The role of the vagal innervation of the gut in insulin release in lactating ewes

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ABSTRACT

The vagal innervation of the pancreas and upper gastrointestinal tract has been implicated in the release of insulin in response to hyperglycaemia in several mammalian species. Therefore the role of the vagal innervation of the abomasum and upper small intestine in the release of insulin and its effect on glucose metabolism was studied in lactating ewes because of the importance of glucose for milk synthesis and secretion in ruminants. Vagal fibres innervating the abomasum, pylorus and duodenum were sectioned in 3 lactating ewes (VAG); 3 control lactating ewes underwent sham-operations (SHAM). Seven and 14 days after the surgery, all animals were given an intravenous injection of glucose (100 mg/kg BW) and jugular blood samples collected for glucose and insulin determinations. Plasma glucose concentration (mean ± standard error) rose in the VAG group from a pre-injection level of 3.9 ± 0.2 mmol/L to 6.2 ± 0.2 mmol/L and in the SHAM group from 3.7 ± 0.2 mmol/L to 6.3 ± 0.2 mmol/L. Maximum glucose concentration occurred at 5 minutes post-injection in both groups but declined more slowly in the VAG group than in the SHAM group and were significantly ($P < 0.05$) different at both 45 and 60 minutes post-injection. This resulted in a larger total area under the curve for the VAG group ($P < 0.01$) compared to the SHAM group. In the SHAM group, plasma insulin concentration rose from 685 ± 193 pg/mL to 3462 ± 649 pg/mL in response to the injection of glucose, whereas in the VAG group, plasma insulin response was smaller and reached a peak of 1530 ± 649 pg/mL from a basal level of 615 ± 193 pg/mL. The mean plasma insulin concentration after glucose injection in the VAG group was significantly ($P < 0.05$) lower than that for the SHAM group at both 5 and 10 minutes post-injection. These results indicate that vagal innervation of the stomach (abomasum), and duodenum modifies the pancreatic release of insulin in response to hyperglycaemia induced by intravenous glucose administration.

Keywords: lactating ewe; vagus; glucose; insulin.

INTRODUCTION

Glucose is an essential metabolite in all mammalian species and its concentration in the blood is maintained within a relatively narrow range by a variety of physiological mechanisms. In this insulin plays a central role and its release from the pancreas in response to elevated glucose concentrations are well known. It is also well known that vagal reflexes affect the functioning of the gastrointestinal tract and its accessory organs, including the pancreas and Woods and Porte (1974) suggested, that the sensitivity of the pancreas to glucose is influenced by the autonomic nervous system. Consequently the role of vagus nerve in regulating insulin secretion during hyperglycaemia has been studied in rhesus monkeys (Daniel and Henderson, 1975), in dogs (Singer *et al.*,1989), and in calves (Bloom and Edwards, 1982). These and other studies (Adrian *et al.*,1983; Berthoud and Powley, 1990; Berthoud *et al.*,1990; Niijima, 1986) strongly indicate a role for the vagal innervation of the gut and its associated structures (i.e. pancreas) in the stimulation of insulin release in response to hyperglycaemia.

The regulation of glucose metabolism in the lactating ruminant is very important since the mammary gland has a large demand for glucose for the synthesis and secretion of milk solids, in particular lactose. Therefore the influence of the autonomic nervous system on the partitioning of the nutrients between the mammary gland and other body tissues is of especial interest.

The experiments described in the present paper were undertaken to study the effects of cutting the vagal innervation to the abomasum, pylorus and duodenum (selective vagotomy) on pancreatic insulin secretion in response to glucose administration in lactating ewes. This is the first study of this kind conducted in a lactating ruminant species; Bloom and Edwards (1981a, 1981b) investigated vagal control of insulin secretion in immature ruminants aged 1-6 months.

MATERIALS AND METHODS

Animals, housing and feeding

Six Romney ewes at 3-4 weeks of lactation (body weight 40-60 kg) were obtained from Massey University farms. They were housed individually in metabolism crates indoors in a temperature controlled room (17-18°C) and given water ad libitum at all times. All animals were fed lucerne pellets and lucerne chaff ad libitum over a one week acclimatization period. They were randomly selected for surgery and either selectively vagotomized (VAG; n=3) or sham-operated (SHAM; n=3). Food was withheld for at least 18 hrs before surgery. After surgery each SHAM ewe was paired with a VAG ewe and fed the *ad libitum* intake of the paired VAG ewe.

