# Chapter 11

# Summarizing discussion and conclusions

The pulsatile secretion of growth hormone (GH) is regulated predominantly by the opposing actions of the hypothalamic peptides GH-releasing hormone (GHRH) and somatostatin (SS), stimulating and inhibiting pituitary GH secretion, respectively (Tannenbaum and Ling, 1984). In addition, the pituitary secretion of GH is regulated by the negative feedback of both insulin-like growth factor-I (IGF-I) (Voss et al., 2000) and GH (Conway et al., 1985; Lanzi and Tannenbaum, 1992). The amplitude and frequency of GH secretory pulses are influenced by a variety of factors such as age, nutrition, body composition, exercise, and several hormones (Hartman, 2000).

In 1999, ghrelin, a 28-amino-acid peptide with strong GH-releasing activity was discovered. It is predominantly produced by the stomach. The action of this peptide is mediated by the activation of the GH secretagogue (GHS) receptor type 1a (Kojima et al., 1999). Before the discovery of ghrelin, this orphan receptor had been shown to be specific for a family of synthetic, peptidyl and nonpeptidyl GHSs such as GH-releasing peptide-6 (GHRP-6), hexarelin, and MK-0677 (Momany et al., 1981; Momany et al., 1984; Bowers, 1993). Apart from a potent GH releasing action, ghrelin has other activities including stimulation of appetite, control of energy balance, control of gastric motility, and influence on glucose metabolism. For a review on pituitary GH secretion and its regulation, and the diverse endocrine and nonendocrine effects of synthetic GHSs and ghrelin, the reader is referred to **Chapter 2** of this thesis.

Several pathological (e.g. obesity and chronic hypercortisolism) and non-pathological (e.g. ageing) states in humans are characterized by a reduction in pituitary GH secretion (Casanueva, 1992; Leal-Cerro et al., 1994). With regard to the effects of chronic glucocorticoid excess on the plasma GH profile, the results of the study described in **Chapter 3** demonstrate that GH is secreted in a pulsatile fashion in dogs with pituitary-dependent hypercortisolism. However, the pulsatile plasma GH profile in these dogs is characterized by less GH secreted per pulse, while the basal plasma GH concentration is similar to that of healthy control dogs of comparable age.

Chronic hypercortisolism in humans is not only associated with reduced pituitary GH release but also with an impaired GH response to various stimuli (Casanueva, 1992; Leal-Cerro et al., 1994). Even a combination of GHRH and GHRP-6, which is a very powerful GH-releasing stimulus, is unable to induce significant GH release in humans with Cushing's syndrome (Leal-Cerro et al., 1994). Also in dogs with pituitary-dependent hyperadrenocorticism (PDH) administration of GHRP-6 results in a blunted GH response compared to healthy dogs of similar age (**Chapter 4**). The GH response after administration

of ghrelin to dogs with PDH was also low but not significantly different from that in healthy dogs.

The impaired pulsatile GH secretion in dogs with PDH is not yet fully understood but may be ascribed to changes in supra-pituitary stimulation. Enhancement of hypothalamic SS release (Wehrenberg et al., 1990; Lima et al., 1993; Wajchenberg et al., 1996; Terzolo et al., 2000), a decrease in hypothalamic GHRH synthesis and secretion (Miell et al., 1991; Senaris et al., 1996; Ohyma et al., 1997), or a combination of both (Leal-Cerro et al., 1998) may be involved. In addition to their effect at the hypothalamic level, glucocorticoids may also influence GH secretion by acting directly at the pituitary level (Leal-Cerro et al., 1994). As mentioned above, chronic glucocorticoid excess results in a decreased response of GH to GH-releasing stimuli (Peterson and Altszuler, 1981; Wehrenberg et al., 1983; Hotta et al., 1988; Burguera et al., 1990; Voltz et al., 1995; Meij et al., 1997; Ohyama et al., 1997; Watson et al., 2000). It has been postulated 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 also result in a lack of priming of the somatotrophs and, subsequently, in reduced GH synthesis and secretion and decreased responsiveness to exogenous GHRH (Thakore and Dinan, 1994). Finally, it has been demonstrated that administration of glucocorticoids to young rats decreases the number of somatotrophs in the pituitary gland (Niimi et al., 1993).

