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Somatotropic Axis and Nutrition in Young Ruminants around Weaning Time*

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ABSTRACT : The somatotropic (GH-IGF-I) axis consists of many hormonal and nutritional factors that control GH release from the somatotrophs in the anterior pituitary. The GH-releasing substances are GHRH and GHS (GHRP or ghrelin), while the GH release-inhibiting substances are somatostatin (SRIF), insulin-like growth factor-I (IGF-I), leptin and glucocorticoids. However, there is evidence showing that nutrition is involved in the control of the somatotropic axis. In addition, weaning is a drastic event for neonates because their alimentary and endocrine circumstances are changed due to the switch, even if gradual, from a liquid milk diet to one composed of such solids as hay and grains. The biological role of ghrelin is one of the hormonal factors that have been focused on ever since ghrelin was discovered at the end of the last century. A 27-amino acid peptide that is mainly synthesized and released from the abomasum epithelium, ghrelin has not been fully evaluated in relation to the somatotropic axis of the ruminant. It has also proven difficult even to investigate the cellular mechanisms of ghrelin action, because this hormone exerts animal-species-dependent actions via a complex set of intracellular signaling pathways. This is also the case for the action of leptin. Another substance, IGF-I, shows a partial inhibitory action on GH secretion in the ruminant. The effect of nutrition is also different among animal species. This is evident by the fact that undernutrition suppresses the circulating GH levels in rodents, but increases it in ruminants and humans. Recently, weaning has been shown to change the postprandial GH responses in ruminants; milk feeding increases, but hay and concentrate feeding suppress, the postprandial circulating GH levels. Even if the postprandial GH level is increased, the ghrelin level is decreased by milk feeding. Macronutrients also possess stimulatory and inhibitory actions on GH secretion *in vivo* and *in vitro*. These findings indicate the complexity of the control mechanisms of the somatotropic axis. In the present review, we summarize recent findings on the factors controlling the axis of the ruminant. (**Key Words :** Ruminant, Somatotropic Axis, Nutrition, Weaning, Development)

INTRODUCTION

The somatotopic (GH-IGF-I) axis in the ruminant, as for other domestic animals, is essential for the control of metabolism and the functions of various tissues and organs, including bone, liver, skeletal and cardiac muscles, adipose tissue, and reproductive organs (Simmen et al., 1998; Clark and Rogol, 1999). The somatotropic axis has been shown to include many hormonal and nutritional factors that control GH release from the anterior pituitary. In addition, we recently found that weaning drastically changes the somatotropic axis (Katoh and Obara, 2001; Katoh et al., 2004a). In this review, we summarize recent findings on the factors that control the somatotropic axis in the ruminant.

THE SOMATOTROPIC AXIS

The overall features of the somatotropic axis are depicted in Figure 1. It has been established that GH secretion from the anterior pituitary is controlled by two major hypothalamic hormones: GH-releasing hormone (GHRH) and somatostatin (SRIF). GH release is stimulated by GHRH, but is inhibited by SRIF. The concentrations of GHRH and SRIF in the hypophyseal-portal circulation, as well as GH in peripheral circulation, alternate in a pulsatile manner (Frohman et al., 1990). The peaks of GHRH are significantly correlated with GH pulses up to 70%, whereas the nadir of the somatostatin (SRIF) concentration does not synchronize with the peaks of the plasma GH concentration (Frohman et al., 1990) or maintain complex, time-varying interactions (Veldhuis et al., 2002). In addition, no sex differences were demonstrated in circulating patterns of SRIF in the ovine hypophyseal-portal vein, despite the

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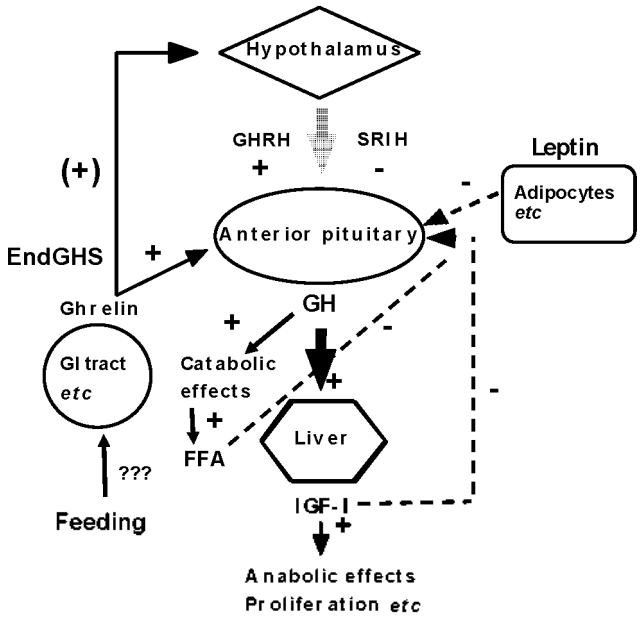

Figure 1. The somatotropic axis. GHRH: GH-releasing hormone, SRIF: somatostatin, IGF-I: insulin-like growth factor I, FFA: free fatty acids, EndGHS: endogenous GH secretagogues, GI tract: gastrointestinal tract. +: stimulation, -: inhibition.

existence of sexually dimorphic patterns of GH secretion (Gatfold et al., 1997). It was also reported that sheep are 10 times less sensitive to SRIF than rats to cause the SRIF-induced inhibition of GH secretion (Briard et al., 1997). These findings suggest that the role of SRIF in controlling GH secretion in sheep is different from that in rats, suggesting that the control mechanisms of the somatotropic axis are different among different animal species.

