Primary hypothyroidism in dogs is associated with elevated GH release

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Abstract

The pulsatile secretion patterns of GH were investigated in seven beagle bitches by collecting blood samples every 10 min for 6 h during euthyroidism and 1.5 years after induction of primary hypothyroidism. Hypothyroidism was induced by surgical removal of the thyroid gland and subsequent destruction of any remnant thyroid tissue by oral administration of sodium [^{131}I]iodide. Some of the physical changes observed in the dogs with primary hypothyroidism mimicked those of acromegaly. During both euthyroidism and hypothyroidism GH was secreted in a pulsatile fashion. The mean ($\pm$ s.e.m.) basal plasma GH concentration was significantly higher ($P=0.003$) in the hypothyroid state ($4.1 \pm 1.6 \mu\text{g/l}$) than in the euthyroid state ($1.2 \pm 0.4 \mu\text{g/l}$). Likewise, the mean area under the curve (AUC) for GH above the zero-level during hypothyroidism ($27.0 \pm 10.0 \mu\text{g/l} \times 6 \text{ h}$) was significantly higher ($P=0.004$) than that during euthyroidism ($11.7 \pm 2.0 \mu\text{g/l} \times 6 \text{ h}$). The mean AUC for GH above

the baseline was significantly lower ($P=0.008$) during hypothyroidism ($2.4 \pm 0.8 \mu\text{g/l} \times 6 \text{ h}$) than during euthyroidism ($4.5 \pm 1.8 \mu\text{g/l} \times 6 \text{ h}$), whereas there was no significant difference in GH pulse frequency. The mean plasma IGF-I level was significantly higher ($P<0.01$) in the hypothyroid state ($169 \pm 45 \mu\text{g/l}$) than in the euthyroid ($97 \pm 15 \mu\text{g/l}$). The results of this study demonstrate that primary hypothyroidism in dogs is associated with elevated basal GH secretion and less GH secreted in pulses. This elevated GH secretion has endocrine significance as illustrated by elevated plasma IGF-I levels and some physical changes mimicking acromegaly. It is discussed that the increased GH release in hypothyroid dogs may be the result of the absence of a response element for thyroid hormone within the canine pituitary GH gene and alterations in supra-pituitary regulation.

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Introduction

Growth hormone (GH) is secreted by the pituitary anterior lobe in a pulsatile fashion under the regulation of two hypothalamic peptides: GH-releasing hormone (GHRH) stimulates GH secretion, while somatostatin (SS) inhibits GH release. This regulatory system is influenced by negative feedback from peripherally formed growth factors, particularly insulin-like growth factor-I (IGF-I) (Hartman *et al.* 1993, Bermann *et al.* 1994). In addition, a recently identified GH-releasing peptide, called Ghrelin, is likely to play a role in the regulation of pituitary GH secretion (Kojima *et al.* 1999). The amplitude and frequency of GH secretory pulses are regulated by a complex array of external and internal stimuli including age (Corpas *et al.* 1993, Arvat *et al.* 1997), gender (Van den Berg *et al.* 1996), oestrous cycle phase (Faria *et al.* 1992), genetic background (Mendlewicz *et al.* 1999), nutritional status (Riedel *et al.* 1995), sleep (Matsuno *et al.* 1998), disease status (Frohman *et al.* 1992) and body composition

(Ahmad *et al.* 1989). In addition, hormones such as glucocorticoids and thyroid hormones (Devesa *et al.* 1992) influence the pulsatile secretion pattern of GH.

