

Characterization of Growth Hormone Secretion to Growth Hormone-releasing Peptide-2 in Domestic Animals - A Review

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ABSTRACT : Growth hormone-releasing peptide-2 (GHRP-2, also named KP102) is a new hexapeptide of a series of synthetic growth hormone-releasing peptides (GHRPs) which stimulates the secretion of growth hormone (GH) *in vitro* and *in vivo* in several species including calf, sheep and pig. The GH-releasing activity of GHRP-2 is two to three times more effective than that of the original GHRP-6, and GHRP-1 in the rats and humans. To date, GHRP-2 seems to be the most potent member of the family of GHRPs. Since the GHRPs are short peptides (5-7 amino acid residues), they are synthesized easily and are not as readily degraded in plasma as GH-releasing hormone (GHRH). These features ameliorate their potential on domestic animals because of their chemical nature the GHRPs are efficacious when administered *i.v.*, orally or orally. However, studies in cow, pig and sheep do not indicate such a close relationship between GHRH, somatostatin (SS) and GH, calling into question the general applicability of the human and rat models. Perhaps there is an important role for an endogenous GHRP in the regulation of GH secretion in domestic animals. This review provides an overview on the current knowledge of physiological role of GHRP-2 in domestic animals. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 5 : 757-766)

Key Words : GHRP, GH, Calf, Pig, Sheep

INTRODUCTION

There is now unequivocal evidence that the secretion of growth hormone (GH), like that of other anterior pituitary hormones, is under the regulatory control of the central nervous system (CNS). Two hypothalamic peptidergic hormones, GH-releasing hormone (GHRH) and somatostatin (SS) which have been identified, isolated and synthesized, are the final mediators of metabolic, endocrine and neural influences on GH secretion in the pituitary. Interposed between the brain and the hypophysiotropic functions are complex networks of neurotransmitter neurons that act to modulate the release of the hypothalamic hormones. GHRH and SS are secreted into the hypothalamic-hypophyseal system for rapid and direct access to the pituitary. The diurnal and nocturnal fluctuations in GH secretion are rapid and unpredictable, with a pattern that makes it difficult to assess the status of GH from single, randomly collected blood samples. Although pulses in secretion of GH occurred coincidentally with pulses in somatostatin in hypophysial-portal blood of rats (Plotsky et al., 1985), approximately 70% of pulses in GH secretion occurred coincident with, or immediately after

pulses in GHRH release in sheep (Frohman et al., 1990). In addition, both GHRH and SS can feedback to the hypothalamus to cause acute down-regulation of their own secretion. Considerable evidence has now been accumulated to indicate that GH can act to inhibit its own secretion through a complex feedback mechanism operating both on the CNS and the pituitary. It was inferred that elevated titers of circulating GH exert an auto-feedback at the level of the still elusive hypothalamic GHRH and SS centers and the pituitary level.

In addition to GHRH and its analogs, there has recently been an upsurge of interest in small molecule GH secretagogues (figure 1) that act through a mechanism different from that of GHRH. These secretagogues stimulate somatotrophs in the pituitary gland to release GH (Cheng et al., 1989; Pong et al., 1996) and act on the arcuate nucleus in the hypothalamus to apparently cause GHRH release (Dickson et al., 1995). Moreover, they potentiate the effects of GHRH and functionally antagonize somatostatin (Gertz et al., 1993).

This review will be focused on our recent data regarding the mechanism by which GHRP-2 (KP102) influences the regulation of GH secretion in domestic animals, e.g. calf, sheep and pig.

GROWTH HORMONE SECRETAGOGUES (GHS)

The GHRPs are a family of synthetic oligopeptides, which specifically stimulate the release of GH in many species as well as in humans. The first generation of GHRP, GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂), was derived from the pentapeptide met-enkephalin through

