ABSTRACT

In monogastric animals, suckling influences the secretion of gastrointestinal hormones during lactation. The aim of the present study was to investigate whether similar effects are induced by milking in cows. Experiments were performed on four cows in midlactation. Blood samples were drawn from a chronic jugular vein catheter and gastrin, and somatostatin were determined by radioimmunoassay. Milking and feeding increased plasma gastrin. Somatostatin increased at morning milking and at feeding, but it decreased at evening milking. Atropine injected subcutaneously 30 min before milking increased resting concentrations of gastrin but decreased resting concentrations of somatostatin. Feeding-induced release of gastrin remained but the milking-induced release disappeared. The milking- and feeding-induced effect on somatostatin became more marked. We suggest that milking influences gastrin and somatostatin via activation of the vagal nerves. The gastrin release caused by milking may be mediated via a cholinergic mechanism, whereas the atropine resistant effect on gastrin caused by feeding and on somatostatin caused by both milking and feeding suggest that a noncholinergic, perhaps peptidergic, transmitter may be involved.

INTRODUCTION

Suckling and milking in lactating animals activates a neuroendocrine reflex where the pituitary hormones oxytocin and prolactin are released. In most species, prolactin is responsible for the maintenance of lactation, and oxytocin is involved in milk removal. However, in ruminants basal concentrations of prolactin can be very low without influencing milk production (6), indicating that this hormone is of minor importance for maintenance of lactation. Further, in some species oxytocin does not appear essential for efficient milk production since cows may have normal milk yields without detectable release occurring during milking (4, 13). Thus, other mechanisms may be involved in the control of milk secretion and milk ejection in ruminants. In monogastrics, suckling increases the concentrations of the gastrointestinal hormones; gastrin, cholecystokinin (CCK),
RELEASE OF GASTRIN AND SOMATOSTATIN

Insulin, and vasoactive intestinal polypeptide (15, 22). Suckling also influences plasma concentrations of gastrointestinally derived somatostatin, but in a more complex way (10, 15, 22).

Gastrin and CCK stimulate gastric and pancreatic secretion and exert trophic effects on the stomach and pancreas, whereas somatostatin counteracts these effects (3, 11, 12, 24). It is possible that the suckling-released gastrointestinal hormones prepare the organism for increased food intake needed during lactation by increasing the size of the gastrointestinal tract (14, 22). Moreover, low plasma concentrations of somatostatin, in response to suckling, appear to be associated with high milk yields in women, indicating the importance of gastrointestinal hormones in milk secretion and ejection (27).

In cows, injections of somatostatin reduce milk production and increase basal, premilking, and milking serum concentrations of prolactin and reduce concentrations of somatotropin (9). However, the mechanisms by which somatostatin influences milk secretion are not known.

There are reasons to assume that the suckling-induced release of gastrointestinal hormones are due to a reflex activation of the vagal nerves. A similar release of these hormones occurs in response to activation of the vagal nerves by electrical stimulation in cats (26) and by feeding in dogs (21). Furthermore, milking induces rumination in lactating goats (a vagally controlled phenomenon) (2). Vagally induced effects on gastrin and somatostatin secretion can be blocked by atropine in rat, but are resistant to cholinergic blockade in species such as cat and human (1, 16, 25). This suggests that both noncholinergic and cholinergic mechanisms are involved in their release.

The objective of the present study was to investigate whether a vagal reflex activation occurs in response to milking in ruminants by measuring milking induced release of gastrin and somatostatin. If so, we also wanted to find out if the release is controlled via cholinergic or noncholinergic mechanisms.

MATERIALS AND METHODS

Experiments were performed on four, nonpregnant Swedish Red and White cows in midlactation. Daily milk yields ranged from 20.3 to 25.7 kg to 18.4 to 21.9 kg for the first and last week of the experiment. The cows were milked twice a day at 0800 and 1700 h. The milking machine was an Alfa Laval Duo-Vac with a main milking vacuum of 50 kPa, pulsation frequency of 1.0 Hz, and pulsation ratio 70:30. The experiment began June 20 and was completed on July 24. During this time, day length decreased by 40 min.

