Pulsatile secretion pattern of growth hormone during the luteal phase and mid-anoestrus in beagle bitches

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The pulsatile secretion pattern of growth hormone was investigated during four stages of the luteal phase and during mid-anoestrus in six cyclic beagle bitches. Plasma samples were obtained via jugular venepuncture at 10 min intervals for 12 h at 19 ± 2 (mean ± SEM; luteal phase 1), 38 ± 2 (luteal phase 2), 57 ± 2 (luteal phase 3), 78 ± 2 (luteal phase 4) and 142 ± 4 days (mid-anoestrus) after ovulation. During all stages, growth hormone was secreted in a pulsatile fashion. The mean basal plasma growth hormone concentration during luteal phase 1 (2.2 ± 0.3 μg l⁻¹) was significantly higher than that during luteal phase 4 (1.5 ± 0.1 μg l⁻¹) and mid-anoestrus (1.4 ± 0.2 μg l⁻¹). The mean area under the curve (AUC) above zero during luteal phase 1 (27.3 ± 2.7 μg l⁻¹ in 12 h) tended to be higher than that during luteal phase 4 (20.8 ± 1.8 μg l⁻¹ in 12 h) and mid-anoestrus (19.2 ± 2.5 μg l⁻¹ in 12 h). In contrast, the mean AUCs above the baseline during luteal phase 1 (1.1 ± 0.5 μg l⁻¹ in 12 h) and luteal phase 2 (1.2 ± 0.5 μg l⁻¹ in 12 h) were significantly lower than that during luteal phase 4 (2.8 ± 0.5 μg l⁻¹ in 12 h).

Conclusion: The pulsatile secretion pattern of growth hormone changes during the luteal phase in healthy cyclic bitches: basal growth hormone secretion is higher and less growth hormone is secreted in pulses during stages in which the plasma progesterone concentration is high. It is hypothesized that this change is caused by a partial suppression of pituitary growth hormone release by progesterone-induced growth hormone production in the mammary gland. The progesterone-induced production of growth hormone in the mammary gland may promote the physiological proliferation and differentiation of mammary gland tissue during the luteal phase of the bitch by local autocrine–paracrine effects. In addition, progesterone-induced mammary growth hormone production may exert endocrine effects, such as hyperplastic changes in the uterine epithelium and insulin resistance.

Introduction

The oestrous cycle of the domestic bitch is characterized by a follicular phase, spontaneous ovulations, a luteal phase of an average duration of about 75 days, and a non-seasonal anoestrus of variable duration of 2–10 months (Concannon, 1993, Schaefers-Okkens, 1996). In contrast to most other mammalian species, the duration of the luteal phase in the bitch is comparable to that of the pregnant bitch (Edqvist et al., 1975; Austad et al., 1976; Concannon et al., 1977a). In addition, plasma progesterone concentrations do not differ significantly between pregnant and non-pregnant bitches (Onclin and Verstegen, 1997). This long exposure to high circulating progesterone concentrations during each oestrous cycle may result in disorders such as cystic endometrial hyperplasia–endometritis (Dow, 1958), insulin resistance and diabetes mellitus (Eigenmann et al., 1983), and acromegaly (Eigenmann et al., 1983).

Acromegaly is a syndrome of tissue overgrowth and insulin resistance due to excessive growth hormone production. In dogs, the excessive production of growth hormone can be induced either by endogenous progesterone or by exogenous progestagens used for prevention of oestrus (Eigenmann et al., 1983). The progestagen-induced growth hormone production in this species originates from foci of hyperplastic ductular epithelium of the mammary gland (Selman et al., 1994; Van Garderen et al., 1997). In contrast to pulsatile growth hormone secretion in healthy dogs (Takahashi et al., 1981; French et al., 1987), in bitches with acromegaly, the progestagen-induced plasma growth hormone concentrations do not have a pulsatile secretion pattern (Watson et al., 1987). In addition, the progestagen-induced overproduction of growth hormone cannot be stimulated with growth hormone-releasing hormone (GHRH) or the α-adrenergic agonist clonidine, and cannot be inhibited by somatostatin (Watson et al., 1987; Selman et al., 1991).

