Chapter 5

Effects of growth hormone secretagogues on the release of adenohypophyseal hormones in young and old healthy dogs

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Abstract

The effects of three growth hormone secretagogues (GHSs), ghrelin, growth hormone-releasing peptide-6 (GHRP-6), and growth hormone-releasing hormone (GHRH), on the release of adenohypophyseal hormones, growth hormone (GH), adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), prolactin (PRL) and on cortisol were investigated in young and old healthy Beagle dogs.

Ghrelin proved to be the most potent GHS in young dogs, whereas in old dogs, GHRH administration was associated with the highest plasma GH concentrations. The mean plasma GH response after administration of ghrelin was significantly lower in the old dogs compared with the young dogs. The mean plasma GH concentration after GHRH and GHRP-6 administration was lower in the old dogs compared with the young dogs, but this difference did not reach statistical significance. In both age groups, the GHSs were specific for GH release as they did not cause significant elevations in the plasma concentrations of ACTH, cortisol, TSH, LH, and PRL.

It is concluded that in young dogs, ghrelin is a more powerful stimulator of GH release than either GHRH or GHRP-6. Ageing is associated with a decrease in GH-releasing capacity of ghrelin, whereas this decline is considerably lower for GHRH or GHRP-6.

Introduction

In 1977, Bowers and co-workers reported the growth hormone (GH)-releasing properties of enkephalin-derived peptides (Bowers et al., 1977). Among these synthetic peptides, GH-releasing peptide-6 (GHRP-6) proved to be a potent releaser of GH, both *in vitro* and *in vivo*, in several species (Bowers et al., 1984; Casanueva and Dieguez, 1999). After the synthesis of GHRP-6, new peptydil (e.g. hexarelin) and non-peptydil (e.g. MK-0677) GH secretagogues (GHSs) with a higher bioavailability and a longer life span were produced (Ghigo et al., 1994; Patchett et al., 1995). These synthetic GHSs have a potent GH-releasing activity in humans, mice, rats (Bowers et al., 1977; Bowers et al., 1984; Ghigo et al., 1994; Casanueva and Dieguez, 1999), swine, goats, and cows (Hayashida et al., 2001).

In humans, nearly all synthetic GHSs are more powerful than growth hormone-releasing hormone (GHRH) in terms of GH release (Casanueva and Dieguez, 1999). Studies in dogs have shown that orally (e.g. MK-0677, capromorelin) and intravenously (e.g. hexarelin) administered GHSs are effective GH releasers as well (Cella et al., 1995; Jacks et al., 1996; Rigamonti et al., 1999; Carpino et al., 2003). However, the action of the synthetic GHSs is not always confined to the promotion of GH release (Smith et al., 1997; Casanueva and Dieguez, 1999; Lamberts, 1999). For instance, in man, synthetic GHSs such as GHRP-6 stimulate the secretion of PRL, ACTH, and cortisol as well (Massoud et al., 1996; Smith et al., 1997; Casanueva and Dieguez, 1999; Lamberts, 1999; Arvat et al., 2001). Additionally in rats, GHRP-6 activates the pituitary-adrenocortical axis (Thomas et al., 1997).

GHSs stimulate GH release from the pituitary somatotrophs by acting on receptors different from those for GHRH (Momany et al., 1981). In the mid-nineties, the GHS-receptor (GHS-R), a G-protein-coupled seven-transmembrane receptor was first detected in the anterior pituitary and hypothalamus of rats and humans (Pong et al., 1996). In 1999, Kojima et al. purified and characterized the endogenous ligand for the GHS-R in rats and humans. The 28-amino-acid peptide with an *n*-octanoyl modification at its third Serine residue was called ‘ghrelin’. Surprisingly, its expression was found to be much higher in the stomach than in any other tissue. Tomasetto and coworkers (2001) identified cDNA encoding ghrelin from the fundus of the canine stomach as well, and found that it is highly conserved with man, mouse and rat. Thus, structural heterogeneity of ghrelin among species seems minor, and we could probably expect a rather functional homogeneity in various mammalian species. Ghrelin’s expression is restricted to the X/A- like cells, or Ghr-cells, of the oxyntic gland (Date et al., 2000; Rindi et al., 2002).

The GH-releasing activity of ghrelin is more marked in humans than in animals (Smith et al., 1997; Kojima et al., 1999; Seoane et al., 2000; Takaya et al., 2000). In humans, the GH response to ghrelin is considerably greater than that observed following administration of GHRH or synthetic GHSs (Seoane et al., 2000; Takaya et al., 2000; Arvat et al., 2001; Bowers, 2001), whereas in rats, the GH-releasing potency of ghrelin is similar to that of GHRH (Kojima et al., 1999). In both isolated pituitary cells and intact (anaesthetized) rats, ghrelin solely stimulates GH release and does not affect the secretion of other adenohypophyseal hormones (Kojima et al., 1999). However, in humans, ghrelin significantly increases circulating concentrations of adrenocorticotrophic hormone (ACTH), cortisol, and prolactin (PRL), without affecting the release of luteinizing hormone (LH), follicle-stimulating hormone (FSH) or thyroid-stimulating hormone (TSH) (Massoud et al., 1996; Arvat et al., 2001).

The ageing process of organisms may be regarded as a progressive fall in bodily functions associated with a diminished ability to maintain homeostasis (Everitt and Meites, 1989). Both basal and stimulated GH secretion and circulating insulin-like growth factor-I (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). Also in dogs, the GH responsiveness to GH stimuli, such as GHRH and hexarelin, decreases with ageing (Cella et al., 1989; Cella et al., 1995). Until now, the effect of ageing on the GH responsiveness to ghrelin has not been studied in dogs. Preliminary results on the effects of GHSs on GH, ACTH, and cortisol concentrations in old dogs have been reported earlier (Bhatti et al., 2002).

