Effect of cholinergic blockade on inhibited GH secretion by feeding and intraruminal SCFA infusion in sheep

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Matsunaga, N., M. Wakiya, S. G. Roh, M. Hirota, M. L. He, S. Hidaka, and H. Hidari. Effect of cholinergic blockade on inhibited GH secretion by feeding and intraruminal SCFA infusion in sheep. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E45–E51, 1998.—The effect of cholinergic blockade on suppressed growth hormone (GH) secretion caused by feeding or the intraruminal infusion of an acetate, propionate, and butyrate mixture (107 and 214 µmol·kg⁻¹·min⁻¹ over 6 h) was examined in ovariectomized ewes. Intraruminal infusion at the rate of 107 µmol·kg⁻¹·min⁻¹ increased peripheral plasma short-chain fatty acid (SCFA) concentrations to approximately the physiological levels noted after feeding. Plasma GH was markedly suppressed by feeding and at both the 107 and 214 µmol·kg⁻¹·min⁻¹ SCFA infusion rates; however, cholinergic blocking agents completely blocked the suppressed GH secretion after feeding and only at the 107 µmol·kg⁻¹·min⁻¹ infusion rate. Plasma glucose increased at both infusion rates, and the plasma free fatty acids decreased after feeding and at both infusion rates. However, both metabolites were unchanged relative to the saline control after the injection of the cholinergic antagonists. It is suggested that the decrease in plasma GH observed after feeding and a near-physiological ruminal SCFA increment is mediated via the parasympathetic nerve and not by pharmacological ruminal SCFA increments attributed to other pathways.

INHIBITORY EFFECTS of feeding on plasma growth hormone (GH) levels as well as GH-releasing factor (GRF)-induced GH responses have been reported in sheep (2, 14, 29) and goats (28). Trenkle (29) and Tindal et al. (28) found that feeding, anticipation of feeding, or artificial distention of the cranial sac of the rumen with a water-filled balloon reduced basal and GRF-induced GH secretion. These findings suggest that visual and mechanical stimuli accompanying feeding are involved in the mechanism causing the postprandial GH decrease. Moreover, because the action is very rapid, these findings also suggest involvement of a neural pathway. The vagus nerve is known to transmit signals from chemosensory and mechanoreceptors existing in the rumen epithelium (11, 19), which could be a possible mechanism. However, Tindal et al. failed to demonstrate the involvement of the vagus nerve in the suppression of GH secretion after feeding after surgical cooling (28). Furthermore, because muscarinic receptor antagonists have been shown to abolish the rise in plasma GH stimulated by GH-releasing stimuli such as GRF in humans (10, 13), dogs (8, 9), and rats (6, 20), it is difficult to demonstrate that these stimuli also pass through the parasympathetic nerve, which can be blocked with cholinergic antagonists by pharmacological methods. However, scopolamine-N-butyl bromide, a muscarinic receptor antagonist that is a quaternary ammonium derivative unable to pass the blood-brain barrier (BBB), failed to alter GH release by GH-releasing stimuli in the dog (8, 9). This result suggests that cholinergic muscarinic receptors located in the central nervous system, inside the BBB, play a promoting role in GH release and that muscarinic receptor antagonists have no direct effect on the pituitary (24).

These conclusions are supported by the fact that, although the existence of muscarinic receptors in the anterior pituitary of sheep (7) and rats (23) has been detected by the binding of [³H]quinuclidinyl benzilate, a potent and specific muscarinic antagonist, acetylcholine does not stimulate the release of GH from perfused rat adenohypophyses (18). Likewise, nicotinic receptors have no effect on the GH secretion mechanism independent of penetrating BBB (24). This conclusion is supported by the fact that nicotine, one of the nicotinic receptor agonists (tertiary amine derivative), and mecamylamine, one of the nicotinic receptor antagonists (secondary amine derivative), have no effect on GH secretion, although these drugs easily penetrate the BBB (8). Therefore, it is possible to block the parasympathetic nerve without effects on the GH secretion mechanism by using scopolamine-N-butyl bromide and a nicotinic antagonist.

