Inhibition by somatostatin of carbamylcholine-induced gastrin and glucagon release from the isolated perfused canine stomach*

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SUMMARY At an arterial plasma concentration of 61 nmol/l (100 ng/ml) synthetic cyclic somatostatin completely abolished basal glucagon and gastrin release as well as carbamylcholine-induced glucagon and gastrin release from the isolated perfused dog stomach. These observations are compatible with the view that endogenous somatostatin previously reported to be released during vagal stimulation might be involved to explain the lack of gastric-glucagon response in this situation. They do not, however, rule out the alternative proposal that the dog fundic A-cell may simply be a non-innervated cell.

We have previously reported that the electrical stimulation of both dorsal and ventral vagal trunks of the isolated perfused dog stomach does not elicit gastric-glucagon release, although gastrin release is markedly stimulated.¹ In contrast, the intra-arterial infusion of acetyl- (or carbamyl-) choline unequivocally stimulated the release of glucagon and gastrin from the isolated stomach.^{2,3} Among the possible explanations for this discrepancy, we have suggested that somatostatin, also released in our system by the electrical stimulation of the vagus nerves, might simultaneously inhibit gastric-glucagon release.^{3,4} The present experiments were performed in order to see if exogenous somatostatin intra-arterial infusion would modify glucagon and gastrin release from the isolated perfused canine stomach, in response to an intra-arterial infusion of carbamylcholine, an analogue of the physiological neurotransmitter, acetylcholine.

Methods

ISOLATION AND PERFUSION OF STOMACH

Stomachs from overnight-fasted normal 8-12 kg mongrel dogs of both sexes were isolated with

their arterial and venous supply and perfused with whole blood collected from large blood-donor dogs according to a procedure previously described in detail.⁵ The perfusing blood was supplemented with 1000 U/ml Trasylol (Bayer, Leverkusen, West Germany) and anti-insulin serum (guinea-pig anti-insulin serum GPAIS 567 from PH Wright, Indianapolis, In., obtained through the courtesy of W Malaisse, Brussels or guinea-pig anti-insulin serum LAA obtained from L Heding, Novo Research Institute, Copenhagen); 50 µl of the original sera diluted in 20 ml saline containing 1 g albumin/dl (lot PL 77 C24 from Institut Mérieux, Lyon, France) were added to the blood reservoir; based on the neutralising capacities of these antisera, the quantity added represented an excess of 20 to 30 times the amount needed to neutralise the endogenous insulin present in the system.⁶ Immediately after the beginning of the perfusion, the glucose concentration of the perfusing blood was measured (see below) and eventually adjusted to 3.6-4.2 mmol/l (65-75 mg/100 ml) by addition to the blood reservoir of a given volume of either saline or a 10% (wt/vol) glucose solution in distilled water. The blood glucose was kept constant throughout the experiment by infusing into the blood reservoir a 66 mmol/l (12 mg/ml) glucose solution at the rate of 0.5 ml/min; this amount was previously found adequate to compensate for glucose consumption by both the stomach and the blood cells.⁶ The blood flow through the isolated stomach at the beginning of the experiment was set by adjusting the perfusion

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rate at a level for which the perfusion pressure was identical with the systolic blood pressure recorded before the experiment in the stomach donor dog. Thereafter, the pump rate was kept constant; further changes in blood flow or pressure thus reflected changes in the resistance of the stomach vessels. Blood sampling started only after a 45–60 minute equilibration period during which the blood was recirculated. After this equilibration period, basal blood samples were simultaneously collected from the artery and the vein. After collecting basal samples, carbamylcholine was infused intra-arterially (see below) and venous samples were collected continuously at intervals of 10–30 seconds, without recirculation of the blood. Samples were also collected at frequent intervals during the two minutes after the end of the carbamylcholine infusion.