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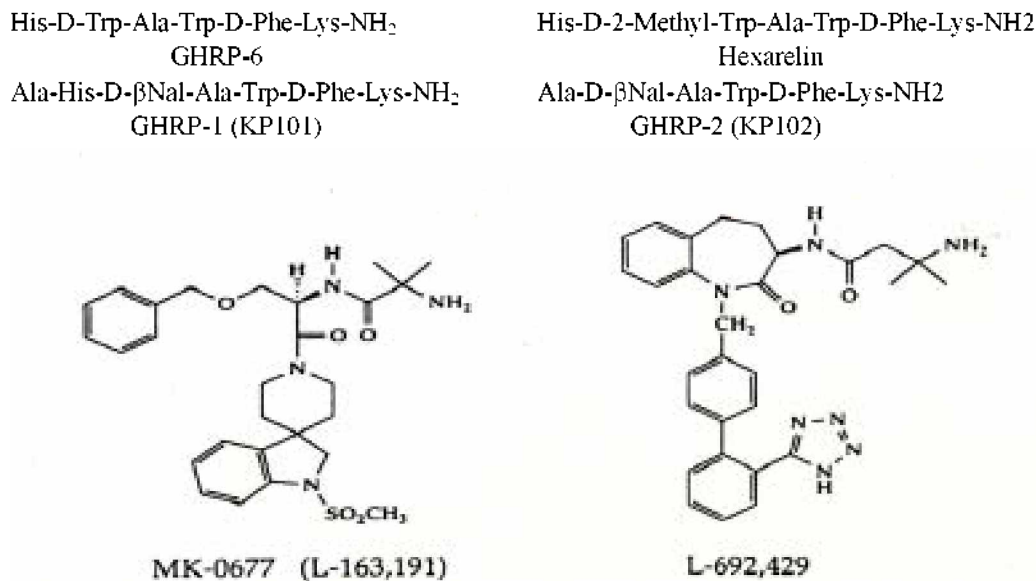


Figure 1. GH secretagogue structures.

theoretic low energy conformational calculations, computer modeling, structural modification and biologic studies (Momany et al., 1984). GHRP-6 stimulates GH release in many species (Bowers et al., 1984; Walker et al., 1990). Based on the structure of GHRP-6, a second generation of GHRP, GHRP-1 (Ala-His-D-βNal-Ala-Trp-D-Phe-Lys-NH₂, KP101), was developed. Recently, a new generation of GHRP, GHRP-2 (Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH₂, KP102), was synthesized and reported to be a potent GH secretagogue *in vitro* (Wu et al., 1994a; Hashizume et al., 1997b; Roh et al., 1997a) and *in vivo* in many different species (Hashizume et al., 1997a; Roh et al., 1996; Roh et al., 1997b; Hashizume et al., 1999; Sawada et al., 1994) as well as in humans (Bowers et al., 1993; Nijland et al., 1998; Van den Berghe et al., 1999) after i.v., s.c., intranasal, and even oral administration. The GH-releasing activity of GHRP-2 has been found to be two to three times more active in rats and humans than GHRP-6 and GHRP-1 (Bowers et al., 1993). Hexarelin, a 2-methyl substitution of D-tryptophan of GHRP-6, has been reported to be active in human (Ghigo et al., 1994). However, these peptides have the disadvantage that they are less than 1% orally bioavailable; therefore, several people sought mimetics that have structures more amenable to chemical modification and optimization of oral bioavailable (Smith et al., 1993). L-692,429, a benzolactam GH secretagogues (Smith et al., 1993; Gertz et al., 1993; Cheng et al., 1993), and L-692,585, a hydroxy propyl derivative of L-692,429 (Jacks et al., 1994), both induce a transient release of GH from rat pituitary cells and in rats and dogs, and L-692,429 is also effective in humans. MK-0677 (L-163,191), being a spiroindoline, belongs to a new structural class of GHRP-6 mimetics that has high *in vitro* and *in vivo* potency (Patchett

et al., 1995).

