Chapter 3

Pulsatile secretion pattern of growth hormone in dogs with pituitary-dependent hyperadrenocorticism

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Abstract

The amplitude and frequency of growth hormone (GH) secretory pulses are influenced by a variety of hormonal signals, among which glucocorticoids play an important role. The aim of this study was to investigate the pulsatile secretion pattern of GH in dogs in which the endogenous secretion of glucocorticoids is persistently elevated, i.e., in dogs with pituitary-dependent hyperadrenocorticism (PDH). Blood samples for the determination of the pulsatile secretion pattern of GH were collected at 10-min intervals between 0800h and 1400h in sixteen dogs with PDH and in six healthy control dogs of comparable age. The pulsatile secretion patterns of GH were analyzed using the Pulsar programme.

Growth hormone was secreted in a pulsatile fashion in both dogs with PDH and control dogs. There was no statistical difference between the mean (± SEM) basal GH level in dogs with PDH (0.7 ± 0.1 µg/l) and the control dogs (0.6 ± 0.1 µg/l). The mean area under the curve above the zero-level (AUC_0) for GH in dogs with PDH (4.6 ± 0.6 µg/lx6h) was significantly lower than that in the control dogs (7.3 ± 1.0 µg/lx6h). Likewise, the mean area under the curve above the baseline (AUC_{base}) for GH in dogs with PDH (0.6 ± 0.1 µg/lx6h) was significantly lower than that in the control dogs (3.7 ± 1.0 µg/lx6h). The median GH pulse frequency in the dogs with PDH (2 pulses/6h, range 0 to 7 pulses/6h) was significantly lower ($P = 0.04$) than that (5 pulses/6h, range 3 to 9 pulses/6h) in the control group

The results of this study demonstrate that PDH in dogs is associated with less GH secreted in pulses than in control dogs, whereas the basal plasma GH concentrations were similarly low in both groups. It is discussed that the impaired pulsatile GH secretion in dogs with PDH is the result of alterations in function of pituitary somatotrophs and changes in supra-pituitary regulation.

Introduction

Like the other hormones of the canine pituitary anterior lobe, growth hormone (GH) secretion is pulsatile in nature (Takahashi et al., 1981; Kooistra et al., 2000; Lee et al., 2001). Pituitary GH secretion is regulated predominantly by the opposing actions of the stimulatory hypothalamic peptide GH-releasing hormone (GHRH) and the inhibitory hypothalamic peptide somatostatin (SS). Each GH secretory episode seems to be initiated by a burst of GHRH into the hypophyseal portal system, preceded by a reduction of somatostatinergic input to the pituitary (Plotsky and Vale, 1985). In addition to these hypothalamic hormones, 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 influenced by a variety of hormonal signals, among which glucocorticoids play an important role (Devesa et al., 1992).

Glucocorticoids are important physiological regulators of GH synthesis and secretion. In humans and rats, glucocorticoids enhance GH gene transcription (Evans et al., 1982; Karin et al., 1990) and increase pituitary GHRH receptor numbers (Seifert et al., 1985; Miller and Mayo, 1997; Ohyama et al., 1998). Consequently, both spontaneous and GHRH-induced GH secretion are stimulated by acute administration of dexamethasone (Wehrenberg et al., 1983; Casanueva et al., 1990; Veldhuis et al., 1992). A minimum level of cortisol is essential for normal GH production. Individuals with hypoadrenocorticism (Addison's disease) may be GH-deficient because of poor GH synthesis (Allen, 1996). In line with this observation, humans with idiopathic adrenocorticotrophic hormone (ACTH) deficiency need appropriate glucocorticoid replacement to re-establish the normal pattern of GH response to stimulatory tests (Giustina et al., 1989). However, when in humans the endogenous secretion of glucocorticoids is persistently elevated, as in Cushing's disease, the spontaneous and stimulated GH secretion are blunted (Takahashi et al., 1992; Magiakou et al., 1994; Borges et al., 1997).

