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Recent Progress on the Effects of Weaning and Nutrition on the Action of Physiologically Active Peptides in the Ruminant

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Summary

In the present mini-review, we will introduce our recent new findings accumulated at the Animal Physiology Laboratory over the last several years, in particular with reference to studies on leptin and ghrelin, which are involved in both the control of the somatotrophic axis (GHRH/GHS-GH-IGF-I system) and in the regulation of the energy balance of domestic animals. Our interest is also focused on weaning because it is the most drastic event that neonates have to experience through their lives. 1) We compared the effect of feeding on basal plasma GH levels in 3- (milk-fed) and 12-week old (concentrates and roughage-fed) Holstein bull calves, and found that feeding caused a rise in GH levels at 3 weeks but a reduction at 12 weeks of age. 2) Plasma leptin levels, which were increased with aging in Japanese Black cattle, were not changed during the 5 hours after feeding. However, the expression of leptin, and CCK_A and CCK_B receptors in the stomachs of calves was abolished after weaning. This finding was confirmed even when calves were maintained on a milk replacer diet with an intragastric injection of VFA until 13 weeks of age. In addition, we demonstrated that VFA increased the expression of leptin, but suppressed that of the leptin receptor (OB-Ra) in calf anterior pituitary cells. 3) Although the basal ghrelin level in the plasma of goats decreases as the animal ages, parallel with basal GH levels as well as the responses of GH to GH secretagogues, milk feeding appears not to reduce the basal ghrelin levels in calves and goats. Weaning significantly decreased the ghrelin content to 30% of that seen pre-weaning in the stomachs.

These findings will substantially contribute to the studies on the control of the somatotrophic axis and the regulation of the energy balance of ruminant animals.

For the agricultural industry to rear domestic animals effectively, it is crucial for the farmer to control the animals' growth and feeding behavior because these determine the commercial value of the resultant animal products. Although the concept of the "hormone" emerged at the start of the last century, two hormones have been discovered much more recently that are critical with respect to growth

and feeding behaviour of farm animals: these hormones have been called leptin and ghrelin. Leptin and ghrelin are involved in both the control of the somatotropic axis (GHRH/GHS-GH-IGF-I system) and in the regulation of the energy balance of the animal. This indicates the intimate interaction between growth and feeding behavior at the hypothalamic level of many animal species. In particular, weaning is the most drastic event that neonates have to experience, because they are obliged to change feed stuffs from liquid (milk) to solid particles (concentrates and roughage), even if this process occurs gradually over several weeks. This, therefore, means that weaning requires animals to alter the digestive and metabolic functions in order to meet the change in the quality and quantity of the diet. In the present mini-review, we will introduce our recent new findings accumulated at the Animal Physiology Laboratory over the last several years.

1. Growth Hormone (GH) and Insulin Responses to Feeding

As for other domestic animals, the somatotropic axis is central in the control of the metabolism and function of various other organs in the ruminant. The axis principally consists of hypothalamic (growth hormone-releasing hormone (GHRH) and somatostatin (SRIF)) and peripheral hormones, such as ghrelin and leptin, as well as insulin-like growth factor-I (IGF-I). Pituitary GH is stimulated mainly by two hormones (GHRH and ghrelin (or GH secretagogues: GHS)), but is suppressed by SRIF and leptin (Roh *et al.*, 1998; Tannenbaum and Epelbaum, 1999).

We compared the effect of feeding on basal plasma GH levels in 3- and 12-week old Holstein bull calves. They were fed with either a milk replacer diet at 3 weeks of age, or concentrates and roughage at 12 weeks of age (weaning was at 6 weeks of age). Feeding caused a rise in GH levels at 3 weeks but a reduction at 12 weeks of age. However, the increased GH levels seen at 3 weeks of age were accompanied by increased insulin levels (Katoh *et al.*, 2004).

In the next experiment, we compared changes in GH responses to the intravenous administration of GHRH or GHRP-6 in bull calves. The increase in plasma GH levels in response to either GHRH or GHRP was significantly greater at 3 weeks than at 12 weeks of age. It is apparent therefore that GH secretion in response to GH secretagogues declines as cattle progress through this aging process. Furthermore, in 12-week old animals, the GHRP administration significantly increased insulin levels. This indicates that GHS receptors may be involved in the control mechanism for the pancreatic hormone secretion in the ruminant (Katoh *et al.*, 2004).

In general, feeding reduces plasma GH levels in sheep (Bassett, 1974) and calves (Moseley *et al.*, 1988). Feeding-induced reduction in plasma GH levels

becomes apparent in animals that are restricted fed or are fasted, because this is known to raise the basal GH levels and the pulse amplitude (Thomas *et al.*, 1991), and increased GHRH but reduced SRIF synthesis in the hypothalamus of sheep (Henry *et al.*, 2001).