EXPERIMENTAL PROTOCOLS

In four experiments, carbamylcholine alone was tested. Carbamylcholine chloride (C 4382, Sigma Chemicals Co, St Louis, Mo) was dissolved in 155 mmol/l (0.9 g/100 ml) NaCl solution; this solution was infused intra-arterially at a rate of 1 ml/min for five minutes, the concentration being adjusted to reach a final concentration of $5 \cdot 10^{-6}$ M in the arterial plasma. In six experiments, somatostatin was infused in combination with carbamylcholine. Synthetic cyclic somatostatin (CM 9357, lot RKA 06, Clin Midy, Paris, France) was dissolved in 155 mmol/l (0.9 g/100 ml) NaCl supplemented with 0.25 g human purified albumin/dl (Lot PL 76 E 12, Institut Mérieux, Lyon, France); this solution was infused intra-arterially at a rate of 1 ml/min, the concentration being adjusted to reach a final concentration of 61 nmol/l (or 100 ng/ml) in the arterial plasma; the somatostatin infusion started 20 minutes before the carbamylcholine infusion, performed under the conditions mentioned above, and lasted till the end of the experiment.

ASSAYS AND CALCULATIONS

As previously described,⁵ hormone production was calculated by multiplying the venoarterial difference in plasma hormone concentration by the plasma flow (the latter being derived from the blood flow and the haematocrit). Blood glucose concentration was determined by the hexokinase method adapted to the Technicon AutoAnalyzer (Technicon Instrument Co, Tarrytown, NY). For hormone assays, 0.4 ml of a solution containing Trasylol, 5000 U/ml, and Na_2EDTA , 35.5 mmol/l (or 12 mg/ml) were added to 3.6 ml 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 a classical immunoassay procedure, using porcine ^{125}I iodoglucagon (NEN Chemicals, D-6072, Dreieichenhain, West Germany) as tracer, 30K antiserum (provided by Dr R H Unger, Dallas, Texas) and dextran-charcoal separation of free and antibody-bound hormone.⁷ Plasma gastrin was assayed in duplicate using a commercial kit (Gask, Institut National des Radioéléments, Fleurus, Belgium); human gastrin 1–17 was used as standard and ^{125}I -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 assayed as previously described.⁸ Statistical evaluation included variance analysis and Student's *t* test for unpaired data. All results are expressed as mean $\pm$ standard error of the mean (SEM).

Results

PERFUSION CONDITIONS

The haemoglobin O_2 saturation of the perfusing blood was over 98% throughout all the experiments. Blood glucose averaged 3.8 ± 0.17 mmol/l or 68.6 ± 3.1 mg/100 ml ($n=10$; range 58–85). Plasma glucagon in the perfusing blood, before introduction of the stomach in the circuit, averaged 82 ± 18 pg/ml ($n=10$, range 5–181), plasma gastrin averaged 10.1 ± 1.8 pg/ml ($n=10$; range 0–21), and plasma insulin averaged 10.1 ± 2.4 $\mu\text{U/ml}$ ($n=10$; range 2.4–25.7). As stated in the Methods section, an excess of anti-insulin serum was added to the perfusing blood in order to neutralise these small amounts of circulating plasma insulin.

BLOOD FLOW

Basal blood flow was 54 ± 4 ml/min/100 g ($n=4$) before carbamylcholine infusion; it was 57 ± 7 ml/min/100 g ($n=6$) during somatostatin infusion alone. As shown in Fig. 1, carbamylcholine induced a 45% increase in blood flow; this carbamylcholine-induced rise in blood flow was not statistically affected by somatostatin.

GASTRIC-GLUCAGON RELEASE

Basal gastric-glucagon release averaged 340 ± 116 pg/100 g/min; in the somatostatin experiments it was abolished to 5 ± 61 pg/100 g/min ($P < 0.005$).