In normal cyclic bitches, mean basal plasma growth
hormone concentrations are higher during the luteal phase than during anoestrus (Selman et al., 1991). Moreover, the responsiveness of growth hormone release to stimulation with both GHRH and clonidine is lower during the luteal phase than during anoestrus (Selman et al., 1991). Thus it can be postulated that, in normal cyclic bitches, there is also circulating growth hormone from the mammary gland during the luteal phase, which suppresses the release of pituitary growth hormone. If this is true, then there should not only be a diminished response to supra-pituitary stimulants but also a loss of growth hormone pulsatility. The present study was designed to investigate the pulsatile secretion pattern of growth hormone in the normal cyclic bitch during different stages of the luteal phase and during mid-anoestrus.

Materials and Methods

Animals and collection of blood samples

Six healthy beagle bitches 2–5 years of age were used in this study. All dogs had been born and raised in the Department of Clinical Sciences of Companion Animals and were accustomed to the laboratory environment and procedures such as collection of blood. They were housed singly or in pairs in indoor–outdoor runs, fed a standard commercial dog food once a day and were given water ad libitum.

All dogs were examined three times per week for the presence of swelling of the vulva and serosanguinous vaginal discharge, which were considered to signify the onset of pro-oestrus. Plasma concentrations of progesterone were determined three times per week from the start of pro-oestrus until the day on which the plasma progesterone concentration exceeded 16 nmol l–1, which is when ovulation is assumed to occur (Concannon et al., 1977b; Wildt et al., 1979; Okkens et al., 1985a).

The secretory profiles of growth hormone were determined at 19 ± 2 (mean ± SEM; luteal phase 1), 38 ± 2 (luteal phase 2), 57 ± 2 (luteal phase 3), 78 ± 2 (luteal phase 4), and 142 ± 4 days (mid-anoestrus) after the estimated day of ovulation. The 12 h secretory profiles of growth hormone were determined from 08:00 h until 20:00 h. In the first blood sample the plasma progesterone concentration was also measured. Blood samples were collected via jugular venepuncture at 10 min intervals, immediately placed in chilled EDTA-coated tubes, and centrifuged at 4°C for 10 min at 3000 g. Plasma was stored at −25°C until assayed.

Ethics of experimentation

This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Utrecht University.

Hormone determinations

Plasma growth hormone concentrations were measured in a homologous radioimmunoassay (Eigenmann and Eigenmann, 1981). The intra-assay and interassay coefficients of variation were 3.8% and 7.2%, respectively, and the sensitivity of the assay was 0.3 μg l–1.

Plasma concentrations of progesterone were determined by a validated radioimmunoassay (Dieleman and Schoenmakers, 1979; Okkens et al., 1985b). The intra-assay and interassay coefficients of variation were 11% and 14%, respectively. The lowest detectable amount was 0.13 nmol l–1.

Data analysis

The secretory profiles of growth hormone were analysed by means of the Pulsar programme developed by Merriam and Wachtler (1982). The programme 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 programme were set at 3.98, 2.40, 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. The smoothing time, a window used to calculate a running mean value omitting peaks, was set at 10 h. The splitting cut-off parameter was set at 0.5 and 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 = 0.0, B = 7.2 and C = 5.0. The values extracted from the Pulsar analyses included the mean of the smoothed baseline, the mean pulse amplitude, the pulse frequency, the mean pulse duration, and the area under the curve (AUC). The AUC was calculated above zero as well as above the baseline.

