CORRELATION OF ENDOGENOUS SOMATOSTATIN, GASTRIC INHIBITORY POLYPEPTIDE, GLUCAGON AND INSULIN WITH GASTRIC FUNCTION IN THE CONSCIOUS CALF

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SUMMARY

Levels of endogenous somatostatin, gastric inhibitory polypeptide (GIP), glucagon and insulin were measured during gastric (abomasal) emptying in the conscious calf. Isotonic NaHCO₃ infused into the duodenum increased rates of emptying of a saline test meal and of gastric acid secretion, but had no effect on basal levels of blood glucose, somatostatin, GIP, insulin or glucagon. By contrast, intraduodenal infusion of 60 mM-HCl caused complete inhibition of gastric emptying, reduction of acid secretion, and an immediate increase in plasma somatostatin from 121·3 ± 9·4 (s.e.m.) to 286·3 ± 16·3 pg/ml (P < 0·01) but levels of GIP, insulin, glucagon and glucose were unaltered. Intravenous injection of somatostatin (0·5 µg/kg) suppressed the antral electromyographic recording and gastric efflux so long as plasma somatostatin levels remained above approx. 200 pg/ml. This suggests that somatostatin can be released by intraduodenal acidification and that it inhibits gastric function by an endocrine effect. Since somatostatin retards gastric emptying it may therefore have an indirect role in nutrient homeostasis by limiting discharge of gastric chyme to the duodenum.

INTRODUCTION

Release of immunoreactive somatostatin following instillation of glucose, peanut oil, casein hydrolysate and 100 mM-HCl into the stomach and intestine has been reported in anaesthetized dogs (Schusdziarra, Harris, Conlon, Arimura & Unger, 1978). Since it has been shown that somatostatin has an inhibitory effect on insulin and glucagon secretion by the pancreas (Barden, Cote, Lavoie & Dupont, 1978; Efendic, Lins & Luft, 1978), somatostatin may have a direct regulatory role in the control of metabolism. It has also been reported that exogenous somatostatin reduces rates of gastric emptying in man (Bloom, Ralphs, Besser, Hall, Coy, Kastin & Schally, 1975) and inhibits gastric antral motility in the dog (Tansy, Martin, Landin & Kendall, 1978). It is possible, therefore, that somatostatin release may affect nutrient homeostasis only indirectly, for inhibition of flow of gastric chyme could reduce the amount of nutrients available for intestinal absorption.

We have shown previously (Bell & Watson, 1976) that 60 mM-HCl maintains an absolute inhibition of gastric emptying when infused into the duodenum of calves even when the stomach is distended. This paper reports our investigation of the circulating levels of
somatostatin, gastric inhibitory polypeptide (GIP), insulin and glucagon during changes in gastric function on the intraduodenal infusion of acid or alkali.

MATERIALS AND METHODS

Animals

Eight Friesian bull calves, 40–50 kg body weight, were maintained as described by Bell & Razig (1973). At 10–14 days of age each animal was fitted with a gastric (abomasal) cannula and re-entrant duodenal cannulae were inserted 3 and 7 cm from the pylorus (Bell & Mostaghni, 1975). The preparation permits the instillation of a non-nutrient ‘test meal’ into the stomach through the abomasal cannula and the direct measurement of gastric effluent from the proximal duodenal cannula. At the same time duodenal infusion can be made through the other cannula without any contamination by gastric effluent.

Five other calves were prepared surgically as above but in addition electrodes were implanted in the pyloric antrum for electromyographic recording (e.m.g.) (Bell & Grivel, 1975).

Experimental procedures

Experimental procedures were as described previously (Bell & Mostaghni, 1975), except that the non-nutritive test meals were 1·5 l isotonic saline at pH 6·0, with 70 mg phenol red/l as a marker for volume measurement. The stomach was always drained and washed out before instillation of the test meal through the gastric cannula. Duodenal infusions were 143 mm-isotonic NaHCO₃ (pH 8·2) or 60 mM HCl (pH 1·22). The solutions were infused at 10 ml/min beginning 10 min before the instillation of a test meal, and continuing throughout the 45 min experimental period.