A cardinal physical feature of PDH in dogs is centripetal obesity with abdominal enlargement (Rijnberk, 1996). As not only chronic hypercortisolism but also obesity is associated with an impaired GH response to GH-releasing stimuli (Bowers, 1993), it can be hypothesized that the suppressed GH release in Cushing's syndrome is related to obesity as well. However, in contrast to the situation of chronic hypercortisolism (Leal-Cerro et al., 1994), intravenous administration of the combination of GHRH and GHRP-6 results in an elevated GH response in obese humans (Bowers, 1993). This indicates that the impaired GH response in individuals with Cushing's syndrome cannot be explained solely by obesity.

Both basal and stimulated GH secretion as well as circulating IGF-I concentrations decline with age in several mammalian species (Finkelstein et al., 1972; Rudman, 1985; Zadik et al., 1985; Corpas et al., 1992; Wilshire et al., 1995; Muller et al., 2002; Lee et al., 2004). Little is known about how age affects the GH response to GH-releasing stimuli in dogs. The results of the study described in **Chapter 5** demonstrate the existence of age-related differences with regard to the GH-releasing activity of intravenously administered GHSs in dogs. In young and old healthy dogs, ghrelin caused a significant rise in plasma GH

concentrations when compared with the administration of 0.9 % NaCl. In young dogs, ghrelin was a more potent stimulator of GH release than GHRH and GHRP-6. In old dogs, however, GHRH administration caused higher elevations in plasma GH concentrations than GHRP-6 or ghrelin. These results also illustrate remarkable species-related differences, as studies in rats demonstrate that the GH-releasing potency of ghrelin is similar to that of GHRH (Kojima et al., 1999), whereas in humans ghrelin is a more potent stimulus of GH secretion than GHRH or the synthetic GHS hexarelin (Takaya et al., 2000; Arvat et al., 2001).

The mean ghrelin-induced plasma GH response was significantly lower in the old dogs than in the young dogs. The mean plasma GH concentration after GHRH and GHRP-6 administration was also lower in the old dogs compared with the young dogs, but this difference did not reach statistical significance. These observations are compatible with findings in humans, indicating that not only the GH-releasing effect of ghrelin (Broglio et al., 2003) but also that of GHRH and peptidyl or nonpeptidyl synthetic GHSs undergoes an age-related decrease (Bowers et al., 1992; Aloi et al., 1994; Chapman et al., 1996; Muccioli et al., 2002; Broglio et al., 2003). In old rats, the GH response to synthetic GHSs is impaired as well (Ceda et al., 1986; Walker et al., 1990). Also in old dogs, the GH responsiveness to the synthetic GHS hexarelin has been reported to be low (Cella et al., 1995). In humans, it has been demonstrated that the age-related reduction of both spontaneous and stimulated GH secretion reflects age-related changes in the neural control of somatotroph function (Giustina and Veldhuis; 1998; Ghigo et al., 1999). These changes include a concomitant reduction in the secretion of GHRH and enhancement in SS release (Kelijman, 1991; Giustina and Veldhuis; 1998; Ghigo et al., 1999; Muller et al., 1999). A recent study of the hypothalamic release of GHRH and SS in monkeys has demonstrated that the GHRH pulse frequency and amplitude and baseline GHRH levels are much lower in aged animals than in young adult animals. In contrast, the amplitude of SS pulses and baseline SS levels are significantly higher in aged monkeys than in young adult monkeys (Nakamura et al., 2003). It seems that an impairment of pituitary function does not play a major role (Muller et al., 1999). Repeated GHRH injections in elderly subjects, combined administration of GHRH and clonidine in old dogs, or GHRH + GHRP-6 injection in aged rats (Walker et al., 1991) significantly increases circulating GH concentrations (Cella et al., 1993; Nicolas et al., 1994). These observations support the idea that the pituitary somatotrophs retain their capacity to synthesize and secrete adequate concentrations of GH during ageing (Corpas et al., 1992; Cella et al., 1993; Muller et al., 1999; Muccioli et al., 2002). This despite the fact that the number and size of GH-producing cells in the human pituitary decrease with increasing age (Sun et al., 1984) and that

the decreased GH secretion in elderly rats is associated with reduced pituitary GH content (Sonntag et al., 1980), reduced pituitary GH mRNA (Takahashi et al, 1990), and low pituitary GHRH-receptor mRNA concentration (Kamegai et al., 1999). The diminished GH response to GHRH in aged humans, rats, and dogs indicates that pituitary somatotrophs also become less sensitive to GHRH in older individuals (Pavlov et al., 1986; Cella et al., 1989; Arce et al., 1990).