Changes in the parameters of the secretory patterns during the different stages of the luteal phase and mid-anoestrus were evaluated by ANOVA for repeated measures for main effect of stage of cycle. Subsequently, multiple comparisons were performed for data with significant (P ≤ 0.05) main effect using the Student–Newman–Keuls test. Since the data were not assumed to be normally distributed, differences in pulse frequency were determined by non-parametric analysis, using the Friedman test, and multiple comparisons were performed using Dunnett’s test. Values are expressed as mean ± SEM. P values ≤ 0.05 were considered significant.

Results

Plasma progesterone concentrations were high during luteal phase 1 and decreased gradually during the subsequent stages of the luteal phase. During mid-anoestrus plasma progesterone concentrations were < 3 nmol l–1 (Table 1).

During all stages of the luteal phase and during mid-anoestrus, the secretion pattern of growth hormone was characterized by a fluctuating baseline with occasional distinct increases, indicating pulsatile secretion of growth hormone. A representative example of the pulsatile secretion of growth hormone during the different stages of the luteal phase and during mid-anoestrus is shown (Fig. 1). The growth hormone pulse frequency ranged from 0 to 10 peaks per 12 h, the growth hormone pulse duration ranged from 10 to 80 min, and the growth hormone pulse amplitude ranged
from 0.5 to 7.2 \( \mu g \cdot l^{-1} \). Pulse characteristics during the successive stages of the luteal phase and mid-anoestrus are shown (Table 1).

The mean basal plasma growth hormone concentration during luteal phase 1 was significantly higher \((P < 0.05)\) than that during luteal phase 4 and mid-anoestrus (Fig. 2). The mean AUC above zero during luteal phase 1 also tended to be higher than that during luteal phase 4 and mid-anoestrus, but this difference did not reach statistical significance. In contrast, the mean AUCs above the baseline during luteal phases 1 and 2 were significantly lower \((P < 0.02)\) than that during luteal phase 4 (Fig. 2). The median growth hormone pulse frequency and the mean growth hormone pulse amplitude did not differ significantly among the different stages of the luteal phase and mid-anoestrus. The mean growth hormone pulse duration during luteal phase 1 was significantly shorter \((P < 0.01)\) than that during mid-anoestrus and luteal phases 3 and 4.

**Discussion**

The results of this study demonstrate that growth hormone secretion in the bitch is pulsatile, as has been found for most of the hormones of the anterior lobe of the pituitary in this species (Kemppainen and Sartin, 1984; Kooistra et al., 1997a, 1999), and that the secretion pattern of growth hormone in the bitch is influenced by the gonadal sex steroid progesterone. High plasma progesterone concentrations were associated with higher basal growth hormone concentrations, a tendency to higher AUCs above zero, lower AUCs above the baseline, and shorter growth hormone pulse duration. Reports on pulsatile growth hormone secretion during the menstrual cycle in normal women also indicate an influence of progesterone on growth hormone secretion (Genazzani et al., 1975; Faria et al., 1992). Moreover, in normal women, growth hormone responses to the indirect cholinergic agonist pyridostigmine are markedly influenced by the menstrual cycle phase, increasing incrementally from the early follicular phase through the mid-cycle to the luteal phase of the menstrual cycle (O’Keane and Dinan, 1992). On the basis of these data, progesterone is considered to be an important modulator of growth hormone secretion in women.

The higher basal growth hormone secretion in the bitches during stages with a high plasma progesterone concentration may originate from the pituitary, an extrapituitary source, or both. The canine mammary gland expresses the gene encoding growth hormone (Mol et al., 1996) and its expression is highly stimulated by progestagens (Selman et al., 1994). As pituitary and mammary growth hormone are identical (Selman et al., 1994), it is likely that at least part of the growth hormone produced during the luteal phase originates from the mammary gland. The lower AUCs above the baseline during luteal phases 1 and 2 compared with that during luteal phase 4 indicate that less growth hormone is secreted in pulses during stages in which the plasma progesterone concentration is high. This finding supports an extra-pituitary origin because it has been reported that progestagen-induced growth hormone excess in the bitch is characterized by a loss of pulsatile growth hormone secretion (Watson et al., 1987). A lack of growth hormone pulsatility has also been reported in women during the second half of pregnancy (Eriksson et al., 1989). In these women, the loss of growth hormone pulsatility was due to the release of a placental growth hormone variant (Eriksson et al., 1989). The loss of growth hormone pulsatility in both humans and bitches may be ascribed to the negative feedback effects of non-episodically secreted extra-pituitary growth hormone. In humans, growth hormone exerts its negative feedback effect by a stimulation of hypothalamic somatostatin secretion (Bereelowitz et al., 1981). In addition, extra-pituitary growth hormone may result in increased circulating concentrations of insulin-like growth factor I (IGF-I), which also inhibits pulsatile pituitary growth hormone secretion (Hartman et al., 1993).