Recently, the GH secretagogue (GHS) family (GHRP or ghrelin) has been discovered to be a stimulant for GH release, and intensive study on GHS is being conducted with respect to the somatotropic axis and orexigenic action. GHRH-6, one of the GHS family, did not change the concentrations of GHRH and SRIF in the ovine hypophyseal-portal vein (Fletcher et al., 1996), despite a marked increase in the peripheral circulating GH level. At the end of the last century, ghrelin, an endogenous GHS, was discovered by Kojima et al. (1999). Ghrelin is mainly synthesized in, and released from, the stomach epithelium of many animal species studied so far, including ruminants and humans. Although this hormone has been shown to stimulate GH release and appetite in rats and humans (Kojima and Kangawa, 2005), the significance of ghrelin has not been fully evaluated in the ruminant.

Other substances such as insulin-like growth factor-I (IGF-I), leptin and glucocorticoids are known to inhibit GH release in the ruminant. In addition, factors such as feeding and nutrition are also important for controlling GH release, which is also changed by weaning. There is a complex relationship among these factors, which is described below.

GHRELIN

History

Bowers and colleagues (Bowers, 1999) found that some opioid peptide derivatives had weak GH-releasing activity. Since the 1970's, these peptides had been referred to as GHRP (GH-releasing peptides). The first generation of GHRP was a pentapeptide derived from a Met-enkephalin derivative, in which the second Gly from the N terminal was replaced by D-Trp. The second generation was a hexapeptide, which was called GHRP-6 and was biologically active *in vivo* and *in vitro* (Argente et al., 1996). In 1983, researchers at the Merck Institute found that non-peptide GHS, L-692,429 and L-163,191 (MK-0677) exerted sufficient activity even when orally administered (Smith et al., 1993; Patchett et al., 1995). It was first shown that GHS stimulates GH release through distinct receptor sites from GHRH (Blake and Smith, 1991), and the GHS receptor (GHSR) was identified by expression cloning in *Xenopus* oocytes (Howard et al., 1996). Since that time, the existence of an endogenous GHS ligand had been expected, and Kojima et al. (1999) successfully isolated a GH-releasing acylated peptide, ghrelin, from the stomach of rats. GHSR, a typical GTP-binding protein-coupled receptor (GPCR), is widely expressed in various tissues, including the pituitary, hypothalamus, hippocampus, kidney and reproductive organs.

Chemical features and synthesis

Ghrelin is a 28-amino acid peptide in humans and rodents. The Ser residue at position 3 from the N-terminal is *n*-octanonylated, and this acylation is essential for ghrelin's activity (Kojima and Kangawa, 2005). When the amino acid sequence of ghrelin is compared among mammalian species, residues at the positions of 1-10, 13, 15-17, 19-21, 25 and 27-28 from the N-terminal are the same within all species in

Table 1. Amino acid sequence of ghrelin in various mammals

Mammals	Amino acid sequence																												
				5						10						15						20						25		
Human	G	S	S	F	L	S	P	E	H	Q	R	V	Q	Q	R	K	E	S	K	K	P	P	A	K	L	Q	P	R
Cow	G	S	S	F	L	S	P	E	H	Q	K	L	Q	*	R	K	E	A	K	K	P	S	G	R	L	K	P	R
Goat/sheep	G	S	S	F	L	S	P	E	H	Q	K	L	Q	*	R	K	E	P	K	K	P	S	G	R	L	K	P	R
Rat	G	S	S	F	L	S	P	E	H	Q	K	A	Q	Q	R	K	E	S	K	K	P	P	A	K	L	Q	P	R
Mouse	G	S	S	F	L	S	P	E	H	Q	K	A	Q	Q	R	K	E	S	K	K	P	P	A	K	L	Q	P	R

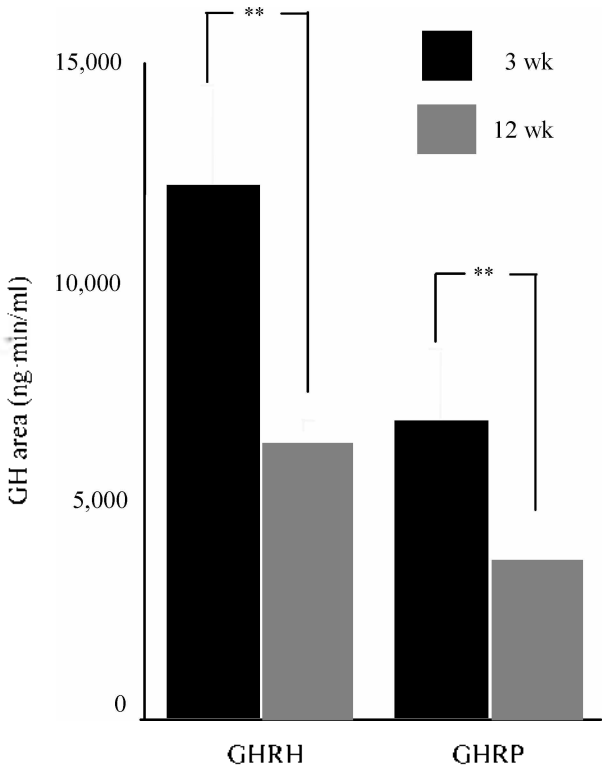

Figure 2. GH area in response to intravenous administration of GHRH or GHRP in young calves at 3 or 12 weeks of age. ** $p<0.01$ (Katoh et al., 2004a).

which the residues were determined. In the ruminant, ghrelin is a 27-amino acid peptide because Gln at position 14 from the N-terminal is lacking. The sequence of ovine ghrelin is exactly the same as that of caprine, whereas one amino acid (Ala) at position 18 from the N-terminal is replaced by Pro in the bovine (Table 1).