GHSs are artificial compounds which release GH in all species tested so far. Up to now, these molecules mimicked an unknown endogenous factor that activates the GHS-receptor (GHS-R). The earlier cloning of GHS-R (Howard et al., 1996) suggested that an endogenous ligand for this receptor might exist. Indeed, after intensive research by different groups, the isolation of an endogenous ligand of the GHS-R, named ghrelin (Kojima et al., 1999), was recently reported. Taking into account that it is secreted predominantly from the stomach and that ghrelin circulates in normal subjects at considerable plasma concentrations, it has been postulated that this molecule is secreted from the stomach, and circulates in the blood stream to stimulate GH secretion by the somatotrophs. Furthermore, ghrelin has been shown to regulate gastric acid secretion and motility in rats, indicating that this peptide may well be involved in the regulation of gastrointestinal function (Masuda et al., 2000)

GROWTH HORMONE SECRETION PATTERN BY GHRP-2 IN CALF

GH response by acute administration of GHRP-2 in calf

We firstly reported the characteristics of GH secretion response to GHRP-2 and showed the usefulness of GHRP-2 in calves (Roh et al., 1996, 1997a,b; Lee et al., 2000). Our study showed that calves responded to acute injections of GHRP-2 in a dose-dependent manner up to a dose of 12.5 μg/kgBW (Roh et al., 1996; Roh et al., 1997a). In the urethan-anesthetized rat, GH response to i.v. GHRP-2 was increased in a dose-dependent manner up to a dose of 1000 mg/kgBW (Sawada et al., 1994). These levels were considerably higher than those seen after maximal doses of

GHRH (81 ± 31 mU/L) (Bowers et al., 1990). These differences may be attributed to GH responsiveness depending on the species, endocrine regulatory system, nutritional body condition, age, weight and breed. The peak pattern of GH secretion following GHRP-2 injection showed similar results to other GHS (Baker et al., 1984; Doscher et al., 1984; Kraft et al., 1984; Bowers et al., 1991; Cheng et al., 1993; Johnson et al., 1993; Smith et al., 1993; Hickey et al., 1994; Walker et al., 1990; Petitclerc et al., 1987; Jacks et al., 1994). The GH response with GHRP-6 peaked at about 10-15 min, and gradually decreased to a basal level within 60 min in lambs (injected i.v. 2 mg/kg) and calves (injected i.v. 0.4 mg/kg) (Bowers et al., 1984). On the other hand, Dubreuil et al. (1990) reported that 10 mg/kg i.v. injected GHRH induced great GH responses for periods longer than 4 h in pigs. The endurance of these peaks appeared to be related to the characteristics of GH secretagogues acting on the hypothalamus-pituitary axis.

The GH secretory responses to multiple injections are important in studying whether chronic GHRP-2 administration induces a long-term increase in GH. Our data show that GH response gradually decreased following injections every 2 h for 8 h (Roh et al., 1996). A decrease in subsequent GH responses to GHRP-6 occurred after a single s.c. injection of GHRP-6 (Sartor et al., 1985). GH secretory responses to human pancreatic GHRH ($1-40$ NH₂) administered at 1 and 2 h intervals decreased with each injection (Della-fera et al., 1986). From these results, several possibilities can be derived: depletion of pituitary GH content, down-regulation and involvement of GHRH and SRIF. Bovine somatotroph cells have the potential to endure exterior stimulation. It is also possible that the duration of GHRP-2 receptor occupancy is an important determinant of the decreased response. However, it is very difficult to explain these possibilities from our results. To study the receptor binding characteristics may be necessary to investigate the down-regulation in GH response to GHRP-2 multiple injections. It is generally accepted that the neuroregulation of GH secretion is involved with hypothalamic GHRH and SRIF. The plasma GH level was higher in rats given GHRP-2+SRIF antiserum (or GHRH) than GHRP-2 alone (Sawada et al., 1994). The effect of GHRP-2 has been postulated to occur via its hypothalamic anatomic site of action.

With GHRP-2 the increased average daily body weight gain for total body growth and improvements in daily food efficiency may be due primarily to increased growth rate (Roh et al., 1996). Cumulative daily food intakes were remarkably similar in GHRP-2-treated and control calves. Thus, performance responses which were due to increased growth rate, may be dependent on GHRP-2. One possible mechanism for GHRP-2 effects could be via an increase in the digestibility of feed. Previous studies suggested that a