The following duodenal infusion experiments were carried out:

1. NaHCO₃ and test meal (seven experiments in seven calves);
2. HCl and test meal (nine experiments in seven calves);
3. HCl without test meal (six experiments in five calves);
4. no infusions, test meal only (six experiments in five calves).

The calves with antral electrodes were used to ascertain the effect on the e.m.g. during i.v. injection and infusion of synthetic somatostatin (Sigma, London) during measurement of gastric emptying.

Statistical comparisons were made using Student’s t-test for unpaired values.

Blood sampling and radioimmunoassay

Blood was taken from the jugular vein through an indwelling cannula (Portex 200/500/040). Samples were taken into fluoride oxalate for glucose estimation by the glucose oxidase method (Boehringer, Mannheim, Germany). Blood for hormone estimations was taken into lithium heparinized tubes containing 10 000 Kallikrein inactivator units aprotinin/10 ml blood. After centrifugation at 4 °C, the plasma was frozen in liquid N₂ and stored at −25 °C.

Somatostatin was measured by radioimmunoassay validated for human somatostatin (Penman, Wass, Lund, Lowry, Stewart, Dawson, Besser & Rees, 1979). Immunoreactive somatostatin in various tissues and in different vertebrates appears to be indistinguishable (King & Millar, 1979). Gastric inhibitory polypeptide was measured by radioimmunoassay (Morgan, Morris & Marks, 1978), values being expressed as porcine GIP equivalents. Sensitivity of the assay for GIP in plasma was 110 pg/ml. The cross-reactivity of bovine GIP with the antiserum (raised against porcine GIP) was established in calves in response to a milk feed. Undetectable values of GIP were treated mathematically as if they were at the limit of assay detection. Insulin was measured by a radioimmunoassay with double antibody separation (Morgan & Lazarow, 1963). Immunoreactive glucagon was measured by a double antibody radioimmunoassay using an antiserum directed against the CO₂H-terminal portion of porcine pancreatic glucagon (Al-Tamer, 1978). The amino-acid
sequence of porcine and bovine pancreatic glucagon is identical. The sensitivity of the assay was 100 pg/ml.

**RESULTS**

**Gastric function**

Approximately 50% of the instilled abomasal volume was discharged from the stomach in 45 min in the absence of duodenal infusion. Infusion of isotonic NaHCO₃ into the duodenum resulted in enhancement of emptying; approximately 30% of the meal remaining in the stomach. By contrast, infusion of 60 mm-HCl into the duodenum caused complete inhibition of gastric emptying.

Acid output in the stomach was significantly inhibited by duodenal HCl infusion (10 ± 1.3 (s.e.m.) mmol/45 min, \( P < 0.01 \)) compared with NaHCO₃ infusion (21 ± 2.9 mmol/45 min) and no infusion of the duodenum (26 ± 3.4 mmol/45 min).

**Somatostatin**

Immunoreactive somatostatin values immediately before the experiment ranged from 86 to 152 pg/ml (Fig. 1). These basal levels were unaffected by duodenal infusion of NaHCO₃. Duodenal infusion of HCl resulted in an immediate rise of somatostatin to 286 ± 16.3 pg/ml (\( P < 0.01 \)) which was sustained as long as duodenal infusion continued, and fell sharply towards the basal level on cessation of infusion (Fig. 1). Somatostatin levels were therefore correlated directly with duodenal HCl infusion and the response was unaffected by the presence or absence of a non-nutritive test meal in the stomach (Fig. 1).

![Graph](image-url)

Fig. 1. Plasma levels of somatostatin (mean ± S.E.M.) before, during and after infusion with 60 mm-HCl (dotted line; six experiments in five calves) or 143 mm-isotonic NaHCO₃ (broken line; four experiments in four calves). In two experiments in two calves no test meal was instilled during the infusion of HCl (solid line). *\( P < 0.02 \), **\( P < 0.01 \) compared with preinfusion values (Student’s t-test). Somatostatin values during HCl infusion were 286 ± 16.3 pg/ml (\( P < 0.01 \) v. preinfusion values).