In the studies on the effects of GHSs on the release of adenohypophyseal hormones other than GH, interesting species-related differences were observed (**Chapter 5**). The action of ghrelin and GHRP-6 is GH-specific in old and young dogs, i.e., both stimulants did neither stimulate the pituitary-adrenocortical axis nor the release of thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and prolactin (PRL). These results are in line with observations in anaesthetized rats reported by Kojima et al. (1999), who found that intravenous administration of ghrelin specifically stimulated GH release but did not affect other adenohypophyseal hormones. However, Thomas et al. (1997) have shown that GHRP-6 mediates the release of ACTH and cortisol in conscious rats. Studies in healthy humans demonstrated that intravenous administration of ghrelin and synthetic GHSs apart from stimulating GH release, also increase circulating concentrations of PRL, adrenocorticotrophic hormone (ACTH), and cortisol (Arvat et al., 2001; Muccioli et al., 2002; Takaya et al., 2002).

The diagnosis of GH deficiency should be based upon the results of a stimulation test because basal plasma concentrations of GH and IGF-I may overlap between pituitary dwarfs and healthy individuals (Gill et al., 1998; Kooistra et al., 2000). In young dogs, ghrelin is a more potent stimulator of GH release than GHRH. Therefore ghrelin might be used to diagnose GH deficiency. We investigated the effects of intravenous administration of ghrelin on the plasma concentration of GH in German shepherd dogs with congenital combined pituitary hormone deficiency and in healthy dogs of a similar age (**Chapter 6**).

In none of the dwarf dogs ghrelin administration resulted in a rise of the plasma GH concentration above 5 µg/l. This finding corresponds with observations in humans with isolated childhood-onset GH deficiency, in whom the GH response to ghrelin is also markedly reduced (Aimaretti et al., 2002). However, in some of the healthy dogs the plasma GH concentration also remained low after ghrelin administration. Thus, while a ghrelin-induced plasma GH concentration higher than 5 µg/l seems to exclude GH deficiency, false-negative results may occur.

Through activation of pathways distinct from those needed for GH secretion, ghrelin causes weight gain by increasing food intake and reducing fat utilization. In several

mammalian species this gastric peptide seems to play a role in meal initiation (Kamegai et al., 2000; Tschop et al., 2000; Wren et al., 2000; Wren et al., 2001). **Chapter 7** is a report on investigations on the effects of food intake and fasting in healthy Beagle dogs. Therefore, the plasma concentrations of ghrelin, GH, IGF-I, glucose, and insulin were measured when food was administered at the usual time, after a 1-day fast, after a 3-day fast, and after re-feeding at the usual time the next day. In agreement with observations in rodents (Tschop et al., 2000; Asakawa et al., 2001), administration of a meal lowered plasma ghrelin concentrations and fasting increased plasma ghrelin concentrations in our dogs. The high plasma ghrelin concentrations during fasting fits in with a physiological role for this hormone in increasing appetite and initiation of food intake. Similar to the situation in rodents, circulating ghrelin concentrations in humans are rapidly suppressed by food intake, and 24-hour plasma ghrelin profiles reveal marked preprandial increases and postprandial decreases associated with every meal (Cummings et al., 2001).

