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STUDIES ON PENTAGASTRIN-INDUCED HYPOCALCAEMIA IN LACTATING COWS

By

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LUTHMAN, J., J. PERSSON and S. O. JACOBSSON: *Studies on pentagastrin-induced hypocalcaemia in lactating cows.* Acta vet. scand. 1975, *16*, 126—133. — During recent years it has been evident that a number of gastrointestinal hormones are potent calcitonin secretagogues, and it has been suggested that a gastrointestinal-thyroid C cell system exists as a part of post prandial calcium homeostasis. In the present study the hypocalcaemic effect of pentagastrin, a synthetic peptide with gastrin effects, was studied in lactating cows. Intravenous infusion of pentagastrin caused marked hypocalcaemia and hypophosphataemia in the cows. Thyroidectomy completely abolished the hypocalcaemic and hypophosphataemic effects of the peptide. The results thus suggested that the effects of the peptide were due to release of endogenous calcitonin.

cow; hypocalcaemia; hypophosphataemia; pentagastrin; thyroidectomy.

A possible role of gastrointestinal hormones in calcium homeostasis was first pointed out by *Cooper & Deftos* (1970) and *Care* (1970). *Cooper & Deftos* found that the calcitonin concentration increased in the thyroid venous effluent plasma in pigs after intragastric administration of calcium chloride in amounts which did not change the plasma calcium level. At that time hypercalcaemia was the only known physiological stimulus for calcitonin secretion, the authors suggested, however, that some factor(s) from the gastrointestinal tract might be responsible for the increased release of calcitonin. *Care* reported that intravenous infusion of pancreozymin increased calcitonin secretion in pigs. The role of gastrointestinal hormones in the regulation of calcitonin secretion was further studied by *Care et al.* (1971 a, b). It

was shown that all hormones with the same C-terminal tetrapeptide, e.g. pancreozymin-cholecystokinin, gastrin and synthetic pentagastrin, were potent calcitonin secretagogues.

Gastrin secretion increases when food enters the stomach. *Swaminathan et al.* (1973) showed in pigs that the secretion of both gastrin and calcitonin increased when the stomach was distended with physiological saline. It was also observed that, when the animals were re-fed after a 48 hrs. fast, the blood concentration of both gastrin and calcitonin increased markedly and plasma calcium showed a decrease of short duration. *Levant et al.* (1973) made the interesting discovery that intragastric administration of calcium carbonate, but not of sodium bicarbonate, caused a significant increase of the blood gastrin concentration in man.

Cooper et al. (1972) reported that intravenous infusion of gastrin and pentagastrin caused hypocalcaemia and hypophosphataemia in intact, but not in thyroidectomized pigs. Similar results were obtained in sheep by *Barlet* (1972), who also observed that electric stimulation of the vagus nerve caused hypocalcaemia and hypophosphataemia. Since vagal stimulation is known to increase the release of gastrointestinal hormones, it was suggested that the effects on plasma calcium and inorganic phosphorus were due to the release of these hormones.

It is thus well established that gastrointestinal hormones serve as calcitonin secretagogues, and several authors e.g. *Care et al.* (1971 b) and *Cooper et al.* have suggested the existence of a gastrointestinal-thyroid C cell system as a part of postprandial calcium homeostasis. The role of gastrointestinal hormones in calcium homeostasis in ruminants has been studied to a very limited extent. The aim of the present investigation was therefore to study the effect of pentagastrin on some blood mineral components in lactating cows.

MATERIAL AND METHODS

Four lactating cows of the Swedish Red and White Breed were used. All cows had calved about 3 weeks previously. Milk production varied from 15 to 30 l/day.

All cows received an intravenous infusion of pentagastrin (Peptavlon, ICI) at a dose of 0.13 μg/kg/min. during 60 min. The drug was diluted with physiological saline, so that each cow

received a total volume of 80 ml. The infusion was given by means of a peristaltic pump.

About a week after the infusion 3 of the cows were thyroidectomized. The animals were tranquilized with Plegicil (Agrivet, Uppsala) and Rompun (Bayer, Leverkusen), surgical thyroidectomy was then performed during local anaesthesia with the cow in lateral position. Pentagastrin was then infused as described above 1—3 days after surgery.

Blood was sampled in heparinized tubes (Heparinrör, Vitrum). Plasma calcium was determined according to *Skerry* (1965). Inorganic phosphorus and magnesium was determined by means of commercial reagents (Sigma Kit 670, Sigma Chemical Company, St. Louis, USA and Merckotest Magnesium, E. Merck, Darmstads, Germany).