In rats, thyroid hormones are necessary to maintain both pulsatile and induced GH secretion (Martin *et al.* 1985). The GH secretory profile has been found to shift from a low-frequency, high-amplitude pattern in euthyroid rats to a high-frequency, low-amplitude pattern in rats with mild hypothyroidism induced by methimazole (Bruhn *et al.* 1992). Severe hypothyroidism in rats is associated with markedly reduced circulating GH levels (Varela *et al.* 1991, Santini *et al.* 1993, Mizobuchi *et al.* 1996, Tam *et al.* 1996, Osorio *et al.* 1998), abolished GH bursts (Takeuchi *et al.* 1978, Katakami *et al.* 1986), blunted GH response to GHRH (Williams *et al.* 1985, Tam *et al.* 1996) and decreased pituitary content of mRNA encoding for GH (Martinoli & Pelletier 1989, Lloyd *et al.* 1990, Tam *et al.* 1996). In addition, hypothyroidism in rats causes a reduction in pituitary GH content (Szabo *et al.* 1985, Katakami *et al.* 1986, Wood *et al.* 1987, Lloyd *et al.* 1990, Varela

et al. 1991), pituitary GHRH receptor mRNA (Tam *et al.* 1996, Korytko & Cuttler 1997), hypothalamic SS content (Tam *et al.* 1996) and hypothalamic SS mRNA (Tam *et al.* 1996).

In humans with primary hypothyroidism, the peak GH values after GHRH administration are low (Root *et al.* 1985, Williams *et al.* 1985, Damjanovic *et al.* 1996, Pimentel-Filho *et al.* 1997). Severe primary hypothyroidism in children is associated with attenuated spontaneous GH secretion (Buchanan *et al.* 1988, Chernauek & Turner 1989, Nishi *et al.* 1989). In line with these observations are the findings of *in vitro* studies in cell lines derived from human GH-secreting pituitary tumours, which revealed that thyroid hormones promote GH mRNA accumulation (Chomczynski *et al.* 1993) and GH release (Lamberts *et al.* 1984). The stimulatory effect of thyroid hormones on GH mRNA accumulation has been ascribed to transcriptional activation of the human GH gene (Chomczynski *et al.* 1993).

Information with regard to changes in pituitary GH secretion during primary hypothyroidism in other mammals is scarce. Low circulating GH levels have been reported in hypothyroid pigs (Morovat & Dauncey 1998). In contrast, hypothyroidism did not affect spontaneous GH secretion in sheep and steers, although the response to stimulation with GHRH was reduced in hypothyroid sheep (Elsasser *et al.* 1992, Fletcher & Clarke 1994).

In dogs, primary hypothyroidism is one of the most common endocrine diseases. The signs and symptoms include weight gain, lethargy and dermatological changes such as truncal alopecia and skin thickening due to mucopolysaccharide accumulation (Feldman & Nelson 1996, Rijnberk 1996). Changes in the GH secretion pattern might contribute to the features observed in dogs with primary hypothyroidism but information on the pulsatile GH secretion pattern in dogs with primary hypothyroidism is lacking. Therefore, we investigated the 6 h pulsatile GH secretion pattern both before and after induction of hypothyroidism in seven beagle dogs.

Materials and Methods

Dogs

Seven neutered (ovariectomized) beagle bitches entered the study at the age of 3 years. All bitches were accustomed to the laboratory environment and procedures such as the collection of blood. They were housed in individual cages, fed a commercial dog food twice a day, and had free access to water. On the days on which blood samples were collected, the dogs were fed their usual commercial dog food between 0900 and 0930 h.

Euthyroidism was confirmed by the finding of basal and bovine thyrotrophin (TSH)-stimulated plasma thyroxine concentrations within their respective reference range, a plasma TSH concentration within the reference range,

a normal image of the thyroid glands obtained by scintigraphy using radioactive pertechnetate ($^{99m}\text{TcO}_4^-$), and, eventually, by the finding of normal thyroid gland tissue by histological examination after thyroidectomy.

Primary hypothyroidism was induced by surgical removal of the thyroid gland, leaving the external parathyroid glands *in situ*. About 3 months 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.

The present study was part of a larger study in which we investigated the effect of long-term primary hypothyroidism on pituitary TSH secretion in dogs.