different subtype of GHRP receptor in CNS is involved in the gastrointestinal mobility to increase the absorption and digestibility (Rigamonti et al., 1999; Masuda et al., 2000). Furthermore, it is interesting to note that average daily body weight gain was greater during the 7 days period than the 14 days period. These findings might be partly due to the different effect of GHRP-2 on hypothalamus-pituitary-axis for longer period of time. The continuous increase of IGF-I on day 2, 7 and 14 with GHRP-2 chronic administration indicates that GHRP-2 stimulated GH secretion. The secretion of IGF-I responds to the hypothalamic-pituitary axis, then inhibits SRIF and in process of time may decrease GH secretion. As continuous administration of GHRP-2 seems to cause the refractoriness of GH, continuation of treatment over several weeks may reduce growth rate progressively. Since refractoriness following GHRP-2 administration occurs in GH secretion, it will be important to determine both the dosage and pattern of administration to closely monitor the GH secretory effect.

No desensitization of GH response between GHRP-2 and GHRH in calf

Secondly we have attempted to demonstrate the involvement of GHRH in the desensitization and mechanisms of action of GHRP-2 *in vivo* and *in vitro* (Roh et al., 1997a,b). The responses to a GHRP-2 or GHRH bolus injection were not attenuated by the concomitant continuous infusion of GHRH or GHRP-2 in the calf (Roh et al., 1997b). GHRP-1 and GHRP-2 have been shown to cause no cross-desensitization with GHRH in an ovine pituitary cell culture (Wu et al., 1994b; Wu et al., 1996). Furthermore, the continuous infusion of GHRP-6 did not attenuate the GH response to GHRH in the rat *in vivo* (Clark et al., 1989). Our study showed that homologous desensitization occurred between GHRPs and GHRH, but the response to the alternative secretagogue was preserved. These results argue for a separate and unique action for the two secretagogues, and suggest that they act through discrete receptor sites in the hypothalamus and/or pituitary. GHRPs have a direct GH-stimulatory action at the pituitary level via receptor sites which are different from those of GHRH (Wu et al., 1994a; Wu et al., 1996; Goth et al., 1992). The review of Chen et al. (1996) suggested that GHRPs could bind to a site on the GHRH receptor in the pituitary different from that employed by GHRH. GHRP-6 has been shown to interact with a novel low-affinity GHRH-binding site in the rat anterior pituitary cell membrane (Lau et al., 1991). GHRPs may have multiple binding sites in pituitary cells (Wu et al., 1994; Chen et al., 1996).

More evidence has been published which demonstrates that GHRPs may not only have a direct action on the pituitary gland but also act on the hypothalamus (Conley et

al., 1995; Fletcher et al., 1996; Codd et al., 1989; Bowers et al., 1990). GHRP-6 has been shown to cause a small, but significant effect on the pulsatile secretion of GHRH (Fletcher et al., 1996). Anti-GHRH antiserum pretreatment caused a decreased responsiveness to both GHRP-6 and hexareline (His-D-2-Methyl-Trp-Ala-Trp-D-Phe-Lys-NH₂) (Conley et al., 1995). It has also been reported that GHRP-6 has a binding site in the hypothalamus (Lau et al., 1991). It has been hypothesized that the actions of GHRP-6 are mediated, at least in part, via a decrease in hypothalamic tone (Bowers et al., 1990). In conclusion, our results showed that prolonged infusion of GHRP-2 did not diminish the GH response of GHRH in calves, and vice versa. However, the continuous infusion and injection of GHRP-2 attenuated GH responsiveness. We therefore suggest that GHRP-2 may cause GH secretion through a different receptor than GHRH in calves *in vivo*.

INFLUENCE OF MUSCARINIC, ADRENERGIC, DOPAMINERGIC AND SEROTONINERGIC BLOCKADE ON GH SECRETION TO GHRP-2 IN SHEEP

Although the mechanisms of action of GHRP-2 have not fully established, there is probably a dual site of action on both pituitary and hypothalamus, possibly involving regulatory factors in addition to GHRH and somatostatin. The secretion of GHRH and SS is governed by a host of neuropeptides and neurotransmitters which provide a functional link between higher CNS centers and hypophysiotropic neurons. Factors that have been implicated include the biogenic amines, e.g. acetylcholine, adrenaline, dopamine, serotonin, histamine etc. (Buonomo and Baile, 1990). Indeed, the facts that GHRP-6 is much more potent *in vivo* than *in vitro* suggested that GHRPs are deeply related to these factors (Chen et al., 1996).

