Vagal stimulation and its role in eliciting gastrin but not glucagon release from the isolated perfused dog stomach¹

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SUMMARY Electrical stimulation (10 V, 10 Hz, 3 min) of both dorsal and ventral vagal trunks of the isolated canine stomach perfused with whole blood induced strong gastric contractions, transient release of cyclic GMP and marked release of gastrin. No gastric-glucagon release was elicited either at 'normal' (4.8 ± 0.1 mmol/l) or at low (1.5 ± 0.1 mmol/l) concentrations of blood glucose. It is concluded that, in conditions effective for the stimulation of gastrin release, electrical stimulation of the vagus nerves does not stimulate glucagon release from the isolated perfused dog stomach. Thus one of the well-accepted mechanisms controlling pancreatic-glucagon secretion, vagal stimulation, is ineffective on gastric-glucagon release.

The isolated perfused dog stomach provides a useful tool for the *in vitro* study of the mechanisms controlling gastric hormone release (Lefebvre *et al.*, 1976; Lefebvre and Luyckx, 1976, 1977). Recent *in vivo* and *in vitro* experiments have demonstrated that gastric-glucagon release was stimulated by intra-arterial (Lefebvre and Luyckx, 1976, 1977; Muñoz-Barragan *et al.*, 1976) or intraluminal (Muñoz-Barragan *et al.*, 1976) arginine, and by insulin deprivation (Blazquez *et al.*, 1976; Vranic *et al.*, 1976a). On the other hand arginine-induced gastric-glucagon release is inhibited by somatostatin (Dobbs *et al.*, 1975; Lefebvre and Luyckx, 1976, 1977); it is not affected by hyperglycaemia alone but is reduced by about 40% when hyperglycaemia is concomitant with a hyperinsulinaemia within the physiological range (Lefebvre and Luyckx, 1976, 1977). As demonstrated by other investigators (Bloom *et al.*, 1973, 1974a, b; Kaneto *et al.*,

1974), pancreatic glucagon secretion is increased in response to vagal stimulation. The aim of the present study was to investigate the effect of an electrical stimulation of the vagus nerves on the release of glucagon from the isolated perfused dog stomach. In view of the well-known effect of blood glucose on glucagon secretion (Review in Unger and Lefebvre, 1972), the experiments were performed both at normal and low glucose concentrations of the perfusing blood. The release of gastrin, which is known to be under vagal control was simultaneously evaluated. As there is evidence that cholinergic stimulation might be associated with an increased intracellular accumulation and extracellular release of guanosine-3', 5' adenosine monophosphate (cyclic GMP) (Butcher *et al.*, 1976), the net gastric output of this nucleotide was also measured.

Methods

The stomachs of overnight-fasted 9.5-12 kg dogs of both sexes were isolated according to a procedure previously described by Lefebvre and Luyckx (1977) with, in addition, careful dissection and isolation of both dorsal and ventral vagal trunks along the oesophagus to a distance of about 5 cm from the cardia. The isolated stomachs were perfused with whole canine blood supplemented with Trasylol (Bayer, Leverkusen, Germany) as previously described (Lefebvre and Luyckx, 1977). In four experi-