In contrast to the basal plasma growth hormone concentration, differences in the AUC above zero just failed to reach statistical significance when luteal phase 4 and mid-anoestrus were compared with luteal phase 1. This may be

<table>
<thead>
<tr>
<th>Days after ovulation</th>
<th>Luteal phase 1</th>
<th>Luteal phase 2</th>
<th>Luteal phase 3</th>
<th>Luteal phase 4</th>
<th>Anoestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 ± 2</td>
<td>38 ± 2</td>
<td>57 ± 2</td>
<td>78 ± 2</td>
<td>142 ± 4</td>
<td></td>
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<tr>
<td>Progesterone (nmol l⁻¹)</td>
<td>123 ± 15b</td>
<td>56 ± 9b</td>
<td>20 ± 3b</td>
<td>9 ± 3b</td>
<td>0.9 ± 0.2b</td>
</tr>
<tr>
<td>Basal GH (µg l⁻¹)</td>
<td>2.2 ± 0.3b</td>
<td>1.9 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.1a</td>
<td>1.4 ± 0.2b</td>
</tr>
<tr>
<td>Area under the curve for GH above zero (µg l⁻¹ in 12 h)</td>
<td>27.3 ± 2.7</td>
<td>23.6 ± 1.9</td>
<td>21.2 ± 1.7</td>
<td>20.8 ± 1.8</td>
<td>19.2 ± 2.5</td>
</tr>
<tr>
<td>Areas under the curve for GH above baseline (µg l⁻¹ in 12 h)</td>
<td>1.1 ± 0.5b</td>
<td>1.2 ± 0.5b</td>
<td>1.5 ± 0.3</td>
<td>3.3 ± 1.0b</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>GH pulse frequency (peaks per 12 h)</td>
<td>2.0 ± 0.7</td>
<td>2.7 ± 1.5</td>
<td>3.0 ± 0.8</td>
<td>4.8 ± 1.2</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>GH pulse duration (min)</td>
<td>11 ± 4ab</td>
<td>23 ± 8</td>
<td>34 ± 8b</td>
<td>34 ± 6b</td>
<td>41 ± 8c</td>
</tr>
<tr>
<td>GH pulse amplitude (µg l⁻¹)</td>
<td>1.8 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>1.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.4</td>
</tr>
</tbody>
</table>

The secretory profiles were determined during four different stages of the luteal phase and during anoestrus (indicated as days after the estimated day of ovulation). Values are expressed as mean ± SEM. Within rows, means with the same superscript are significantly different \((P < 0.05)\).

Table 1. Plasma progesterone concentration and pulse characteristics of the 12 h secretory profile of growth hormone (GH) in six beagle bitches.
due to the fact that a high plasma progesterone concentration is associated with two opposite effects: a high basal plasma growth hormone concentration and a lower AUC above the baseline. In other words, during stages in which the plasma progesterone concentration is high, the higher basal growth hormone secretion is accompanied by less growth hormone secreted in pulses, that is, of pituitary origin.