Pretreatment with atropine (0.2 mg/kg, i.v.) and saline injection did not modify resting GH concentration, but suppressed the GH release caused by GHRP-2 (Roh et al., 1997c). It is now widely accepted that cholinergic muscarinic pathways play a major role in GH secretion (Müller, 1987). Increased concentration of GH after GHRP-2 injection results from the activation of the cholinergic muscarinic receptor, since it was abolished by the anticholinergic agent atropine. The muscarinic blocker atropine (100 µg, i.v.) completely abolished the stimulatory action of GHRP-6 in adult beagles (Muruais et al., 1993). Administration of atropine (1 mg, i.m.) completely prevented the GH responses to GHRP-6 in human (Peñalva et al., 1993). Administration of the cholinergic muscarinic blocker atropine, which acts by increasing hypothalamic SS release, completely blunted the GH responses to GHRP-2. These results suggested that the GH release to GHRP-2 was

regulated by cholinergic muscarine receptor via hypothalamus, although GHRP-2 acts directly on pituitary tissue. Theoretically, this could be due to either the possibility that GHRP-2 does not act via SS or alternatively the hexapeptide is unable to counteract the stimulatory effect of atropine on SS. Muscarinic cholinergic agonist drugs, such as pyridostigmine, stimulate basal GH release and GH responses to GHRH (Ross et al., 1987; Peñalva et al., 1990). Since the inhibitory effect of atropine in the GH response to GHRH is abolished by anti-somatostatin antibodies (Locatelli et al., 1986), it is widely accepted that acetylcholine regulates GH secretion by inhibition of SS release from the hypothalamus.

The blockade of α -adrenergic receptor, phentolamine, had no effect on basal concentration of GH. Infusion of phentolamine did not change the GH secretion elicited by GHRP-2 (Roh et al., 1997c) and GH secretion pattern by the injection of GHRP-2 during the administration of propranolol (0.25 mg/kg, i.v.). Propranolol following the injection of saline did not change the basal concentration of GH, and pretreatment of propranolol attenuates the GH release caused by GHRP-2 (Roh et al., 1997c). There was no significant difference on the GH AUC between propranolol plus GHRP-2 and propranolol plus saline. Adrenergic mechanisms participate in the regulation of GH secretion. α - and β -adrenergic receptors having, respectively, stimulatory or inhibitory influences (Müller, 1987). While the α_1 - (clonidine) and α_2 - (methoxamine) adrenergic agonist did not significantly increase GH responses to GHRP-6, administration of the α_1 - adrenoceptor antagonist prazosin (20 mg, i.v.) reduced GHRP-6-induced GH secretion (Muruais et al., 1993).

Chlorpromazine, a phenothiazine derivative, is known to block dopamine receptors and α -adrenergic receptors in the central nervous system (Müller, 1987). Administration of chlorpromazine (0.5 mg/kg, i.v.) slightly increased the basal plasma oGH levels (Roh et al., 1997c). Mianserine (1 mg/kg) did not alter GHRP-2-induced GH release, and modify the basal concentration of GH (Roh et al., 1997c). Serotonergic receptors in the hypothalamus may affect the secretion of GH by altering the synthesis or release of either GHRH or SS (Murakami et al., 1986; Willoughby et al., 1987; Conway et al., 1990). However, mianserine did not change the basal GH concentration and the GHRP-2-induced GH release. In rat, stimulation of serotonergic receptors is associated with increased GHRH and GH release (Murakami et al., 1986; Willoughby et al., 1987; Conway et al., 1990). In steer, Sartin et al (1987) showed that stimulation of 5-HT receptors with quipazine increased the release of GH, whereas the 5-HT receptor antagonist cyproheptadine suppressed the release of GH. The stimulation of serotonergic receptors with quipazine increased release of GH, while the serotonergic receptors

antagonist cyproheptadine suppressed the release of GH (Gaynor et al., 1996; Sartin et al., 1987). These results indicated that serotonergic receptors are involved in the stimulation of GH secretion. However, in our study the plasma GH response to GHRP-2 was not suppressed by serotonin receptor antagonist, mianserin. Furthermore, Mianserin did not decrease the concentration of GH in plasma compared with saline. These showed that although serotonergic system participates in physiological regulation of GH secretion, GHRP-2 does not have a direct action via this pathway.