In dogs, pituitary-dependent hyperadrenocorticism (PDH) is one of the most common endocrine diseases and has many similarities with Cushing's disease in humans (Kemppainen and Peterson, 1994). Consequently, changes in pituitary GH secretion may also be expected in dogs with PDH. Indeed, it has been reported that basal plasma GH levels are lower and stimulated GH secretion is blunted in dogs with PDH (Peterson and Altszuler, 1981; Regnier and Garnier, 1995; Meij et al., 1997). However, information on changes in the spontaneous pulsatile secretion pattern of GH in dogs with PDH is lacking. Therefore, we investigated the

6-h pulsatile secretion pattern of GH in sixteen dogs with PDH and compared the characteristics of these secretory profiles with those of six healthy control dogs of comparable age.

Materials and methods

Dogs

A total of six female and ten male dogs with PDH and six healthy Beagle bitches were used in this study. The mean (± SEM) age (8.3 ± 0.5 years; median 8 years, range 5-12 years) and the mean (± SEM) body weight (15.7 ± 1.7 kg, median 14 kg, range 7-30 kg) of the dogs with PDH did not differ significantly from those of the control dogs (7.8 ± 0.2 years, median 8 years, range 7-8.5 years, and 14.7 ± 0.6 kg, median 15 kg, range 12-17 kg, respectively).

The suspicion of hyperadrenocorticism was based upon medical history, physical examination, and results of routine haematological and biochemical testing. Hyperadrenocorticism was diagnosed when the mean corticoid/creatinine (C/C) ratio in two consecutive morning urine sample exceeded 10 x 10^{-6} (Rijnberk et al., 1988). Differentiation between PDH and hyperadrenocorticism due to an adrenocortical tumour was accomplished by administering, after collection of the second urine sample, three oral doses of dexamethasone 0.1 mg/kg body weight at 8-h intervals. When the C/C ratio in the third urine sample was less than 50 % of the mean of the first two samples, the dog was categorized as being responsive to dexamethasone and PDH was diagnosed (Rijnberk et al., 1988). In the dogs with less suppression of the third urinary C/C ratio, pituitary dependency was established by the finding of non-suppressed plasma ACTH levels (≥10 ng/l). The latter is justified because ectopic ACTH- or CRH-producing tumours have not been reported in dogs. In addition to these biochemical function tests, the diagnosis of PDH was supported by visualization of the adrenals by ultrasonography (Voorhout et al., 1990) and computed tomography of the pituitary gland (Kooistra et al., 1997; Meij et al., 1998).

Blood sample collection

Blood samples for the determination of the plasma concentration of GH were collected at 10-min intervals between 0800h and 1400h. Blood samples were collected by jugular venipuncture and immediately placed in chilled EDTA-coated tubes, and centrifuged. Plasma was stored at -20° C until assayed.

Hormone determination

Plasma ACTH concentrations were measured using a commercially available two-site immunoradiometric assay (IRMA) (Nichols Institute, Wijchen, The Netherlands). The intra- and interassay coefficients of variation were 3.2 % and 7.8 %, respectively, and the sensitivity was 1 ng/l. There was no cross-reaction between the antiserum and α-melanocyte-stimulating hormone or ACTH precursors (Findling and England, 1990).

Urinary corticoid concentrations were measured by radioimmunoassay (RIA) as described previously (Rijnberk et al., 1988). The intra- and interassay coefficients of variation were 6 % and 8 %, respectively, and the sensitivity was 1 nmol/l. The urinary corticoid concentration was related to the urinary creatinine concentration (Jaffé kinetic method, initial rate reaction) and the C/C ratio was calculated (Stolp et al., 1983; Rijnberk et al., 1988).

Plasma GH concentrations were measured by a homologous RIA as described by Eigenmann and Eigenmann (1981). The intra- and interassay coefficients of variation were 3.8 % and 7.2 %, respectively. The sensitivity of the assay was 0.3 μg/l.

