH level. There was a significant positive correlation ($r = 0.84$, $P < 0.05$) between the mean basal 6-hr plasma TSH concentration and the mean amplitude of the TSH pulses. The mean basal 6-hr plasma TSH concentration was negatively correlated ($r = -0.88$, $P < 0.05$) with the TSH pulse frequency.

4. Discussion

The results of this study demonstrate pulsatile secretion of TSH in dogs during hypothyroidism. Pituitary TSH secretion is controlled by the stimulatory action of hypothalamic TRH and inhibition via central dopaminergic and somatostatinergic mechanisms, as well as by an inhibitory action of thyroid hormones at the central, hypothalamic, and pituitary levels [23,24]. Studies in humans have demonstrated that continuous infusions with dopamine and somatostatin suppress mean circulating TSH levels but the pulsatile pattern of TSH secretion remains [25]. Thyroid hormones, with their long metabolic half-lives are also unlikely to act as rapid modulators of TSH secretion. Therefore, in humans, and probably also in dogs, TRH is most likely to be the driving force for the pulsatile TSH secretion pattern [23,25].

In contrast to the findings during primary hypothyroidism, the results of this study failed to demonstrate irrefutably that TSH is secreted in a pulsatile fashion in euthyroid dogs. Although there were fluctuations in circulating TSH levels in 5 euthyroid dogs, in only 2 was significant pulsatility detected by the Pulsar program. Similar observations have been reported in rats [16] and ewes [26]. However, in humans pulsatile secretion of TSH has been reported in both primary hypothyroidism and euthyroidism [14,27]. The difference between euthyroid humans and euthyroid dogs with regard to TSH pulsatility may be the relatively low plasma TSH levels in euthyroid dogs, which could be related to the high iodine content of commercial dog food. The minimum daily iodine requirement of the adult beagle dog has been estimated to be 140 μg [28]. Ad libitum consumption of most commercially manufactured dry dog foods provides the average dog with a daily iodine intake of at least 500 μg and some foods provide considerably more [29]. In dogs receiving less than 140 μg iodine per day enlarged and sparsely granulated pituitary TSH-secreting cells were found, suggesting high secretory activity [28]. In contrast, the high iodine content of commercial dog food implies a relatively low stimulus for TSH secretion. Consequently, the mean basal TSH levels are low in euthyroid dogs and this may prohibit the detection of significant TSH pulses, because of the positive correlation demonstrated in the present study and in several studies in humans [14,24,27,30,31] between the mean basal plasma TSH concentration and the mean amplitude of the TSH pulses. In addition, the significant TSH pulses detected in the dogs during hypothyroidism had a relatively low amplitude compared to the basal plasma TSH levels. Therefore, the amplitudes of possible TSH pulses in the euthyroid dogs may have been so low that, due to the variability of the TSH assay in the range of euthyroid TSH levels, TSH pulses could not be detected by the Pulsar program.

Blood samples for the determination of the plasma TSH concentration were collected at 10-min intervals for 6 hr. Although this 6-hr period was sufficient to demonstrate the pulsatile secretion of TSH in hypothyroid dogs, this 6-hr period did not allow an accurate estimation of the TSH pulse frequency. In the present study the mean TSH pulse frequency was approximately 2 pulses per 6 hr. However, the range of the TSH pulse frequency in the 6 dogs was considerable, indicating that sampling for a longer time period is required to determine accurately the TSH pulse frequency.

The minor fluctuations observed in the 6-hr plasma profiles of thyroxine in hypothyroid dogs have been reported before [32]. However, the fluctuations which we observed were not significantly different from the intraassay variation. In addition, the plasma concentration of

a hormone with a biologic half-life of about a day, such as thyroxine [33], cannot be expected to vary strongly.

In normal regulation of the hypothalamic-pituitary-thyroid axis, even small decrements in circulating thyroid hormone concentration result in an increase in basal TSH and augmentation of the TSH response to TRH [34]. Nevertheless, neither during euthyroidism nor during hypothyroidism were the mean basal plasma TSH concentration and the mean plasma thyroxine level significantly correlated. This suggests that the set-point for basal TSH secretion and sensitivity to plasma thyroid hormone concentrations may vary from dog to dog. The role of thyroid hormones in the generation of pulsatile TSH secretion is probably not great, since in humans no major influence of thyroid hormones on TSH pulse frequency has been observed [14,22]. The role of thyroid hormones in regulating pulsatile TSH secretion in humans appears to be restricted to modulation of the TSH pulse amplitude [22].

Studies in humans have demonstrated that ovarian sex steroids and androgens are not critically involved in the generation of pulsatile TSH secretion [15,35]. Therefore, the present results with neutered bitches may most likely apply for intact dogs and neutered male dogs as well. All dogs were 3 years of age, which is an age at which primary hypothyroidism is common. Because Greenspan et al. [31] have reported that TSH pulse frequency in men is not age dependent, our observations may presumably be extrapolated to other ages.

Despite the fluctuations in the 6-hr plasma profile of TSH during hypothyroidism, plasma TSH levels in euthyroidism and hypothyroidism did not overlap. This underlines the discriminatory power of the determination of the TSH concentration in a single plasma sample in distinguishing these 2 subgroups. However, the dogs used in the present study were healthy beagle dogs examined both before and after induction of hypothyroidism by a combination of surgical thyroidectomy and ^{131}I treatment. Similar to the results in other studies [7,36], this model of primary hypothyroidism resulted in consistent increases in the plasma TSH concentration. These changes are in contrast to the much more moderate increases in plasma TSH concentrations observed in most dogs with spontaneous primary hypothyroidism [2–8]. Moreover, in as many as one-third of the dogs with naturally occurring primary hypothyroidism, plasma TSH concentrations have been found to be within the reference range for euthyroid dogs [3–8]. The reason for this difference between experimental and spontaneous primary hypothyroidism is still unknown but it raises the question of whether dogs with experimentally induced primary hypothyroidism are an adequate model for naturally occurring canine primary hypothyroidism. Nevertheless, the fluctuations around the TSH baseline in experimentally induced primary hypothyroid dogs in the present study may in part explain why in some spontaneous primary hypothyroid dogs a plasma TSH concentration within the reference range for euthyroid dogs is found. The mean plasma TSH concentration in dogs with spontaneous primary hypothyroidism has been reported to exceed the upper limit of the reference range for TSH in euthyroid dogs only marginally [3,6,7]. Consequently, fluctuations around the TSH baseline at this level may occasionally result in plasma TSH concentrations within the reference range for euthyroid dogs. This line of reasoning is supported by the findings of Bruner et al. [7], who reported that dogs with spontaneous primary hypothyroidism may have random fluctuations in plasma TSH concentrations, with some of the values being outside the reference range for euthyroid dogs and some within.

In summary, the results of this study demonstrate pulsatile secretion of TSH in dogs during hypothyroidism and only small fluctuations in plasma TSH concentration during euthyroidism. The pulsatile secretion pattern of TSH was characterized by a pulse frequency of approximately 2 pulses/6 hr and TSH pulses with a relatively low amplitude compared with basal levels. The results of this study also suggest that the low TSH values occasionally found in dogs with spontaneous primary hypothyroidism may in some cases in part be the result of ultradian fluctuations.

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