In mammals, ghrelin is mainly synthesized in the stomach. It is known that ghrelin-containing cells are more abundant in the fundus than in the pylorus (Date et al., 2000). Four types of endocrine cells that contain ghrelin have been identified, and X/A-like cells were found to be filled with ghrelin (Kojima et al., 1999). In goats and calves, ghrelin peptide and mRNA are expressed mainly in the abomasum (Hayashida et al., 2001).

Actions

The intravenous injection of ghrelin as well as GHRP increases GH secretion in goats and calves (Fletcher et al., 1996; Hayashida et al., 2001; Katoh et al., 2004a; Hashizume et al., 2005; Itoh et al., 2005) as well as humans (Takaya et al., 2000) *in vivo*. The potency of ghrelin was less than one-tenth that of GHRH in goats (Hashizume et al., 2005) and in calves (Itoh et al., 2005). It has been accepted that the somatotropic axis is not completely established at the fetus and neonate stages (Blum and Hammon, 1999). In our study with pituitary cells isolated from a bovine fetus

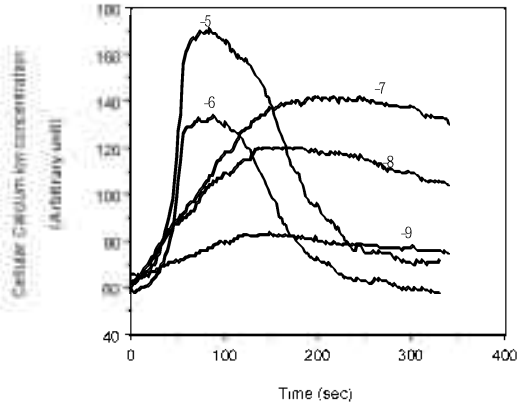

Figure 3. Time courses of increase in $[Ca^{2+}]c$ in response to KP-101 (one of the GHRP (GH-releasing peptides)) given at time 0 in ovine anterior pituitary cells. The number near the traces means the power of the concentration used: ex) -5 means 10^{-5} M.

(3-month gestation, term = 280 days) and cultured for 3 days in DME medium, GHRH or GHRP-1 stimulation caused significant increases in GH release. However, the amount of GH release was one-tenth that of adults. Pituitary cells isolated from a 70-day-old ovine fetus (term = 147 days) also responded to the administration of GHRH and SRIF (Blanchard et al., 1988; Silverman et al., 1989).

We compared GH secretion in response to GHRH and GHRP around weaning time. The intravenous injection of GHRH and GHRP-6 (0.25 and 2.5 g/kg BW, respectively) significantly increased the plasma GH concentrations, despite the fact that the calves were in the process of being weaned. However, responsiveness to the GHRH and GHS challenge was reduced after weaning, as shown in Figure 2 (Katoh et al., 2004a). The GH area was significantly smaller at 12 weeks of age than at 3 weeks of age. It has been accepted that aging suppresses the circulating GH levels in many animal species. The intravenous injection of GHRP significantly increased the circulating insulin levels in young calves (Katoh et al., 2004a).

In the cellular signal transduction system for the action of GHRH and GHS, it is well established that GHRH exerts GH release through the cyclic AMP (PKA) system followed by an increased cellular calcium concentrations ($[Ca^{2+}]c$), while GHS exerts its action through the IP_3-$[Ca^{2+}]c$ (PKC and PKG) system. In sheep anterior pituitary cells cultured for 3 days, stimulation with GHRP-1 and GHRP-2 (KP-101 and -102, respectively) increased GH release (Wu et al., 1994a, b) as well as $[Ca^{2+}]c$ in a dose-dependent manner (Figure 3). Dr. Chen's group in Australia recently showed that ghrelin reduces the voltage-gated K^+ current (Han et al., 2005), whereas SRIF, an inhibitor for ghrelin secretion (Shimada et al., 2003; Silva et al., 2005) and ghrelin-induced GH release, increases voltage-gated K^+ current in GH_3 cells (Yang et al., 2005). The increase in $[Ca^{2+}]c$ and GH release evoked by ghrelin or GHRP stimulation was