GH SECRETION BY GHRP-2 IN PIG

GHRP-2 on the release of GH and growth performance in swine

The single i.v. injection of GHRP-2 over the dose range of 2, 10, 30 and 100 mg/kg BW to cross-bred castrated male swine significantly stimulated plasma GH release in a dose-dependent manner (Phung et al., 2000), though there was no significant difference in the response between the 30 and 100 mg of GHRP-2/kg BW doses. The chronic s.c. injection of GHRP-2 once daily at a dose of 30 mg/kg BW for 30 days significantly increased peak plasma GH concentrations and GH AUCs for 300 min after the injections at all sampling days and consistently increased GH release during the entire treatment period (for at least 30 days). These findings are in agreement with previously reported results for human that s.c. injection of 100 mg of GHRP-2/kg BW once daily over 5 days to healthy young men significantly increased serum GH concentration (Nijland et al., 1998). Similarly, the daily s.c. administration of GHRP-6 (30 mg/kg BW) to rats for 9 days increased the GH-releasing effect of the peptide (Sartor et al., 1985). A point of interest in chronic GHRP-2 administration is that there was a partial attenuation of GH response to GHRP-2 between d 1 and 10, and a trend toward an increase in the response between d 10 and 30 of treatment in GHRP-2-treated swine. These observations suggest that it seems to have a desensitization of somatotrophs in chronic administration of GHRP-2 for the first treatment period, and after having reached a certain level, desensitization will not increase or that responsiveness to GHRP-2 is restored after chronic repeated administration.

In addition, our report also showed that chronic s.c. administration of 30 mg of GHRP-2/kg BW once daily for 30 days to swine improved average daily gain for the entire treatment period and feed efficiency (feed/gain) without affecting daily feed intake (Phung et al., 2000). An improvement of growth rate by chronic administration of GHRP-6 was also documented in rats (Bowers et al., 1984). Although there was no significant difference in daily feed intake between treatments in this experiment, an increase in

feed intake was reported after the acute administration of GHRPs in rats (Locke et al., 1995; Okada et al., 1996; Toisello et al., 1998). However, in a recent study, Rigamonti et al. (1999) have reported that the acute feeding response to hexarelin in a six-week treatment with hexarelin in young dogs was abolished at the 3rd week and returned to normal at the 6th week. At present, the underlying cause of this temporal pattern of feeding response was not known; however, the probable existence of different subtypes of central nervous system GHRP receptors engaging in the manifold actions of GHRPs was suggested (Rigamonti et al., 1999). It is difficult to interpret completely the mechanisms, which are responsible for the improvement of growth rate in swine from our present experiment. However, the findings that GH responses to chronic administration of GHRP-2 were consistently enhanced for the entire treatment period in the current experiment might be related to the acceleration of body weight gain observed after administration of GHRP-2.

Oral administration on GH release in swine

Another study in swine demonstrated that GHRP-2 stimulates the release of GH after being orally administered as a bolus in saline and when mixed in feed, and that the administration of GHRP-2 in feed does not desensitize the GH responsiveness of the peptide, at least after short-term treatment (Phung et al., 2001). This result showed that administration of GHRP-2 orally at doses of 1, 4.5 and 9 mg/kg BW to cross-bred castrated male swine significantly stimulated the release of GH at all doses, with dose-related increases in peak concentrations of GH. This suggests that swine respond to the oral administration of GHRP-2, even at a relatively low dose. This could be explained by a difference in the magnitude of GH response between individuals in each treatment dose. In fact, the oral activity of GHRP-6, the original GHRP, on the release of GH was seen at doses as low as 3 mg/kg BW in rats (Walker et al., 1990). The oral administration of GHRP-6 at a dose of 300 mg/kg BW in humans elicited a GH-releasing effect similar to that induced by i.v. administration of its maximal effective dose (1 mg/kg BW) (Hartman et al., 1992; Bowers et al., 1992). Oral bioactivity of GHRPs is unusual for the peptide (Hartman et al., 1992). However, the high GH-releasing potency of GHRPs, their small size, and partial protection from proteolytic degradation of the peptide by D-amino acids substitutions in the chemical structure, support the oral bioactivity of these peptides (Walker et al., 1990; Bowers et al., 1992). However, administration of GHRP-2 in feed at the same dose range only stimulated the release of GH with doses of 4.5 and 9 mg/kg BW (Phung et al., 2001). In addition, peak concentrations of GH and GH AUCs for 180 min after the treatments were considerably lower when GHRP-2 was given in feed rather than as an oral bolus. These results