In our dogs, the highest plasma ghrelin concentrations were observed immediately before the administration of food on the first day. Possibly this preprandial rise occurs as an anticipatory response to feeding as the dogs received their food for several years at the same time of the day. Sugino et al. (2002) demonstrated in sheep that expectation of food may stimulate ghrelin secretion . The transient increase in ghrelin secretion just before feeding is most likely elicited by a conditioned emotional response. It is well known that secretion of saliva and gastric acid preceding food intake is induced by a conditioned emotional response through the stimulation of the vagal nerve (Harding and Leek, 1973). Ghrelin secretion may be induced by the vagal system in the same manner as the secretion of saliva and gastric acid. Like in humans and rodents, also in our dogs the plasma ghrelin concentrations decreased shortly after food intake, but this decline did not reach statistical significance. In a very recent study in dogs this post-prandial decrease was found to be statistically significant. (Yokoyama et al., 2005). The mechanism by which nutrients suppress ghrelin concentrations are beginning to be elucidated; changes in plasma insulin concentrations, intestinal osmolarity, and enteric neural signalling probably play a role, whereas gastric distension, vagal nerve activity, and glucagon-like peptide-I are not required (Williams et al., 2003; Gelling et al., 2004).

In contrast with studies in humans and sheep (Cummings et al., 2001; Sugino et al., 2002), the results of the present study, did not provide evidence for an association between a preprandial rise in plasma ghrelin concentrations and a GH surge in dogs. Similar to the

situation in our dogs, a link between plasma ghrelin and plasma GH concentrations has not been demonstrated in cows (Miura et al., 2004).

In our dogs the plasma profiles of ghrelin on the one hand and the profiles of insulin and glucose on the other hand were reciprocal after food intake and fasting. These findings are in agreement with a study in humans, in which plasma ghrelin concentrations evolved oppositely to plasma insulin concentrations (Cummings et al., 2001). This raises the question whether insulin negatively regulates ghrelin or *vice versa*. The former hypothesis has been investigated by several groups (Saad et al., 2002; Flanagan et al., 2003; Kamegai et al., 2004). Taken together, these studies demonstrated that while insulin can suppress ghrelin release when administered in supraphysiologic doses or at high-normal concentrations for prolonged periods of time, physiological concentrations of insulin do not appear to regulate ghrelin release (Caixas et al., 2002; Schaller, et al., 2003; Soriano-Guillen et al., 2004). It has also been suggested that ghrelin may act as a counter-regulatory hormone blocking insulin secretion and insulin action to maintain blood glucose concentrations (Broglio et al., 2001; Cummings et al., 2005). Indeed, several studies have shown that ghrelin can inhibit glucose-mediated insulin secretion, both *in vitro* and *in vivo* (Egido, et al., 2002; Colombo, et al., 2003; Reimer, et al., 2003). Similarly, exogenous ghrelin administration decreases circulating insulin concentrations in mice (Reimer, et al., 2003) and humans (Broglio et al., 2003).

The production and release of GH has been demonstrated in a variety of human extra-pituitary tissues such as the central nervous system (Render et al., 1995) and the immune system (Clark, 1997; Van Buul-Offers and Kooijman, 1998). Expression of GH mRNA has also been found in bone marrow (Kooijman et al., 1997) and testis (Untergasser et al., 1997).

In dogs, a pre-eminent example of extra-pituitary GH production is the progestin-induced synthesis in the mammary gland (Selman et al., 1994a; Mol et al., 1995 a,b; Mol et al., 1996; van Garderen et al., 1997). In this species, mammary GH reaches the systemic circulation and may give rise to a syndrome of GH excess (Selman et al., 1994b). The progestin-induced elevations of plasma GH concentrations do not have a pulsatile pattern (Watson et al., 1987). Additionally, the progestin-induced GH overproduction can neither be stimulated with GHRH, nor can it be inhibited by SS (Watson et al., 1987; Selman et al., 1991). Endogenous progesterone and synthetic progestins, such as medroxyprogesterone acetate (MPA), primarily induce the expression of GH in areas of hyperplastic mammary epithelium, suggesting that locally produced GH promotes epithelial proliferation and differentiation in an autocrine and/or paracrine fashion (van Garderen et al., 1997).