RESULTS AND DISCUSSION

The physiological role of calcitonin has been a matter for discussion ever since the discovery of the hormone. It is, however, clearly shown that calcitonin prevents from hypercalcaemia after a meal in monogastric animals (*Gray & Munson* 1969, *Lederer et al.* 1969, *Swaminathan et al.* 1973). It is now evident that the secretion of calcitonin is not only regulated by the blood calcium level, but also by certain gastrointestinal hormones.

In a previous study it was observed that protamine, an agent inhibiting bone resorption, showed a pronounced hypocalcaemic effect in cows about 3 weeks after calving. Plasma calcium decreased about 3 mg/100 ml in some cows (*Persson & Luthman* 1975). As seen from Fig. 1 pentagastrin caused marked hypocalcaemia and hypophosphataemia in cows in the same stage of lactation. Plasma magnesium increased slightly in all animals. The decrease in calcium and inorganic phosphorus was of the same order as that obtained in swine and sheep after pentagastrin infusion (*Cooper et al.* 1972, *Barlet* 1972). The dose of pentagastrin used in the present study was rather large (about 8 μg/kg/hr.). *Barlet* used a dose of 1 μg/kg/hr. *Cooper et al.* showed that a single injection of 2.6 μg/kg caused a 40-fold increase of the calcitonin concentration in thyroid venous plasma, and that even a dose of 0.026 μg/kg significantly increased the calcitonin secretion.

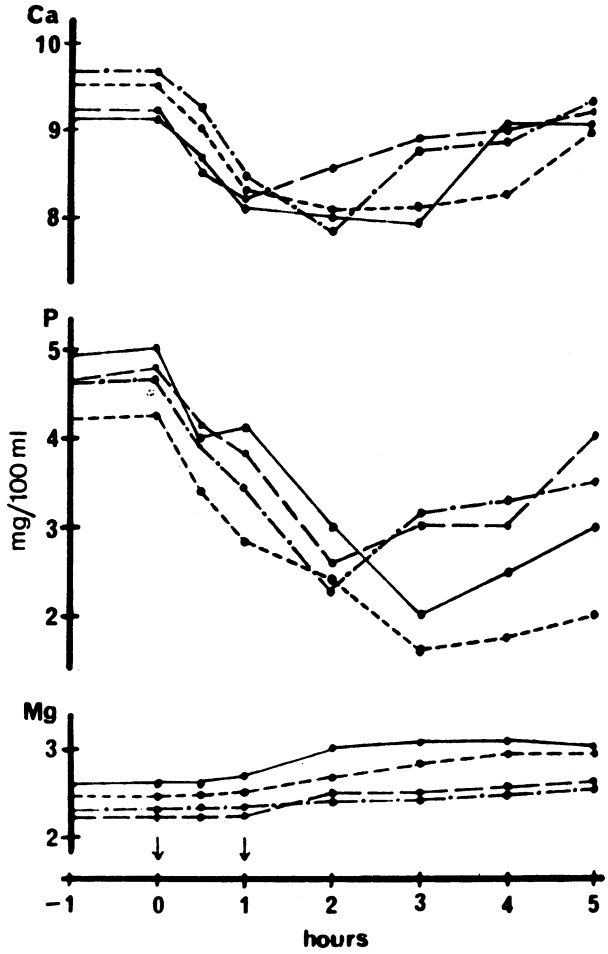

Figure 1. The effect of intravenous infusion of pentagastrin (0.13 μg/kg/min.) on the plasma levels of calcium, magnesium and inorganic phosphorus in intact cows. The arrows indicate the start and the end of the infusion.

We observed previously that plasma calcium remained unchanged or increased slightly in sheep after thyroidectomy (*Luthman & Persson* 1974, *Luthman* 1974). In the cows plasma calcium was lowered by thyroidectomy. Immediately before the operation the plasma levels were 9.9, 9.4 and 10.0 mg/100 ml, at the time of the second pentagastrin infusion the levels were 8.8, 8.5 and 9.2 mg/100 ml. The animals showed reduced appetite after the operation, and it is possible that the decrease in plasma