The aim of this study was to compare the effects of ghrelin, GHRP-6, and GHRH on the release of GH, ACTH, cortisol, TSH, LH, and PRL in both young and old healthy dogs.

Materials and methods

Dogs

Four young female and four young male Beagle dogs aged between 13-17 months (median 15 months) and four old female and four old male Beagle dogs aged between 7-12 years (median 10 years) were used. The mean body weight of the young dogs (11.8 kg) was significantly lower ($P = 0.01$, Mann-Whitney test) than that of the old dogs (17.9 kg). The dogs were accustomed to the laboratory environment and procedures such as collection of blood samples. They were housed in pairs in indoor-outdoor runs, had free access to tap water

and were fed on a commercial dog food. They were healthy and had no history of illnesses or treatments. All studies were carried out in conscious animals after an overnight fast. The bitches were in anoestrus during the study.

Study design and blood sample collection

Two 4x4 cross-over studies (young and old dogs, respectively) were conducted at different times. Each dog received four treatments sequentially on four different days (day 1 to 4) with a washout period of at least four days in between. The dogs were two by two randomly assigned to one of the four treatment sequences. The four treatments consisted of an intravenous injection of either human ghrelin (MW 3370.9) in a dose of 2 µg/kg body weight (Peninsula Laboratories Inc), GHRP-6 [(His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2)^3] (MW 872.4) in a dose of 2 µg/kg body weight, human GHRH (MW 5036.6) in a dose of 2 µg/kg body weight (hGHRF; Peninsula Laboratories Inc), or NaCl 0.9 % (control).

Blood samples for the determination of plasma concentrations of GH, ACTH, cortisol, TSH, LH, and PRL were collected by jugular venipuncture at 15 min before and 0, 5, 10, 20, 30, and 45 min after intravenous administration of the treatments and immediately transferred to ice-chilled EDTA-coated tubes (GH, ACTH, cortisol, LH, and PRL) or heparin-coated tubes (TSH). Samples were centrifuged at 4^o C for 10 min. Plasma was stored at -25^o C until assayed.

Hormone determination

Plasma GH concentrations were determined with a homologous radioimmunoassay (RIA) (Eigenmann and Eigenmann, 1981). The intra- and interassay coefficients of variation were 3.8 % and 7.2 %, respectively, and the sensitivity of the assay was 0.3 µg/l. The degree of cross-reaction with canine PRL was 2 %.

Plasma ACTH concentrations were determined with an immunoradiometric assay (Nichols Institute, Wijchen, The Netherlands). The inter-assay coefficient of variation was 7.8 % and the sensitivity was 0.2 pmol/l.

Plasma cortisol concentrations were determined with a commercially available RIA (Diagnostic Products Corporation), validated for the dog. The intra- and interassay coefficients of variation ranged from 3.0 to 5.1 % and from 4.0 to 6.4 %, respectively. The sensitivity of the assay was 5.5 nmol/l.

Plasma TSH concentrations were determined with a homologous solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite canine TSH, Diagnostic Products Corporation [DPC]) according to 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 2.60 µg/l, respectively. The interassay coefficients of variation were 6.3 % and 8.2 % at TSH levels of 0.16 and 2.80 µg/l, respectively. The sensitivity of the assay was 0.03 µg/l. Cross-reactivity with FSH and LH was negligible.

Plasma LH concentrations were determined with a heterologous RIA as described previously by Nett et al. (1975). A rabbit antiserum raised against ovine LH (CSU-204; kindly supplied by G.D. Niswender, Colorado State University), radioiodinated NIAMDD-bLH-4, and canine pituitary standard LER 1985-1 (a gift from Dr. L.E. Reichert, Albany Medical College, NY) were used in this assay. The intra- and interassay coefficients of variation for values higher than 0.5 µg/l were 2.3 % and 10.5 % respectively. The sensitivity of the assay was 0.3 µg/l.

Plasma concentrations of PRL were determined with a previously validated heterologous RIA (Okkens et al., 1985). The intra- and interassay coefficients of variation were 3.5 % and 11.5 %, respectively. The sensitivity of the assay was 0.8 µg/l.

Statistical analysis

1. Cross-over studies

Mixed models with dog as random effect were fitted to study the association between treatment (ghrelin, GHRP-6, GHRH, and NaCl 0.9 %) and the plasma hormone concentrations (GH, ACTH, cortisol, TSH, LH, and PRL) in the young and the old dogs. Treatment, day of treatment (day 1-4), and time (repeated measures of adenohypophyseal hormones and cortisol starting with the basal concentration) were included as fixed effects. The four treatments were compared pair wise adjusting for multiple comparisons (Tukey's correction). In addition, difference between the plasma hormone concentration (GH, ACTH, cortisol, TSH, LH, and PRL) just before (0 min) injection of the GHSs or NaCl 0.9 % and the maximal plasma hormone concentration after injection was calculated (referred to as the maximal increment) and used to capture the effect of treatment. Therefore, a mixed model was fitted to the maximal increments as response variables with dog as random effect and day of treatment and treatment as fixed effects. The four treatments were compared pair wise (Tukey's correction).

2) Comparison between young and old dogs

The basal plasma hormone concentrations (mean of -15 and 0 min, collected in the cross-over studies) of the young and the old dogs were compared using an independent samples t-test to study differences between age groups before treatment. Additionally, if the analyses based on the cross-over studies indicated a significant treatment effect in one or both age groups, a repeated measures analysis was performed in order to compare the respective hormone response between old and young dogs. Therefore, models were fitted including dog as random effect, and age (young versus old dogs), time (-15, 0, 5, 10, 20, 30, and 45 min), and the interaction between age and time as categorical fixed effects. A first-order autoregressive covariance structure was used to model the correlation in the repeated measures of the response variables.