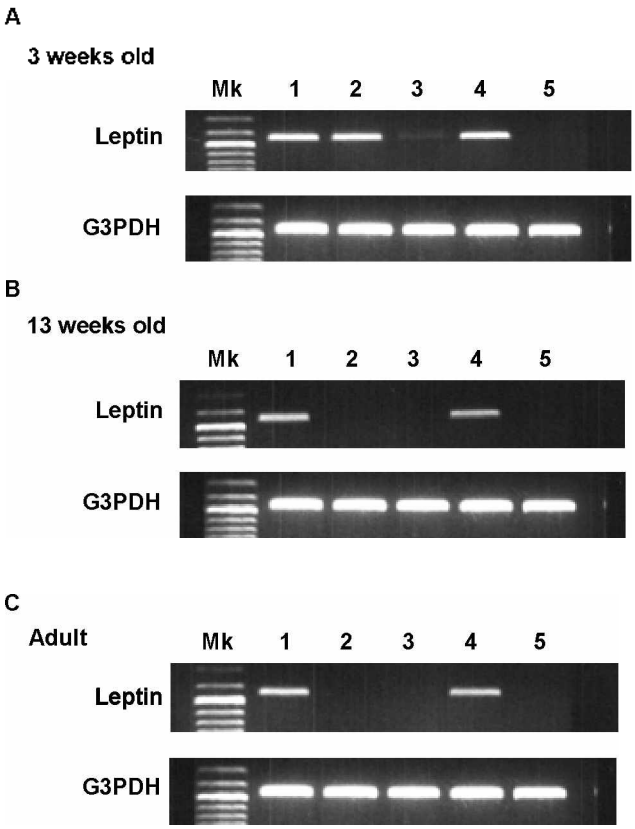

Figure 4. Effects of aging on leptin mRNA expression in various GI tracts of young and adult cattle. Lane 1: adipose tissue (positive control), lane 2: rumen, lane 3: abomasums, lane 4: duodenum, lane 5: liver (negative control) (Yonekura et al., 2002).

significantly inhibited by the addition of TTX, nifedipine, SRIF, dexamethasone or fatty acids (our unpublished data). The inhibitory actions of these substances were also observed for GH release evoked by GHRH stimulation.

Unexpectedly, GH release induced by KP-102 was completely abolished, but that by KP-101 stimulation was not affected (Wu et al., 1994b), by a GHRH antagonist ([Ac-Try^1, D-Arg^2] GHRH1-29). Additionally, KP-102 is reported to increase cAMP concentration in ovine somatotrophs (Wu et al., 1996). Also in porcine somatotrophs, ghrelin increased GH secretion and cellular cyclic AMP concentrations (Malagon et al., 2003). However, Chen et al. (1998a, b) clearly showed that GHRP-2 does not act through the GHRH receptor in human somatotrophs and rat pituitary GC cells transfected cDNA coding for the human GHRH receptor. Although this confusion remains to be clarified, it is plausible that there might be partially sharing molecules between GHSR and GHRHR on the cell membrane of the somatotrophs of the artiodactyla.

Secretion

Aging decreases the circulating concentrations of ghrelin and GH. In rats, however, aging enhanced the ghrelin mRNA expression and increased the circulating concentration and accumulation in the stomach (Englander et al., 2004). Although it was reported that the circulating ghrelin level was 20-35% lower in older than in young adults (Rigamonti et al., 2002; Sturm et al., 2003), this finding should be carefully analyzed because it is known that increased body fat, commonly seen in elderly adults, decreases the circulating ghrelin levels. Ghrelin secretion is suppressed by somatostatin, as is GH secretion (Shimada et al., 2003; Silva et al., 2005).

Sugino et al. (2004), based on their excellent series of studies, concluded that ghrelin secretion is not stimulated by direct contact with ingested feed or nutrients, but by anticipation of a meal in adult sheep. This is because the plasma ghrelin concentrations increased just before a scheduled meal, but thereafter decreased. In addition, the preprandial ghrelin surge that was followed by a GH surge was enhanced by the intravenous infusion of cholinergic antagonists. These findings mean that the transient surge of ghrelin is caused by a conditioned emotional response, but not by ingested nutrients. Therefore, the preprandial ghrelin surge was thought to be a signal for controlling feeding behavior. However, Iqbal et al. (2006) demonstrated that the IV or ICV infusion of ghrelin did not stimulate voluntary food intake, but did change GH and LH secretion in ovariectomized ewes, indicating that ghrelin does not appear to be a significant signal for appetite in this animal species.

It is obvious that nutrition is related to ghrelin secretion. First, mRNA in the stomach (Toshinai et al., 2001) and the circulating levels (Hayashida et al., 2001; Toshinai et al., 2001) of ghrelin are increased by fasting but reduced by feeding in rats and ruminants. Second, circulating ghrelin levels are reduced by macronutrient administration or by the ingestion of glucose or lipids, but increased by a high-protein diets in rats and humans (Broglio et al., 2002; Nakagawa et al., 2002; Erdmann et al., 2003; Vallejo-Cremades et al., 2004). However, more detailed studies are needed in the future on the somatotropic axis.

LEPTIN

General features

Leptin, the product of the *ob* gene, is expressed in and released mainly from mature adepocytes (Zhang et al., 1997) as well as other tissues, including the hypothalamus, anterior pituitary gland, mammary gland and gastrointestinal tract (Senaris et al., 1997; Morash et al., 1999; Yonekura et al., 2002a, b, 2006). The leptin receptors, which consist of at least five isoforms (Lee et al., 1996), are also widely expressed in various tissues (Fei et al., 1997). Messenger RNA of the leptin receptor (Ob-Rb) exists in the two major neuronal groups in the arcuate nucleus (Mercer