Other mechanisms for this inhibitory effect after feeding have been proposed. In our previous study we demonstrated that intravenous infusion of propionate or butyrate suppressed GRF-induced GH secretion in sheep (22). In addition to this, intraruminal infusion of short-chain fatty acids (SCFA) at a physiological rate, as high as the levels observed after a meal, suppressed plasma GH in sheep (21). Thus SCFA are also supposed to play a role in regulating GH secretion after feeding in ruminants.

We therefore examined in detail the effects of blocking the parasympathetic nerve, using cholinergic antagonists, on suppressed GH secretion by feeding and intraruminal SCFA infusion.

MATERIALS AND METHODS

Animals. Six adult ovariectomized ewes (54–64 kg) were used. The ewes were housed in metabolic cages and offered alfalfa pellets at 2% of body weight in a single meal at 1200. Water was available continuously. At least 1 mo before experiments began, a rumen cannula (Flexible Rumen Cannula, no. 7C, Bar Diamond, Yokohama, Japan) was fitted to each animal under general anesthesia with pentobarbital sodium (25 mg/kg, Nembutal injection, Abbott, North Chicago, IL). At least 1 wk before the experiments, polyethylene catheters (IVH catheter kit, Terumo, Tokyo, Japan) for sampling and injection were inserted into each jugular vein.
through a hypodermic needle. The catheters were kept patent by daily flushing with a sterile solution of trisodium citrate (3.8 g/100 ml). All animal-based procedures were in accordance with the “Guidelines for the Care and Use of Experimental Animals of Obihiro University of Agriculture and Veterinary Medicine,” which were formulated from the “Declaration of Helsinki and Guiding Principles in the Care and Use of Animals”(1).

Feeding experiment. Experiments were carried out from 1000 (−2 h) to 1600 (4 h) and performed at 7-day intervals. The ewes were fed at 1200 (0 h). All ewes completely finished eating within 1 h of being fed. At 1400 (2 h) the ewes were intravenously injected through one catheter with a 3-ml volume of scopolamine-N-butyl bromide (0.08 mg/kg, Sparicon, Yamanouchi Pharmacy, Tokyo, J apan) (9) and hexamethonium bromide (10 mg/kg, Wako Pure Chemical, Osaka, J apan) (5) dissolved in sterile saline. For the control, injections were carried out using the same volume of saline. Samples of venous blood were collected at 15-min intervals. In addition, ruminal fluid samples were collected at 0900 and 1600 to avoid mechanical stimuli for the rumen during blood sampling.

Intraruminal infusion experiment. Experiments were carried out from 1000 (−2 h) to 1800 (6 h) and performed at 7-day intervals. Cholinergic receptor antagonists were intravenously injected at the same rate as for the feeding experiment at 1600 (4 h). The control injection was carried out using the same volume of saline. To assess the effect of the ruminal infusion of the SCFA mixture on GH secretion, a mixture of sodium acetate, propionate, and n-butyrinate (molar ratio of each acid respectively 70:20:10, adjusted to pH 4.5 with sodium hydroxide) were infused over a period of 6 h, starting at 1200 (0 h), at the rates of 107 or 214 µmol·kg$^{-1}$·min$^{-1}$ with the use of a constant infusion pump (1 ml/min). The 107 µmol·kg$^{-1}$·min$^{-1}$ infusion rate causes a rumen SCFA concentration similar to physiological levels (21). Samples of venous blood were collected at 15-min intervals. In addition, ruminal fluid samples were collected at 0900 and 1800.