Sample collection

Blood samples for determining the plasma concentration of GH were collected at 10 min intervals between 0800 and 1400 h. This was done 1 month prior to the surgical thyroidectomy and again 1.5 years later. Blood samples were collected by jugular venepuncture and immediately placed in chilled EDTA-coated tubes, and centrifuged. Plasma was stored at -20°C until assayed.

Hormone determination

Plasma TSH concentrations were determined by a homologous solid-phase, two-site chemiluminescence enzyme immunometric assay (Immulite canine TSH; Diagnostic Products Corporation (DPC), Los Angeles, CA, USA) in accordance with the instructions of the manufacturer. The intra-assay coefficients of variation were 5.0, 4.0 and 3.8% at TSH levels of 0.20, 0.50 and 2.6 $\mu\text{g/l}$ respectively. The intra-assay coefficients of variation were 6.3 and 8.2% at TSH levels of 0.16 and 2.8 $\mu\text{g/l}$ respectively. The lowest detectable amount of TSH was 0.03 $\mu\text{g/l}$.

Plasma total thyroxine concentrations were determined by a homologous solid-phase, chemiluminescence enzyme immunoassay (Immulite canine Total T4; DPC) in accordance with the instructions of the manufacturer and validated for the dog by Bruner *et al.* (1998). The intra-assay coefficients of variation were 13.8 and 8.2% at thyroxine levels of 8 and 25 nmol/l respectively. The lowest detectable amount of thyroxine was 1.5 nmol/l.

Plasma GH concentrations were measured by a homologous RIA as described previously (Eigenmann & Eigenmann 1981). The intra- and inter-assay coefficients of variation were 3.8 and 7.2% respectively, and the sensitivity of the assay was 0.3 $\mu\text{g/l}$.

Plasma IGF-I concentrations were measured by a heterologous RIA as described previously (Nap *et al.* 1993). The intra- and inter-assay coefficients of variation were 4.7

and 15.6% respectively, at an IGF-I concentration of 175 µg/l. The sensitivity of the assay was 6 µg/l.

Data analysis

The 6 h secretion patterns of GH were analysed using the Pulsar program developed by Merriam & Wachter (1982). The program identifies secretory peaks by height and duration from a smoothed baseline, using the assay s.d. as a scale factor. The cut-off parameters G1–G5 of the Pulsar program were set at 3.98, 2.40, 1.68, 1.24 and 0.93 times the assay s.d. as criteria for accepting peaks 1, 2, 3, 4 and 5 points wide respectively, resulting in a false-positive error rate of less than 5%. The smoothing time, a window used to calculate a running mean value, was set at 5 h. 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, B=7.2 and C=5.0. The values extracted from the Pulsar analyses included the overall mean of the smoothed baseline, the number of peaks, the area under the curve (AUC) above the zero-level and the AUC above the baseline.

Differences in parameters during euthyroidism and hypothyroidism were evaluated by Student's *t*-test for related samples (two-tailed). Since the data were not assumed to be normally distributed, differences in pulse frequency were determined by non-parametric analysis, using Wilcoxon's signed rank test. Values are expressed as means $\pm$ s.e.m. $P < 0.05$ was considered significant.

Ethics of experimentation

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

Results

The mean body weight during hypothyroidism (20.1 ± 1.4 kg) was significantly higher ($P = 0.002$) than that during euthyroidism (14.5 ± 0.7 kg). In all dogs, induction of primary hypothyroidism also resulted in marked exercise intolerance and skin changes such as thick folding. In all dogs, alopecia and hyperpigmentation were noticeable especially on the bridge of the nose. In four of the dogs there appeared to be some maxillary prognathia with widening of the interdental spaces.

At 1.5 years after induction of primary hypothyroidism plasma thyroxine concentrations were low (< 2 nmol/l) as compared with the euthyroid state (18.3 ± 1.7 nmol, $P < 0.001$). The mean plasma TSH level rose ($P = 0.01$) from 0.11 ± 0.03 µg/l prior to thyroidectomy to 1.4 ± 0.38 µg/l during hypothyroidism.

