Effects of [Asu^{1,7}]-eel calcitonin on gastric somatostatin and gastrin release

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SUMMARY Effects of [Asu^{1,7}]-eel calcitonin on gastric somatostatin and gastrin secretion were studied by using the isolated perfused rat stomach. [Asu^{1,7}]-eel calcitonin (10^{-9} – 10^{-7} M) caused a simultaneous dose-dependent increase of gastric somatostatin release and decrease of gastrin secretion, with a significant correlation between these two. The demonstration of calcitonin stimulation of gastric somatostatin release raises the possibility of somatostatin-mediated suppression of gastrin secretion by calcitonin.

In addition to its effects on bone and kidney as a hypocalcaemic agent, calcitonin was found to suppress various gastrointestinal functions,^{1–5} including secretion of gastrin and gastric acid.^{6–8}

Recently, significant amounts of somatostatin, a potent inhibitor of gastric acid and gastrin secretion,^{9,10} were demonstrated in the stomach by radioimmunoassay^{11,12} as well as by immunocytochemical technique.¹³ More recently, we demonstrated somatostatin release from the stomach.¹⁴ In view of the location of somatostatin-producing D cells in the mucosa of the fundus and the antrum in the vicinity of the parietal cells and gastrin-producing G cells,^{15,16} an influence of gastric somatostatin on gastric acid and gastrin secretion was suggested. It therefore appeared worth while to investigate the effect of calcitonin on gastric somatostatin release with reference to gastrin secretion.

In the present study, we studied the effects of [Asu^{1,7}]-eel calcitonin, an analogue of eel calcitonin,¹⁷ on gastric somatostatin and gastrin secretion from the isolated perfused rat stomach.

Methods

Male Wistar rats weighing 300–350 g were maintained in a temperature-controlled and air-conditioned room under light-dark cycle, fed Oriental laboratory chow containing 0.71% calcium and 2 IU/g vitamin D (Oriental Yeast Company, Tokyo) and given tap water *ad libitum*. The perfusion of rat stomach was performed by the method

of Lefévre *et al.*¹⁸ with minor modifications.¹⁴ Animals were anaesthetised with pentobarbital (40 mg/kg) after an overnight fast. After a midline laparotomy, polyethylene cannulae were inserted into the left gastric artery and gastric vein. All other vessels and pancreas were carefully excluded by ligation. After cardinal ligation, a catheter was inserted into the stomach through the pyloric ring to drain gastric juice. Infusion was then started with 4.6% dextran (mean mol.wt.70,000) Krebs-Ringer bicarbonate buffer containing 5.5 mM glucose (DKRBG) into the left gastric artery at a flow rate of 2 ml/min without recirculation. The perfusate was equilibrated with a gas phase of 95% O₂/5% CO₂ immediately before use and maintained at pH 7.4 and PaO₂ 350 mmHg. The entire perfusion system including the perfusate was kept at 37°C throughout the experiment. After the pre-stimulation period (20 minutes) perfused with DKRBG alone, the perfusate with or without [Asu^{1,7}]-eel calcitonin (Toyo Jozo Research Laboratories, Shizuoka) was infused for 15 minutes at the concentrations of 10^{-9} , 10^{-8} , and 10^{-7} M. The number of animals was six for each dose of [Asu^{1,7}]-eel calcitonin, and each stomach was exposed to only one perfusion. The venous effluents were collected every minute into chilled tubes containing Bacitracin (Sigma Chemical Company, St. Louis)-Trasylol (Bayer Leverkusen, Germany) mixture (2×10^{-5} M and 1000 KIU/ml, respectively), frozen immediately, and stored at –20°C until the assay.

Immunoreactive somatostatin levels were measured by a specific radioimmunoassay using antiserum RA-823 described previously.¹⁴ Anti-

serum RA-823 was found not to cross-react with [Asu¹⁻⁷]-eel calcitonin and synthetic human gastrin I (Imperial Chemical Industries, England). The minimal detectable quantity of somatostatin was 10 pg/ml in this system. The coefficients of variation within and between assays were 5.4% and 8.5%, respectively.