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ments, the blood perfusing each stomach was supplemented with a glucose solution (10% W/V) in order to maintain a 'normal' blood glucose concentration (about 4.7 mmol/l or 85 mg%), while, in four other experiments, no glucose was added, thus permitting the blood glucose concentration to fall during the equilibration period, because of consumption of glucose by stomach and blood cells. After a 50-60 minute equilibration period during which the blood was recirculated, blood samples were collected continuously from the vein at intervals of 10-30 seconds, without recirculation of the blood before, during, and after an electrical stimulation of both vagal trunks. Two arterial blood samples were collected before and after the electrical stimulation. The electrical stimulation was performed for three minutes, at an amplitude of 10 V and a frequency of 10 Hz using Tektronix Pulse Generators (Types 162 and 163, Tektronix, Inc, Portland, Oregon, USA). Arterial blood glucose concentration was determined using the method of Hoffman (1937) adapted to the Technicon Auto-Analyzer. For hormone assays, 0.4 ml of a solution containing Trasylol, 5000 U/ml, and Na₂EDTA, 12 mg/ml, were added to 3.6 ml of blood. The mixture was immediately centrifuged at +4°C and the separated plasma was stored at -20°C. Plasma glucagon was determined in duplicate assays by an established radioimmunoassay procedure (Luyckx, 1972), using porcine ¹²⁵I-labelled-glucagon as tracer, 30K antiserum (provided by Dr R. H. Unger, Dallas), and a dextran-charcoal separation of free and antibody-bound hormone. Plasma gastrin was assayed in duplicate by radioimmunoassay using a commercial kit (Gask, Institut National des Radio-éléments, Fleurus, Belgium); human gastrin 1-17 was used as standard and ¹²⁵I-labelled human gastrin as tracer; an anti-human gastrin was used as antibody and the separation of free and antibody-labelled hormone was performed using dextran-charcoal. The identity of reactivity between dog and human gastrin was assayed by verifying the parallelism of dilution curves. Plasma insulin was determined as previously described (Quabbe, 1969; Lefebvre and Luyckx, 1972). For cyclic GMP assay 10 µl Na₂EDTA 0.5 M were added to 1 ml blood. After centrifugation, plasma cyclic GMP was assayed by radioimmunoassay (cyclic GMP/RIA kit, the Radiochemical Center, Amersham, England). Gastric-glucagon, gastrin, and cyclic-GMP production by the stomach were calculated by multiplying the venoarterial difference in their plasma concentrations by the plasma flow (the latter being derived from the blood flow and the haematocrit). Statistical analysis was performed using Student's *t* test for paired and non-paired data.

Results

PERFUSING CONDITIONS AND BASAL VALUES
The haemoglobin O₂ saturation of the perfusing blood remained over 98% throughout the experiment and the stomach produced 18.9 ± 4.2 ml/h/100 g of stomach of an acidic juice (pH 4.4 ± 0.9) during the equilibration period. Arterial blood glucose was 4.8 ± 0.1 mmol/l or 86 ± 2 mg % (*n* = 4) in the 'normal glucose' experiments and 1.5 ± 0.1 mmol/l or 27 ± 2 mg % (*n* = 4) in the 'low glucose' experiments. Plasma glucagon and gastrin concentrations in the perfusing blood, before the introduction of the stomach in the circuit, averaged 126 ± 19 and 11 ± 3 pg/ml (*n* = 8), respectively. Basal plasma insulin was 3.7 ± 0.7 µU/ml (*n* = 8). Basal gastric blood flow was 77 ± 3 ml/min/100 g of stomach. Basal gastric-glucagon release was not statistically different from zero at both normal or low blood glucose concentration. Basal gastrin release was similar at normal and low blood glucose concentration and averaged 134 ± 31 pg/min/100 g stomach, a value significantly different from zero (*P* < 0.01). Basal cyclic GMP output averaged 9.2 ± 8.0 pmol/min/100 g, a value not statistically different from zero.

Fig. 1 Effect of the electrical stimulation of the dorsal and ventral vagal trunks on gastrin release from the isolated perfused dog stomach. The results of the experiments are grouped, as no difference was observed in the gastrin response between the 'normal' and the 'low' blood glucose experiments. Results are expressed as mean $\pm$ SEM. *P* < 0.02 (**) and < 0.01 (***) versus prestimulation values.

ELECTRICAL STIMULATION OF THE VAGUS NERVES

The electrical stimulation of both dorsal and ventral vagal trunks for three minutes induced violent contractions of the gastric musculature, the gastric blood flow remaining unchanged. It induced a transient release of cyclic GMP: 19.7 ± 8.5 pmol/min/100 g during the first minute of stimulation. As shown in Fig. 1, electrical stimulation of both vagi markedly stimulated gastrin release. The first significant rise was detected 40-50 seconds after the beginning of the stimulation, the release remained high up to the end of the stimulation and started to decline 30-60 seconds later. In contrast (Fig. 2), the electrical stimulation of the vagus nerves did not elicit any change in gastric-glucagon release either at 'normal' or at 'low' blood glucose concentrations.