GH was secreted in a pulsatile fashion both before and after induction of primary hypothyroidism. Examples of

Figure 1 The 6 h secretory profiles of GH in a beagle bitch during euthyroidism (before) and during hypothyroidism (after). Significant GH pulses, calculated by the Pulsar program, are indicated by an asterisk.

the pulsatile secretion patterns of GH during euthyroidism and hypothyroidism are shown in Fig. 1. The mean basal plasma GH level in the hypothyroid state (4.1 ± 1.6 µg/l) was significantly higher ($P = 0.003$) than that during euthyroidism (1.2 ± 0.4 µg/l). Likewise, the mean AUC for GH above the zero-level during hypothyroidism (27.0 ± 10.0 µg/l $\times$ 6 h) was significantly higher ($P = 0.004$) than that during euthyroidism (11.7 ± 2.0 µg/l $\times$ 6 h). In contrast, the AUC for GH above the baseline was significantly lower ($P = 0.008$) during hypothyroidism (2.4 ± 0.8 µg/l $\times$ 6 h) than during euthyroidism (4.5 ± 1.8 µg/l $\times$ 6 h). The GH pulse frequency in the 6 h secretory patterns varied from one to ten peaks prior to thyroidectomy, and from two to five peaks after induction of hypothyroidism (Table 1). The mean GH pulse frequency in the euthyroid state (6 ± 3 pulses/6 h) was higher than that in the hypothyroid (4 ± 1 pulses/6 h), but this difference was not statistically significant ($P = 0.24$). The mean plasma IGF-I level was significantly higher

Table 1 Characteristics of the 6 h secretory profiles of GH and the plasma IGF-I levels in seven beagle bitches during euthyroidism and hypothyroidism. Basal GH, basal plasma GH level; AUC(0), AUC above zero-level; AUC (baseline), AUC above baseline; frequency, GH pulse frequency per 6 h. The values are expressed as means±S.E.M.

	Basal GH (µg/l)	AUC(0) (µg/l × 6 h)	AUC(baseline) (µg/l × 6 h)	Frequency (pulses/6 h)	IGF-I (µg/l)
Dog 1					
Euthyroid	1.2	9.0	1.8	1	91
Hypothyroid	2.6	17.9	2.2	5	169
Dog 2					
Euthyroid	0.8	10.1	5.4	8	121
Hypothyroid	3.5	23.3	2.5	5	193
Dog 3					
Euthyroid	1.8	13.2	2.7	4	91
Hypothyroid	4.6	29.1	1.7	4	169
Dog 4					
Euthyroid	1.5	13.4	4.2	3	111
Hypothyroid	3.1	20.8	2.2	5	100
Dog 5					
Euthyroid	1.4	13.3	5.1	8	100
Hypothyroid	7.4	47.2	3.1	2	227
Dog 6					
Euthyroid	1.0	13.1	7.3	10	76
Hypothyroid	4.4	30.1	3.6	5	202
Dog 7					
Euthyroid	0.9	9.8	4.6	9	92
Hypothyroid	3.2	20.3	1.4	4	121

($P=0.009$) in the hypothyroid state (169 ± 45 µg/l) than in the euthyroid (97 ± 15 µg/l).

Discussion

The results of this study demonstrate that in dogs primary hypothyroidism is associated with elevated GH secretion. In contrast to observations in other mammals, in dogs both basal and integrated GH levels were significantly higher in hypothyroidism than in euthyroidism. In some mammals, such as sheep and steers, primary hypothyroidism does not seem to affect GH secretion (Elsasser *et al.* 1992, Fletcher & Clarke 1994), whereas in rats (Varela *et al.* 1991, Santini *et al.* 1993, Mizobuchi *et al.* 1996, Tam *et al.* 1996, Osorio *et al.* 1998) and pigs (Morovat & Dauncey 1998) primary hypothyroidism is associated with low circulating GH levels.