Statistical significance was defined at $P \leq 0.05$. Values are expressed as mean concentrations of all hormone measurements ± SEM. Analyses were performed with SAS version 8.02 (SAS Institute Inc) and S-Plus version 6.1 for Windows (Insightful Corp).

Ethics of the study

This study was approved by the Ethical Committees of the Faculty of Veterinary Medicine, Utrecht University, the Netherlands, and the Faculty of Veterinary Medicine, Ghent University, Belgium.

Results

No side effects were observed during or after administration of the GH-releasing agents.

The mean plasma GH concentration in the young dogs when treated with ghrelin was 15.0 ± 5.1 µg/l, which was significantly higher than the concentration in the dogs when treated with GHRP-6 (2.7 ± 1.2 µg/l) or NaCl 0.9 % (1.8 ± 1.1 µg/l). The mean plasma GH concentration in the young dogs when treated with ghrelin did not differ significantly from this concentration in dogs when treated with GHRH (7.5 ± 3.8 µg/l). The mean GH concentration in the young dogs when treated with GHRH did not differ significantly from that in dogs when treated with GHRP-6 (Figure 1a). Analysis of the maximal increment indicated that the mean plasma GH response was significantly higher after treatment with ghrelin when compared with this response after GHRP-6 ($P = 0.01$) or NaCl 0.9 % ($P =$

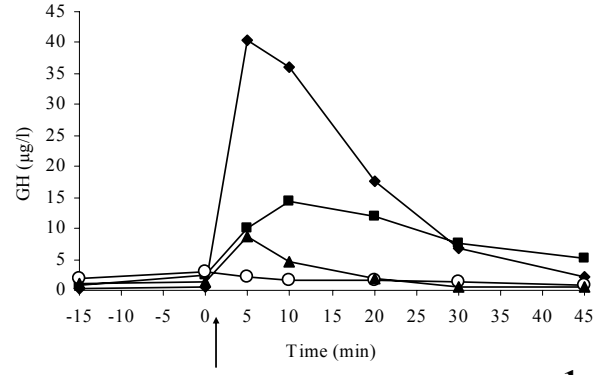
0.007) injection, and tended to be higher than the response induced by administration of GHRH (P = 0.06) (Figure 2a).

The mean plasma ACTH concentration in the young dogs when treated with ghrelin, GHRP-6, GHRH, and NaCl 0.9 % was 68 ± 22 ng/l, 61 ± 17 ng/l, 33 ± 7 ng/l, and 38 ± 9 ng/l, respectively. Although plasma ACTH concentrations were higher when the dogs were injected with ghrelin or GHRP-6, the concentrations were not significantly different from these measured when GHRH or NaCl 0.9 % were administered (Figure 1b). A treatment effect could not be demonstrated based on comparison of the maximal increments (Figure 2b).

The mean plasma cortisol concentration in the young dogs when treated with ghrelin, GHRP-6, and GHRH was 102 ± 23 nmol/l, 109 ± 22 nmol/l, and 87 ± 21 nmol/l, respectively. These levels were not significantly different from that obtained when NaCl 0.9 % was administered (84 ± 21 nmol/l) (Figure 1c). No treatment effect was present based on comparison of the maximal increments (Figure 2c).

In the young dogs, the mean plasma TSH, LH, and PRL concentrations did not differ significantly when treated with the GH-releasing agents or NaCl 0.9 % (Figures 1d-f, respectively), and no treatment effects were present based on comparison of the maximal increments (Figures 2d-f, respectively).

The mean plasma GH concentration in the old dogs when treated with ghrelin, GHRP-6, and GHRH was 1.8 ± 0.5 µg/l, 2.7 ± 1.0 µg/l, and 5.5 ± 1.0 µg/l, respectively, which was significantly higher than the concentration in the dogs when treated with NaCl 0.9 % (0.9 ± 0.2 µg/l). The mean plasma GH concentration in dogs when treated with GHRH was significantly higher than in dogs when treated with ghrelin or GHRP-6 (Figure 3a). Moreover, analysis of the maximal increment indicated that the plasma GH response was significantly higher after treatment with GHRH than the response after administration of ghrelin or NaCl 0.9 % (Figure 4a).



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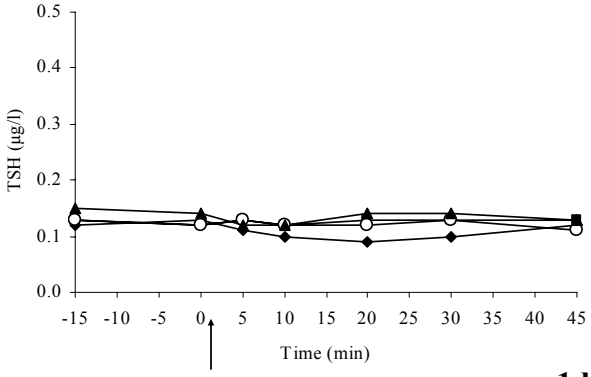

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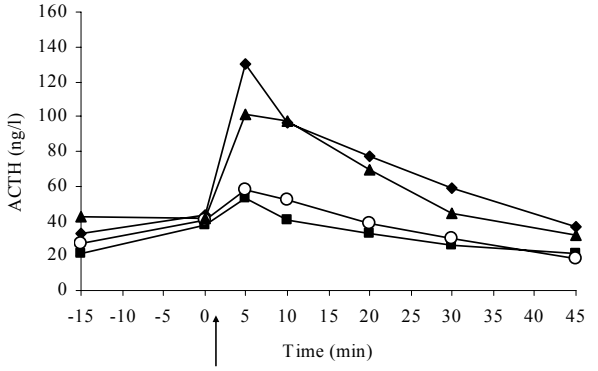