Analyses. Blood samples collected in heparinized syringes were immediately transferred into polyethylene test tubes, cooled on ice water, and centrifuged at 4°C. A portion of plasma was stored at −25°C for GH, glucose, and free fatty acid (FFA) assays. The GH assay was performed as described previously (21, 22). Briefly, GH was assayed by a double-antibody method using ovine GH antiserum [National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) anti-GH-2, AFP-C0123080] and GH standards (NIDDK oGH-I-4, AFP-8758C). Ovine GH (NIDDK oGH-I-4, AFP-8758C) was iodinated using the chloramine T method. The assay had a minimum-detectable concentration of 0.098 ng/ml. Inter- and intra-assay coefficients of variation were 6.3 and 6.3%, respectively. Plasma glucose was determined by the glucose-oxidase method (glucose CII-Test Wako, Wako Pure Chemical). Plasma FFA were determined by the acyl-CoA synthase-acyl-CoA oxidase method [nonesterified fatty acids (NEFA) C-Test Wako, Wako Pure Chemical]. Plasma and ruminal fluid were mixed with a one-half volume of a 25% metaphosphoric acid solution containing crotonic acid (25 mmol/l) as internal standard and were stored at −25°C until analysis. NEFA concentrations were determined using a gas chromatographic technique (163, Hitachi, Tokyo, J apan). The length and internal diameter of the column used for the analysis were 2 m and 3 mm, respectively. The rate of flow of the carrier gas (N$_2$) was 20 ml/min, and the oven temperature was 145°C.

Statistics. Mean values as well as the standard errors of the means were calculated. The area under the curve (AUC) of GH and the mean for the other data were calculated for the three (feeding experiment) or four (intraruminal infusion experiment) 2-h periods [for the feeding experiment: the period before feeding (between −2 and 0 h), the period after feeding before injection of the cholinergic antagonists (between 0 and 2 h), and the period after feeding after cholinergic antagonist injection (between 2 and 4 h); and for the infusion experiment: the period before infusion of SCFA (between −2 and 0 h), the first period of SCFA infusion before injection of the cholinergic antagonists (between 0 and 2 h), the second period of SCFA infusion before cholinergic antagonist injection (between 2 and 4 h), and during SCFA infusion after cholinergic antagonist injection (between 4 and 6 h)]. Significant differences between periods within each treatment were analyzed by analysis of variance, using the general linear model procedure of the SAS program package (SAS Institute, Cary, NC) on the GH AUC or the mean for the other data followed by Dunnett’s test for comparing all treatments with the control test. The significance of differences between the control and treatments within the same period for the increase in GH AUC and mean values above basal values (−2 to 0 h) were analyzed by analysis of variance. The ruminal and plasma SCFA concentration data were analyzed by analysis of variance comparing all infusions with feeding by using Dunnett’s test.

RESULTS

Feeding experiment. The time course for the GH response is represented in Fig. 1. The mean basal
concentration of plasma GH before the onset of feeding (−2–0 h) was 3.1 ± 0.5 and 3.2 ± 0.7 ng/ml for the control and cholinergic antagonist injection experiments, respectively. GH concentration was reduced to <1 ng/ml until 2 h after the onset of feeding. The mean concentration of GH was immediately enhanced to 5.2 ± 0.9 ng/ml between 2 and 4 h after the injection of cholinergic antagonists. However, the plasma GH concentration remained at low levels after the saline injection (1.6 ± 0.4 ng/ml). The GH AUC for the antagonist injection was significantly reduced to 121.8 ± 18.0 ng·ml⁻¹·min⁻¹ (P < 0.05) between 0 and 2 h relative to the basal levels (−2–0 h; 370.8 ± 60.1 ng·ml⁻¹·min⁻¹). However, GH AUC was significantly increased to 600.6 ± 110.4 ng·ml⁻¹·min⁻¹ by injection of the cholinergic antagonists between 2 and 4 h postfeeding, whereas the GH AUC for the control injection was significantly reduced (100.2 ± 14.3 and 187.4 ± 50.4 ng·ml⁻¹·min⁻¹ for the 0- to 2-h and 2- to 4-h periods, respectively; P < 0.05) relative to the basal value (−2–0 h; 369.5 ± 89.7 ng·ml⁻¹·min⁻¹). Additionally, the AUC above basal values after injection of the antagonists was significantly higher than that of the control injection value (P = 0.0032). After feeding there was an immediate tendency for the glucose concentration to decrease, followed by a gradual increase (Fig. 1). However, these trends did not reach statistical significance between periods within each treatment. In addition, there was no significant difference (2–4 h) between control and cholinergic antagonist treatment with respect to the increase in the mean value above basal values. Plasma FFA concentration gradually decreased after feeding, regardless of the injection treatment (Fig. 1). The mean concentration for the control injection significantly decreased to 0.107 ± 0.007 and 0.058 ± 0.005 meq/l (P < 0.05) compared with the basal value (−2–0 h; 0.221 ± 0.020 meq/l) for the 0- to 2-h and 2- to 4-h periods, respectively. Similarly, plasma FFA for the cholinergic antagonist injection was significantly reduced to 0.197 ± 0.035 and 0.092 ± 0.026 meq/l (P < 0.05) compared with the basal value (−2–0 h; 0.265 ± 0.033 meq/l) at the 0- to 2-h and 2- to 4-h periods, respectively. However, there was no significant difference (2–4 h) between the control and cholinergic antagonist injection for the AUC above basal values.