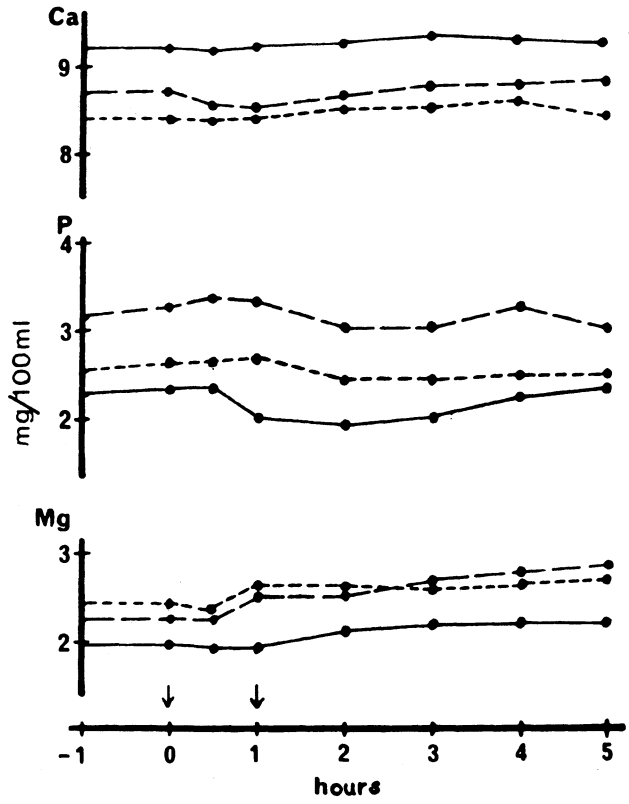

Figure 2. The effect of intravenous infusion of pentagastrin (0.13 μg/kg/min.) on the plasma levels of calcium, magnesium and inorganic phosphorus in thyroidectomized cows. The arrows indicate the start and the end of the infusion.

calcium was due to reduced intake of calcium. *Lomba et al.* (1972) showed that lactating cows were unable to maintain normal plasma calcium levels during starvation.

As shown in Fig. 2 thyroidectomy abolished the hypocalcaemic and hypophosphataemic effect of pentagastrin, which is in agreement with the results obtained in other species.

There are now several facts suggesting that there exists a gastrointestinal-thyroid C cell system in monogastric animals. Gastrin regulates the gastric acid secretion and is released when the stomach is distended. Pancreozymin-cholecystokinin is released when food passes out in the duodenum. The primary

function of this hormone is to regulate the pancreatic enzyme secretion and the contractions of the gall bladder. Besides their primary functions both gastrin and pancreozymin-cholecystokinin also increase the secretion of calcitonin. Extracts of the upper gastrointestinal tract contain polypeptides which react with antisera against pancreatic glucagon. These substances are generally referred to as enteroglucagon. Enteroglucagon is released by glucose, but *Böttger et al.* (1972) showed that enteroglucagon was released also after intraduodenal administration of calcium salts, and *Swaminathan et al.* showed that enteroglucagon increased calcitonin secretion in pigs. The passage of food through the gastrointestinal tract is thus associated with the release of a number of hormones which increase the secretion of calcitonin in order to prevent hypercalcaemia. The thyroid C cells can obviously be activated despite an unchanged blood calcium level.

Gastrin is not only released after oral administration of calcium, but also after intravenous injection. It is verified in several species including man that intravenous injection of calcium is associated with elevated blood gastrin levels and increased gastric acid secretion (*Becker et al.* 1973).

In monogastric animals the intake of food is followed by increased levels of gastrin and subsequently also calcitonin. In ruminants the passage of food is delayed in the fore stomachs, and there is an almost continuous flow of digesta through the abomasum. Nothing is hitherto known about the regulation of the blood gastrin level in ruminants. Both bovine and ovine gastrins have, however, been isolated from the abomasal mucosa. *Agarwal et al.* (1968) found ovine and bovine gastrins to be identical, differing from human and porcine gastrins at only 2 amino acid residues. The acid secretion by the abomasum was studied in sheep by *Ash* (1961 a, b). In animals fed ad libitum there was no effect of feeding. When the animals were fed only twice daily, feeding had a definite effect on acid secretion. It was further shown that distention of abomasum stimulated acid secretion. The introduction of rumen fluid and buffer solutions of pH 5—7 had similar effects. *Phillippo et al.* (1972) obtained a very short-lived increase in the blood calcitonin concentration in sheep in association with feeding. The authors suggested that the increased calcitonin secretion was due to the excitement of receiving food.

Ruminants are the domestic animals in which disturbances in calcium metabolism are most common. During recent years the physiological significance of parathyroid hormone and calcitonin has been intensely discussed. The role of gastrointestinal hormones also seems worth further study.

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SAMMANFATTNING

Studier över pentagastrininducerad hypokalcemi hos lakterande kor.

Under senare år har det visats att flera gastrointestinala hormoner kan frisätta calcitonin och det har föreslagits att gastrointestinala hormoner spelar en viss roll i kalciumhomeostasen, framför allt när det gäller att förhindra hyperkalcemi efter en måltid. Den hypokalcemiska effekten av pentagastrin, en syntetisk peptide med gastrineffekt, studerades hos lakterande kor. Intravenös infusion av pentagastrin gav påtagliga sänkningar av plasmakoncentrationerna av kalcium och oorganisk fosfor. Dessa effekter kunde dock helt elimineras av thyroidektomi. Resultaten talade således för att de observerade effekterna orsakades av en ökad frisättning av endogent calcitonin.

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