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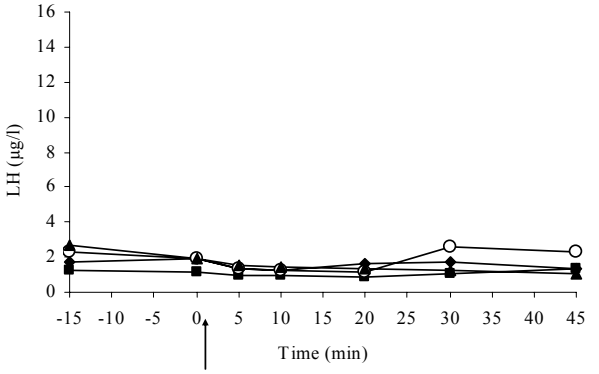

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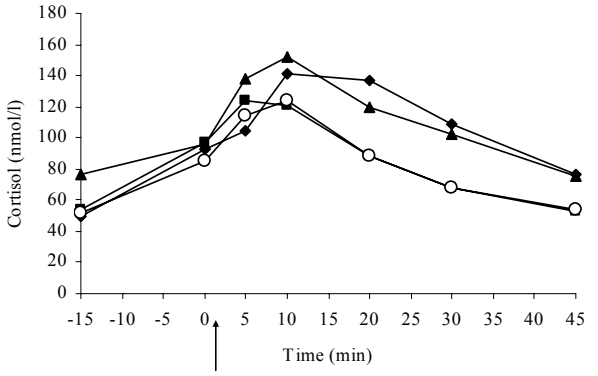

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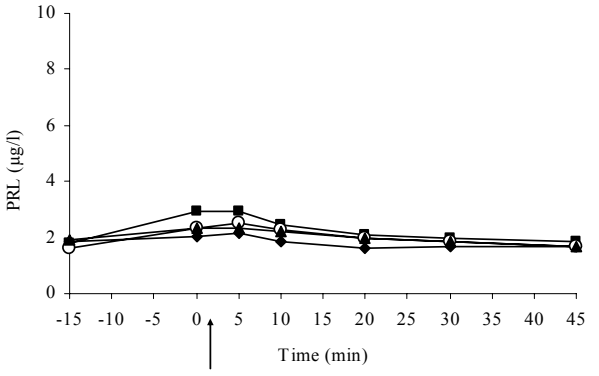
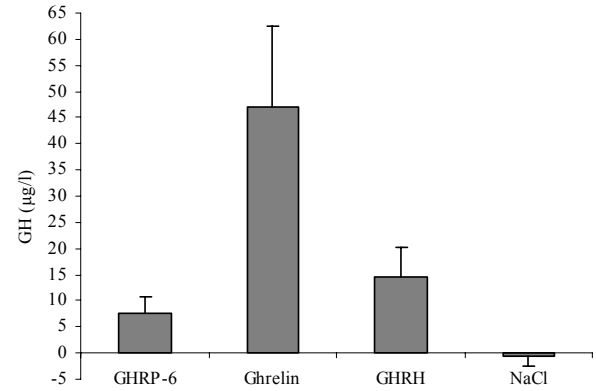


1f

Figure 1. (a-f) Mean plasma concentrations of GH, ACTH, cortisol, TSH, LH, and PRL before and after intravenous administration of ghrelin (♦), GHRP-6 (▲), GHRH (■), or NaCl 0.9 % (○) in eight healthy young dogs. The arrows indicate the intravenous administration of the treatment.



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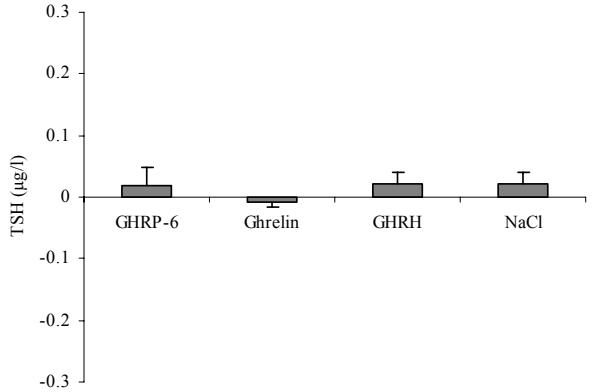

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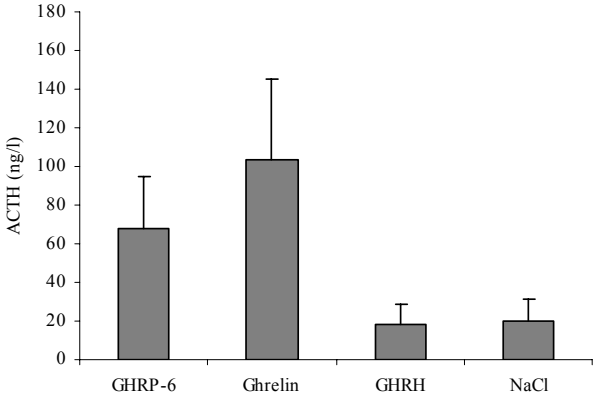

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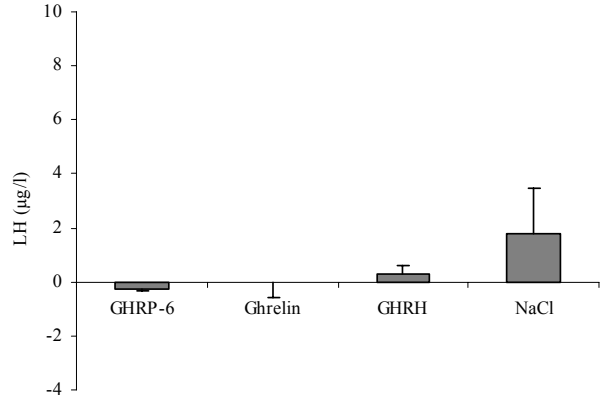