The total plasma SCFA concentrations for the samples taken at 1200 (0 h) and 1600 (4 h) are shown in Table 1. The prefeeding and preinfusion total acetic, propionic, and n-butyric acid SCFA concentrations were pooled because there was no significant difference between the feeding and infusion experiments. The pooled plasma concentration was 2.133 ± 0.223 mmol/l. Because the cholinergic antagonist injection did not affect the plasma SCFA concentration, the saline and cholinergic antagonist injection data were pooled. The average SCFA concentrations became 2.881 ± 0.301, 4.097 ± 0.540, and 12.770 ± 3.561 mmol/l, respectively, after feeding and the infusion of the 107 and 214 µmol·kg⁻¹·min⁻¹ SCFA mixtures. The total plasma SCFA concentration that followed the 214 µmol·kg⁻¹·min⁻¹ infusion was significantly greater than that observed after feeding (P < 0.05). The total SCFA concentrations in the ruminal fluid sampled before and after feeding or SCFA infusion are shown in Table 1. The prefeeding and preinfusion total acetic, propionic, and n-butyric acid SCFA concentrations were pooled because there was no significant difference between the feeding and infusion experiments. The pooled ruminal concentration was 67.0 ± 3.6 mmol/l. Because the saline and cholinergic antagonist injection had no significant effect on ruminal SCFA concentration, the pooled concentrations of SCFA for only the two injections are presented. The values were 134.5 ± 12.2, 182.6 ± 24.6, and 248.2 ± 12.8 mmol/l, respectively, for the feeding and the infusion of the 107 and 214 µmol·kg⁻¹·min⁻¹ SCFA mixtures. The total SCFA concentration for the 214 µmol·kg⁻¹·min⁻¹ infusion was significantly higher than that of the feeding experiment (P < 0.05).

SCFA infusion (107 µmol·kg⁻¹·min⁻¹) plus cholinergic blocker. The time course for the GH response for the 107 µmol·kg⁻¹·min⁻¹ infusion experiment is presented in Fig. 2. The mean basal concentrations of plasma GH before 107 µmol·kg⁻¹·min⁻¹ SCFA infusion began (−2–0 h) were 5.3 ± 1.0 and 4.5 ± 0.9 ng/ml for the control and cholinergic antagonist injections, respectively. GH concentrations were gradually suppressed during the 4 h of the SCFA infusion. After the cholinergic antagonist injection, however, the plasma GH concentration immediately increased to 6.4 ± 2.1 ng/ml. GH concentrations remained in the suppressed state after the saline injection (2.8 ± 0.7 ng/ml). The GH AUC was not significantly different between the periods for the cholinergic antagonist injection. However, the GH AUC for the control injection significantly decreased to 372.7 ± 79.7 and 322.8 ± 73.4 ng·ml⁻¹·min⁻¹ (P < 0.05) from the basal value (−2–0 h; 618.9 ± 115.9 ng·ml⁻¹·min⁻¹) during the 2- to 4-h and 4- to 6-h periods, respectively. The incremental area minus basal values for the cholinergic antagonist treatment after injection was significantly greater than that for the saline injection value (P = 0.0455). Plasma glucose concentrations gradually

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Table 1. Changes in total plasma and ruminal fluid SCFA