Surgical procedures

Surgery was carried out using aseptic procedures under general anaesthesia induced with Saffan (Pitman-Moore)

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and maintained by Halothane inhalation. Sections (0.5-2.0 cm) of the abomasal, pyloric, and duodenal branches of the ventral vagus nerve, but excluding those branches supplying the rumen, reticulum, and omasum, were removed from each of the animals in the VAG group. The operations on the ewes in the SHAM group, during which all surgical procedures were carried out except the severing of the nerves, were performed on the same day as the selective vagotomies. The excised sections of nerve were fixed in Bouins solution and transferred to 70% alcohol 6-8 hrs later. They were later examined histologically to confirm the presence of neural tissue. An antibiotic (4-5 mL of Streptopen) was given to all ewes and rectal temperature recorded for 2 consecutive days after surgery. Catheters (polyvinyl tubes: ID-0.8 mm; OD-1.2 mm), previously filled with heparinized saline (100 i.u./mL of 0.9% NaCl), were inserted into each jugular vein under local anaesthesia (2% lignocaine) two days before the start of the experiment. The catheters were used for glucose injection and for the collection of blood samples. All catheters were flushed and filled with heparinized saline (100 i.u/mL) every morning.

MEASUREMENTS

Glucose challenge experiments were carried out on days 7 and 14 following surgery. All ewes were given a bolus injection of glucose (100 mg/kg body weight) through one jugular catheter. Sterile 40% (w/v) glucose solution (Baxter Healthcare Pty Ltd, NSW, Australia) was used for i.v. injections. Blood samples (10 ml) were collected into heparinized tubes at -10, -5, 5, 10, 15, 20, 30, 45, 60, and 90 minutes relative to glucose injection from the contra–lateral jugular catheter. Blood samples were stored in ice and centrifuged at 4000 rpm for 15 min at 4°C immediately after completing sampling. The resulting plasma was stored at -20°C until analysed for glucose and insulin.

Analyses

Plasma glucose concentrations (Trinder, 1969) were measured using a Cobas Fara II autoanalyser (Hoffman LA Roche Ltd, Switzerland). Intra- and interassay coefficients of variation were 1.9% and 4.6%, respectively. Plasma insulin levels were determined by radio–immunoassay (RIA) using crystalline bovine insulin (Sigma 1 5500, Lot 55F - 0536, 26.2 iu/mg Sigma Chemical Co., St Louis, Mo., 63178, USA) standards following the method described by Flux *et al.* (1984). Intra- and interassay coefficients of variation were 8.4% and 12.4%, respectively. The sensitivity of the assay ranged from 50 to 12800 pg/mL.

Statistics

Data are presented as means ± standard errors. Data from 1st and 2nd glucose challenges were pooled and statistical significance between the means was determined using ANOVA designed to account for repeated measures. A probability value less than 0.05 was considered significant. Statistical analyses were carried out using the computer packages 'SAS' (SAS, 1987: version 6.04) and 'REG' (Gilmour, 1990: version 90.12).

RESULTS

General animal effects of vagotomy

It took 24-48 hours following surgery for all ewes to return to pre-surgical levels of food intake and milk production. Body temperature was normal before and after surgery.

Plasma glucose

During the first glucose challenge at day 7, mean basal glucose concentration in the VAG group (3.9 ± 0.2 mmol/L) was not significantly different from that of the SHAM group (3.6 ± 0.2 mmol/L). At 5 min following glucose injection plasma glucose concentrations in the VAG and SHAM groups were 5.9 ± 0.3 mmol/L and 6.1 ± 0.3 mmol/L respectively (Fig. 1). On day 14 after surgery, the basal glucose concentrations were 3.9 ± 0.2 mmol/L and 3.7 ± 0.2 mmol/L in the VAG group and SHAM group respectively. The glucose concentration 5 min after the glucose challenge at day 14 was 6.5 ± 0.3 mmol/L in the VAG group whereas in the SHAM group it was 6.4 ± 0.3 mmol/L (Fig. 2).

The glucose removal rate from the blood, however, was slower in the VAG group compared to the SHAM group (Fig. 1 and Fig. 2) and the concentrations were significantly different ($P < 0.05$) at 45 min and 60 min post-injection (Table 1). Baseline corrected areas under the curve at 20-30 min and 30-45 min ($P < 0.05$) and the total area under the curve ($P < 0.01$) were significantly larger in the VAG group than in the SHAM group.

Plasma insulin responses

During the first glucose challenge, the SHAM ewes responded as expected to the glucose bolus injection; the

FIGURE 1: Changes in mean plasma glucose and insulin concentrations of lactating vagotomized (selective vagotomy) ewes (●; n = 3) and control, sham-operated, ewes (○; n = 3) in response to an intravenous glucose bolus injection (100 mg/kg BW) on day 7 post-surgery. Vertical bars: S.E. (±) of each mean value. *P < 0.05.