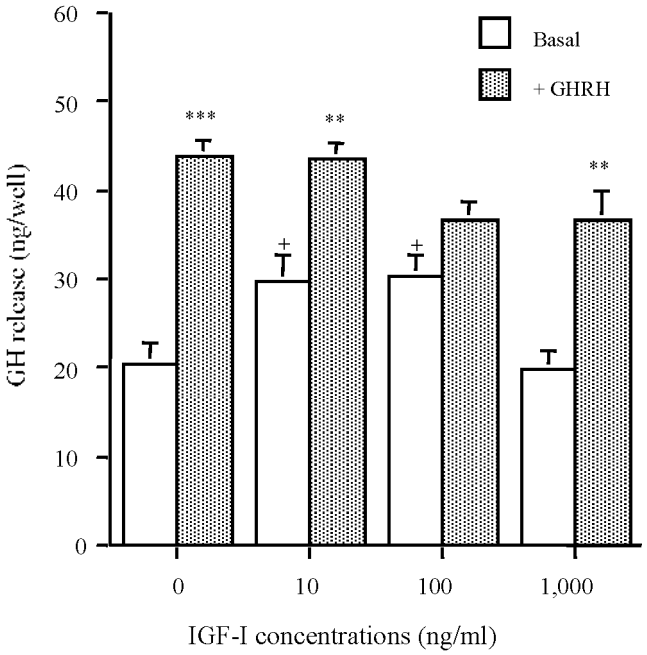

Figure 5. Inhibitory effects of IGF-I on GHRH-induced GH release in goat anterior pituitary cells. **, *** $p<0.01$, $p<0.001$ vs. Basal at each IGF-I concentration; +: $0.05<p<0.10$ vs. Basal value at IGF-I concentration of 0 ng/ml (Katoh et al., 2004b).

et al., 1996; Cheung et al., 1997). This finding suggests the possibility that leptin, as well as voluntary food intake, is involved in the somatotropic axis. The circulating leptin level increases with aging in calves (Yonekura et al., 2002b) and dietary energy levels change the plasma leptin levels in sheep (Tokuda et al., 2002). However, feeding does not cause any significant change in the plasma leptin levels.

Weaning

Leptin mRNA expression in the rumen and abomasum of calves was abolished, but that in the adipocytes and duodenum was still observed after weaning (Figure 4)(Yonekura et al., 2002a). However, leptin mRNA was observed in the rumen and abomasum isolated from animals that were maintained on milk feeding until 13 weeks of age, but not in the animals that were maintained on milk and VFA feeding until 13 weeks of age. These findings suggest that the reason why the leptin mRNA expression in the stomachs was abolished after weaning may be because of VFA production in the forestomach along with changes in diet caused by weaning. It is not known, however, whether or not the inhibitory effects of leptin on the somatotropic axis change with aging.

Actions

It was reported that serum leptin concentrations are inversely correlated with circulating GH concentrations in humans (Tuominen et al., 1997). This is also likely in young calves. Roh et al. (1998) demonstrated that leptin significantly suppressed GHRH-induced, but not basal, GH secretion in the ovine pituitary cells. However, leptin enhanced GH secretion and GHmRNA expression in pig pituitary cells (Saleri et al., 2005). The difference in animal species has not been clarified.

Leptin administration in peripheral circulation was demonstrated to suppress voluntary food intake in sheep and rats (Henry et al., 1999; Stanley et al., 2005). The intracerebroventricular infusion of leptin also reduced voluntary food intake only in sheep fed *ad lib*, but it increased the circulating GH level in sheep fed *ad lib*, and under long-term food-restriction (Henry et al., 2001). This finding indicates that an increased circulating GH level in food-restricted sheep is not caused by leptin, but by suppressed SRIF secretion, as previously demonstrated by Thomas et al. (1991).

IGF-I

Insulin-like growth factor I, which is particularly contained at a high concentration in colostrum, is one of the essential factors for neonatal calves because orally administered IGF-I stimulates epithelial cell proliferation in the small intestine (Baumrucker et al., 1994; Blum and Hammon, 1999). The synthesis and release of IGF-I are mainly undertaken by the liver, and are accelerated by stimulation with GH as well as by amino acids. The plasma IGF-I levels increase with aging, although the GH levels are reduced in calves. This means that control of the basal plasma IGF-I levels diminishes with aging.

Although IGF-I partially mediates GH action on animal growth, it also exerts an inhibiting action on GH secretion by a negative feedback mechanism. IGF-I suppresses GHRH-stimulated GH release *in vivo* and *in vitro* (Yamashita and Melmed, 1986; Fletcher et al., 1995; Wehrenberg and Giustina, 1999; Katoh et al., 2004b). Fletcher et al. (1995) found that IGF-I possesses a short-loop feedback action on the anterior pituitary gland, but does not have a long-loop feedback action to the hypothalamus. We attempted to prove by an *in vitro* study that this short-loop feedback occurs, in which the effects of various concentrations of IGF-I on basal and GHRH-induced GH release were assessed in goat anterior pituitary cells (Katoh et al., 2004b). A significant increase in GH release induced by GHRH stimulation was abolished when the cells were incubated in a medium containing IGF-I at 100 ng/ml (Figure 5). This abolishment of GH release was due to the increased basal GH release by IGF-I treatment without changing the GHRH-induced responses. IGF-I was also demonstrated to suppress the increase in the cellular calcium ion concentrations induced by GHRH stimulation. Additionally, the inhibitory action of IGF-I was caused by a process, which was acute because it did not cause the

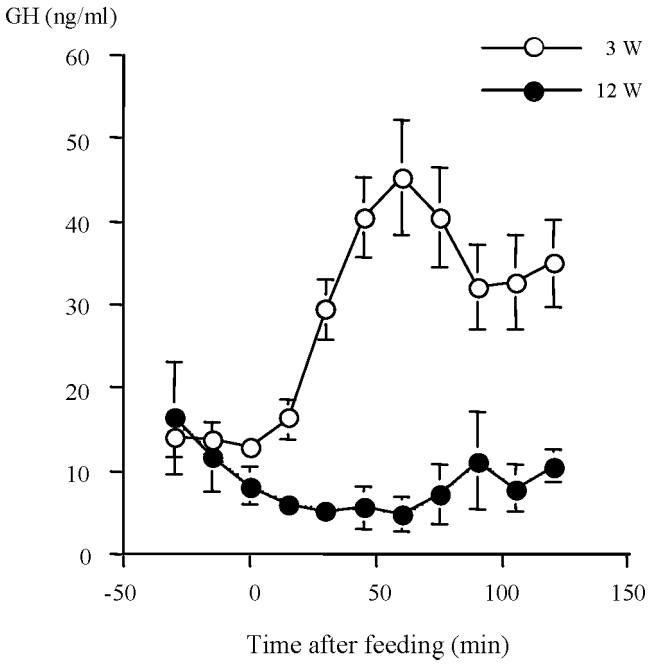

Figure 6. Postprandial changes in venous GH concentrations in young calves at 3 or 12 weeks of age (Katoh et al., 2004a).