<table>
<thead>
<tr>
<th>SCFA</th>
<th>Before Treatment</th>
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<th>After Treatment</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td>2.133 ± 0.223</td>
<td>2.881 ± 0.301</td>
<td>4.097 ± 0.540</td>
</tr>
<tr>
<td>Ruminal fluid</td>
<td>67.0 ± 3.6</td>
<td>134.5 ± 12.2</td>
<td>182.6 ± 24.6</td>
</tr>
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</table>

Values represent means ± SE and are in mmol/l. Total plasma and ruminal fluid short-chain fatty acid (SCFA) concentrations are the sum of acetic, propionic, and butyric acids. Because there were no significant differences between treatments, all samples were pooled for basal period. In addition, because there were no significant differences between injections within feeding or the same dose of SCFA, samples for saline and cholinergic antagonists injection treatments were pooled after the injection. Significant differences were analyzed by analysis of variance between treatment, using the general linear model procedure of SAS on total mean concentration of plasma and ruminal fluid SCFA and using Dunnett’s test comparing all treatments to control experiment. *Significantly different from feeding value (P < 0.05).
increased after the start of the SCFA infusions (Fig. 2). However, cholinergic receptor antagonists had little effect on the plasma glucose concentration. The mean plasma glucose concentration for the control injection increased to 59.3 ± 2.5 and 64.0 ± 3.1 mg/100 ml relative to the basal value (−2–0 h; 51.9 ± 2.4 mg/100 ml) during the 2- to 4-h and 4- to 6-h periods for the 107 µmol·kg⁻¹·min⁻¹ SCFA infusion, respectively. Similarly, the mean concentration of glucose significantly increased to 63.4 ± 3.7 mg/100 ml (4–6 h) compared with the basal value (−2–0 h; 54.2 ± 1.6 mg/100 ml) for the cholinergic antagonist injection during the 107 µmol·kg⁻¹·min⁻¹ SCFA infusion. However, there was no significant difference (4–6 h) between the control and cholinergic antagonist injection in the difference of the mean value from the baseline.

 Plasma FFA concentrations were gradually reduced after the start of the SCFA infusion, and this trend continued soon after the control and treatment injections (Fig. 2). Plasma FFA concentrations for the saline injection significantly decreased to 0.062 ± 0.015 meq/l (4–6 h; P < 0.05) compared with the basal value (−2–0 h; 0.314 ± 0.053 meq/l). Similarly, the mean FFA concentration for the cholinergic antagonists experiment was significantly reduced to 0.095 ± 0.017 and 0.060 ± 0.012 meq/l (P < 0.05) compared with the basal value (−2–0 h; 0.201 ± 0.025 meq/l) in the 2- to 4-h and 4- to 6-h periods, respectively. However, there was no significant difference (2–4 h) between the control and cholinergic antagonist injection in the difference of the mean value from the baseline.

