ELSEVIER

European Journal of Pharmacology 364 (1999) 199–203

ejp

Biological activity of carboxy-terminal gastrin analogs

Arne K. Sandvik [a,*], Graham J. Dockray [b]

[a] *Department of Physiology and Biomedical Engineering, Norwegian University of Science and Technology, N-7005 Trondheim, Norway*
[b] *Department of Physiology, University of Liverpool, Liverpool L69 3BX, UK*

Received 6 August 1998; revised 6 November 1998; accepted 13 November 1998

Abstract

Amidated forms of gastrin are derived by post-translational processing of a large precursor peptide and stimulate gastric acid secretion via the gastrin/CCK_B receptor. Non-amidated biosynthetic intermediates may exert biological effects through other mechanisms, but their effect on gastric acid secretion is unclear. Amidated gastrins stimulate acid secretion mainly by releasing histamine from mucosal enterochromaffin-like cells. This study examines the effects on histamine release from the vascularly perfused rat stomach of amidated gastrin-17, COOH-terminal glycine-extended gastrin-17, gastrin-17 extended at the COOH-terminal including the remaining progastrin sequence, and carboxy-terminal progastrin fragments (SAEDEN and GRRSAEDEN). Carboxy-terminal extended gastrins induced histamine release which was inhibited by the gastrin/CCK_B antagonist L-740,093, but had to be given in concentrations 100-fold higher than amidated gastrin-17 to produce comparable effects. These progastrin-derived peptides are found in high concentrations in some patients with the Zollinger–Ellison syndrome and may contribute to acid hypersecretion and other gastrin/CCK_B receptor mediated responses.

Keywords: CCK_B receptor; Gastrin; Glycine-extended gastrin; Histamine; (Rat)

1. Introduction

Like many other peptide hormones, biologically active gastrin is derived by postranslational processing of a larger precursor peptide (Dockray et al., 1996). The major proteolytic events are removal of the signal peptide and cleavage of three internal dibasic amino acid sequences, followed by removal of C-terminal amino acids leaving glycine-extended gastrin. The final processing step is the conversion of the carboxy-terminal phenylalanine–glycine dipeptide to phenylalanine amide, thereby generating the biologically active amidated forms of gastrin (Varro et al., 1995).

The amidated gastrins are recognized to stimulate gastric acid secretion via the gastrin/CCK_B receptor. Non-amidated gastrins have low affinity at these receptors. However, a body of evidence has emerged recently to suggest that the non-amidated gastrins may have biological activities mediated by different receptors. Thus glycine-extended gastrin promotes proliferation in the rat exocrine pancreatic cell line AR4-2J probably through a specific receptor (Kaise et al., 1995). Moreover, gastrin-precursor peptides have been reported to stimulate growth in human cancer cell lines through a receptor other than CCK_B or CCK_A (Singh et al., 1996; Iwase et al., 1997), while glycine-extended gastrins stimulated [^{3}H]thymidine uptake in isolated enterochromaffin-like (ECL) cells (Park et al., 1997). This growth-promoting effect is interesting since progastrin and glycine-extended gastrins have been found in increased concentrations in for example colorectal carcinomas (Nemeth et al., 1993; Hollande et al., 1997).

The gastric acid secretory actions of non-amidated gastrins are less clear-cut. Glycine-extended gastrin has been found to induce H^+,K^+-ATPase α-subunit gene expression, probably through a specific receptor, and to enhance subsequent acid secretion during stimulation with amidated gastrin (Kaise et al., 1995). Glycine-extended gastrin did not induce acid secretion during infusion in humans (Hilsted et al., 1988), and was 10^4- to 10^5-fold less potent than amidated gastrin in stimulating aminopyrine uptake in isolated parietal cells (Matsumoto et al., 1987). On the other hand, when glycine-extended gastrin was given to conscious fistula rats prior to a bolus dose of amidated gastrin, this peptide potentiated the acid secretagogue effect of amidated gastrin (Higashide et al., 1996). Glycine-

* Corresponding author. Tel.: +47-73-59-8808; Fax: +47-73-59-8986; E-mail: arne.sandvik@medisin.ntnu.no

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00846-2

extended gastrin did not stimulate histamine release from isolated canine ECL cells (Park et al., 1997). The biological effects of other gastrin precursors like progastrin and gastrin with intact carboxy-terminal flanking peptide have not been studied.