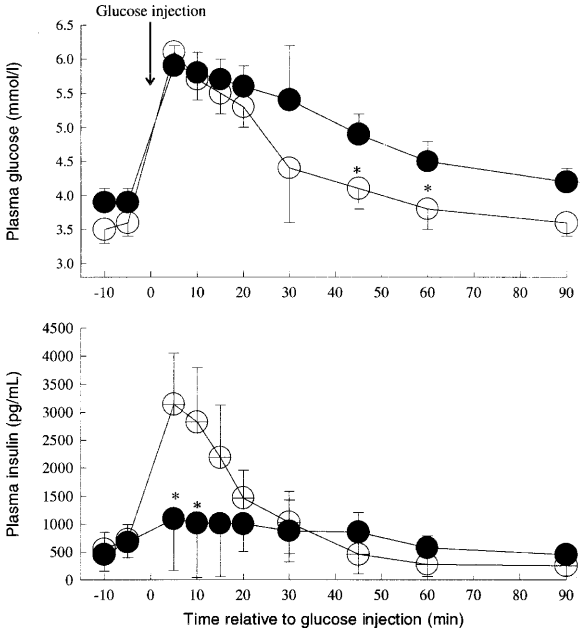
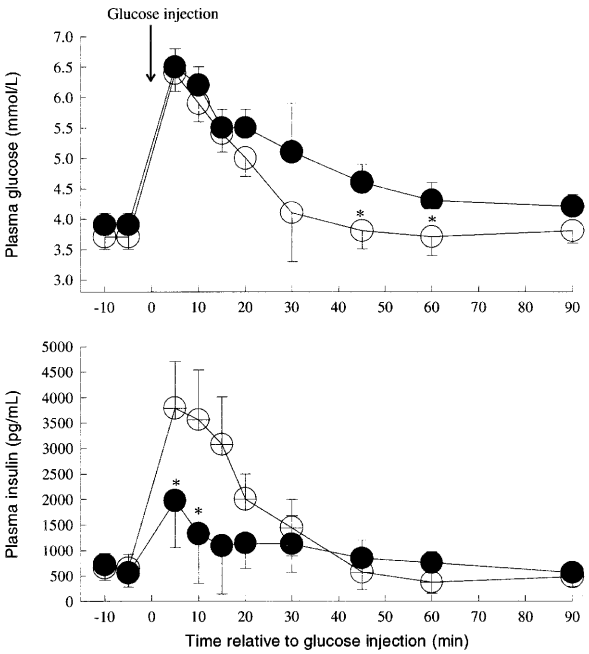


FIGURE 2: Changes in mean plasma glucose and insulin concentrations of lactating vagotomized (selective vagotomy) ewes (●; n = 3) and control, sham-operated, ewes (○; n = 3) in response to an intravenous glucose bolus injection (100 mg/kg BW) on day 14 post-surgery. Vertical bars: S.E. (±) of each mean value. *P < 0.05.



mean plasma insulin concentration rose from 718 ± 274 pg/mL to 3139 ± 918 pg/mL. The mean plasma insulin response in the VAG group was smaller and reached a peak of 1088 ± 918 pg/mL from its basal concentration of 677 ± 274 pg/mL (Fig. 1). The basal plasma insulin concentrations before the second glucose challenge were 555 ± 274 pg/mL and 652 ± 274 pg/mL in the VAG group and SHAM group respectively. On day 14 a bolus injection of glucose resulted in an increase in mean plasma insulin concentration up to 3787 ± 918 pg/mL in the SHAM group whereas it increased only up to 1973 ± 918 pg/mL in the VAG group.

The VAG group had a significantly lower (P < 0.05) plasma insulin response (baseline corrected) than the SHAM group at 5 min and 10 min post-injection (Table 1). Baseline corrected areas under the curve were significantly smaller

(P < 0.05) in the VAG group compared to the SHAM group at 5-10 min and 10-15 min post-injection. During the second glucose challenge on day 14, the plasma insulin response in the VAG group increased by 81% compared to the response during the first glucose challenge on day 7; the percentage increment of the total response in the SHAM group was 20%.