Statistical analysis

The 6-h secretion patterns of GH were analyzed using the Pulsar programme developed by Merriam and Wachter (1982). The programme identifies secretory peaks by height and duration from a smoothed baseline, using the assay standard deviation (SD) as a scale factor. The cut-off parameters G1-G5 of the Pulsar programme were set at 3.98, 2.4, 1.68, 1.24, and 0.93 times the assay SD 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 5h. The weight assigned to peaks was 0.05. The A, B, and C values of the Pulsar programme used to calculate the variance of the assay, were set at A=0, B=7.2, and C=5. The values extracted from the Pulsar analyses included the overall mean of the smoothed baseline, the number of peaks, the area under the curve above the zero level (AUC_0), and the AUC above the baseline (AUC_{base}).

Differences in parameters between control dogs and dogs with PDH were evaluated by the unpaired Student's t-test (two-tailed). Since the data were not assumed to be normally distributed, differences in GH pulse frequency were determined by non-parametric analysis, using Wilcoxon-Mann-Whitney test. Values are expressed as mean ± SEM or range or as median and range. $P < 0.05$ was considered significant.

Ethics of the study

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

Results

The mean basal urinary C/C ratio ranged from 23 to 301 x 10^{-6} in the dogs with PDH. In thirteen of the sixteen dogs with PDH oral dexamethasone suppressed the urinary C/C ratio to less than 50 % of the mean basal urinary C/C ratio of the first two days (range 3 to 33 %). In the remaining three dogs the urinary C/C ratios after oral dexamethasone were 57 %, 85 %, and 105 % of the mean basal urinary C/C ratio of the first two days. The plasma ACTH concentrations in these three dogs were 90 ng/l, 111 ng/l, and 129 ng/l, respectively.

Growth hormone was secreted in a pulsatile fashion in both dogs with PDH and control dogs (Figure 1). There was no statistical difference ($P = 0.57$) between the mean (± SEM) basal GH level in dogs with PDH (0.7 ± 0.1 µg/l) and the control group (0.6 ± 0.1 µg/l). The mean AUC_0 for GH in dogs with PDH (4.6 ± 0.6 µg/lx6h) was significantly lower ($P < 0.05$) than that in the control group (7.3 ± 1.0 µg/lx6h). Likewise, the mean AUC_{base} for GH in dogs with PDH (0.6 ± 0.1 µg/lx6h) was significantly lower ($P = 0.03$) than that in the control group (3.7 ± 1.0 µg/lx6h). The median GH pulse frequency in the dogs with PDH (2 pulses/6h, range 0 to 7 pulses/6h) was significantly lower ($P = 0.04$) than that (5 pulses/6h, range 3 to 9 pulses/6h) in the control group (Table 1).