Locally produced GH may also play a role in tumourigenesis in the mammary gland. GH expression has been found in benign and malignant mammary tumours of dogs, and in fibroadenomatous hyperplasia of the mammary gland of cats that have been treated with progestins (Mol et al., 1995a). In cats mammary GH does not seem to reach the systemic circulation. In woman GH is expressed in unaffected mammary tissue and in mammary neoplasms (Mol et al., 1995a). The GH genes expressed in mammary tissues of dogs and women are identical to the genes encoding GH in the pituitary gland (Mol et al., 1995 a,b). For an overview on the effects of progesterone and synthetic progestins in the bitch, the reader is referred to **Chapter 2** of this thesis.

In agreement with previous publications (Takahashi et al., 1981; French et al., 1987; Kooistra et al., 2000), the results of the study reported in **Chapter 8** demonstrate that GH is secreted in a pulsatile fashion in the bitch. Administration of MPA during one year resulted in higher basal plasma GH and IGF-I concentrations, higher area under the curves (AUCs) above the zero-level for GH, and lower AUCs above the baseline for GH (i.e., less GH secreted in pulses) in the healthy control dogs compared to dogs with a complete excision of the mammary gland. The findings in the control dogs are consistent with partial suppression of pituitary GH release by progestin-induced mammary-derived GH secretion and by elevated plasma IGF-I concentrations. Before treatment with MPA, in the anoestrous phase of the ovarian cycle, the mammary tissue of our dogs was inactive on histological examination. After one year of MPA administration, most of the glandular tissue had differentiated into lobulo-alveolar structures in which milk synthesis occurred, except in one dog where nodular epithelial proliferation resulting in ductal buds was present. These findings are in agreement with the the observations of van Garderen et al. (1997).

In canine mammary tissue immunoreactive GH (iGH) and GH gene expression is found predominantly in ductal epithelial buds during the early and midluteal phase of the ovarian cycle. The GH gene expression is diminished in differentiated lobulo-alveolar glandular tissue, and in the inactive tissue during the anoestrous phase of the canine ovarian cycle (van Garderen et al., 1997). Similarly, iGH was not detected in the mammary gland tissue of the anoestrous dogs before treatment with MPA. Additionally, iGH was absent in the mammary gland tissue of all control bitches treated for one year with MPA, except for one dog. In this dog, iGH appeared to be present only in hyperplastic ductular epithelium that consisted of more than 2 cell layers, i.e. epithelial cells in budding structures.

RT-PCR analysis demonstrated that MPA administration increased the GH gene expression and decreased the GH receptor (GHR) gene expression in mammary tissue of the

control dogs. Increased GH mRNA concentrations in mammary gland tissue of dogs after prolonged treatment with progestins have been reported earlier (Mol et al., 1995a). Immunohistochemical expression of the GHR may be down regulated in completely differentiated alveolar epithelial cells at the end of the luteal phase (van Garderen et al., 1999).

In this study it was hypothesized that progesterone-induced mammary GH production may have endocrine effects on other tissues such as the uterine epithelium. Cystic endometrial hyperplasia (CEH) is frequently seen in bitches treated repeatedly with progestins for prevention of oestrus (Capel-Edwards et al., 1973; Sokolowski and Zimbelman, 1973; Goyings et al., 1977). Cystic endometrial hyperplasia may also develop spontaneously during the luteal phase of the oestrous cycle of middle-aged and elderly bitches (Dow, 1958). Because of the similarity of the progestin-induced epithelial changes in both the mammary gland and the uterus, it was hypothesized that GH is also involved in the development of progestin-induced CEH. Although iGH has been found in uterine epithelial cells of progestin-treated dogs, the absence of GH mRNA in uterine tissue suggests that it does not originate in the uterus (Kooistra et al., 1997). Both the control dogs and the mastectomized dogs developed CEH, macroscopically and histologically, with treatment of MPA. After MPA administration, iGH was present in uterine epithelial cells of both dog groups, whereas no uterine GH immunoreactivity was observed in these groups before MPA administration. These findings indicate that progestin-induced mammary GH does not play an essential role in the development of CEH in the bitch. Nevertheless, the presence of iGH in the cytoplasm of hyperplastic glandular uterine epithelial cells of dogs with CEH suggests that GH may be involved in the pathogenesis of CEH.