SCFA infusion (214 µmol·kg⁻¹·min⁻¹) plus cholinergic blocker. The time course for the GH response for the 214 µmol·kg⁻¹·min⁻¹ infusion experiment is presented in Fig. 2. The mean basal concentrations of plasma GH before the 214 µmol·kg⁻¹·min⁻¹ SCFA infusion began (−2–0 h) were 5.2 ± 0.8 and 5.0 ± 0.6 ng/ml for the control and cholinergic antagonists injections, respectively. GH concentrations gradually decreased during the 4 h of the SCFA infusion. However, cholinergic receptor antagonists had little effect on the plasma GH concentration. The mean concentrations were 1.1 ± 0.3 and 1.3 ± 0.2 ng/ml for the saline and cholinergic blocker injections, respectively. The GH AUC for the control injection was significantly reduced to 147.6 ± 45.6 ng·ml⁻¹·min⁻¹ (4–6 h; P < 0.05) relative to the basal value (−2–0 h; 585.1 ± 104.6 ng·ml⁻¹·min⁻¹). Similarly, the GH AUC for the blocker injection was also significantly reduced to 161.6 ± 28.4 ng·ml⁻¹·min⁻¹ (4–6 h; P < 0.05) relative to the basal value (−2–0 h; 554.6 ± 92.9 ng·ml⁻¹·min⁻¹). The incremental area minus basal values after the control and antagonists injections were not significantly different. The mean plasma glucose concentration gradually increased after
the onset of the SCFA infusion (Fig. 2). However, the cholinergic antagonists had no significant effect on this increase. The mean glucose concentration for the control experiment increased to 66.7 ± 2.0 and 75.2 ± 3.3 mg/100 ml compared with the basal value (−2–0 h; 60.0 ± 2.3 mg/100 ml) at the 2- to 4-h and 4- to 6-h periods, respectively. Similarly, the mean concentration of glucose for the antagonist experiment was significantly increased to 65.9 ± 2.2 and 72.1 ± 3.4 mg/100 ml compared with the basal value (−2–0 h; 54.2 ± 1.6 mg/100 ml) at 2- to 4-h and 4- to 6-h periods, respectively. However, there was no significant difference (4–6 h) between saline and cholinergic antagonist injections for the increment of mean FFA values compared with the baseline. Plasma FFA concentrations were gradually reduced after the start of the SCFA infusion, and injection of the cholinergic blocker did not change the concentration of plasma FFA. This is in contrast to the saline injection, which apparently caused a small increase in FFA concentrations (Fig. 2). The mean FFA concentration for the control experiment significantly decreased to 0.101 ± 0.021 and 0.070 ± 0.011 meq/l (P < 0.05) compared with the basal value (−2–0 h; 0.163 ± 0.017 meq/l) in the 2- to 4-h and 4- to 6-h periods, respectively. Plasma FFA for the antagonist experiment was also significantly reduced to 0.080 ± 0.011 meq/l (4–6 h; P < 0.05) compared with the basal value (−2–0 h; 0.155 ± 0.012 meq/l). In addition, there was no significant difference (2–4 h) between control and antagonist experiments for the increment of mean FFA values compared with the baseline.

**DISCUSSION**

This experiment demonstrates that feeding and the intraruminal infusion of SCFA to cause a rumen SCFA content at near-physiological levels except for above pharmacological levels caused suppression of GH secretion via the parasympathetic nerve.

Inhibition of plasma GH levels and the GRF-induced GH response after feeding have been reported in sheep (2, 14, 29) and goats (28). Trenkle (29) demonstrated a clear and chronic reduction in GH secretion within 30 min after feeding in sheep by estimating the magnitude of the GH response to GRF. He further demonstrated that both distention of the rumen and the anticipation of being fed each rapidly reduced the GH response to the intravenous injection of GRF but that basal plasma GH concentrations remained unchanged in sheep. Tindal et al. (28) also observed that artificial distention of the cranial sac of the rumen with a water-filled balloon and the anticipation of being fed each caused an immediate decline in the plasma GH concentration in goats. Additionally, when lambs drank milk diluted with water or vigorously ate chopped lucerne hay or when adult wether sheep ate dry feed rapidly or were fitted with an esophageal fistula and were sham fed, the plasma GH level declined rapidly (4). These data suggest that mechanical or visual stimuli at ingestion of feed via some neural pathway may be one of the factors causing the immediate suppression of GH secretion by the somatotroph, either directly and/or by other neuropeptides released in the hypothalamus after feeding. The vagus nerve is known to transmit signals from chemo- and mechanoreceptors existing in the rumen epithelium (11, 19), which could be a possible mechanism. Tindal et al. investigated the role of visceral stimuli in the regulation of GH release in castrated male goats by bilateral cooling of exteriorized vagi. However, it was not possible to determine whether bilateral cooling of the vagi was able to block transmission of afferent impulses that inhibit release of GH, because the stress associated with cooling-induced paralysis of the swallowing mechanism itself may have suppressed GH release. In addition, the stress associated with accidental embolism was also found to inhibit GH release. Therefore, it is difficult to demonstrate the control GH release by this mechanism with surgical methods. However, in the present experiment, we clearly demonstrated that GH release is mediated via the parasympathetic nervous system by a pharmacological method, although we cannot stipulate that this mechanism is mediated via the vagus nerve.