With regard to the physiological role of the progesterone-induced mammary growth hormone production, local autocrine and paracrine effects in the mammary gland as well as systemic endocrine effects have to be considered. It is thought that the progesterone-induced growth hormone production in the mammary gland leads to local production of IGFs, whereby the growth promoting effect is modulated by IGF-binding proteins synthesized locally (Mol et al., 1996). Thus a proliferative environment for the glandular epithelium is created, that is, the autocrine–paracrine background for the physiological proliferation and differentiation of mammary tissue during the luteal phase of the oestrous cycle of the bitch. This contention is in agreement with studies in rats (Feldman et al., 1993; Walden et al., 1998), which demonstrated that growth hormone and growth hormone-induced IGFB-I are necessary for mammary development. Furthermore, in ewes, mammogenesis is partly dependent upon growth hormone (Kann, 1997). However, until now it has not been demonstrated in these species that this growth

Fig. 1. The secretory profile of growth hormone in a 4-year-old beagle bitch. Blood samples were collected at 10 min intervals for 12 h at (a) 16 days (luteal phase 1), (b) 37 days (luteal phase 2), (c) 54 days (luteal phase 3), (d) 74 days (luteal phase 4) and (e) 136 days (anoestrus) after the estimated day of ovulation. Plasma progesterone concentrations were (a) 173.0, (b) 66.0, (c) 25.0, (d) 9.0 and (e) 1.2 nmol l⁻¹. Asterisks show peaks identified by the Pulsar programme.
hormone originates from the mammary gland. The significance of growth hormone from the mammary gland in the bitch also extends beyond the cyclic effects on mamogenesis and thereby on galactopoiesis. High growth hormone concentrations are present in canine mammary gland secretions and particularly in colostrum, through which growth hormone may promote gastric and intestinal development in newborns (Schoenmakers et al., 1997).

Studies on progestagen-induced release of growth hormone from the mammary gland have highlighted the concept that the mammary gland should be regarded as an endocrine organ (Peaker, 1995). Two possible endocrine effects of mammary gland growth hormone will be discussed. Firstly, there is the effect on uterine epithelium. During each luteal phase, irrespective of whether the bitch is pregnant or not, progesterone-dependent uterine epithelial changes occur. In progestagen-treated dogs, the hyperplastic changes in the uterine epithelium are associated with the presence of immunoreactive growth hormone. However, local uterine production of growth hormone could not be demonstrated in these bitches and therefore the growth hormone in the uterine epithelium is likely to be of mammary origin (Kooistra et al., 1997b).

Secondly, there are metabolic effects. As indicated above, progestagen-induced growth hormone excess leads to insulin resistance (Eigenmann et al., 1983). The small increase in growth hormone exposure during the first half of the luteal phase may also cause some insulin resistance. For an animal of prey such as the dog, it may have been evolutionarily advantageous to have this temporary insulin resistance in a critical phase of life. Insulin resistance may serve as an extra safeguard against hypoglycaemia, especially when there are long intervals between feeding. Insulin resistance is the mechanism for coping with a shortage of dietary glucose (Miller and Colagiuri, 1994). It allows blood glucose concentrations to be maintained immediately after eating a low carbohydrate meal (prey), when insulin is secreted in response to other food components such as amino acids.

In conclusion, the results of this study indicate that the pulsatile secretion pattern of growth hormone changes during the luteal phase in normal cyclic bitches; basal growth hormone secretion is higher and less growth hormone is secreted in pulses during stages in which plasma progesterone concentration is high. The loss of growth hormone pulsatility is probably caused by partial suppression of pituitary growth hormone release by progesterone-induced growth hormone production in the mammary gland. The present results indicate that progesterone-induced growth hormone production in the mammary gland is not an aberration in middle-aged and older dogs but is a normal physiological event during the luteal phase of the oestrous cycle in healthy cyclic bitches.

The authors are grateful for the technical assistance of D. M. Blankenstein, Y. M. E. A. Pollak, M. E. van Wolfereen and H. G. H. van Engelen. The critical reading of the manuscript by B.E. Belshaw is highly appreciated.