Volume 236, number 1, 119–122 FEB 06177 August 1988

Effect of truncated glucagon-like peptide 1 on cAMP in rat gastric glands and HGT-1 human gastric cancer cells

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Received 6 June 1988

We tested the truncated 7–37 glucagon-like peptide 1 (TGLP-1), a naturally occurring porcine intestinal peptide, and other members of the glucagon family, including pancreatic glucagon (G-29), GLP-1 and GLP-2 for their ability to activate the cAMP generating system in rat gastric glands and HGT-1 human gastric cancer cells. In rat fundic glands, TGLP-1 was about 100 times more potent ($EC_{50} = 2.8 \times 10^{-9}$ M) than GLP-1 of G-29, and 10 times more potent than G-29 in the HGT-1 cell line. Our results support the notion that TGLP-1 plays a direct role in the regulation of acid secretion in rat and human gastric mucosa.

Enteroglucagon; Proglucagon 78–107; cyclic AMP; (Gastric mucosa, Fundic gland)

1. INTRODUCTION

The mammalian glucagon precursor is a 180 amino acid peptide [1,2]. Besides pancreatic glucagon (G-29), glicentin (G-69) and oxyntomodulin (G-37), it contains two glucagon-like sequences, GLP-1 and GLP-2, which share 50% homology with G-29. A truncated form of GLP-1, lacking the N-terminal 6-amino acid sequence, has been isolated from the porcine intestinal mucosa [3]. This peptide (TGLP-1) is strongly insulinotropic [3,4], as opposed to GLP-1, and specific receptors for TGLP-1 have recently been demonstrated on rat insulinoma cells [5]. In man, G-29 is a potent inhibitor of meal- and pentagastrin-induced gastric acid secretion [6] and stimulates mucus secretion [7]. GLP-1 and TGLP-1 both inhibit pentagastrin-induced gastric acid secretion in man [8]. Consistent with these physiological data, G-29 has been shown to activate membrane receptors in rat gastric glands [9] and in the human gastric cancer cell line HGT-1 [10]. We therefore studied the effects of G-29, GLP-1, TGLP-1 and GLP-2 on the receptor-cAMP systems previously evidenced in these two models.

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2. MATERIALS AND METHODS

2.1. *Peptides*

TGLP-1, GLP-1, GLP-2 and oxyntomodulin were from Peninsula Europe (St. Helens, Merseyside, England). Crystalline, pure porcine pancreatic glucagon (G-29) was from Novo Research Institute (lot 42306, Bagsvaerd, Denmark). Synthetic porcine secretin was prepared by Professor E. Wünsch (Max-Planck-Institut für Peptidchemie, Martinsried, FRG).

2.2. *Tissues*

Adult male Wistar rats (200–250 g) were from our own colony. Mucosal glands were isolated from the rat fundus and antrum, using EDTA as chelator of divalent cations [11]. The HGT-1 cell line was routinely cultured, as described [12].

2.3. *cAMP assay*

In a standard cAMP assay, 150 μl from the HGT-1 cell suspension ($1-2 \times 10^6$ cells/ml) or from the rat gastric gland preparation (50–150 μg cell protein/ml) was preincubated at 20°C for 10 min in 250 μl KRP buffer containing 1% bovine serum albumin (BSA, fraction V) and IBMX as a cAMP phosphodiesterase inhibitor [11,13]. Cyclic AMP was determined by our radioimmunoassay method [14].

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2.4. *Calculations*

The apparent EC_{50} was the concentration required to produce 50% of the maximal stimulation produced by peptides. The significance of the differences observed was assessed using Student's *t*-test.