**Levels of gastric inhibitory polypeptide, insulin and pancreatic glucagon**

Immunoreactive GIP levels in calves 3 h after the last feed were 340 ± 42.7 pg/ml. These levels were affected neither by the introduction of test meal nor by duodenal infusion of NaHCO₃ or HCl.
Insulin levels in calves were 8.8 ± 0.5 μu./ml. Mean values were not consistently affected by the presence of a test meal in the stomach or by the infusion of NaHCO₃ or HCl into the duodenum.

Pancreatic glucagon values were 417.3 ± 91.8 pg/ml. Glucagon values were unaffected by acid or alkaline infusion of the duodenum, or by the presence of a test meal in the stomach.

Blood glucose levels were unaffected by the introduction of an inert test meal into the stomach, or by the duodenal infusion of HCl or NaHCO₃.

Somatostatin injection and gastric emptying

In five calves injection of somatostatin (0.25–0.5 μg/kg) induced a dose-dependent reduction of gastric emptying and abolished the action potentials of the antral e.m.g. (Fig. 2). In six experiments in five calves, after injection of 0.5 μg somatostatin/kg, mean plasma levels were 922.3 ± 174.2 pg/ml (2 min after injection), 303.8 ± 47.7 pg/ml (4 min after), 188.5 ± 23.8 pg/ml (6 min after) and 111.3 ± 7.4 pg/ml (14 min after) (compare Fig. 1). Infusion of somatostatin (0.25–0.5 μg/kg per min) during experiments giving a test meal with NaHCO₃ infusion reduced gastric emptying significantly (P < 0.05).

DISCUSSION

In the conscious calf, perfusion of the duodenum with isotonic NaHCO₃ solution caused rapid emptying of a saline test meal from the stomach without any rise in assayable somatostatin. By contrast, duodenal infusion with 60 mm-HCl suppressed gastric emptying completely, gastric acid secretion was much reduced and a significant increase in endogenous somatostatin occurred. The same result was obtained whether the stomach was empty or
charged with a meal. These results in the conscious calf corroborate earlier evidence that somatostatin is implicated in mechanisms activated by acidification of the duodenum which evoke inhibition of gastric function (Schusdziarra et al. 1978). It is known that the antrum and duodenal bulb in vivo can develop a pH of 1.0-1.8 in man (James & Pickering, 1949; Andersson & Grossman, 1965) and ruminants (Hill, 1968). The acid infusates in our experiments were within this range and were less acidic than the 100 mM-HCl used in the anaesthetized dog by Schusdziarra et al. (1978). The output of somatostatin coincided precisely with the onset of acid duodenal infusion, persisted during the infusion and fell abruptly on termination of infusion.

The inhibitory action of injected synthetic somatostatin on gastric function seen in man (Bloom et al. 1975) and dog (Tansy et al. 1978) has now been demonstrated in the calf. In the calf, injection of 0.5 µg somatostatin/kg body weight caused inhibition of action potentials in the antral e.m.g. and abolition of efflux identical to the effects reported previously to occur with duodenal acidification (Bell & Grivel, 1975). The level of circulating somatostatin fell within 6 min to <200 pg/ml at which stage normal electromyographic recordings re-developed gradually and gastric emptying recommenced. This finding suggests that the somatostatin level seen on duodenal acidification was sufficient to inhibit contraction of gastric smooth muscle and thus restrain gastric emptying. The rapid disappearance of both endogenous and exogenous somatostatin from the blood is in accord with the short half-life of this hormone demonstrated by Schusdziarra, Harris & Unger (1979).

Our results suggest that endogenous somatostatin evoked by acid in the duodenum inhibits gastric function as an endocrine effect by inhibiting antral motility and reducing gastric efflux. It is possible therefore, that any role in nutrient homeostasis ascribed to somatostatin in suppressing glucose, insulin, glucagon and GIP could also occur indirectly by reducing the amount of gastric chyme passing to the duodenum for subsequent absorption.

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REFERENCES