Feeding also reduces GHRH-induced GH release. This is mimicked in sheep by the animals' anticipation of being fed, distension of the rumen with a water-filled balloon or sham-feeding (Trenkle, 1989). However, Moseley *et al.* (1988) reported that the amplitude and area under the GH response induced by GHRH stimulation was not significantly different following sham-feeding compared with before (animals were not given food, but expected to be fed). In addition, hypophysial stalk transection reduced the GH increase induced by GHRH stimulation, but feeding further reduced the GHRH-induced response in calves (Plozek *et al.*, 1988). These findings imply that a postprandial reduction in GH

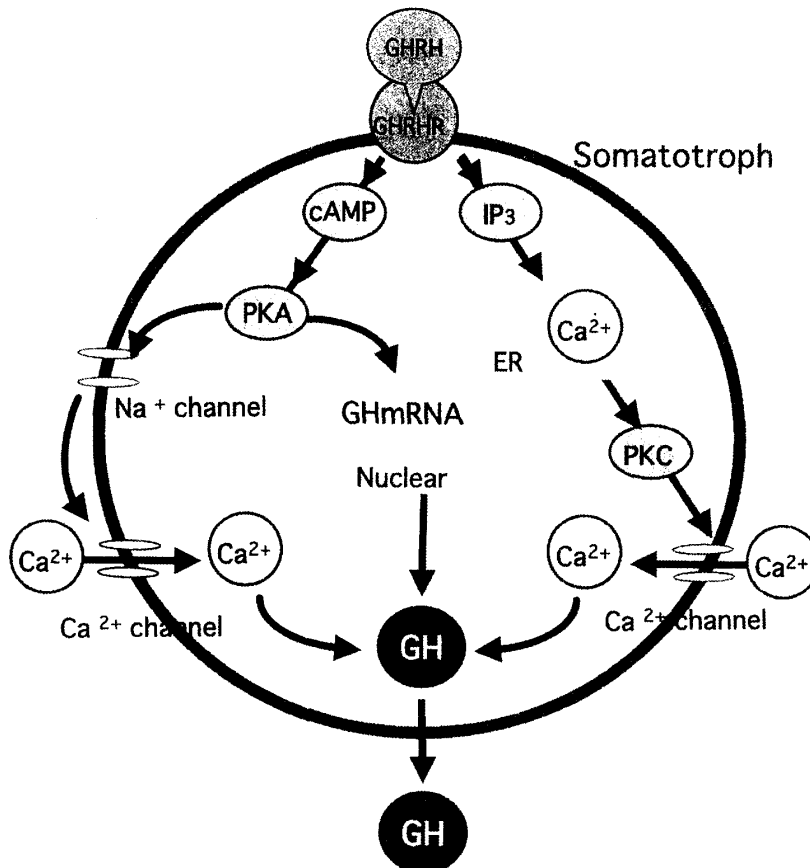


FIG. 1. Suppressing actions of butyrate on the intracellular signal transduction system in ovine somatotrophs. Note that all the pathways shown by the arrows are suppressed by butyrate (Ishiwata, Katoh and Obara, unpublished). GHRH: GH-releasing hormone; GHRHR: GHRH receptors; cAMP: cyclic AMP; PKA: Protein kinase A; IP3: Inositol trisphosphate; PKC: Protein kinase C.

level is mediated largely by peripheral factors, and not by the central nervous system. As a peripheral factor suppressing GH level, we have postulated that VFA, the main energy source for the ruminant, is able to suppress GH secretion in goat' somatotrophs (Ishiwata *et al.*, 2000). The suppressing actions of VFA have been widely demonstrated, i.e., GH release and synthesis, GHmRNA expression, intracellular calcium and cyclic AMP dynamics, and activation of protein kinases (Fig. 1).

2. Leptin and Ghrelin

Leptin was found in 1994. It is a cytokine, which is released from the adipocytes into the circulating system and is recognized at the central nervous system. Amongst a number of other functions, it is known to suppress appetite, acting over the longer term. "Leptin" is derived from the Greek word "leptos", which means "thin". Ghrelin was isolated in 1999 from human and rat stomachs as an agent that stimulates the secretion of GH from the anterior pituitary cells (Kojima *et al.*, 1999). Ghrelin-secreting cells have been identified as X/A-like cells, and receptors for it have been widely demonstrated in the digestive and central nervous systems. The secretion of both of these peripheral hormones is involved in controlling GH secretion and is altered by nutritional status in many animal species.

1) *Leptin*

Plasma leptin levels increased with aging in Japanese Black cattle, which is thought to parallel the animals' adipose tissue accumulation (Yonekura *et al.*, 2002a). However, plasma leptin levels do not change during the 5 hours after feeding.