RT-PCR analysis revealed that the GH mRNA content was only increased in uterine tissue of the mastectomized dogs and not in the control dogs after MPA treatment. Comparable with the progestin-induced GH gene expression in canine mammary tissue during development of ductal epithelial buds (van Garderen et al., 1997), MPA treatment also resulted in more GH gene expression in the uterine epithelial tissue. Probably in the control dogs the elevated circulating concentrations of GH of mammary origin and the consequently elevated plasma IGF-I concentrations suppressed uterine GH gene expression, as has been reported for the pituitary (Hartman et al., 1993). MPA treatment also resulted in increased expression of the IGF-I gene in uterine tissue, but this increase was significant only in the control dogs. This may be explained by the stimulating effect of the elevated circulating concentrations of GH, originating from the mammary gland, on uterine IGF-I gene expression

in these dogs. MPA treatment did not promote the expression of GHRs in uterine epithelium. This makes it unlikely that increased numbers of GHRs can explain the presence of iGH in uterine cells, as proposed earlier (Kooistra et al., 1997).

In **Chapter 9**, an integral picture of the effects of progestins on the function of the adenohypophysis in the bitch is reported. The effects of supra-pituitary stimulation, using a combined anterior pituitary function test (Meij et al., 1996), on the release of seven adenohypophyseal hormones was studied in Beagle bitches before and several times during one year of MPA treatment. The prevention of oestrus by MPA in our bitches cannot be ascribed to a significant reduction in circulating concentrations of neither follicle-stimulating hormone (FSH) nor LH. On the contrary, during the first months of MPA treatment basal plasma FSH concentrations increased, without a concomitant change in the basal plasma LH concentrations. This elevated basal plasma FSH concentration may be due to a direct inhibitory effect of MPA at the ovarian level, resulting in suppression of the ovarian secretion of oestradiol and/or inhibin or stimulation of activin release (Couzinet and Schaison, 1993; Poindexter et al., 1993; Heikinheimo et al., 1996; Shupnik, 1996). With progression of the MPA treatment, basal plasma FSH concentrations declined to pre-treatment concentrations, while the pituitary FSH response to supra-pituitary stimulation decreased. These observations may be explained by down-regulation of the pituitary GnRH receptors due to continuous GnRH stimulation (Belchetz et al., 1978).

The results of the study in **Chapter 9** confirmed previous findings that progestins alter the GH-IGF-I axis in the bitch (Eigenmann et al., 1983; Selman et al., 1994b). Basal plasma GH concentrations tended to increase gradually during the course of the MPA treatment, although this rise was not statistically significant. This is in agreement with results of an earlier study, in which in 27 out of 36 MPA treated bitches plasma GH concentrations did not rise significantly (Concannon et al., 1980). However, the significant increase in circulating IGF-I concentrations during MPA treatment in our study indicates indirectly excessive exposure to GH (Selman et al., 1994b). Plasma IGF-I concentrations may thus be a more sensitive indicator than plasma GH concentrations for the effect of progestin treatment on the GH-IGF-I axis.

Besides an interaction with the progesterone receptor, MPA also has a relatively high affinity for the glucocorticoid receptor (Selman et al., 1996). Consequently, suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis was expected during MPA treatment, as has been reported before in both humans (Willemse et al., 1990) and dogs (Selman et al., 1994c, Selman et al., 1996). However, the results of the study reported in **Chapter 9** indicate

that the effects on ACTH secretion characteristics were limited. Because the supra-pituitary stimulation test was carried out four weeks after the injection of MPA, ACTH release most likely had returned to pre-treatment values within this timeframe. The suppression of the adrenocortical component of the HPA axis was more pronounced and comparable to previous observations (Selman et al., 1996). Apparently the suppression of the ACTH secretion was severe enough to cause atrophy of the adrenocortical zona fasciculata.

The basal plasma TSH concentrations were elevated at 8 months after the start of the MPA treatment, although they were still within the reference range for TSH in our laboratory. Our results conflict with those of others, who found no effect of MPA treatment on mean circulating TSH concentrations (Frank et al., 1979). One may speculate that MPA had a direct effect on the thyroid gland as a result of its inherent glucocorticoid properties, leading to a (slight) rise of the plasma TSH concentrations (Kemppainen et al., 1983).