suggest that administering GHRP-2 in feed is less effective than oral administration as a bolus in saline. There are several probable explanations for the decline in GH response when GHRP-2 was administered in feed. It is possible that feeding immediately after each treatment with GHRP-2 decreased the secretion of GH and reduced GHRP-2-induced GH responses. Secretion of GH, as well as GH-releasing factor-induced GH response, has been shown to decrease during and for several hours after feeding (Moseley et al., 1988; Trenkle et al., 1989), although the precise mechanism underlying the decrease in the response is still unclear (McMahon et al., 1999). Another possible explanation is that the absorption of the peptide was limited and delayed by the presence of feed in the gastrointestinal tract. After the administration of GHRP-2 in feed, plasma concentrations of GH peaked within 60-75 min, and then gradually declined to the basal concentrations between 120 and 135 min. Although there was no significant difference in peak concentrations of GH and GH AUCs between the 4.5 and 9 mg/kg BW GHRP-2 doses on any sampling day, the concentrations of GH increased earlier after treatment with GHRP-2 in feed at a dose of 9 mg/kg BW rather than with GHRP-2 at a dose of 4.5 mg/kg BW.

The administration of GHRP-2 in feed also revealed that there was no significant difference in peak concentrations of GH and GH AUCs between the initial and final treatments with GHRP-2 within each dose level after a 3 d treatment period. This suggests that the GH-releasing effect of GHRP-2 in swine does not become desensitized after the administration of the peptide in feed, at least after a 3 d treatment period. In fact, a partial attenuation in GH responsiveness to GHRP-2 after a 5 d treatment with a daily s.c. injection of 100 mg of the peptide in healthy young men was reported (Nijland et al., 1998). Also, the continuous infusion of GHRP-6 caused homologous desensitization both in animals and in humans (Badger et al., 1984; Sartor et al., 1985; Debell et al., 1991; Huhn et al., 1993; Jaffe et al., 1993). However, corresponding with our results, a 4 d oral treatment with GHRP-6 administration twice daily in normal aging, maintained the GH-releasing effect of the peptide, with a trend toward an increase in the response after treatment (Ghigo et al., 1994). In addition, the GH-releasing effect of Hexarelin, a GHRP, did not change after a 15 d oral treatment with the peptide (Ghigo et al., 1996). Taken together, these studies along with our present data appear to indicate that desensitization of the GH-releasing effect of GHRPs depends on the mode of administration of the peptides, and that it does not occur after intermittent oral treatment (Ghigo et al., 1994; Ghigo et al., 1996) or after short-term administration in feed.

IGF-1 SECRETION BY GHRP-2 IN STEERS

Nutritional status plays an important role in the regulation of circulating levels of GH, IGF-1 and IGFBPs