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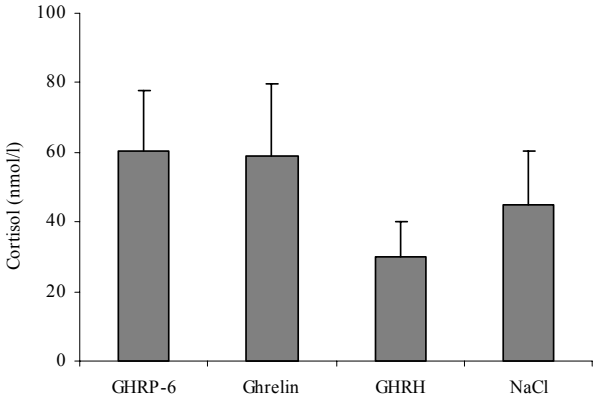

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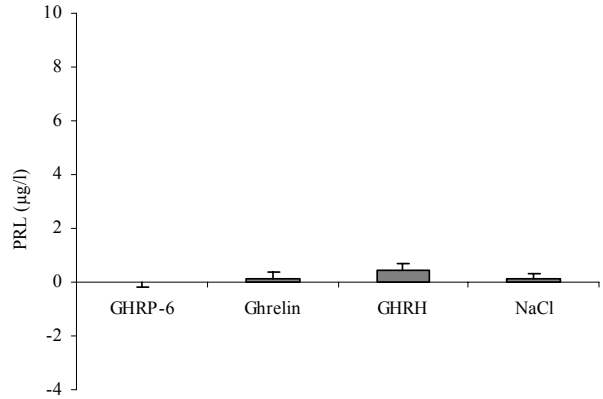


2f

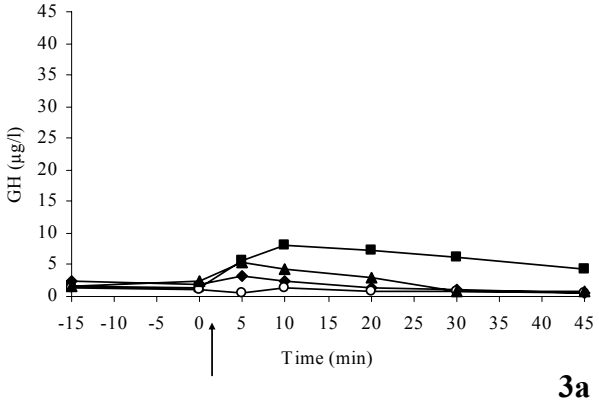
Figure 2. (a-f) The mean (+SEM) difference in plasma concentrations of GH, ACTH, cortisol, TSH, LH and PRL between 0 min and the maximal increment after administration of the GHSs and NaCl 0.9 % in eight healthy young dogs.

The mean plasma ACTH concentration in the old dogs when treated with ghrelin (25 ± 5 ng/l), GHRP-6 (30 ± 6 ng/l), and GHRH (21 ± 4 ng/l) was not significantly different from that in the dogs when treated with NaCl 0.9 % (25 ± 5 ng/l) (Figure 3b). Also, the mean plasma cortisol concentration in the old dogs when treated with ghrelin (64 ± 19 nmol/l), GHRP-6 (80 ± 24 nmol/l), or GHRH (55 ± 8 nmol/l) did not differ significantly from that in the dogs when treated with NaCl 0.9 % (73 ± 13 nmol/l) (Figure 3c). Additionally, no treatment effects were present based on comparison of the maximal increments (Figures 4b and 4c, respectively).

In the old dogs, the mean plasma TSH, LH, and PRL concentrations did not differ significantly when treated with the GH-releasing agents or NaCl 0.9 % (Figures 3d-f, respectively), and no treatment effects were present based on comparison of the maximal increments (Figures 4d-f, respectively).

The mean basal plasma GH concentration in the young dogs (1.4 ± 0.5 µg/l) did not differ significantly from that in the old dogs (1.6 ± 0.3 µg/l). The mean basal plasma concentrations of ACTH and cortisol were significantly lower in the old dogs (26 ± 3 ng/l and 55 ± 4 nmol/l, respectively) than in the young dogs (36 ± 4 ng/l and 76 ± 7 nmol/l, respectively). The mean basal plasma concentrations of TSH, LH, and PRL were significantly higher in the old dogs (0.32 ± 0.05 µg/l, 4.4 ± 0.7 µg/l, 4.2 ± 0.4 µg/l, respectively) than in the young dogs (0.13 ± 0.01 µg/l, 1.9 ± 0.2 µg/l and 2.1 ± 0.2 µg/l, respectively).

The mean plasma GH response after treatment with ghrelin, GHRP-6, and GHRH, respectively, was compared between young and old dogs. The mean plasma GH response after administration of ghrelin was significantly lower in the old dogs compared with the young dogs. In addition, this plasma GH response evolved significantly different over time between both groups (Figure 1a versus Figure 3a). The mean plasma GH response after treatment with GHRP-6 or GHRH was lower in the old dogs when compared with the young dogs, but this was not statistically significant. Additionally, this plasma GH response did not evolve significantly different over time when both groups were compared (Figure 1a versus Figure 3a).