inhibitory action on GH release if the medium did not contain IGF-I, even in the cells cultured for 48 h before stimulation with GHRH with IGF-I at a 10 times greater concentration (1,000 ng/ml). Furthermore, IGF-I even at this concentration did not alter the cellular GH content. Therefore, it is unlikely that IGF-I reduces the GH mRNA expression in ruminant somatotrophs, although IGF-I suppresses the expression of mRNA for GH and the GHS receptor in rats (Yamashita and Melmed, 1986; Kamegai et al., 2005). Eventually, these findings may indicate a limited role of IGF-I in the somatotropic axis of the ruminant.

FEEDING

Feeding reduces the plasma GH levels in sheep (Bassett, 1974a, b; Driver and Forbes, 1981; Trenkle, 1989; Thomas et al., 1991; Matsunaga et al., 1998, 1999), calves (Moseley et al., 1988) and humans (Merimee and Fineberg, 1974; Ishizuka et al., 1983; Jaffe et al., 1998), but not in rats (Tannenbaum et al., 1976). Postprandial reduction in the plasma GH levels is apparent in animals under food restriction, because food restriction is known to raise the basal GH levels and pulse amplitude (Thomas et al., 1990, 1991). Chronic food restriction increases GHRH but reduces SRIF synthesis in the hypothalamus of sheep (Henry et al., 2001). Feeding is accompanied by a reciprocal increase in the plasma insulin, glucagon and SRIF concentrations (Bassett, 1974b; Matsunaga et al., 1999).

Feeding also reduces GHRH-induced GH release, and the action was mimicked by the anticipation of being fed, distension of the rumen with a water-filled balloon, and sham feeding (Trenkle, 1989). However, Moseley et al. (1988) reported that the amplitude and area under the GH response curve in response to GHRH stimulation were not significantly different after sham feeding compared with before (the animals were not given food, but expected to be fed). Interestingly, the GH responses induced by activation of the 5-HT receptors with quipazine were not significantly different between before and after feeding, whereas blockade of the 5-HT receptors with cyproheptadine decreased both the basal GH levels before and after feeding in steers (Gaynor et al., 1995). In addition, hypophyseal stalk transection reduced the GH increase induced by GHRH stimulation, but feeding further reduced the GHRH-induced response in calves (Plouzek et al., 1988). These findings imply that a postprandial reduction in the GH level is mediated mainly by peripheral factors, but not entirely by the central nervous system.

After birth, weaning is the most drastic event that neonates have to experience, because they are forced to change from a liquid milk diet to a solid particle one, even if this process occurs gradually. Since weaning causes changes in the digestive and metabolic functions to meet the altered quality of the diet and nutrients, it is plausible that the somatotropic axis changes around weaning time. In practice, we recently found that the plasma GH levels do change around weaning time (Figure 6)(Katoh et al., 2004a). We compared the effect of feeding on the plasma basal GH level in 3- (before weaning) and 12- (after weaning) week-old Holstein male calves. They were fed with either milk at 3 weeks of age, or roughage and milk replacer at 12 weeks of age, respectively. Weaning was at 6 weeks of age. Eventually, there was a significant difference in GH areas induced by feeding between before and after weaning. The milk-feeding-induced increase in the plasma GH levels was also reported in 3-month-old lambs (Bassett, 1974a). It is apparent that the digestive tract is able to detect and respond to the chemical and physical components of diets, resulting in changes in the GH levels.

It would be interesting to find out whether or not the milk-feeding-induced GH increase is parallel with changes in the plasma ghrelin level. The postprandial plasma level of ghrelin was, however, decreased by milk feeding, even though the plasma GH level was increased in young calves. Therefore, the plasma ghrelin level does not seem to be related to the postprandial level of GH.

The precise reason why the postprandial plasma GH concentration was different relative to weaning time remains to be clarified. However, in calves maintained on a milk-replacer diet until 12 weeks of age, the postprandial plasma GH concentrations and GH area were not different