The human pituitary GH gene contains a positive thyroid hormone response element (TRE) (Chomczynski *et al.* 1993). In line with this observation, experimentally induced thyroid hormone excess leads to increased GH secretion in healthy men (Lovejoy *et al.* 1997). It is likely that the low thyroid hormone levels of hypothyroidism will result in a low expression of pituitary GH in species with a positive TRE in the GH gene. Indeed, in hypothyroid rats both decreased pituitary content of GH mRNA

(Martinoli & Pelletier 1989, Lloyd *et al.* 1990, Tam *et al.* 1996) and reduced pituitary GH content (Szabo *et al.* 1985, Katakami *et al.* 1986, Wood *et al.* 1987, Lloyd *et al.* 1990, Varela *et al.* 1991) have been reported. The lack of a decrease in GH biosynthesis and release in dogs with primary hypothyroidism may be explained by the absence of a positive TRE within the canine GH gene. Alternatively, the presence of a negative TRE within the GH gene would result in a reduced suppression of GH expression, i.e. elevated GH production and secretion in hypothyroid animals. The presence of negative TREs has been demonstrated in different genes, for example in the TSH-releasing hormone (TRH) gene (Hollenberg *et al.* 1995), the TSHbeta gene (Breen *et al.* 1997) and in keratin genes (Radoja *et al.* 1997). Sequence analysis of the promoter region and intron 1 of the canine GH gene (Lantinga-van Leeuwen & Mol 2000) has not demonstrated the existence of a TRE in this species. However, gel shift assays or footprints have not been performed yet in the dog.

Apart from direct effects in transcription of the GH gene, it is possible that hypothyroidism affects GH release via an influence on the hypothalamic hormones GHRH and SS. In rats, primary hypothyroidism is associated with a reduced hypothalamic content of SS mRNA and SS (Tam *et al.* 1996). Moreover, thyroidectomy in rats leads to decreased SS release from the hypothalamus (Mizobuchi

et al. 1996). Therefore, it may be postulated that the hypothalamic SS secretion is also reduced in dogs with primary hypothyroidism, which may in turn explain the elevated basal GH secretion in this species.

Another option that should be discussed concerns the feedback signal of the low thyroxine concentration on the release of hypothalamic TRH. In healthy humans administration of TRH does not result in appreciable changes in plasma GH levels (Faggiano *et al.* 1985, Coiro *et al.* 1994, Hanew *et al.* 1995), whereas in euthyroid rats stimulation with TRH elicits no (Varela *et al.* 1991) or only a small transient (Szabo *et al.* 1985) rise in plasma GH levels. In contrast, in humans and rats with primary hypothyroidism TRH administration results in significantly increased plasma GH levels (Faggiano *et al.* 1985, Varela *et al.* 1991, Coiro *et al.* 1994, Hanew *et al.* 1995). In line with these observations, Konaka *et al.* (1997) have demonstrated the expression of TRH receptor mRNA in rat somatotrophs. The paradoxical response of GH to TRH in primary hypothyroidism may be caused by the relative deficiency of SS discussed in the previous paragraph, as SS infusion inhibits the GH response to TRH in hypothyroid human patients (Baldini *et al.* 1992). Also, in healthy dogs TRH administration does not result in changes in the plasma GH level (Rutteman *et al.* 1987). However, so far there is no information on the effect of TRH on GH secretion in dogs with primary hypothyroidism. Nevertheless, it may be hypothesized that also in hypothyroid dogs the elevated release of TRH not only causes increased TSH secretion but may also give rise to elevated GH secretion.

In agreement with previous publications (Takahashi *et al.* 1981, French *et al.* 1987, Kooistra *et al.* 2000), in the present dogs GH was secreted in a pulsatile fashion. The results demonstrate that primary hypothyroidism in dogs is associated with changes in the pulsatile secretion pattern of GH. The mean AUC for GH above the baseline was significantly lower during hypothyroidism than in the euthyroid state. In combination with an unchanged GH pulse frequency this indicates that in hypothyroid dogs less GH is released per pulse. This may be explained by a blunted response of the pituitary somatotrophs to GHRH. Indeed, administration of GHRH results in a markedly reduced GH response in hypothyroid humans (Root *et al.* 1985, Williams *et al.* 1985, Damjanovic *et al.* 1996, Pimentel-Filho *et al.* 1997), hypothyroid rats (Williams *et al.* 1985, Tam *et al.* 1996) and hypothyroid sheep (Fletcher & Clarke 1994). Moreover, in hypothyroid rats a reduced pituitary content of GHRH receptor mRNA has been reported (Tam *et al.* 1996, Korytko & Cuttler 1997).