It is now generally appreciated that the gastric acid secretory effects of gastrin are mediated, at least in part, by histamine release from enterochromaffin-like (ECL) cells. The totally isolated, vascularly perfused rat stomach with a single-pass perfusate provides an excellent model for studies of the action of gastrin on histamine secretion. Histamine release is stimulated by gastrin-17 concentrations as low as 2 pM in the arterial perfusate (Sandvik and Waldum, 1990). The histamine release has been characterized as a clear response to gastrin/CCK_B receptor stimulation (Sandvik and Waldum, 1991). In the present study we have examined the effects on histamine release of glycine-extended gastrin-17, gastrin-17 extended to the carboxy-terminus of progastrin (i.e., CFP/gastrin-17), and the progastrin carboxy-terminal fragments SAEDEN and GRRSAEDEN. The results suggest that acute secretory responses of the ECL cell to this range of peptides are mediated through the gastrin/CCK_B receptor.

2. Materials and methods

2.1. Materials

The peptides studied were human amidated gastrin-17 (Sigma, St. Louis, MO); Glycine-extended gastrin-17 (Gly/gastrin-17), gastrin-17 extended to the COOH-terminus of progastrin (CFP/gastrin-17), the progastrin COOH-terminal fragments GRRSAEDEN and SAEDEN (all synthesised by Dr. J. Smith, University of Liverpool, Dept. of Biochemistry). The gastrin-CCK_B receptor antagonist, L-740,093, was a gift from Merck, Sharpe and Dohm. Dextran T70 was obtained from Pharmacia, Uppsala, Sweden; histamine radioimmunoassay kits from Immunotech, Marseilles, France; and all other chemicals from Sigma. Male Wistar rats weighing 240–260 (mean 250) g were purchased from Møllegaard (Skensved, Denmark).

2.2. Handling of animals

The animal experiments were approved by the Animal Welfare Committee of the University Hospital of Trondheim. The rats were housed in wire-mesh cages at 24°C and constant humidity with a 12:12-h light–dark cycle, and fed ad libitum with a commercial rat diet and tap water. The animals were fasted for 36 h prior to surgery, and anaesthetized with 0.2 ml per 100 g body weight of a combination of (per ml) 2.5 mg fluanison, 0.05 mg fentanyl, and 1.25 mg midazolam. Totally isolated, vascularly perfused rat stomachs were prepared as previously described (Kleveland et al., 1985) and transferred to an organ bath filled with Krebs-Ringer buffer. The vascular bed was perfused (one-pass perfusion without recirculation) through the aorta with 2 ml per min of a Krebs-Ringer buffer with ionized calcium 1.12 mM (at pH 7.25), glucose 5 mM, pyruvate 5 mM and dextran T70 40 g/l as colloid. The vascular perfusate was gassed with O_2 (96%) and CO_2 (4%) using a membrane oxygenator. The gastric lumen was perfused with 1 ml per min distilled water (pH 7.0) and gassed with 100% O_2. All perfusates and the organ bath were kept at 37°C.

2.3. Study design

An outline of the different experimental procedures is shown in Fig. 1. All isolated stomach preparations had an initial 20-min stabilization period before any substances were added to the arterial perfusate. In the studies with L-740,093 or gastrin-17 background, those substances were infused continuously into the arterial perfusate from 20 min. The isolated stomachs were stimulated with the different gastrin analogs during three 3-min periods at 37–40, 57–60 and 77–80 min after start of perfusion. L-740,093 is a noncompetitive CCK_B receptor antagonist (Dunlop, 1998), and the concentration used (1 nM) was chosen according to studies on the binding properties of this substance to cortical membranes (Patel et al., 1994). The venous effluent was collected in the 1-min period immediately preceding stimulation, and in the third 1-min period during administration of peptide. This mode of administration has previously been shown to induce an instantaneous, rapidly reversible histamine release when gastrin is given (Sandvik and Waldum, 1990). The venous effluent was collected on ice, frozen and kept at −20°C until analysis for histamine.

2.4. Histamine and gastrin radioimmunoassays

Histamine analysis was done with a previously evaluated histamine radioimmunoassay kit which is very specific and has a sensitivity for histamine down to 0.5 nM (Sandvik et al., 1987) and a coefficient of variation of

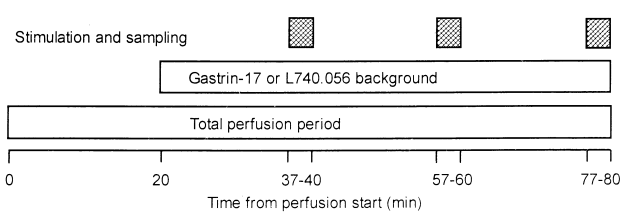

Fig. 1. Outline of the experimental procedures. Total perfusion time 80 min, with gastrin-17 or L-740,093 background those substances were administered continuously to the arterial perfusate from 20 min. Stimulation with gastrin analog was done during three 3-min periods with 1-min sampling of perfusate before stimulation and during the last 1-min period of stimulation.

