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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), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), luteinising 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.

Keywords: Ageing; Canine; Ghrelin; Growth hormone-releasing hormone; Growth hormone-releasing peptide-6

1. 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, 1984; Casanueva and Dieguez, 1999;

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Ghigo et al., 1994), swine, goats and cows (Hayashida et al., 2001).

In humans, nearly all synthetic GHSs have been shown to be 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 (IV) (e.g., hexarelin) administered GHSs are also effective GH releasers (Carpino et al., 2003; Cella et al., 1995; Jacks et al., 1996; Rigamonti et al., 1999). However, the action of synthetic GHSs is not always confined to the promotion of GH release (Casanueva and Dieguez, 1999; Lamberts, 1999; Smith et al., 1997). In man, synthetic GHSs such as GHRP-6 also stimulates the secretion of Prolactin (PRL), adrenocorticotropic hormone (ACTH), and cortisol (Arvat et al., 2001; Casanueva and Dieguez, 1999; Lamberts, 1999; Massoud et al., 1996; Smith et al., 1997). Moreover in rats, GHRP-6 activates the pituitary–adrenocortical axis (Thomas et al., 1997).

GHSs stimulate GH release from the pituitary somatotropes by acting on receptors different from those for GHRH (Momany et al., 1981). In the mid-1990s, 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. (1999) purified and characterised 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 et al. (2001) also identified cDNA encoding ghrelin from the fundus of the canine stomach, and found that it was highly conserved with man, mouse and rat. Thus, structural heterogeneity of ghrelin among species seemed minor, and a rather functional homogeneity in various mammalian species could be expected. 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 (Kojima et al., 1999; Seoane et al., 2000; Smith et al., 1997; Takaya et al., 2000). In humans, the GH response to ghrelin was considerably greater than that observed following administration of GHRH or synthetic GHSs (Arvat et al., 2001; Bowers, 2001; Seoane et al., 2000; Takaya et al., 2000), whereas in rats the GH-releasing potency of ghrelin was similar to that of GHRH (Kojima et al., 1999). In both isolated pituitary cells and intact (anaesthetised) rats, ghrelin only stimulated GH release and did not affect the secretion of other adenohypophyseal hormones (Kojima et al., 1999). However, in humans, ghrelin significantly increased circulating concentrations of ACTH, cortisol and PRL, without affecting the release of luteinising hormone (LH), follicle-stimulating hormone (FSH) or thyroid-stimulating hormone (TSH) (Arvat et al., 2001; Massoud et al., 1996).

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-1) concentrations decline with age in several mammalian species (Corpas et al., 1992; Finkelstein et al., 1972; Muller et al., 2002; Rudman, 1985; Wilshire et al., 1995; Zadik et al., 1985). In dogs too the GH responsiveness to GH stimuli, such as GHRH and hexarelin, decreases with ageing (Cella et al., 1989, 1995). Until now, the effect of ageing on the GH responsiveness to ghrelin has not been studied in the dog. Preliminary results on the effects of GHSs on GH, ACTH, and cortisol concentrations in old dogs have however been reported earlier (Bhatti et al., 2002).

The aim of the present 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.

2. Materials and methods

2.1. Dogs

Four young female and four young male Beagle dogs aged between 13 and 17 months (median 15 months) and four old female and four old male Beagle dogs aged between 7 and 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.

2.2. Study design

Two 4×4 cross-over studies (young and old dogs, respectively) were conducted at different times. Each dog received four treatments sequentially on four different days (day 1–4) 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 IV injection of either human ghrelin (MW 3370.9) in a dose of 2 μg/kg body weight (Peninsula Laboratories Inc.), GHRP-6 [(His-D-