The reduction in GH secreted in pulses in the hypothyroid dogs may also be explained by increased IGF-I secretion. The results demonstrate that the plasma IGF-I levels were significantly higher in hypothyroidism than in euthyroidism. Elevated circulating levels of IGF-I have been reported to inhibit pulsatile pituitary GH secretion

(Hartman *et al.* 1993), mainly by attenuation of spontaneous GH pulse amplitude (Bermann *et al.* 1994).

Finally, the reduction in GH secreted in pulses in hypothyroid dogs may be ascribed to extra-pituitary secretion of GH. Decreased GH pulsatility has been reported in women during the second half of pregnancy (Eriksson *et al.* 1989). In these women the loss of GH pulsatility is due to the release of a placental GH variant (Eriksson *et al.* 1989). In addition, a reduction in GH secreted in pulses has been reported in bitches during the progesterone phase of the oestrous cycle (Kooistra *et al.* 2000) and in bitches treated with progestagens (Watson *et al.* 1987). Endogenous progesterone and exogenous progestagens in these bitches result in excessive GH secretion (Eigenmann *et al.* 1983) originating from foci of hyperplastic ductular epithelium of the mammary gland (Selman *et al.* 1994, Van Garderen *et al.* 1997). The loss of GH pulsatility in both humans and bitches has been ascribed to the negative feedback effects of non-episodically secreted extra-pituitary GH. However, it is very unlikely that this mechanism was operational in the present dogs as only ovariectomized bitches were used.

The endocrine effects of GH can be divided into rapid catabolic actions, mainly due to insulin antagonism, and into slow anabolic actions. The slow hypertrophic effects of GH are mediated via IGF-I. In this respect it is interesting to note that children with primary hypothyroidism have low plasma IGF-I levels compared with concentrations during therapy with L-thyroxine (Chernausk & Turner 1989). Reduced circulating IGF-I levels have also been reported in hypothyroid rats (Osorio *et al.* 1998, Ramos *et al.* 1998). The low circulating IGF-I levels probably reflect the reduced GH secretion in these children and rats. In contrast, the results of this study demonstrate that the plasma IGF-I levels in dogs with primary hypothyroidism were significantly higher than those in the euthyroid state. The significantly elevated plasma IGF-I levels indicate that the elevated GH secretion in the hypothyroid dogs has systemic endocrine effects. Another indication that the elevated GH secretion has endocrine significance concerns the physical changes observed in dogs with primary hypothyroidism. Both in dogs with acromegaly (Rijnberk 1996) and in dogs with primary hypothyroidism, the physical features are characterized by thick skin folds. In acromegaly the skin thickening has been ascribed to mucopolysaccharide accumulation (Matsuoka *et al.* 1982). Similarly, in dogs with primary hypothyroidism the thickening of the skin is also due to mucopolysaccharide accumulation (Muller *et al.* 1989, Doliger *et al.* 1995). Thus, some of the physical changes observed in dogs with primary hypothyroidism might very well be acromegalic changes.

To summarize, the results of this study demonstrate that primary hypothyroidism in dogs is associated with elevated basal GH secretion and less GH secreted in pulses. This elevated GH secretion has endocrine significance as

illustrated by elevated plasma IGF-I levels and some physical changes mimicking acromegaly. It is discussed that the increased GH release in hypothyroid dogs is the result of the absence of a response element for thyroid hormone at the canine pituitary GH gene and alterations in supra-pituitary regulation.

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