Immunoreactive gastrin was measured by the dextran-coated charcoal radioimmunoassay reported previously.¹⁹ The antisera raised in rabbits against synthetic human gastrin I was used for this assay. Synthetic human gastrin I was used as the standard and also as the labelled tracer prepared by a modification of the chloramine T method.²⁰ The minimal detectable quantity by this assay was 20 pg/ml. Intra- and interassay variations were 6.5% and 8.8%, respectively. In our radioimmunoassay system [Asu¹⁻⁷]-eel calcitonin and somatostatin (Protein Research Institute, Osaka) did not cross-react with the antigastrin antisera. The serial dilution curve of gastrin-like immunoreactivity in the perfusate was completely superimposable on the standard curve.

Statistical analysis was performed by Duncan's new multiple range test.

[Asu¹⁻⁷]-eel calcitonin, used in this study, has been shown to have an equal hypocalcaemic potency to that of natural eel calcitonin,²¹ with a high physico-chemical stability.²²

Results

In the control experiments perfused only with DKRBG, somatostatin levels ranged from 130 ng/l to 218 ng/l (mean $\pm$ SEM: 176 $\pm$ 5 ng/l), and gastrin levels from 165 ng/l to 250 ng/l (mean $\pm$ SEM: 223 $\pm$ 7 ng/l), neither of which changed significantly throughout the perfusion period (Fig. 1).

The infusion of [Asu¹⁻⁷]-eel calcitonin caused a significant and dose-related increase of gastric somatostatin secretion (Table, Fig. 1). The peak values of 246 $\pm$ 14 ng/l (P < 0.05 vs. control), 254 $\pm$

Fig. 1 Gastric somatostatin and gastrin responses to the 10^{-9} M ($\times-\times$), 10^{-8} M ($\blacksquare-\blacksquare$), and 10^{-7} M ($\bullet-\bullet$) of [Asu¹⁻⁷]-eel calcitonin for 15 minutes. The light solid line (—) also indicates the control group. Each value represents the mean $\pm$ SEM.

10 ng/l (P < 0.05), and 340 $\pm$ 21 ng/l (P < 0.01) were obtained by 10^{-9} , 10^{-8} , and 10^{-7} M [Asu¹⁻⁷]-eel calcitonin, respectively. In most experiments, the peak values were attained at 11–13 minutes after the start of [Asu¹⁻⁷]-eel calcitonin infusion (Fig. 1). Conversely, [Asu¹⁻⁷]-eel calcitonin elicited a significant decrease of gastrin secretion also in a dose-dependent fashion (Table, Fig. 1). The nadir of gastrin release caused by 10^{-9} , 10^{-8} , and 10^{-7} M eel calcitonin was 184 $\pm$ 11 ng/l (P < 0.05 vs. control), 180 $\pm$ 10 ng/l (P < 0.05), and 152 $\pm$ 6 ng/l (P < 0.05), respectively. The most prominent suppression of gastrin release

Table Total amounts of gastric somatostatin and gastrin release during infusion of [Asu¹⁻⁷]-eel calcitonin for 15 minutes

	Dose of peptide (M)	Gastric somatostatin* release (pg/15 min)	Relative amounts of† somatostatin release (%)	Gastrin release* (pg/15 min)	Relative amounts† of gastrin release (%)
Control	0	5330 $\pm$ 120	100.0	6660 $\pm$ 70	100.0
Calcitonin	10^{-9}	6350 $\pm$ 230 ^a	119.1	6080 $\pm$ 60 ^a	91.3
	10^{-8}	7220 $\pm$ 220 ^{b, c}	135.4	5560 $\pm$ 260 ^{b, c}	83.8
	10^{-7}	9110 $\pm$ 450 ^{b, d}	170.9	4780 $\pm$ 210 ^{b, d}	71.8

*All values are the mean $\pm$ SEM (six animals).

†The relative amounts of somatostatin and gastrin release are expressed as a percentage of the amounts released by the control.

a: P < 0.05 compared with the control.

b: P < 0.01 compared with the control.

c: P < 0.05 compared with 10^{-9} M.

d: P < 0.05 compared with 10^{-8} M.