6.4%. Gastrin analogs in the concentrations used did not cross-react in the histamine assay. The concentrations of Gly/gastrin-17 and CFP/gastrin-17 in the perfusates were determined by radioimmunoassay as previously described (Varro et al., 1995).

2.5. Statistics and calculations

Portal venous histamine concentrations during stimulation were expressed as percentages of prestimulatory venous histamine concentrations, and the differences evaluated for significance using the Wilcoxon matched pairs test or the Mann–Whitney *U*-test.

3. Results

3.1. Histamine release with gastrin analogs

Radioimmunoassays detected the gastrin analogs in correct concentrations in the arterial perfusate. Gastrin-17 induced a significant ($P < 0.05$ for 16 pM, $P < 0.01$ for all other concentrations), dose-dependent histamine release over the entire range of concentrations tested, from 16 pM ($145 \pm 19\%$, mean $\pm$ S.E.M.) to 1040 pM ($869 \pm 65\%$) (Fig. 2). The histamine concentrations in the venous effluent were for 520 pM gastrin-17 prestimulatory in the range of 20–68 nM, and after gastrin 146–324 nM. Gly/gastrin-17 did not consistently stimulate histamine release at low concentrations, but at 52 nM there was a significant increase ($608 \pm 172\%$, $P < 0.01$). Similarly, CFP/gastrin-17 had no significant effect at low concentrations but at 5.2 nM it stimulated histamine release ($256 \pm 55\%$, $P < 0.01$) increasing to $879 \pm 97\%$ ($P < 0.01$) at 52 nM CFP/gastrin-17 (Fig. 3).

3.2. Histamine release with gastrin analogs and L-740,093

Since the histamine response to 520 pM gastrin-17, 52 nM Gly/gastrin-17 and 52 nM CFP/gastrin-17 were comparable, those concentrations of gastrin analogs were tested with concomitant continuous infusion of the gastrin/CCK_B receptor antagonist L-740,093 (1 nM). The latter attenuated the histamine responses to $348 \pm 89\%$ ($P < 0.05$), $285 \pm 72\%$ ($P < 0.01$) and $390 \pm 95\%$ ($P < 0.01$) above control levels, respectively, which is approximately 50% of the histamine outputs obtained with gastrin analogs alone (Fig. 4).

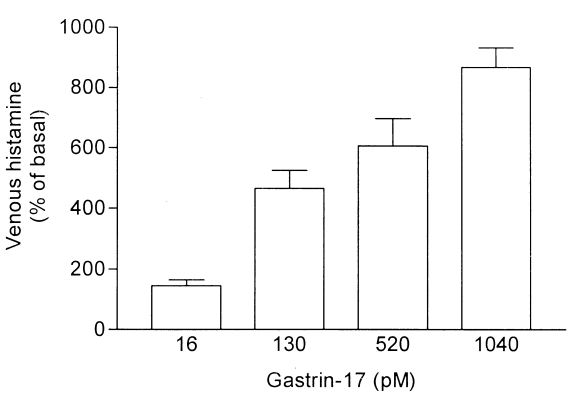

Fig. 2. Venous histamine output (percent of prestimulatory concentrations) with increasing intraarterial concentrations of gastrin-17 (mean $\pm$ S.E.M., * $P < 0.05$, ** $P < 0.01$).

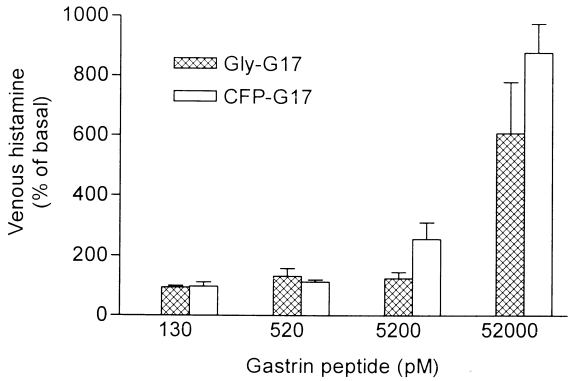

Fig. 3. Venous histamine output (percent of prestimulatory concentrations) with increasing intraarterial concentrations of glycine-extended gastrin-17 (Gly/gastrin-17), or carboxy-flanking peptide gastrin-17 (CFP/gastrin-17) in the arterial perfusate (mean $\pm$ S.E.M., * * $P < 0.01$).