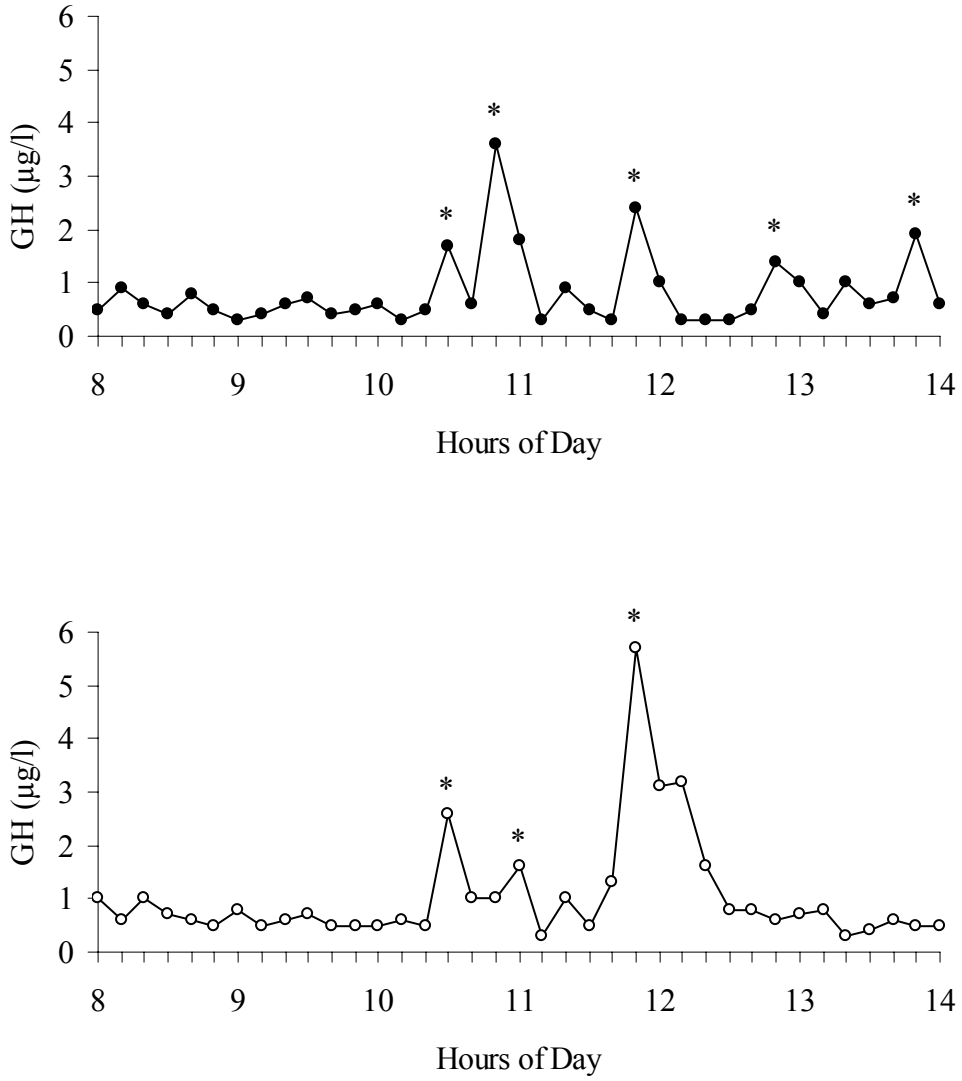

Figure 1. The 6-h secretory profile of GH in a healthy Beagle dog (●) and a dog with pituitary-dependent hyperadrenocorticism (○). Significant pulses, calculated by the Pulsar programme, are indicated with an asterisk.

Table 1. Characteristics of the 6-h secretory profiles of GH in sixteen dogs with pituitary-dependent hyperadrenocorticism and in six control dogs of comparable age. Basal GH = mean basal plasma GH level; AUC_0 = area under the curve above the zero-level for GH; AUC_{base} = area under the curve above the baseline for GH; Frequency = number of GH pulses per 6h.

	Basal GH (µg/l)	AUC_0 (µg/lx6h)	AUC_{base} (µg/lx6h)	Frequency (pulses/6h)
Dog with PDH				
Dog 1	1.2	7.5	0.2	0
Dog 2	0.5	4.1	1.2	5
Dog 3	0.3	3.0	1.1	6
Dog 4	0.3	2.2	0.4	2
Dog 5	0.5	2.9	0.1	0
Dog 6	0.5	3.4	0.3	2
Dog 7	0.6	3.6	0.4	2
Dog 8	0.4	4.2	1.8	7
Dog 9	1.2	7.9	0.6	3
Dog 10	0.8	5.6	0.8	5
Dog 11	0.3	2.0	0.2	1
Dog 12	0.5	4.9	1.8	5
Dog 13	0.5	3.3	0.4	2
Dog 14	1.2	7.2	0.2	0
Dog 15	0.3	2.5	0.6	3
Dog 16	1.6	9.8	0.2	0
Control dogs				
Dog 1	0.5	7.5	4.6	5
Dog 2	0.5	3.2	0.4	3
Dog 3	0.6	6.3	2.7	3
Dog 4	0.8	7.5	2.6	9
Dog 5	0.7	8.7	4.3	7
Dog 6	0.5	10.5	7.7	5