DISCUSSION

The present study investigated the role of the vagal innervation of just the abomasum and upper small intestine (duodenum) in mediating the release of pancreatic insulin during hyperglycaemia in lactating ewes. In ruminants the stomach is differentiated into several compartments (rumen, reticulum, omasum, and abomasum) all of which are innervated by both the ventral (left vagus) and dorsal (right vagus) vagal trunks (Habel, 1989). Because the contractions of the reticulum and rumen are under control of the vagus nerve it was necessary to leave the nerve supply to this part of the intestine intact. By contrast the abomasum physiologically resembles the simpler stomach found in monogastric animals and exhibits considerable intrinsic myogenic activity. The absence of any long lasting effects of the surgery on food intake and milk production indicates that the surgical procedures did not cause major disruptions to digestion in the partially vagotomized or sham-operated animals.

The finding that the release of pancreatic insulin in response to exogenous glucose in ruminants is suppressed by vagal disruption to the abomasum, pylorus and duodenum is supported by the observations of Bloom and Edwards (1981a) who showed that atropine, a muscarinic receptor antagonist, completely blocked the release of insulin following an intravenous infusion of glucose in 2-3 months old calves. Further pre-treatment of calves with atropine suppressed the rise in plasma insulin concentration in response to electrical stimulation of the peripheral ends of cut vagus nerves. Together these data suggest that the vagus nerve plays an important role in mediating the pancreatic insulin response. However, the use of systemi-

TABLE 1. Blood glucose and plasma insulin responses to intravenous glucose (Pooled across day 7 & day 14)

	Time relative to glucose injection (min)								
	-5	**5**	**10**	**15**	**20**	**30**	**45**	**60**	**90**
Controls (SHAM) (n = 3)									
BG (mmol/L)	3.7 ± 0.2	6.3 ± 0.2	5.8 ± 0.2	5.4 ± 0.2	5.1 ± 0.2	3.5 ± 0.5	3.9 ± 0.2	3.7 ± 0.2	3.7 ± 0.2
IRI (pg/mL)	685 ± 193	3462 ± 649	3190 ± 687	2629 ± 665	1731 ± 350	1233 ± 392	516 ± 246	322 ± 149	369 ± 129
Vagotomized (n = 3)									
BG (mmol/L)	3.9 ± 0.2	6.2 ± 0.2	6.0 ± 0.2	5.6 ± 0.2	5.6 ± 0.2	5.2 ± 0.5[a]	4.8 ± 0.2*	4.4 ± 0.2*	4.2 ± 0.2[a]
IRI (pg/mL)	615 ± 193	1530 ± 649*	1170 ± 687*	1044 ± 665	1072 ± 350	1001 ± 392	856 ± 246	666 ± 149	507 ± 129

Values are means ± SE; Abbreviations: BG- blood glucose, IRI-plasma immunoreactive insulin.
*P < 0.05, significantly different from controls (SHAM).
[a]P < 0.06, tend to be significantly different from controls.

cally administered blockers does not shed light on the way in which vagal pathways are involved.

This can be more readily determined by studying insulin release in response to exogenous glucose (either as systemic infusion or as intestinal perfusion) in selectively vagotomized animals. In our study only the vagal supply to the abomasum, pylorus and duodenum was sectioned; the hepatic branch was left intact. Studies using either conscious rats allowed to ingest to provide cephalic stimulation (Berthoud and Powley, 1990) or anaesthetised rats in which the cervical vagal nerves were electrically stimulated (Berthoud and Powley, 1990; Berthoud *et al.*, 1990) when only the gastric or hepatic vagal branches to the pancreas were left intact have shown that the gastric, rather than the hepatic, branches play the predominant role in the vagal control of the endocrine functions of the pancreas. These same authors have shown that the vagal efferent fibres reaching the pancreas by the gastric and hepatic nerves originate in the medial cell columns of the dorsal motor nucleus of the vagus in the medulla oblongata.

Anatomical studies have shown that in rats the gastric branches of the vagus contribute fibres to a nerve bundle that connects with the perivascular plexus of the superior pancreaticoduodenal artery (Kirchgessner and Gershon, 1989). A similar branch supplying the first part of the duodenum has been described in the dog (Matsuo and Seki, 1978). In dogs with only the gastric branches intact, bilateral electrical stimulation of the anterior vagal trunk produces a large contraction of the first part of the duodenum (Matsuo and Seki, 1978). These results are in accordance with the findings of Stavney *et al*. (1963) who demonstrated that bilateral electrical stimulation of the thoracic vagi causes a prominent duodenal contraction in dogs with only the gastric branches intact. It is possible that some of the efferent fibres projecting through the gastric and duodenal branches of the vagus, reach the pancreas via the numerous nerve bundles reported to connect the duodenum and the pancreas (Tiscornia, 1977). Our results are consistent with this hypothesis in that severing the gastric and duodenal branches of the vagus nerve modified the pancreatic beta cell response to an intravenous glucose load.