3. RESULTS

As shown in fig.1, TGLP-1 (10^{-10}–10^{-8} M) increased cAMP production in rat fundic glands 80-fold with a potency $EC_{50} = 2.8 \pm 0.7 \times 10^{-9}$ M ($n = 6$). The glucagon-related peptides GLP-1 and G-29 produced similar and parallel dose-response curves at a much lower potency: $EC_{50} = 3.4 \pm 1.5 \times 10^{-7}$ M and $2.3 \pm 0.4 \times 10^{-7}$ M, respectively ($n = 6$). In contrast, GLP-2 at concentrations as high as 4×10^{-7} M was ineffective in increasing basal cAMP levels (3.5 ± 0.8 pmol cAMP/mg protein, $n = 6$). We verified that the kinetics of cAMP generation induced by either 10^{-7} M G-29 or 10^{-8} M TGLP-1 were similar in rat gastric glands (not shown). The three peptides also increased cAMP generation in rat antral glands, from 4.0 ± 0.4 to 97 ± 10 pmol cAMP/mg protein in the presence of 10^{-7} M TGLP-1 (20-fold increase), to 58 ± 10 and 59 ± 14 pmol cAMP/mg protein in the presence of 10^{-7} M G-29 and GLP-1, respectively ($n = 3$–5 experiments). At maximally effective doses, secretin and TGLP-1 produced additive stimulations in rat fundic glands (fig.2). In contrast, combinations of G-29 or oxyntomodulin with TGLP-1 did not elevate significantly the cAMP values measured in the presence of TGLP-1 alone.

In HGT-1 human gastric cancer cells, TGLP-1 increased basal cAMP levels 3.7-fold, from 4.3 ± 0.6 to 16 ± 2.3 pmol/10^6 cells, with $EC_{50} = 1.8 \times 10^{-9}$ M (fig.3). Pancreatic glucagon was much less potent ($EC_{50} = 1.6 \times 10^{-8}$ M) but more efficient than TGLP-1 in the system (7.5-fold increase over basal). The intact peptide GLP-1 and GLP-2 were ineffective.

4. DISCUSSION

We have shown here that TGLP-1 is the most potent glucagon-like peptide so far examined in stimulating cAMP production in rat fundic glands and the HGT-1 cell line. These peptides included G-29 [9,10], oxyntomodulin [15,16], GLP-1 and GLP-2 in the present study. TGLP-1 exhibits the same potency in the two systems, being 10 times more potent than G-29 in HGT-1 cells and 100 times more potent than intact GLP-1 and G-29 in rat fundic glands. According to its high potency in these two models, the present data suggest that TGLP-1 is the specific ligand for the glucagon-like receptor in gastric mucosa. No additive effect was

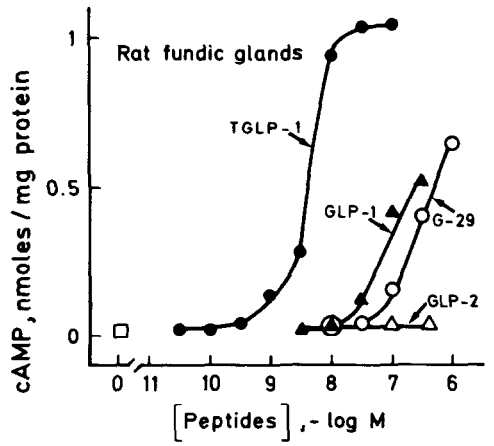

Fig.1. Effects of the glucagon-like peptides, TGLP-1 (●), G-29 (○), GLP-1 (▲) and GLP-2 (△), on cAMP generation in rat fundic glands. Cyclic AMP was determined after 1 h incubation at 20°C, in the presence of 0.5 mM IBMX [9]. The results are from one experiment, typical of 5 others. Data are means of duplicate determinations of cAMP production.