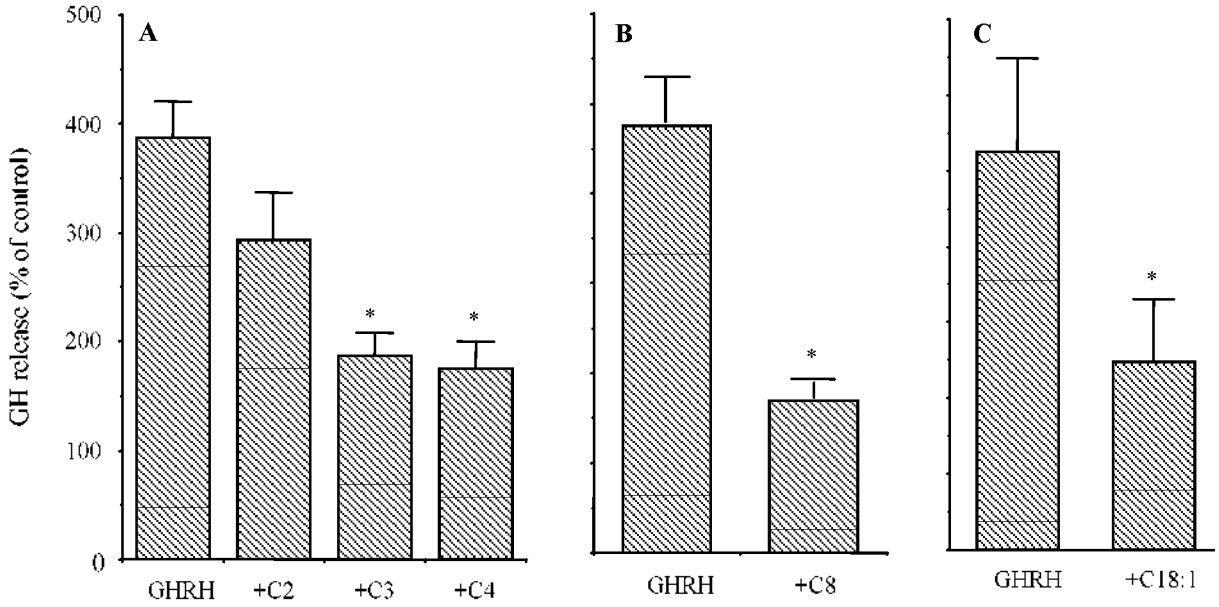

Figure 7. Inhibitory effects of fatty acids on GHRH-induce GH release in ovine anterior pituitary cells. C2: acetate (10 mM), C3: propionate (10 mM), C4: butyrate (10 mM), C8: octanoate (10 mM), C18:1: oleate (10 μM). * $p<0.05$ vs. GHRH stimulation alone.

from those in an age-matched weaned group (Katoh et al., 2004a). This finding indicates that aging is the definitive factor responsible for the postprandial GH changes.

NUTRITION

It is also obvious that nutrition affects the somatotropic axis, because energy restriction or starvation increases the plasma GH levels in sheep (Thomas et al., 1991) and humans (Hartman et al., 1992), respectively. Restricted feeding suppressed the circulating SRIF level, but did not change the GHRH level, in the ovine hypophyseal-portal vein, resulting in an enhanced circulating GH level in sheep (Thomas et al., 1991). Obesity, on the other hand, is characterized by low circulating GH levels and a suppressed GH secretion in response to secretagogues (Baranowska et al., 2003).

The actions of nutrients on secretory functions have been less established for pituitary somatotrophs than for pancreatic endocrine cells. Recently, however, a large amount of evidence suggests that the function of somatotrophs can be modified by various kinds of nutrients (Katoh and Obara, 2001).

Glucose

An increase in circulating glucose concentrations increases the GH levels in goats and cows (Reynaert et al., 1975; Sartin et al., 1985). However, insulin-induced hypoglycemia increases the GH level in humans (Hanew, 2000), but not in sheep (Frohman et al., 1990). These findings *in vivo* are difficult to interpret, because intravenous glucose infusion commonly declines the concentration of NEFA (Reynaert et al., 1975), a nutrient that is known to inhibit GH secretion in somatotrophs. A doubled concentration of glucose in the medium slightly increased GH release (Katoh and Ishiwata, 1998) without changing the cellular cAMP and $[Ca^{2+}]c$ levels (Katoh and Obara, 2001). This result implies that the glucose-induced GH increase is due to elevated cellular metabolism.

Proteins and amino acids

As GH possesses nitrogen-sparing actions, it would be interesting to find out whether or not protein (or amino acids) feeding affects the somatotropic axis. High protein diets reduce the plasma GH levels in sheep (Clarke et al., 1993) and horses (Sticker et al., 1995), although these diets increase the IGF-I levels in sheep. The accumulation of GH proteins in the pituitary gland was reported in rams fed with high protein diets (Clarke et al., 1993).

However, some amino acids act as a potent GH releaser. Excitatory amino acids and their analogue (NMDA) were reported to increase GH secretion in sheep (Estienne et al., 1989b; Kuhara et al., 1991; Downing et al., 1996), horses (Sticker et al., 2001), pigs (Barb et al., 1992, 1996) and rats (Lindström and Ohlsson, 1992) *in vivo* and *in vitro*. It was reported that ghrelin-induced GH secretion is mediated by excitatory amino acids in the nervous system in rats (Aguiler et al., 2005). Estienne et al. (1999) and Estienne and Barb (2005) showed that the NMDA-induced GH secretion is attributable, at least in part, to an enhanced GHRH secretion from the central nervous system. In sheep, aspartate, not glutamate, was a potent agonist for GH release when administered in venous circulation (Kuhara et

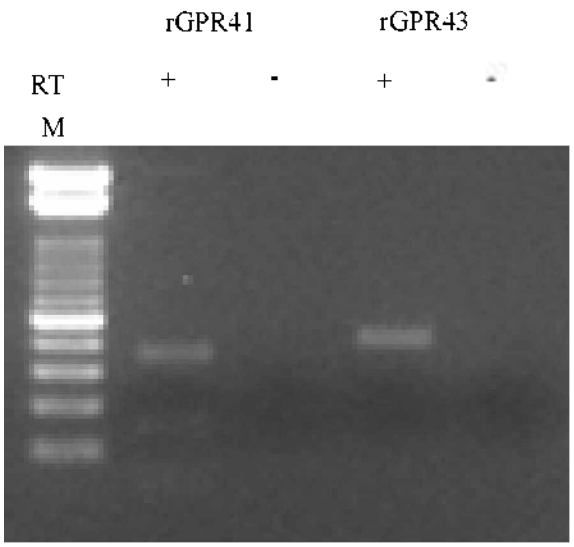

Figure 8. Expression of GPR41 and 43 mRNA in rat anterior pituitary cells (Ishiwata et al., 2005).