3.3. Effects of Gly / gastrin-17 and CFP / gastrin-17 with gastrin-17 background stimulation

To test for interactions between gastrin-17 and Gly/gastrin-17 or CFP/gastrin-17, the latter peptides were given during continuous background stimulation with 130 pM gastrin-17. This concentration of gastrin-17 increased baseline histamine output by $466 \pm 59\%$. In the presence of a maximally effective concentration of Gly/gastrin-17 or CFP/gastrin-17 (52 nM) the response to gastrin-17 was

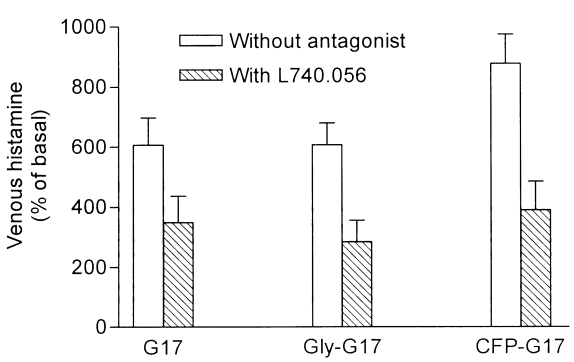

Fig. 4. Venous histamine output (percent of prestimulatory concentrations) with maximally effective arterial concentrations of gastrin-17 (520 pM), glycine-extended gastrin-17 (Gly/gastrin-17) (52 nM), and carboxy-flanking peptide gastrin-17 (CFP/gastrin-17) (52 nM) with or without the gastrin (CCK_B) receptor antagonist L-740,093 (1 nM) (mean $\pm$ S.E.M., * $P < 0.05$, ** $P < 0.01$).

not significantly different to that evoked by gastrin-17 alone, i.e., histamine outputs of 115 ± 7% and 107 ± 4% vs. control.

3.4. Effects of SAEDEN and GRRSAEDEN

The two carboxy-terminal flanking peptides were tested at 52 nM and did not induce histamine release (100 ± 16% and 104 ± 12% compared with control, respectively).

4. Discussion

Histamine release from the isolated, vascularly perfused rat stomach provides a sensitive bioassay for gastric CCK_B receptor activation. In the present study amidated gastrin-17 induced a significant dose-dependent release of histamine at concentrations above 16 pM. The response was inhibited by the specific antagonist L-740,093 and so confirmed that gastrin/CCK_B receptor activation mediated the response. The main findings of the present study are that Gly/gastrin-17 and CFP/gastrin-17 also evoked histamine release via a mechanism sensitive to L-740,093 although concentrations approximately 100-fold higher than those of gastrin-17 were required. The concentrations of Gly/gastrin-17 and CFP/gastrin-17 required to stimulate histamine release were higher than those normally found in circulation. However, these peptides are found in increased concentrations in some patients with the Zollinger–Ellison syndrome or with pernicious anaemia (Pauwels et al., 1986; Varro et al., 1997). There is considerable variation between gastrinoma patients in the production of different progastrin-derived peptides and in some cases the Gly-gastrins and partial progastrin-cleavage products occur in concentrations that are about 100-fold higher than those of amidated gastrin. It is possible therefore that in these circumstances glycine-extended gastrins or carboxy flanking peptide gastrins stimulate gastric histamine release. Since our data indicate that these effects are mediated via gastrin/CCK_B receptors it seems reasonable to suppose that non-amidated gastrins might contribute to gastric secretory responses in hypergastrinaemic conditions.

Previous studies suggest that glycine-extended gastrin may potentiate the acid secretagogue effect of amidated gastrin in the rat (Higashide et al., 1996). We also performed a series of experiments to examine whether this peptide or carboxy flanking peptide extended gastrin could augment histamine release stimulated by a gastrin-17 concentration in the upper end of the physiological range. No effect was found. This excludes an interaction at the level of the ECL cell between the gastrin/CCK_B receptor mediated histamine-releasing action of amidated gastrin and the putative receptor for glycine-extended gastrin. Taking the available evidence as a whole it seems likely that progastrin-derived peptides activate ECL cell secretory responses at least in part via the gastrin/CCK_B receptor. Physiologically, the amidated gastrins are the primary agonists but in hypergastrinaemic states C-terminal extended gastrins may also be agonists. The latter may also act elsewhere in the gastric mucosa to regulate growth and responsiveness to amidated gastrin.