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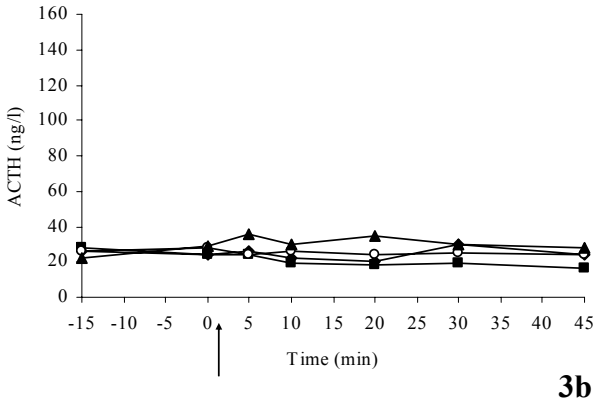

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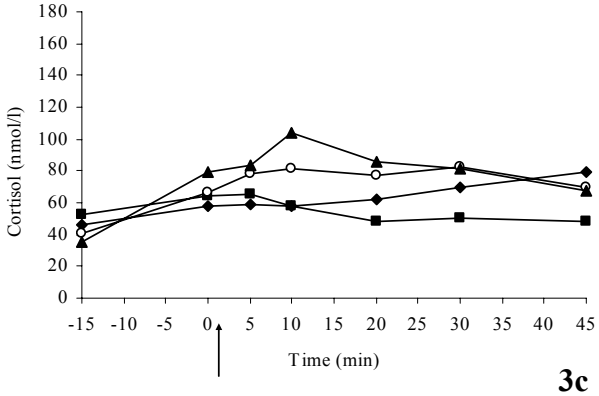

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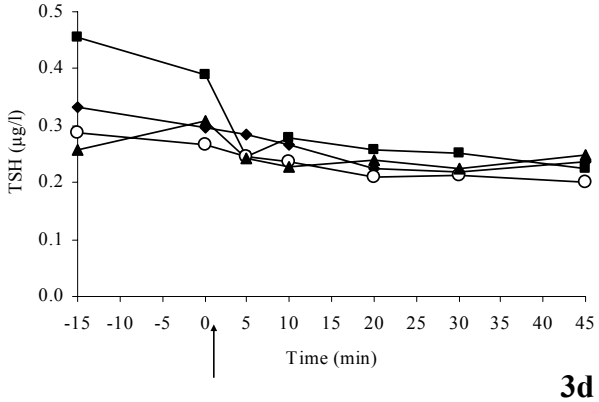

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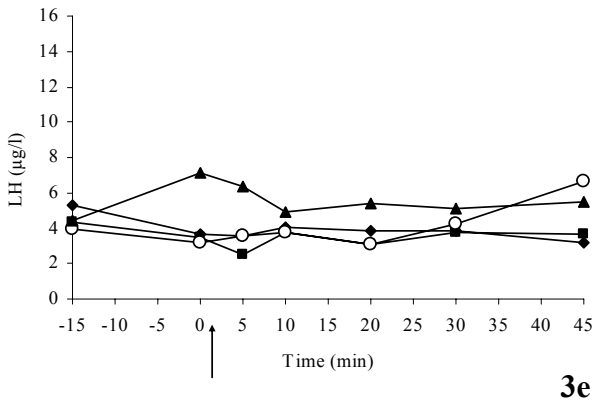

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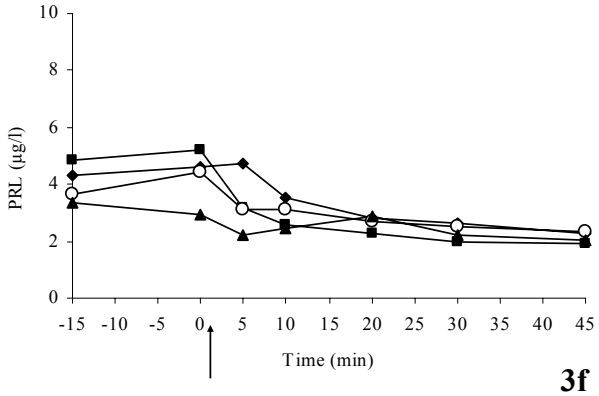

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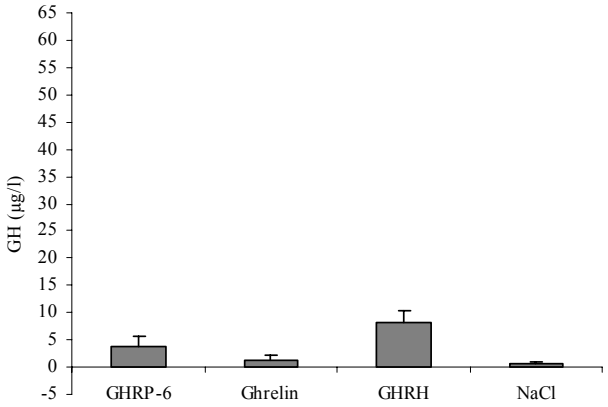
Figure 3. (a-f) Mean plasma concentrations of GH, ACTH, cortisol, TSH, LH, and PRL before and after intravenous administration of ghrelin (♦), GHRP-6 (▲), GHRH (■), or NaCl 0.9 % (○) in eight healthy old dogs. The arrows indicate the intravenous administration of the treatment.