Fig. 2 Correlation between the increment of total amounts of somatostatin release and the decrement of total amounts of gastrin secretion during the infusion of 10^{-9} M ($\times$), 10^{-8} M ($\blacksquare$), and 10^{-7} M ($\bullet$) of [Asu^{1,7}]-eel calcitonin compared with the amounts released by the control.

coincided with the peak of gastric somatostatin release (Fig. 1).

The total amounts of gastric somatostatin release and gastrin secretion during the infusion of [Asu^{1,7}]-eel calcitonin (15 min) are summarised in the Table.

The increment in total somatostatin release and decrement in total gastrin secretion during the infusion of 10^{-9} , 10^{-8} , and 10^{-7} M [Asu^{1,7}]-eel calcitonin were plotted in Fig. 2. A significant correlation ($r=0.60$, $P<0.01$) was found between these two values.

Discussion

Calcitonin has been shown to suppress gastrin and gastric acid secretion in mammals,^{6,7} and it is suggested that it is a physiological regulator of gastrin and gastric acid secretion.⁸ However, the exact mechanism of calcitonin action on gastrin secretion has not been clarified. In view of the hypocalcaemic action of the peptide, the inhibitory action of calcitonin on gastrin secretion could be mediated by calcium depletion around the antral G cells. This is, however, unlikely, as such an effect of calcitonin was shown to occur without any significant change of the serum calcium levels,⁶ so that factors other than the changes in calcium concentration should be considered as the mechanism of calcitonin-induced suppression of gastrin secretion.

The present study clearly demonstrated the potent

suppressive effect of calcitonin on gastrin secretion, confirming previous reports.⁷ As a constant level (2.5 mM) of calcium was supplied to the isolated perfused stomach in the present study, the suppressive effect of calcitonin on gastrin secretion was apparently not mediated by the change in the calcium concentration, though the subtle intra- and extracellular movement of calcium ions in the secretory cells cannot be excluded.

The most striking observation in the present study is the dose-related increase of gastric somatostatin release in response to [Asu^{1,7}]-eel calcitonin. Somatostatin-producing D cells have been demonstrated within the antral mucosa in the vicinity of the G cells.^{15,16} Furthermore, significant amounts of somatostatin have been found to be released from the stomach of dogs²³ and rats in response to various stimuli. These findings¹⁴ raise the possibility that gastrin secretion may be regulated by gastric somatostatin within the local area of the antral mucosa. In view of the simultaneous increase of somatostatin release and decrease of gastrin secretion with a significant mutual correlation, as well as the known potent inhibiting action of somatostatin on gastrin secretion, the suppression of gastrin secretion by calcitonin may, at least in part, be mediated by endogenous gastric somatostatin, though other possibilities independent of these two phenomena cannot be completely excluded.

The fact that both calcitonin and somatostatin inhibit histamine- and pentagastrin-induced gastric acid secretion^{6,10} may suggest a similar mechanism of action of these two substances in inhibiting gastric acid secretion. While somatostatin-secreting D cells are distributed both in the fundic and antral portions of the stomach,¹¹ somatostatin released from the fundic region may act directly on parietal cells to inhibit acid secretion. It is thus possible that calcitonin-induced increase of somatostatin release demonstrated in the present study inhibits gastric acid secretion not only by decreasing gastrin release but also by a direct action on the parietal cells.

Recently, somatostatin-like immunoreactivity has been found to be present in the parafollicular cells of the rat thyroid gland²⁴ known as the site of production of calcitonin, suggesting the intimate functional relationship between calcitonin and somatostatin, while the effect of somatostatin on calcitonin secretion still remains controversial.²⁵⁻²⁷

The present data would suggest a new angle for the study of the interrelationship between gastrin and calcitonin.

This study was supported in part by grants from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare, Japan. We wish to

thank Toyo Jozo Research Laboratories, Shizuoka, for the generous gift of [Asu^{1,7}]-eel calcitonin. We also thank Mr. T. Ichii and Miss C. Shiba for technical assistance and Miss M. Usui and Miss S. Yanagihara for typing the manuscript.

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