Cows were fed individually, 1.5 h postmilking, with hay and concentrate balanced for milk production (5 MJ metabolizable energy -kg⁻¹ FCM + maintenance 60 MJ). Morning feeding was at 0930 h. During the 35-d trial, cows were managed and milked by the same person. All treatments were performed in the same order at the same time for all cows.

Blood Sampling

In an attempt to alleviate stress and discomfort of frequent blood sampling, a semipermanent polyvinyl chloride-catheter (o.d. 2.5 mm) was inserted into the jugular vein and attached to the back of the animal. When not in use, the catheter was filled with .9% NaCl solution containing heparin (50 IU·ml⁻¹) to avoid blood clotting.

Blood samples were drawn at 45, 30, and 0 min before milking and at 5, 15, and 30 min after start of the milking procedure. Sampling was carried out at both morning and evening milkings. Directly after the 0 time sample was taken, the udder and teats were cleaned with a wet paper towel, and control milk was drawn from each quarter. After 30 to 45 s of presimulation, the machine was applied. Further blood samples were collected at 20 and 10 min before the morning feeding and at 5, 20, and 60 min after cows were offered feed. Cows were fed concentrate and hay at the same time, and exactly 5 min after the cows were offered feed a new blood sample was drawn. Means of the samples taken 45 and 30 min before milking and 20 and 10 min after start of the milking procedure. Sampling was carried out at both morning and evening milkings. Directly after the 0 time sample was taken, the udder and teats were cleaned with a wet paper towel, and control milk was drawn from each quarter. After 30 to 45 s of presimulation, the machine was applied. Further blood samples were collected at 20 and 10 min before the morning feeding and at 5, 20, and 60 min after cows were offered feed. Cows were fed concentrate and hay at the same time, and exactly 5 min after the cows were offered feed a new blood sample was drawn. Means of the samples taken 45 and 30 min before milking and 20 and 10 min before feeding were calculated and were used for resting concentrations. The samples taken 5 min after the milking procedure began were at high milk flow rates. The samples taken 5 min after the cows were offered feed, when cows were eating.

During sampling the first 5 ml of the blood was discarded. Then, 10 ml of blood were collected and placed in ice-cold tubes, containing heparin (50 IU·ml⁻¹) and trasylool (400

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IU·ml⁻¹). Samples were centrifuged for 20 min, and then the plasma was removed and immediately stored in a freezer (−20°C).

**Atropine Treatments**

The experiment lasted for 35 d and was divided in four periods, where all cows were treated identically.

Atropine was injected daily s.c 30 min before the morning and evening milkings, according to the following schedule (μg atropine·kg BW⁻¹): d 1 to 14, 0 μg; d 15 to 21, .5 μg; d 22 to 28, 2.3 μg; and d 29 to 35, 11.5 μg. These doses of atropine did not influence rumination, heart rate, respiration rate, or salivary secretion.

**Hormone Analyses**

Hormone concentrations were measured in samples collected at the end of each period. Gastrin was measured in unextracted plasma samples by radioimmunoassay (17). Plasma concentrations of somatostatin were measured by radioimmunoassay (7) after purification using SEP-PAK C₁₈ cartridges (S. Stock and K. Uvnäs-Moberg, unpublished).

**Statistics**

All data were subjected to least squares analysis of variance using the general linear model procedure (18). Results were expressed as least square means. The model included the effect of treatment (atropine), the effect of blood sampling time in relation to milking or feeding, the random effect of cow, and the interactions between these effects.

Contrast of means was used to test for differences in time (compared with resting plasma concentrations) within treatment and differences in resting concentrations between treatments (27). Standard error of the contrast were calculated by hand, because SAS does not give the correct standard error for models with more than one random effect.