Discussion

The results of this study demonstrate that GH is secreted in a pulsatile fashion in both healthy dogs and dogs with PDH. However, the low AUC for GH, both above the zero-level and above the baseline, and the low GH pulse frequency compared with healthy dogs indicate that less GH is secreted in pulses in dogs with PDH. Spontaneous pulsatile GH secretion has also been reported to be suppressed in humans with PDH and rats treated for four weeks with high doses of glucocorticoids (Magiakou et al., 1994; Wajchenberg et al., 1996; Ohyama et al., 1997). The impaired pulsatile GH secretion may be the result of alterations in supra-pituitary regulation and changes at the level of the somatotroph.

The inhibitory effect of chronic hypercorticism on pituitary GH secretion involves, at least in part, enhancement of hypothalamic SS release (Wehrenberg et al., 1990; Wajchenberg et al., 1996; Terzolo et al., 2000). Support for the concept of the enhancement of SS tone by glucocorticoids comes from observations in rats that the hypothalamic content of immunoreactive SS (Nakagawa et al., 1987) and hypothalamic SS mRNA levels (Nakagawa et al., 1992) are increased following chronic dexamethasone administration. Further evidence for a regulatory role of glucocorticoids on GH secretion acting at the hypothalamic level is derived from *in vivo* studies in rats after passive immunization with anti-SS antibodies. This immunization reverses the inhibitory effect of high levels of circulating glucocorticoids on stimulated GH response (Wehrenberg et al., 1990; Mallo et al., 1993). However, pyridostigmine, which activates cholinergic synapses and thus suppresses hypothalamic SS release, does not modify plasma GH levels in humans with Cushing's syndrome (Leal-Cerro et al., 1990). Thus enhancement of hypothalamic SS release is not the only factor suppressing pituitary GH secretion in chronic hypercorticism. Inhibition of pituitary GH secretion may also be explained by a decrease in hypothalamic GHRH synthesis and secretion. Hypothalamic GHRH mRNA levels were indeed reduced in rats treated with high doses of glucocorticoids (Miell et al., 1991; Senaris et al., 1996; Ohyama et al., 1997).

In addition to their effect at the hypothalamic level, glucocorticoids may also influence GH secretion by acting directly at the pituitary level. Studies in humans, rats, and dogs have demonstrated that chronic glucocorticoid excess inhibits the GH response to GH-releasing stimuli, such as GHRH (Peterson and Altszuler, 1981; Wehrenberg et al., 1983; Hotta et al., 1988; Burguera et al., 1990; Voltz et al., 1995; Ohyama et al., 1997; Meij et al., 1997; Watson et al., 2000). In humans with Cushing's syndrome, blunted GH responses were also found to a synthetic hexapeptide (GHRP-6) which releases GH by a direct effect at the pituitary level through receptors other than GHRH receptors (Leal-Cerro et al., 1994). These observations suggest that chronic hypercorticism may also have a direct effect on pituitary somatotrophs, although the impaired response might also be the result of the persisting inhibitory effect of SS.

Although the GH response to GHRH is markedly impaired, an increase in the number of pituitary GHRH receptors has been reported in rats treated for four weeks with high doses of dexamethasone (Ohyama et al., 1997). The increase in the number of pituitary GHRH receptors may be caused by decreased GHRH secretion, since Miki et al. (1996) reported an increase in pituitary GHRH receptor mRNA levels in rats after immunoneutralization for GHRH. Therefore it may be hypothesized that post GHRH receptor signalling is impaired in

somatotrophs exposed to high doses of dexamethasone for long periods (Ohyama et al., 1997). The decrease in hypothalamic GHRH secretion may result in a lack of priming of the somatotrophs and, subsequently, in reduced GH synthesis and secretion and decreased responsiveness to exogenously administered GHRH (Thakore and Dinan, 1994). This concept is supported by the observation that there was a clear GH response to GHRH plus pyridostigmine in humans with Cushing's disease treated for one week with GHRH (Leal-Cerro et al., 1993). This suggests that blunted GH secretion in patients with Cushing's syndrome is at least partially mediated by decreased priming of the somatotrophs with endogenous GHRH. In addition, it has been demonstrated in young rats that administration of cortisone acetate decreases the number of somatotrophs in the pituitary gland (Niimi et al., 1993).