Fig. 1 Changes in stomach blood flow in response to carbamylcholine in control ($n=4$) and somatostatin ($n=6$) experiments. Results are expressed as mean $\pm$ SEM. Basal blood flow was 54 ± 4 ml/min/100 g in the control experiments and 57 ± 7 ml/min/100 g in the somatostatin experiments.

Fig. 2 Changes in gastric-glucagon release in response to carbamylcholine in control ($n=4$) and somatostatin ($n=6$) experiments. Results are expressed as mean $\pm$ SEM. ** $p < 0.02$, *** $p < 0.01$.

As illustrated in Fig. 2, carbamylcholine induced a progressive rise in gastric-glucagon release which averaged 4500–5000 pg/100 g/min in the post infusion period; in contrast, no rise at all in

gastric-glucagon release in response to carbamylcholine was observed during somatostatin infusion.

GASTRIN RELEASE

Basal gastrin release was 117 ± 33 pg/100 g/min; in the somatostatin experiments, it was abolished to 0 ± 10 pg/100 g/min ($p < 0.001$). Carbamylcholine induced a massive gastrin release which averaged 5000–10 000 pg/100 g/min; this response was totally abolished by somatostatin (Fig. 3).

Fig. 3 Changes in gastrin release in response to carbamylcholine in controls ($n=4$) and somatostatin ($n=6$) experiments. Results are expressed as mean $\pm$ SEM. All values in the control experiments are statistically higher than those observed in the somatostatin experiments.

Discussion

The electrical stimulation of the vagus nerves increases the release of both gastrin and somatostatin by the isolated perfused dog stomach;^{1,3} in contrast, such stimulation does not affect gastric-glucagon release.¹ We have previously suggested that an absence of innervation of the canine gastric A cell probably best explains this situation.^{3,4} However, the close interrelationships between A and D cells in the canine stomach (Polak, personal communication), suggesting a paracrine

interplay, allows us to formulate an alternative mechanism in which somatostatin released by the vagal stimulation would inhibit gastric-glucagon release.³ In fact, somatostatin has already been demonstrated to be a potent inhibitor of the gastric A cell under various conditions *in vitro*⁵ and *in vivo*.^{9,10} In the present series of experiments, we have shown that exogenous somatostatin at a plasma concentration of 61 nmol/l (100 ng/ml) completely abolished basal glucagon release as well as the gastric-glucagon response to carbamylcholine. This observation supports the suggestion that endogenous somatostatin, released by vagal stimulation, might be involved in the lack of gastric-glucagon response observed in this condition.

The situation with gastrin is apparently somewhat different. Here (1) both electrical vagal stimulation¹ and acetyl- (or carbamyl-) choline infusion² markedly stimulate gastrin release, (2) exogenous somatostatin infusion suppresses basal and carbamylcholine-induced gastrin release (present results), and (3) somatostatin-producing D cells and gastrin producing G cells are closely interrelated,¹¹⁻¹⁴ the former having long cytoplasmic processes terminating on the latter suggesting also a paracrine regulatory function.¹⁵ If a paracrine secretion of somatostatin is to be involved to explain the lack of gastric-glucagon response to an electrical stimulation of the vagus nerves, such a mechanism is apparently insufficient to inhibit the release of gastrin. A differential sensitivity to endogenous somatostatin of the glucagon and gastrin producing cells or a more complete paracrine control of the gastric A cells, than of the gastric G cells, by the D cells may explain these findings. In any case, high concentrations of somatostatin, such as those used in the present experiments, completely suppress the release of glucagon and of gastrin in response to high concentrations of the cholinergic neurotransmitter analogue, carbamylcholine. In conclusion, at a plasma concentration of 100 ng/ml, exogenous somatostatin completely inhibits carbamylcholine-induced glucagon and gastrin release from the isolated perfused dog stomach. This observation is compatible with the view that endogenous somatostatin might be involved to explain the lack of gastric-glucagon response to vagal stimulation, but does not rule out the alternative proposal¹ that the gastric A cell may simply be a non-innervated cell.

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