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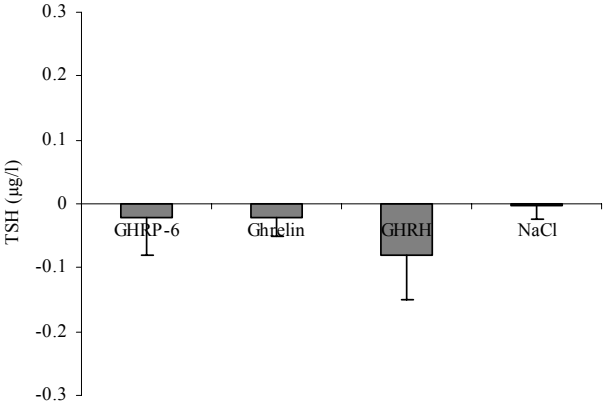

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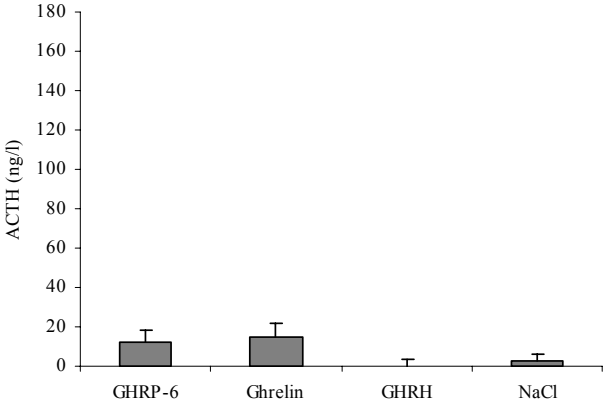

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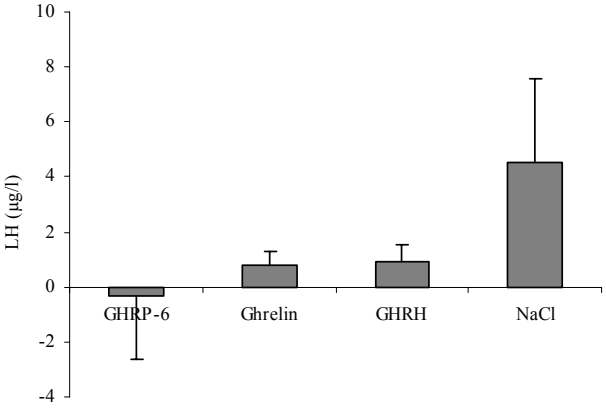

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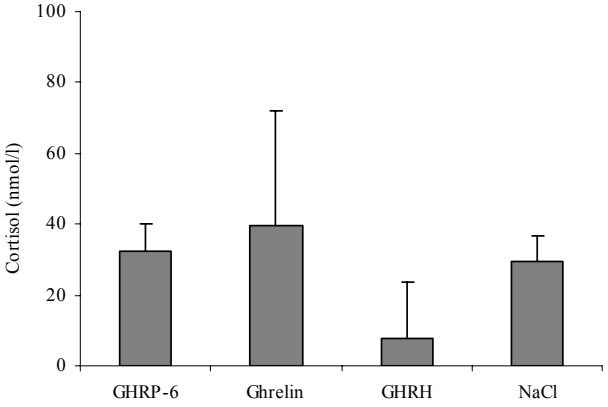
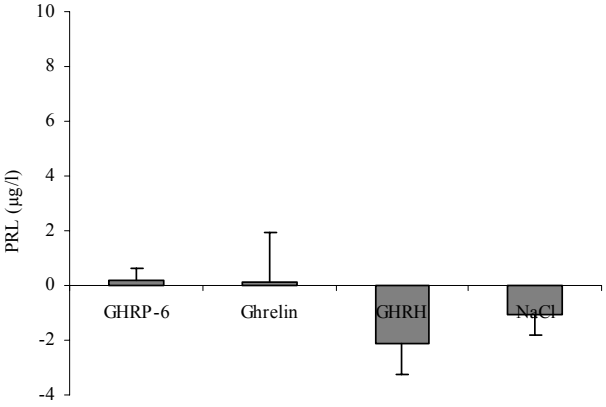


4c



4f

Figure 4. (a-f) The mean (+SEM) difference in plasma concentrations of GH, ACTH, cortisol, TSH, LH and PRL between 0 min and the maximal increment after administration of the GHSs and NaCl 0.9 % in eight healthy old dogs.

Discussion

The results of the present study demonstrate that the natural ligand of the GHS-R, ghrelin, causes a significant rise in plasma GH levels in healthy young and old dogs. In the young dogs, ghrelin is a potent releaser of GH when compared with the other stimulants used in our study. However, in the old dogs, GHRH elicited higher plasma GH levels than ghrelin, GHRP-6, or NaCl 0.9 % administration. With regard to the GH-releasing potency of GHSs, these findings further substantiate the existence of remarkable species-related differences. In rats, 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).

Our findings also indicate the existence of age-related differences in the GH-releasing potency of GHSs. The ghrelin-induced GH response was much lower at old age than at young age. In addition, the GHRP-6- and GHRH-induced release of GH was lower in the old versus the young dogs, although this difference was not statistically significant. These observations are compatible with findings in humans, showing 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).

The GH-releasing activity of ghrelin and synthetic GHSs depends on the functional integrity of the hypothalamus-pituitary unit (Muccioli et al., 2002). 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 somatotrope function (Giustina and Veldhuis; 1998; Ghigo et al., 1999). These changes include a concomitant reduction in the secretion of GHRH and enhancement in somatostatin release (Kelijman, 1991; Giustina and Veldhuis; 1998; Ghigo et al., 1999; Muller et al., 1999). It seems that an impairment of pituitary function does not play a major role (Muller et al., 1999). Indeed, 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 levels (Cella et al., 1993; Nicolas et al., 1994). Additionally, the age-related decrease of the GH response to ghrelin and synthetic GHSs agrees with the well-known *in*

vitro hyporesponsiveness of the aged somatotroph cells to the majority of provocative stimuli, including GHRH, despite the availability of a remarkable GH-releasable pool (Giustina and Veldhuis, 1998; Ghigo et al., 1999; Muccioli et al., 2002)

These observations support the idea that the somatopause is driven primarily by the hypothalamus and that the pituitary somatotrophs retain their capacity to synthesize and secrete adequate levels of GH (Franchimont et al., 1989; Walker et al., 1990; Corpas et al., 1992; Cella et al., 1993; Muller et al., 1999; Muccioli et al., 2002).