The suppressed pulsatile GH secretion in dogs with PDH may also be ascribed to obesity. Under the influence of glucocorticoid excess energy derived from protein catabolism is increased and the contribution from lipid oxidation is decreased. This effect leads to characteristic changes in body habitus that are frequently associated with glucocorticoid excess. Indeed, one of the cardinal physical features of dogs with hyperadrenocorticism is centripetal obesity (Rijnberk, 1996). Like chronic hypercorticism, obesity is associated with insulin-like growth factor-I (IGF-I)-mediated GH suppression (Magiakou et al., 1994) and blocked GH response to GH-releasing stimuli (Cordido et al., 1993). Elevated plasma free fatty acid levels (Leal-Cerro et al., 1997) and changes in circulating leptin levels (Pombo et al., 1999) may be contributing factors to the deranged GH secretion observed in humans with Cushing's syndrome. Leal-Cerro et al. (1998) demonstrated that in humans with Cushing's syndrome hyporesponsiveness of the somatotrophic cells to GHRH is improved after a short-term hypocaloric diet. However, in contrast to chronic hypercorticism (Leal-Cerro et al., 1994) intravenous administration of a combination of GHRH and GHRP-6 resulted in an elevated GH response in obese humans (Cordido et al., 1993), indicating that obesity is not the only explanation for the blocked GH response in chronic hypercorticism.

In contrast to observations of Meij et al. (1997), in the present study the basal plasma GH concentrations were not different in the dogs with PDH compared to the control dogs. However, the basal plasma GH levels in the study of Meij et al. (1997) were derived from only three blood samples collected at 15-min intervals. Therefore, it is possible that pulses may have contributed to the higher basal plasma GH levels in the control dogs in the study of Meij et al. (1997). In addition, in the study of Meij et al. (1997) the basal plasma GH levels in dogs with PDH (median age 10 years) were compared with those of control dogs with a

median age of two years. The significantly lower basal plasma GH levels in dogs with PDH may therefore have been caused by the effects of ageing. Indeed, it has been reported that GH secretion is decreased in elderly humans (Finkelstein et al., 1972; Zadik et al., 1985; Wilshire et al., 1995) and aged rats (Sonntag et al., 1980; De Gennaro Colonna et al., 1994). Also in the dog there are indications that ageing is associated with impaired GH secretion. The basal plasma GH levels in the control dogs in the present study were lower than those in young adult bitches (Kooistra et al., 2000; Lee et al., 2001) employing the same GH assay. The decreased GH secretion in elderly humans has been ascribed to the blunted response of GH to GHRH (Shetty and Duthie, 1995) and feedback disruption of the regulatory GH-IGF-I system (Veldhuis, 1997). The decreased GH secretion in elderly rats is associated with decreased pituitary GH content (Sonntag et al., 1980), reduced pituitary GH mRNA (Takahashi et al., 1990), and reduction of hypothalamic GHRH mRNA levels (De Gennaro Colonna et al., 1994).

With regard to the similar basal plasma GH levels in dogs with PDH and control dogs, it is important to notice that these values were close to the sensitivity of the GH assay used in the present study. Although the results of this study indicate that the basal plasma GH levels were similarly low in both groups, an ultrasensitive GH assay may be needed to confirm this observation.

In conclusion, the results of this study demonstrate that PDH in dogs is associated with less GH secreted in pulses, whereas the basal plasma GH concentrations were similarly low in both groups. The impaired pulsatile GH secretion in dogs with PDH may be the result of alterations in function of pituitary somatotrophs and changes in supra-pituitary regulation.

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