References

Dockray, G.J., Varro, A., Dimaline, R., 1996. Gastric endocrine cells: gene expression, processing targeting of active products. Physiol. Rev. 76, 767–798.

Dunlop, J., 1998. CCK receptor antagonists. Gen. Pharmacol. 31, 519–524.

Higashide, S., Gomez, G., Greeley, G.H., Townsend, C.M., Thompson, J.C., 1996. Glycine-extended gastrin potentiates gastrin-stimulated gastric acid secretion in rats. Am. J. Physiol. 270, G220–G224, Gastrointest. Liver Physiol. 33.

Hilsted, L., Hint, K., Christiansen, J., Rehfeld, J.F., 1988. Neither glycine-extended gastrin nor the 1–13 fragment of gastrin 17 influences gastric acid secretion in humans. Gastroenterology 94, 96–102.

Hollande, F., Imdahl, A., Mantamadiotis, T., Ciccotosto, G.D., Shulkes, A., Baldwin, G.S., 1997. Glycine-extended gastrin acts as an autocrine growth factor in a nontransformed colon cell line. Gastroenterology 113, 1576–1588.

Iwase, K., Evers, M.B., Hellmich, M.R., Guo, Y.-S., Higashide, S., Kim, H.J., Townsend, C.M., 1997. Regulation of growth of human gastric cancer by gastrin and glycine-extended progastrin. Gastroenterology 113, 782–790.

Kaise, M., Muraoka, A., Seva, C., Takeda, H., Dickinson, C.J., Yamada, T., 1995. Glycine-extended progastrin processing intermediates induce H^+,K^+-ATPase α-subunit gene expression through a novel receptor. J. Biol. Chem. 270, 11155–11160.

Kleveland, P.M., Haugen, S.E., Waldum, H.L., 1985. The preparation of bioactive ^{125}I-gastrin, using Iodo-gen as oxidizing agent, and the use of this tracer in receptor studies. Scand. J. Gastroenterol. 20, 569–576.

Matsumoto, M., Park, J., Sugano, K., Yamada, T., 1987. Biological activity of progastrin posttranslational processing intermediates. Am. J. Physiol. 252, G315–G319, Gastrointest. Liver Physiol. 15.

Nemeth, J., Taylor, B., Pauwels, S., Varro, A., Dockray, G.J., 1993. Identification of progastrin derived peptides in colorectal carcinoma extracts. Gut 34, 90–95.

Park, J., Dickinson, C.J., Post, L., DelValle, J., 1997. Characterization of glycine extended gastrin (G-GLY) biological effects on canine gastric ECL cells. Gastroenterology 112, A1179.

Patel, S., Smith, A.J., Chapman, K.L., Fletcher, A.E., Kemp, J.A., Marshall, G.R., Hargreaves, R.J., Ryecroft, W., Iversen, L.L., Iversen, S.D., Baker, R., Showell, G.A., Bourrain, S., Neduvelil, J.G., Matassa, V.G., Freedman, S.B., 1994. Biological properties of the benzodiazepine amidine derivative L-740,093, a cholecystokinin$_B$/gastrin receptor antagonist with high affinity in vitro and high potency in vivo. Mol. Pharmacol. 46, 943–948.

Pauwels, S., Desmond, D., Dimaline, R., Dockray, G.J., 1986. Identification of progastrin in gastrinomas, antrum and duodenum by a novel radioimmunoassay. J. Clin. Invest. 77, 376–381.

Sandvik, A.K., Waldum, H.L., 1990. Rat gastric histamine release: a sensitive gastrin bioassay. Life Sci. 46, 453–459.

Sandvik, A.K., Waldum, H.L., 1991. CCK_B (gastrin) receptor regulates gastric histamine release and acid secretion. Am. J. Physiol. 260, G925–G928, Gastrointest. Liver Physiol. 23.

Sandvik, A.K., Waldum, H.L., Kleveland, P.M., Schulze Søgnen, B.,

1987. Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. Scand. J. Gastroenterol. 22, 803–808.

Singh, P., Owlia, A., Varro, A., Dai, B., Rajaraman, S., Wood, T., 1996. Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells. Cancer Res. 56, 4111–4115.

Varro, A., Voronina, S., Dockray, G.J., 1995. Pathways of processing of the gastrin precursor in rat antral mucosa. J. Clin. Invest. 95, 1642–1649.

Varro, A., Dockray, G.J., Bate, G.W., Vaillant, C., Higham, A., Armitage, E., Thompson, D., 1997. Gastrin biosynthesis in the antrum of patients with pernicious anemia. Gastroenterology 112, 733–741.