Fig. 2 Lack of modification of gastric-glucagon release in response to the electrical stimulation of the dorsal and ventral vagal trunks of the isolated perfused dog stomach. Four stimulations were effected at 'normal' and 'low' blood glucose (BG) concentrations.

Discussion

Several groups recently reported the presence in the gastrointestinal tract of a material identical by all criteria to pancreatic glucagon (Sasaki *et al.*, 1975; Holst, 1977) as well as the presence of 'true A cells' in the dog gastric fundus (Baetens *et al.*, 1976). The isolated perfused dog stomach provides a useful tool for studying gastric-glucagon release in an

animal species where the quantity of this polypeptide in the stomach is particularly abundant (Morita *et al.*, 1976). Until now, data have been available only on the metabolic or hormonal factors modifying gastric-glucagon release (Dobbs *et al.*, 1975; Muñoz-Barragan *et al.*, 1976; Lefebvre *et al.*, 1976; Lefebvre and Luyckx, 1976, 1977). The present investigation aimed to determine whether or not vagal stimulation, a well-known stimulus of pancreatic glucagon secretion (Kaneto *et al.*, 1974; review in Bloom, 1976), also stimulated the release of glucagon from the stomach. In our experimental conditions the efficiency of the vagal stimulation was attested by (1) the induction of visible gastric contractions, (2) a transient release of cyclic GMP, a phenomenon usually associated with cholinergic stimulation (Butcher *et al.*, 1976), and, mainly, (3) an unequivocal release of gastrin. Under these conditions of effective stimulation of the vagus nerves, no release of glucagon by the stomach was observed. This was true even at low glucose concentrations and in the presence of extremely low circulating concentrations of insulin. We, therefore, conclude that gastric-glucagon secretion in the dog, contrary to pancreatic glucagon secretion (Bloom *et al.*, 1973, 1974a, b), is not under the control of the parasympathetic nervous system. This suggests that gastric-glucagon secretion is more dependent on metabolic or hormonal control rather than on neural factors. Vranic *et al.* (1976b) have indeed observed that strenuous exercise, which is known to be a potent stimulus of pancreatic-glucagon secretion probably mainly through stimulation of the adrenergic system (Luyckx and Lefebvre, 1974; Luyckx *et al.*, 1975), does not induce any rise in the plasma glucagon levels of depancreatized dogs, a situation in which circulating glucagon is known to be essentially of gastric origin.

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References

- Baetens, D., Rufener, C., Srikant, C. B., Dobbs, R., Unger, R., and Orci, L. (1976). Identification of glucagon-producing cells (A cells) in dog gastric mucosa. *Journal of Cell Biology*, **69**, 455-464.
- Blazquez, E., Muñoz-Barragan, L., Patton, G. S., Orci, L., Dobbs, R. E., and Unger, R. H. (1976). Gastric A-cell function in insulin-deprived depancreatized dogs. *Endocrinology*, **99**, 1182-1188.