It is interesting that the blocking action of the cholinergic antagonists was observed only during the 107 µmol·kg⁻¹·min⁻¹ infusion rate. As plasma glucose significantly increased during the 107 µmol·kg⁻¹·min⁻¹ infusion rate compared with the basal value (−2 to 0 h), the 107 µmol·kg⁻¹·min⁻¹ infusion rate obviously exceeded physiological concentrations after feeding (12). However, in sheep, feeding once per day, as in the present study, commonly raises the ruminal SCFA concentration up to 120–200 mmol/l at 2 h after the onset of feeding (16). In addition, because the SCFA concentration of the rumen and especially that of the plasma after the 107 µmol·kg⁻¹·min⁻¹ SCFA infusion were not significantly different from that observed after feeding, it is suggested that this infusion rate only slightly exceeded what would be physiologically normal. However, the plasma glucose concentration was significantly increased by the 214 µmol·kg⁻¹·min⁻¹ SCFA infusion rate relative to the basal values (−2–0 h) and the SCFA concentration of ruminal fluid and plasma after infusion was significantly increased by this infusion rate relative to after feeding. In this case, the ewes were infused with SCFA at a pharmacological rate. Thus there was a clearly significant difference in the rumen fluid and plasma SCFA concentration between the two infusion rates. Recently, Ishiwata et al. (17) suggested that propionate and butyrate have an inhibitory effect on GRF-induced GH release from goat primary cultured anterior pituitary cells. Therefore, it is possible that the increase of SCFA in peripheral blood had a direct effect on the pituitary during the 214 µmol·kg⁻¹·min⁻¹ infusion rate. In addition, the pituitary is not the site of action of the cholinergic antagonist, because muscarinic antagonists in particular are known to have no effect on pituitary GH secretion (8, 9). This assumption was supported by the fact that scopolamine-N-butyl bromide, which was used in this study, has been shown to have no direct effects on the pituitary outside of the BBB with respect to GH secretion (24). Consequently, antagonists may be unsuccessful in
blocking the direct suppression of GH secretion from the pituitary, apparently caused by the increase in peripheral blood SCFA after a SCFA infusion in the pharmacological range. Furthermore, it is likely that the direct inhibitory effects of peripheral blood SCFA on pituitary GH secretion overcame the GH-releasing effect of the cholinergic antagonists blocking the parasympathetic nervous system. However, it is possible that the signals stimulated by the intraruminal SCFA infusion were transmitted by the vagus nerve from chemoreceptors in the rumen epithelium to brain centers above the pituitary level during the 107 μmol·kg⁻¹·min⁻¹ infusion rate, as for the feeding experiment. Therefore, these results may indicate that increment of SCFA concentration in the rumen acts as one of the factors on the suppression of GH secretion after feeding in sheep.

In the present experiment, plasma glucose was unchanged after feeding, although there was a trend for an initial decrease that was then followed by an increase. Plasma FFA was significantly reduced by feeding. These trends and significant difference agree with the previous study of Bassett (3). In the case of glucose, it is possible that the sampling time in the present study was too short for the fermentation in the rumen to provide the glucose precursors required to cause an increase in glucose levels for the fermentation in the rumen after feeding (3, 16). On the other hand, intraruminal infusion of SCFA caused hyperglycemia and suppression of the FFA concentration. These changes in metabolite concentration also agree with previous studies (4, 12), although the increase in plasma glucose was not significant in the experiment of de Jong (12) using goats infused with SCFA at an infusion rate similar to that of the present study. Glucose and FFA concentrations have been reported to affect GH secretion in ruminants (15, 26). However, the cholinergic blocker had no significant effect on the plasma glucose and FFA concentrations, although it increased GH secretion in the present investigation. Therefore, the possible involvement of these metabolites in the suppression of GH secretion after feeding and intraruminal infusion of SCFA can be excluded.

In ruminant animals, it has been reported that long-term nutritional status affects GH and IGF-I secretion in addition to the effect of acute feeding. Although the reports detailing these mechanisms are conflicting, it has been suggested that the mechanism is mediated by neural peptides such as GRF or somatostatin at the level of the hypothalamus (3a, 27). From the results of the present study, we suggest that the stimulus of acute feeding in the alimentary tract is transmitted to the pituitary level. Therefore, a further detailed study of the mechanism by which GH secretion is suppressed by feeding in ruminants above the level of the pituitary is required.

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