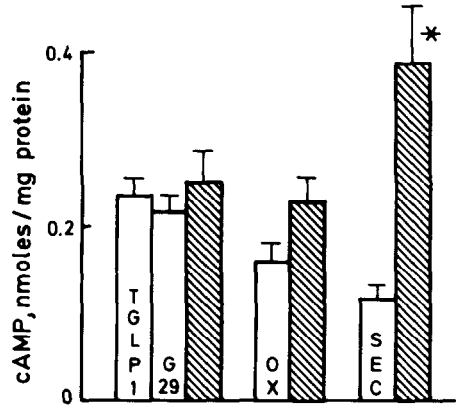

Fig.2. Effects of TGLP-1, G-29, oxyntomodulin and secretin alone (□) or in combination with TGLP-1 (▨) on cAMP generation in rat fundic glands. The peptides were tested at the following concentrations: 10^{-8} M TGLP-1, 10^{-6} M G-29, 10^{-7} M oxyntomodulin (OX) or 10^{-7} M secretin (SEC). Values shown are the means ± SE of 6 experiments. Each determination was performed in duplicate.

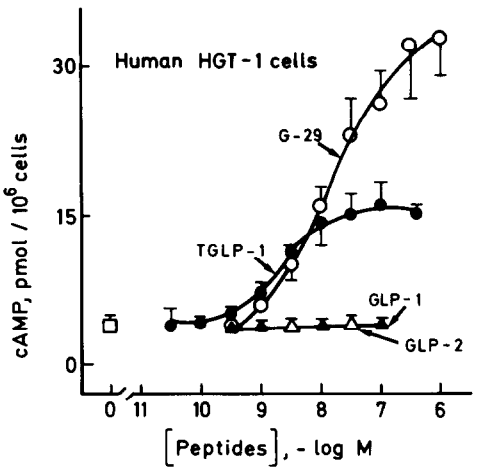

Fig.3. Effects of the glucagon-like peptides, TGLP-1 (●), G-29 (○), GLP-1 (▲) and GLP-2 (△), on cAMP generation in HGT-1 cells. Cyclic AMP was determined after 15 min incubation at 20°C in the presence of 1 mM IBMX [13]. Values shown are the means ± SE of 5 experiments. Each determination was performed in duplicate.

found when TGLP-1 was combined with the other natural glucagon analogs, G-29 and oxyntomodulin. Our data are compatible with the hypothesis that TGLP-1 acts on the acid-secreting parietal cells in rat fundic glands: (i) the actions of TGLP-1 and secretin are additive; (ii) somatostatin selectively inhibits cAMP generation induced by TGLP-1 and histamine [9,17]. Also consistent with these results, is the observation that TGLP-1 is a potent inhibtor of in vivo pentagastrin-induced gastric acid secretion in man [8]. Truncated GLP-1 might also exert an effect on mucus secretion since glucagon-like peptides elevate cAMP generation in rat antral glands and stimulate mucus secretion from the surface epithelial cells in the fundic part of the human stomach [7]. Similar to its effect on insulin secretion [3,4], the truncated GLP-1 peptide showed a higher potency than the intact GLP-1 in rat gastric glands. Thus, from the cleavage of active peptides by proteolytic enzymes (arginine vasopressin, pancreatic glucagon and GLP-1) truncated derivatives arise which have potent and new biological activities [3,4,18,19].

The present data support the notion that TGLP-1 plays an important role in acid and mucus secretions in gastric mucosa. In this connection, it is interesting that glucagon containing A-like cells are present in the oxyntic part of the rat stomach [9], suggesting a paracrine action of glucagons, together with endocrine secretion from the distal gut [3,20]. We and other authors have observed that both histamine and glucagon-like peptides increase cAMP levels in rat parietal cells [9,21]. Since histamine and glucagons exert an opposite effect on acid secretion [6,8,15,21–23], and regarding the central role of cAMP in acid secretion [23], it is therefore likely that other intracellular messengers are involved in the inhibition of gastric acid secretion by TGLP-1. In the liver, pancreatic glucagon has been shown to produce its biological effects on two different receptor types [24]. At low concentrations, the GR-1 receptor stimulates the production of inositol phosphates (0.25×10^{-9} M), at higher concentrations, the GR-2 receptor activates the adenylate cyclase system ($K_a = 6.3 \times 10^{-9}$ M). Further studies are therefore required to delineate the actual contribution of cAMP and inositides in the biological actions of the glucagon-like peptides on gastric secretion.

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