- Bloom, S. R. (1976). Blood glucose control by direct islet innervation. In *Hypoglycemia*, pp. 85-90. Edited by D. Andreani, P. Lefebvre and V. Marks. Georg Thieme: Stuttgart.
- Bloom, S. R., Edwards, A. V., and Vaughan, N. J. (1973). The role of the sympathetic innervation in the control of plasma glucagon concentrations in the calf. *Journal of Physiology*, **233**, 457-466.
- Bloom, S. R., Edwards, A. V., and Vaughan, N. J. (1974a). The role of the autonomic innervation in the control of glucagon release during hypoglycemia in the calf. *Journal of Physiology*, **236**, 611-623.
- Bloom, S. R., Vaughan, N. J. A., and Russell, R. C. G. (1974b). Vagal control of glucagon release in man. *Lancet*, **2**, 546-549.
- Butcher, F. R., McBride, P. A., and Rudich, L. (1976). Cholinergic regulation of cyclic nucleotide levels, amylase, and K⁺ efflux from rat parotid glands. *Molecular and Cellular Endocrinology*, **5**, 243-254.
- Dobbs, R. E., Sakurai, H., Sasaki, H., Faloona, G. R., Valverde, I., Baetens, D., Orci, L., and Unger, R. H. (1975). Glucagon: role in the hyperglycemia of diabetes mellitus. *Science*, **187**, 544-547.
- Hoffman, W. S. (1937). A rapid photoelectric method for the determination of glucose in blood and urine. *Journal of Biological Chemistry*, **120**, 51-55.
- Holst, J. J. (1977). Extraction, gel filtration pattern, and receptor binding of porcine gastrointestinal glucagon-like immunoreactivity. *Diabetologia*, **13**, 159-169.
- Lefebvre, P. J., and Luyckx, A. S. (1972). Effect of ouabain on insulin secretion in the anesthetized dog. *Biochemical Pharmacology*, **21**, 339-345.
- Lefebvre, P. J., and Luyckx, A. S. (1976). Control of gastric glucagon secretion (Abstract). *Diabetologia*, **12**, 405.
- Lefebvre, P. J., and Luyckx, A. S. (1977). Factors controlling gastric-glucagon release. *Journal of Clinical Investigation*, **59**, 716-722.
- Lefebvre, P. J., Luyckx, A. S., Brassinne, A. H., and Nizet, A. H. (1976). Glucagon and gastrin release by the isolated perfused dog stomach in response to arginine. *Metabolism*, **25**, suppl. 1, 1477-1479.
- Luyckx, A. S. (1972). Immunoassays for glucagon. In *Glucagon, Molecular Physiology, Clinical and Therapeutic Implications*, pp. 285-298. Edited by P. J. Lefebvre and R. H. Unger. Pergamon Press: Oxford.
- Luyckx, A. S., Dresse, A., Cession-Fossion, A., and Lefebvre, P. J. (1975). Role of catecholamines in exercise-induced glucagon secretion and free fatty acid mobilization in rats. *American Journal of Physiology*, **229**, 376-383.
- Luyckx, A. S., and Lefebvre, P. J. (1974). Mechanisms involved in the exercise-induced increase in glucagon secretion in rats. *Diabetes*, **23**, 81-93.
- Morita, S., Doi, K., Yip, C., and Vranic, M. (1976). Measurements and partial characterization of immunoreactive glucagon in gastro-intestinal tissues of dogs. *Diabetes*, **25**, 1018-1025.
- Muñoz-Barragan, L., Blazquez, E., Patton, G. S., Dobbs, R. E., and Unger, R. H. (1976). Gastric A-cell function in normal dogs. *American Journal of Physiology*, **231**, 1057-1061.
- Quabbe, H. J. (1969). Modifikation der radioimmunologischen insulinbestimmung nach Hales und Randle. *Diabetologia*, **5**, 101-107.
- Sasaki, H., Rubalcava, B., Baetens, D., Blazquez, E., Srikant, C. B., Orci, L., and Unger, R. H. (1975). Identification of glucagon in the gastrointestinal tract. *Journal of Clinical Investigation*, **56**, 135-145.
- Unger, R. H., and Lefebvre, P. J. (1972). Glucagon physiology. In *Glucagon, Molecular Physiology, Clinical and Therapeutic Implications*, pp. 213-244. Edited by P. J. Lefebvre and R. H. Unger. Pergamon Press: Oxford.
- Vranic, M., Engerman, R., Doi, K., Morita, S., and Yip, C. (1976a). Extrapancreatic glucagon in the dog. *Metabolism*, **25**, suppl. 1, 1469-1473.
- Vranic, M., Kawamori, R., Pek, S., Kovasevic, N., and Wrenshall, G. A. (1976b). The essentiality of insulin and the role of glucagon in regulating glucose utilization and production during strenuous exercise in dogs. *Journal of Clinical Investigation*, **57**, 245-255.