al., 1991). However, the action of arginine is interesting, because this amino acid is an ineffective stimulant for GH release in rams (Kuhara et al., 1991) and calves, although it is a potent stimulant in humans (Müller et al., 1999; Hanew, 2000). Despite the negative action on GH release, arginine is still as potent a stimulant for insulin secretion in ruminants as it is in humans. In horses, curiously, the peak concentration of GH after the venous infusion of arginine appeared 30 min later than that for aspartic acid (Sticker et al., 2001). There is, unfortunately, no information available on the effects of weaning on the actions of excitatory amino acids in the ruminant, although these amino acids showed age-dependent variable effects on GH responses in the rat (Aguilera et al., 2005).

Branched chain or non-essential amino acids (NEAA) also stimulated GH secretion in goat somatotrophs (Ohata et al., 1997) as well as in the baboon (Stewart et al., 1984). The increase in the GH release induced by NEAA depended on the amino acid concentrations, and was abolished by a reduced Ca^{2-} concentration in medium or by blockade of the membrane Ca^{2-} channels by nifedipine (Ohata et al., 1997).

As described above, there is discrepancy in the actions between protein diets and individual amino acids, which remains to be clarified in the future.

Fatty acids

Fatty acids are the nutrient that inhibits GH release *in vivo* and *in vitro*, and the action is principally independent of their chemical structure, carbon lengths, or saturated or unsaturated groups.

Long-chain fatty acids (LCFA) inhibit GH release in sheep (Hertelendy and Kipnis, 1973; Estienne et al., 1989a; Katoh and Ishiwata, 1998), cattle (Coxam et al., 1989; Romo et al., 1997), and humans and rats (Casanueva et al., 1987; Peino et al., 1996).

Whether short-chain fatty acids (SCFA) were administered in the vein or in the forestomachs, they reduced the GH concentrations in the plasma of sheep (Matsunaga et al., 1993, 1997a, b, 1998, 1999). It is likely that activation of the parasympathetic nervous system is involved in the mechanism for the reduced GH release seen postprandially, or induced by the infusion of SCFA in the forestomachs, because the effects of SCFA are abolished by the administration of anticholinergic agents (Matsunaga et al., 1998).

The inhibitory action of SCFA as well as LCFA on GH release was also confirmed in anterior pituitary cells isolated from goats and rats *in vitro* (Figure 7) (Katoh et al., 1999; Ishiwata et al., 2000; Ishiwata et al., 2005). The inhibitory actions of SCFA on the cellular signal transduction system are diverse in rat somatotrophs, because butyrate inhibited cellular cyclic AMP and IP3 production, GH mRNA expression, an increase in the $[Ca^{2+}]c$ level, and Ca^{2-} channel opening. In addition, in anterior pituitary cells isolated from the bovine fetus and neonate, the addition of propionate or butyrate to the medium significantly reduced the cell number compared with cells cultured without SCFA (our unpublished data). In addition, the expression of GTP-binding protein-coupled receptors for fatty acids (GPR41 and 43) was discovered in rat anterior pituitary cells (Figure 8) (Ishiwata et al., 2005). It is likely that the inhibitory actions induced by short-chain fatty acids on pituitary hormone secretion are mediated through these receptors.

SCFA seems to be a likely candidate causing the postprandial decline in the plasma GH levels. However, the physiological importance for the involvement of SCFA may not necessarily be confined to ruminants, because the postprandial reduction of the GH level has also been reported in humans (see FEEDING), and this reduction is not necessarily caused by the afternoon feeding when animals are fed twice per day (our unpublished data).

CONCLUSIONS

The somatotropic (GH-IGF-I) axis consists of many hormonal and nutritional factors: GHRH and GHS (GHRP or ghrelin) as GH-releasing substances, and somatostatin (SRIF), insulin-like growth factor-I (IGF-I) and leptin as GH release–inhibiting substances. In addition, it was shown that nutrition and weaning are strongly involved in the control of the somatotropic axis.

In this review on the control of the somatotropic axis in the ruminant, the biological role of ghrelin, IGF-I and leptin as well as weaning and nutrition was examined. Ghrelin,

which is mainly synthesized and released from the abomasum epithelium in the ruminant, has been shown to stimulate GH release from the anterior pituitary in a similar way to GHS. However, ghrelin as well as leptin seem to exert animal-species-dependent actions via a complex set of intracellular signaling pathways. The effect of nutrition is also different among animal species, particularly the ruminant and rodent. We recently found that weaning changes the postprandial GH responses in ruminants because milk feeding increases, but hay and concentrate feeding suppresses, the postprandial circulating GH levels. Even if the postprandial GH level was increased by milk feeding in young calves, the ghrelin level was decreased. Macronutrients also demonstrate stimulatory and inhibitory actions on GH secretion *in vivo* and *in vitro*. It is likely that the inhibitory action of SCFA is mediated by G-protein-coupled receptors.

In summary,the control mechanisms of the somatotropic axis in the ruminant, in particular around weaning time, are complex.

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