**RESULTS**

**Hormones Released During Milking**

*Gastrin.* Basal plasma concentrations of gastrin averaged 47.8 pM in the morning and decreased significantly to 40.1 pM in the afternoon. Plasma gastrin increased significantly 5 min after onset of the morning milking and tended to increase in the evening. The increased gastrin concentrations disappeared 15 min after milking was completed (Figure 1a and b).

*Somatostatin.* Basal plasma concentrations of somatostatin averaged 29.8 pM in the morning and 29.5 pM in the afternoon. Plasma somatostatin increased significantly in the morning immediately before prestimulation, and they decreased significantly at the evening milking. After milking was completed, somatostatin returned to basal concentrations within 15 min (Figure 1d and e).

**Hormones Released During Feeding**

*Gastrin.* Plasma gastrin showed a significant increase 5 min after the cows were fed, but within 20 min the basal concentrations were almost attained (Figure 1c).

*Somatostatin.* Somatostatin also increased, but unlike gastrin, the elevated concentration remained throughout the following hour (Figure 1f).

**Effects of Atropine Injections: Resting Concentrations**

*Gastrin.* In the experiments with atropine, the resting concentration of gastrin increased significantly at morning milking, at afternoon milking, and at feeding with the two highest atropine doses (Figure 1a, b, and c).

*Somatostatin.* The milking-related resting concentration of somatostatin decreased significantly in the morning with the lowest and the highest doses of atropine. All three doses of atropine significantly decreased somatostatin concentrations in the afternoon (Figure 1d and e).

**Hormones Released During Milking**

*Gastrin.* There was no milking-induced gastrin response either at morning or at evening milking after atropine treatment (Figure 1a and b).

*Somatostatin.* The milking-induced somatostatin release was enhanced by atropine. Increases were obtained both in the morning and in the evening at all three doses of atropine,
significantly with the two highest doses. This effect persisted for at least 30 min. Significantly elevated somatostatin was also recorded immediately before prestimulation (Figure 1d and e).

**Hormones Released During Feeding**

**Gastrin.** Plasma gastrin increased significantly due to feeding in the atropine experiments. The most marked effect was observed after the atropine dose of 2.3 μg·kg·BW⁻¹ was given, but the effect was short-lived. Resting concentrations were resumed within 20 min after feeding (Figure 1c).

**Somatostatin.** Feeding induced significant increases in plasma somatostatin with doses of .5 and 2.3 μg·atropine·kg·BW⁻¹. This effect remained throughout the following hour (Figure 1f).

**DISCUSSION**

Resting concentrations of gastrin were significantly higher in the morning than in the
afternoon. Gastrin rose in response to milking, as well as in response to feeding. Plasma somatostatin showed an increase due to morning milking and to feeding, but they decreased following evening milking. These hormonal responses during the milking process are in agreement with reported results from monogastric animals, where suckling induces a release of gastrin in dogs, pigs, and humans (15, 22, 27). A varying pattern of suckling-induced somatostatin responses has also been observed with somatostatin. Somatostatin fell in response to suckling in pigs and humans but rose in dogs (10, 15, 22).

In monogastric animals, gastrin is produced in the antrum, and its release is stimulated in response to food in the stomach and vagal nerve activity. Gastric somatostatin, which constitutes the majority of the circulating somatostatin, is derived from two pools—one in the corpus and one in the antrum. The latter is activated at low intragastric pH, an effect that may be supported by vagal nerve stimulation. Because the antral somatostatin cells are located close to the gastrin cells, and because somatostatin inhibits gastrin release, gastrin secretion is inhibited when somatostatin secretion from the antrum increases. The release of somatostatin from the corporeal pool of somatostatin cells is inhibited by vagal nerve stimulation and increased in response to sympathetic nerve activity (20). In ruminants, gastrin is produced in the antrum and duodenum. Products of protein digestion lead to its release. Further, it is possible that peptides such as bombesin and epinephrine also cause a release of gastrin. Similar to monogastric animals, the release of gastrin is under vagal control, as observed in calves and sheep. In ruminants, antral acidification and substances such as somatostatin and norepinephrine also lead to gastrin suppression. The presence of somatostatin has been demonstrated in the pancreas of the sheep and in the body of the abomasum and the small intestine. The release of somatostatin from the gastrointestinal tract occurs in response to nervous stimulation, as demonstrated in experiments with stimulation of the vagi (19).