A decline of GHS-Rs in the ageing brain (Arvat et al., 1998; Muccioli et al., 2002) could further explain the reduced GH response to ghrelin/GHSs in elderly humans. Based on the relative great age-related decline in the GH response to ghrelin compared with that to GHRH in dogs, it may be hypothesized that also in dogs GHS-R expression decreases considerably with age.

In the present study, the old dogs were significantly heavier than the young dogs. Obesity, a condition commonly observed in adulthood, is associated with an impaired GH response to GH-releasing stimuli (Daughaday and Rotwein, 1989; Bowers, 1993; Arvat et al., 1998). This may have played a contributing role in the age-associated decline of the GH response. A longitudinal study, in which the GHS-induced hormone responses in dogs in function of age are investigated over several years while maintaining a constant body weight in all dogs, could lead to more reliable conclusions.

Also with regard to the effects of GHSs on the release of adenohypophyseal hormones other than GH, there are interesting species-related differences. In this study, the action of ghrelin and GHRP-6 appeared to be GH-specific in dogs, i.e., the stimulants did not induce a significant rise in plasma concentrations of ACTH, cortisol, TSH, LH, and PRL in either the young dogs or the old dogs. The absence of a TSH and LH response to GHSs is compatible with the results of previous studies of the hormone-releasing effects of ghrelin in humans and rats (Arvat et al., 1997; Kojima et al., 1999). In contrast, the stimulatory effect of ghrelin and synthetic GHSs on PRL secretion varies with the species studied.

In humans, ghrelin and synthetic GHSs induce a significant release of PRL which is independent of both gender and age and probably results from direct stimulation of somatomammotroph cells (Renner et al., 1994; Arvat et al., 1997; Takaya et al., 2000; Muccioli et al., 2002). In dogs (Hickey et al., 1994) and rats (Kojima et al., 1999), synthetic GHSs do not stimulate PRL release. This species-related difference may be explained by different numbers of somatomammotrophs in various species (Raun et al., 1998), with humans having a high proportion of these cells (Frawley and Boockfor, 1991).

Furthermore, administration of the GHSs did not elicit a significant activation of the pituitary-adrenocortical axis in the dogs. In contrast, intravenous administration of ghrelin or synthetic GHSs, such as hexarelin, considerably increases circulating levels of ACTH and cortisol in healthy humans (Massoud et al., 1996; Arvat et al., 1997; Takaya et al., 2000). Kojima et al. (1999) reported that intravenously administered ghrelin specifically stimulates GH release in anaesthetized rats and in isolated rat pituitary cells, but does not affect the release of other adenohypophyseal hormones. However, Thomas et al. (1997) have shown that GHRP-6 mediates the release of ACTH and cortisol in conscious rats. The mechanism by which ghrelin and synthetic GHSs stimulate the pituitary-adrenocortical axis is still unknown, but seems to be mediated via the hypothalamus as it is lost after cutting the pituitary stalk (Loche et al., 1995). They may interact with hypothalamic peptides (e.g. corticotrophin-releasing hormone, arginine vasopressin, and neuropeptide Y) controlling ACTH release (Dickson and Luckman, 1997; Thomas et al., 1997; Korbonits et al., 1999; Broglio et al., 2003), most probably primarily via arginine vasopressin (Korbonits et al., 1999).

In swine, some recently developed selective GHSs, such as ipamorelin, induce massive GH secretion without any elevation in ACTH, cortisol, or PRL release (Raun et al., 1998), whereas GHRP-6 and GHRP-2 administration in this species cause a strong activation of the pituitary-adrenocortical axis. This suggests the existence of subtypes of GHS-Rs with differential effects on GH, ACTH, and PRL release. Furthermore, intravenous bolus administration of ghrelin or synthetic GHSs results in high blood levels and reflects a pharmacological rather than a physiological action of the peptides. It is possible that, at physiological concentrations, these GHSs do not increase ACTH, cortisol, or PRL concentrations (Svensson et al., 1998).

Most basal plasma hormone concentrations did differ significantly when the young and old dogs were compared. Although it is generally accepted that basal GH secretion decreases in humans and animals with increasing age (Finkelstein et al., 1972; Sonntag et al., 1980; Zadik et al., 1985; Borst et al., 1994; De Gennaro Colonna et al., 1994; Wilshire et al., 1995), the basal plasma GH concentration in our study was not significantly different in the young and the old dogs. For identifying age-related differences in GH secretion, determination of the pulsatile secretion pattern of GH is much more sensitive. Indeed, the pulsatile secretion pattern of GH is significantly lower in old humans, rats, and dogs than in young individuals (Zadik et al., 1985; Cella et al., 1989; Borst et al., 1994). The higher basal plasma concentrations of ACTH and cortisol in the young dogs may indicate that the stress of handling caused a stronger activation of the pituitary-adrenocortical axis in these young dogs

than in the old dogs. In some species, ageing is associated with a decrease in stress-induced activation of adrenocortical function (Van Eekelen et al., 1995).

In conclusion, the results of this study demonstrate the existence of age-related differences with regard to the GH-releasing activity of GHSs. Ghrelin is, compared to GHRH and GHRP-6, the most potent stimulator of GH release in young dogs. In old dogs, GHRH administration is associated with the highest elevations in plasma GH levels when compared to administration of GHRP-6 or ghrelin. The GH-releasing capacity of ghrelin decreases with age whereas this decline is considerably lower for GHRP-6 or GHRH. Furthermore, ghrelin and GHRP-6 are specific releasers of GH and do not stimulate the pituitary-adrenocortical axis or the release of TSH, LH, or PRL in dogs.

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