No changes in PRL or α-melanocyte-stimulating hormone secretion were observed. The absence of an effect of MPA treatment on plasma PRL concentrations is in agreement with previous studies (Concannon et al., 1980; Rutteman et al., 1987) and may be explained by the absence of a clearcut decrease in progestational activity, that is known to be a trigger of PRL release (Galac et al., 2000).

The presence of progesterone receptors in mammary gland tissue allows for a targeted endocrine therapy with progesterone receptor blockers in dogs with progestin-induced mammary-derived GH excess. The results of the study reported in **Chapter 10** indicate that administration of the progesterone receptor blocker aglépristone (RU 46534) results in a significant decrease of plasma GH and IGF-I concentrations in dogs with progestin-induced hypersomatotropism. Our findings are in agreement with those of Watson et al. (1987) who found that administration of the antiprogestin mifepristone (RU 38486) decreases plasma GH concentrations and normalizes plasma IGF-I concentrations in bitches with progestin-induced acromegaly.

Analysis of the plasma GH profiles revealed that the mean basal plasma GH concentration and AUC above the zero-level for GH tended to decrease at the end of the treatment period with the progesterone receptor blocker compared with these values before aglépristone administration. In addition, the AUC above the baseline for GH, i.e., the amount of GH secreted in pulses, increased again during aglépristone treatment, although this difference did not reach statistical significance. Thus, treatment with aglépristone resulted in partial restoration of the normal pulsatile GH secretion. Higher dosages of aglépristone may result in complete normalization of the secretion pattern of GH.

Three and a half and 5.5 weeks after the last administration of aglépristone the plasma IGF-I concentrations had increased again, suggesting recurring high GH exposure. The recurrence of IGF-I hypersecretion after withdrawal of aglépristone treatment is not surprising as all dogs received injections of a depot progestin preparation for a period of one year, and the progestin effect of this depot preparation is much longer than the duration of aglépristone treatment in the present study. This indicates that treatment with an antiprogestin is required as long as the action of the synthetic progestin is present.

The following conclusions can be drawn for dogs:

- Pituitary-dependent hyperadrenocorticism is not only associated with less GH secreted per pulse but also with an impaired response to synthetic GHSs.
- In young dogs, ghrelin is a more potent stimulator of GH release than GHRH or GHRP-6. In old dogs, GHRH administration causes higher elevations of plasma GH concentrations than ghrelin or GHRP-6 administration.
- The GH-releasing capacity of ghrelin decreases with age whereas this decline is considerably lower for stimulation with GHRP-6 or GHRH.
- Ghrelin and GHRP-6 are specific releasers of GH. They do not stimulate the pituitary-adrenocortical axis nor the release of TSH, LH, or PRL.
- A ghrelin-stimulation test may be used in the diagnosis of canine pituitary dwarfism.
- Fasting and food intake lead to higher and lower circulating ghrelin concentrations, respectively.
- During food intake and fasting, the changes in plasma ghrelin concentrations are not associated with similar changes in plasma GH concentrations.
- During food intake and fasting, circulating insulin and glucose concentrations change reciprocally with the ghrelin concentrations.
- In healthy dogs, treatment with medroxyprogesterone acetate (MPA) results in a higher basal plasma GH secretion and less GH secreted in pulses compared to dogs with surgically excised mammary gland tissue. In mastectomized dogs however, MPA treatment does not change basal plasma GH concentrations, the AUC above the zero-level for GH, the AUC above the baseline for GH, and the GH pulse frequency.
- In both healthy dogs and mastectomized dogs, cystic endometrial hyperplasia (CEH) develops after one year treatment with MPA. Thus, progestin-induced mammary-derived GH is not a requirement for the development of CEH.

- The presence of immunoreactive GH in the cytoplasm of hyperplastic glandular uterine epithelial cells of dogs with CEH suggests that GH may play a role in the pathogenesis of CEH.
- The effect of MPA on gonadotrophin secretion is confined to FSH secretion. MPA treatment increases basal plasma FSH concentration during the first months of treatment, while the pituitary FSH response to supra-pituitary stimulation decreases during MPA administration.
- The progesterone receptor blocker aglépristone allows for treatment of progestin-induced hypersomatotropism.

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