Differences in resting concentrations of gastrin were observed at morning and evening milking with concentrations of 50 and 40 pM, respectively. These data may indicate that the resting concentrations of plasma gastrin have a daily rhythm, a phenomenon that has also been observed with somatostatin (23).

Suckling in lactating animals influences the secretion of gastrointestinal hormones. It is assumed these effects are mediated via a reflex activation of the vagal nerves, an assumption that is strengthened by the observations that similar hormonal responses were induced when the vagal nerves were activated by electrical stimulation in cats (26) or by feeding in dogs (21). Vagal stimulation invariably leads to enhanced gastrin, whereas somatostatin may increase or decrease, depending on species and the intragastric milieu (20). Because plasma gastrin and somatostatin were influenced by milking in cows, we propose that milking also activates a vagal reflex in cows. That suckling stimulates vagal nerve activity in ruminants is supported by the observations that milking induces rumination in lactating goats (2).

Different somatostatin responses, with an increase at morning milking and a decrease at evening milking, were observed. However, since the cows were fed 15 to 16 h before the a.m. milking, and only 8 to 9 before, the p.m. milking, there may have been a lower abomasum pH at the p.m. milking. Such fluctuations in pH might explain why somatostatin behaved differently at the p.m. milking, compared with the a.m. milking, because the release of somatostatin from pH-sensitive cells is potentiated at low pH (24).

Noteworthy is the high somatostatin at prestimulation in experiments with and without atropine. Gastrin concentrations also tended to increase when prestimulation began. These results indicate the existence of a conditioned release of these hormones due to milking. The oxytocin mediated milk-ejection reflex can be conditioned in cows (5).

Atropine treatment was associated with increased resting plasma concentrations of gastrin during milking, as well as feeding, whereas somatostatin concentrations decreased. In humans, atropine inhibits a cholinergic mechanism, which inhibits release of gastrin (8). In rats, vagally induced release of somatostatin is blocked by atropine, leading to enhanced concentrations of gastrin (1). It is therefore possible that the increased resting concentrations of gastrin and lowered somatostatin seen after atropine treatment in our experiments are due to
an inhibition of cholinergic mechanism, which stimulates somatostatin release.

The milking-induced gastrin response, but not that caused by feeding, was blocked by atropine treatment. In contrast, the effect on somatostatin seemed to be enhanced in both cases. The gastrin release caused by milking may therefore be mediated via a cholinergic mechanism. The atropine-resistant effect on gastrin, caused by milking, as well as the effects caused by both milking and feeding on somatostatin suggest that another vagal transmitter, e.g., bombesin or gastrin releasing peptide, may be involved.

Obviously, the milking- and feeding-induced hormonal responses reflect different functions. Gastrin is involved in the digestive process where it stimulates acid and pepsin secretion. However, we can only speculate as to the function of the milking induced gastrin. Because in monogastrics the gastrointestinal tract increases in size during pregnancy as well as during lactation (12, 14), and because gastrin exerts trophic effects on the stomach (11), it is likely that gastrin stimulates growth of the maternal gastrointestinal tract. Therefore, the milking-induced release of gastrin is involved in adaption of maternal gastrointestinal function. This occurs during lactation as a result of increased energy intake. The possibility that gastrin might also be involved in the control of milk secretion can not be neglected. The release of this hormone is stimulated by protein-rich food, and a protein-rich diet increases milk yield. Moreover, it has been indicated that gastrin may also take part in the milk removal process, because it is possible to induce the milk ejection reflex in dogs by giving pentagastrin (20).

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References


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