We demonstrated that leptin expression in the stomachs of calves was abolished after weaning (Fig. 2). However, when a milk replacer diet was fed until 13 weeks of age, leptin expression in the stomach epithelium was still detected (Yonekura *et al.*, 2002b). Interestingly, even when a milk replacer diet was fed with VFA (SCFAs: a mixture of acetate, propionate and butyrate) until 13 weeks of age, leptin expression was not detected. This indicates that VFA produced by microbes in the fore-stomachs may be involved in the cessation of leptin expression after weaning (Yonekura *et al.*, 2002b).

It is known that CCK (cholecystokinin) controls leptin synthesis and secretion in the rodent. In calves, weaning suppressed the expression of CCK_A and CCK_B receptors in the stomachs (Yonekura *et al.*, 2002b). This finding coincides with the previous result that feeding increased plasma CCK level in neonatal, but not in weaned, calves (Toullec *et al.*, 1992). This suggests that the expression and functions of leptin and CCK receptors in the stomachs are well synchronized in

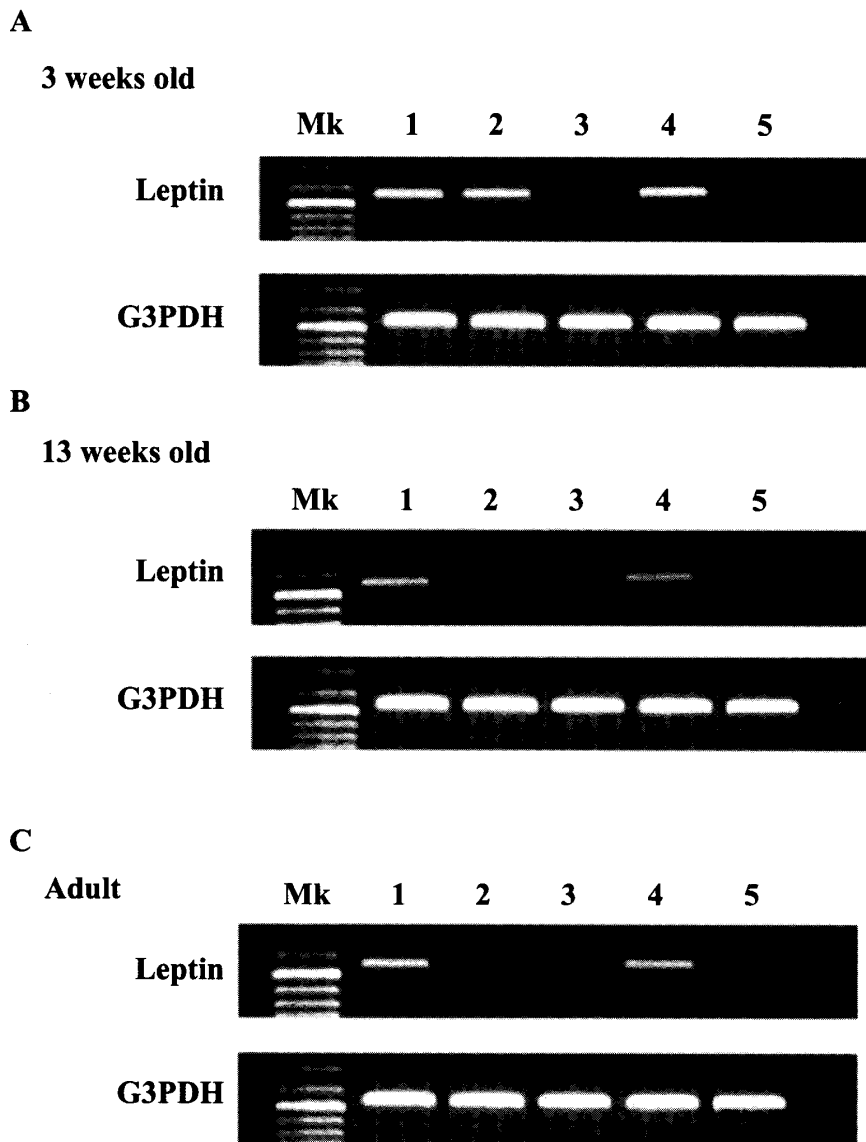


Fig. 2. Effects of aging on the leptin expression in adipose tissue (lane 1), rumen (lane 2), abomasum (lane 3), duodenum (lane 4) and liver (lane 5). The upper panel represents RT-PCR analysis of the mRNA expression for leptin and the lower panel for G3PDH at 3 weeks of age (A), 13 weeks of age (B) and adult (C) calves. Mk: Molecular weight marker. Note that the leptin expression in the rumen and abomasum disappeared after weaning (after 13 weeks of age) (Yonekura *et al.*, 2002b).

calves.