The insulin response to glucose at day 14 was higher in all ewes, compared to day 7; but the increase was greatest in selectively vagotomized ewes (81% increase) compared to a 20% increase in sham-operated ewes. The reason for this apparent increased insulin response a week after the first glucose challenge in selectively vagotomized ewes is not known. At day 14, the differences between means of the two groups in glucose clearance (at 45 min and 60 min post-injection) and insulin response (at 5 min and 10 min post-injection) were still significant ($P < 0.05$). It is possible that the pancreatic beta cells became sensitized to plasma glucose in the presence of the underfunctioning neural control mechanism in selectively vagotomized ewes. This, however, requires further investigation.

CONCLUSIONS

These results indicate that the vagal innervation of the stomach (abomasum) and upper small intestine (duodenum) modifies the pancreatic release of insulin in response to exogenous glucose in lactating ewes. It is concluded that the vagal innervation of the gut is important in controlling insulin secretion in lactating ruminant species. Research is currently in progress to define further the nature of this neuro-endocrine axis and its role in nutrient partitioning during lactation.

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REFERENCES

Adrian, T. E.; Bloom, S. R. and Edwards, A. V. 1983: Neuroendocrine responses to stimulation of the vagus nerves in bursts in conscious calves. *Journal of Physiology*, **344:** 25-35.

Berthoud, H.R.; Fox, E. A. and Powley, T. L. 1990: Localization of vagal preganglionics that stimulate insulin and glucagon secretion. *American Journal of Physiology*, **258:** R160-R168.

Berthoud, H.R. and Powley, T.L. 1990: Identification of vagal preganglionics that mediate cephalic phase insulin response. *American Journal of Physiology*, **258:** R523-R530.

Bloom, S.R. and Edwards, A.V. 1981a: Pancreatic endocrine responses to stimulation of the peripheral ends of the vagus nerves in conscious calves. *Journal of Physiology*, **315:** 31-41.

Bloom, S.R. and Edwards, A.V. 1981b: The role of the parasympathetic system in the control of insulin release in the conscious calf. *Journal of Physiology*, **314:** 37-46.

Daniel, P.M. and Henderson, J.R. 1975: The effect of atropine on insulin release caused by intravenous glucose in the rhesus monkey. *Acta Endocrinologica*, **78:** 736-745.

Flux, D.S.; Mackenzie, D.D.S. and Wilson, G.F. 1984: Plasma metabolites and hormone concentrations in Friesian cows of differing genetic merit measured at two feeding levels. *Animal Production*, **38:** 377-384.

Gilmour, A.R. 1990: REG A generalized Least Squares Program. version 90.12, New South Wales, Department of Agriculture and Fisheries.

Habel, R.E. 1989: Part two 'In Guide to the Dissection of Domestic Ruminants'. 1529 Ellis Hollow Road, Ithaca, NY, 14850.

Kirchgessner, A.L. and Gershon, M.D. 1989: Innervation of the rat's pancreas: Analysis of direct projections from neurons in myenteric ganglia of the duodenum and stomach and intrapancreatic ganglia. *Gastroenterology*, Abstract: **96(5):** A258.

Matsuo, Y. and Seki, A. 1978: The coordination of gastrointestinal hormones and the autonomic nerves. *American Journal of Gastroenterology*, **69:** 21-50.

Niijima, A. 1986: Neural control of blood glucose level. *Japanese Journal of Physiology*, **36:** 827-841.

SAS, 1987: Statistics, version 6.04, Cary, NC: SAS Institute Inc., U. S. A.

Singer, M.V.; Niebel, W. and Jansen, J.B.M.J. 1989: Pancreatic secretory response to intravenous caerulein and intraduodenal tryptophan studies: before and after stepwise removal of the extrinsic nerves of the pancreas in dogs. *Gastroenterology*, **96:** 925-934.

Stavney, L.S.; Kato, T.; Griffith, C.A.; Nyhus, L.M. and Harkins, H.N. 1963: A physiological study of motility changes following selective gastric vagotomy. *Journal of Surgical Research*, **3:** 390-394.

Tiscornia, O.M. 1977: The neural control of exocrine and endocrine pancreas. *American Journal of Gastroenterology*, **67:** 541-560.

Trinder, P. 1969: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry*, **6:** 24-27.

Woods, S.C. and Porte, D. 1974: Neural control of the endocrine pancreas. *Physiological Reviews*, **54:** 596-619.