in sheep and cattle (Vicini et al., 1991; Gallaher et al., 1992; Gallaher et al., 1995). We reported that a low feed intake (LI; 1.22% DM of body weight/day) decreased plasma IGF-1, 38-43 kDa IGFBP-3 and 24 kDa IGFBP-4, but plasma GH and 34 kDa IGFBP-2 were increased compared with a high intake group (HI; 2.43% DM of body weight/day; Lee et al., 2000). These results provide evidence that endogenous GH/IGF-1/IGFBPs axis in circulation can be regulated by nutrition in steers. The maximum peak and AUC (0-180 min) of plasma GH after saline injection in the LI group were higher than in the HI group, but maximum post-injection plasma GH response to GHRP-2 was higher in the HI group than the LI group, although GH AUC (ng/ml.min) for 180 min of plasma GH was not significantly different in the two steer groups (Lee et al., 2001). The increased plasma GH in the LI group may be associated with an increase in GHRH (Armstrong et al., 1993), a decrease in hypophyseal concentration of somatostatin (Thomas et al., 1991) and inhibition of the negative feed back effect of a decreased IGF-1 on the hypothalamic-pituitary synthesis and secretion of GH (Kirby et al., 1993). GH response to GHRH administration has been shown to be higher in poorly fed than in well-fed animals. In sheep, the mean post-injection plasma GH response to rhGHRH-29-NH₂ was increased in the food deprived group rather than the well-fed group (Hart et al., 1985). In addition, the GHRPs stimulate GH release through a G-protein-linked receptor that has an endocrine pathway distinct from that described for GHRH (Howard et al., 1996; Pong et al., 1996), and these act directly on the pituitary somatotrophs as well as on the hypothalamus in sheep (Fletcher et al., 1994). Thus, we suppose that the GHS-R for GHRP-2 may be sensitive to nutrition, as is the GH-receptor in the liver. Serum IGF-1 typically increases 11 to 18 h after administration of ST or elevation of endogenous ST via GHRH in cattle (Enright et al., 1989; Cohick et al., 1998; Simpson et al., 1992).

GHRP-2 treatment increased plasma 38-43 kDa IGFBP-3 in only the HI group, but no significant difference was observed with GHRP-2 treatment in the LI group (Lee et al., 2000). Plasma IGFBP-3 levels showed a positive correlation with IGF-1 concentration in the normal adult human (Rajaram et al., 1997) and sheep (Hodgkinson et al., 1991; Gallaher et al., 1992). Furthermore, the IGFBP-3 in blood decreases clearance of IGF-1 from serum and prolongs the half-life of IGF-1 (Guler et al., 1989). Thus the increase in plasma IGF-1 concentration with GHRP-2 treatment in the HI group may have resulted from an increase in plasma 38-43 kDa IGFBP-3. We clearly noticed no changes in plasma 38-43 kDa IGFBP-3 in response to GHRP-2 treatment appeared on a low nutritional plane, although it was increased with GHRP-2 treatment on a high one. This result demonstrates that resistance to endogenous GH stimulation of 38-43 kDa IGFBP-3 with GHRP-2 administration may be influenced by poor nutrition, because

hepatic GH binding sites decreased in animals on restricted diets, as discussed earlier. The relationship between GH and plasma IGFBP-2 has not been understood in ruminants, but bovine GHRH or GH administration decreased serum IGFBP-2 (Vicini et al., 1991; Vanderkool et al., 1995). Plasma 34 kDa IGFBP-2 did not show any change with GHRP-2 treatment in either feed treatment group. In general, IGFBP-2 inhibits the action of IGF *in vitro*, although it can enhance the mitogenic activity of IGF-1 in porcine smooth muscle cells (Camacho-Hubner et al., 1991); if the IGFBP-2 inhibits IGF-1 activity and prevents IGF-1 association with cell surface receptors, an increased plasma IGF-1 with GHRP-2 may have less biopotency than that increased with GHRH in steers. Plasma 24 kDa IGFBP-4 showed the same response as plasma 38-43 kDa IGFBP-3 in feed treated as well as GHRP-2 treated growing steers. The circulating amounts of IGFBP-4 were not altered by nutritional restriction in ewes (Gallagher et al., 1992) and adult cows (Roberts et al., 1997), or with exogenous GH administration in lactating dairy cows (Cohick et al., 1992). However, immunization against GHRH or feed deprivation for 72 h resulted in decreased serum IGFBP-4 in heifers (Cohick et al., 1996) and 1 year old castrated lambs (Gallagher et al., 1992).

CONCLUDING REMARKS

Although slow to be introduced into domestic endocrine physiology, the concept of the regulation of GH secretion by GHRP-2 is now indisputably established, and the complex mechanisms involved in this function are being clarified. In this manuscript, we clearly show that GHRP-2 stimulates GH secretion and improves the growth performance with the modulation of endocrine profiles in domestic animals. However, there is need to carry out more research before application of GHRP-2 in domestic animals.

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