With regard to digestive and metabolic functions, the maintenance of neonatal calves on a milk replacer diet until 13 weeks of age sustained carbonic anhydrase activity and ion secretion in the parotid gland at pre-weaning levels (Kitade *et al.*, 2002). Surprisingly, however, it drastically and significantly increased the basal and postprandial levels of plasma glucose and insulin, compar-

ed with of the levels of these seen in weanlings (Katoh *et al.*, 2004).

We investigated in vitro the effects of VFA on leptin mRNA expression in calf and rat anterior pituitary cells in vitro (Yonekura *et al.*, 2003). In this study, we demonstrated that VFA (acetate and butyrate) increased the expression of leptin, but suppressed that of the leptin receptor (OB-Ra). This finding is reasonable because leptin as well as VFA are known as inhibitory factors for GH release in sheep (Roh *et al.*, 1998). However, the treatment of rat cells with butyrate suppressed the expression of leptin. The reason for the difference shown in two animal species is not known.

2) Ghrelin

Feeding may change ghrelin levels in the ruminant as in the rodent, because GH is a catabolic and orexigenic hormone and its secretion may be partly controlled by ghrelin. However, this may not necessarily be the case for the ruminant.

We recently investigated the effects of weaning on the ghrelin content of the goat gastrointestinal tract. We found that the ghrelin content of the abomasum was highest in the epithelium of all the stomachs and the duodenum. Furthermore, we found that the ghrelin content after weaning was significantly decreased to 30% of that seen pre-weaning (Kobayashi, Katoh and Obara, unpublished data).

The basal ghrelin level in the plasma of goats decreases as the animal ages, parallel with basal GH levels as well as the responses of GH to GH secretagogues (Kobayashi, Katoh and Obara, unpublished data). Although the contribution of GHRH is estimated to be 66% of the total GH-secreting activity of blood-borne factors, the contribution of ghrelin for basal GH levels is not known. In the ruminant, as in other animal species, ghrelin administration into the blood or into the ventricle increased plasma GH levels in a dose-dependent manner (Hayashida *et al.*, 2001).

In sheep and goats, fasting or restricted feeding increased plasma ghrelin levels, while feeding suppressed ghrelin levels (Hayashida *et al.*, 2001; Sugino *et al.*, 2002a). The ghrelin surge demonstrated in some individuals just before feeding is due to the emotional autonomic nervous reflex associated with enhanced salivary or gastric acid secretion. However, it remains to be established whether or not feeding reduces the level of ghrelin in neonates, because feeding appears not to reduce the basal ghrelin levels in calves and goats.

In conclusion, we summarize a new concept for the somatotrophic axis in the ruminant (Fig. 3).

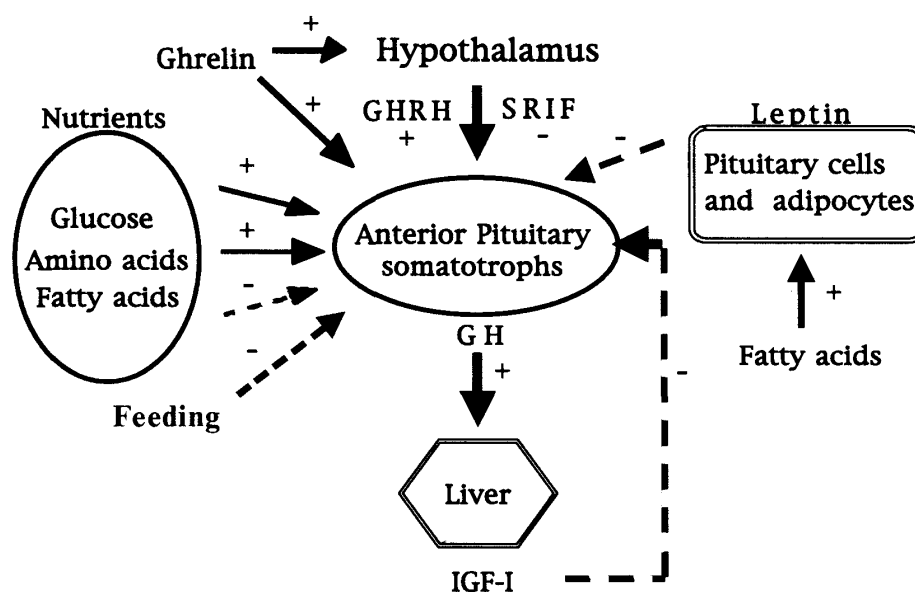


FIG. 3. A scheme summarizing the interaction of endocrine factors and nutrients involved in the control of somatotrophic axis in the ruminant (Katoh and Obara, unpublished).

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