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Characterization of high-affinity receptors for truncated glucagon-like peptide-1 in rat gastric glands

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The truncated form of glucagon-like peptide-1 (TGLP-1, or proglucagon 78–108), secreted by the mammalian intestine, has potent pharmacological activities, stimulating insulin release and inhibiting gastric acid secretion. We have characterized high-affinity receptors for this peptide in rat isolated fundic glands. Scatchard analysis of binding studies using mono-^{125}I-TGLP-1(7–36) amide as tracer showed a single class of binding site of K_d (4.4±(SE) 0.8) × 10^{-10} M, with a tissue concentration of 1.0 ± 0.1 fmol sites/μg DNA. Whole GLP-1 was approximately 700 times less potent in displacing tracer, while human GLP-2 and pancreatic glucagon produced no significant displacement at concentrations up to 10^{-6} M. The data support a physiological role for TGLP-1 in the regulation of gastric acid secretion.

Receptor; Proglucagon 78–108; Gastric mucosa; Fundic gland

1. INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is one of two glucagon-like peptide sequences found in the C-terminal portion of mammalian proglucagon [1,2]. Whereas these peptides are produced in the free form by post-translational processing in human, porcine [3,4] and rat [5] intestine, the pancreas produces mainly a larger fragment consisting of the two peptides linked by a spacer peptide [3,6]. However, only a fraction (about 40%) of the total GLP-1 produced by human and rat intestine occurs as the entire GLP-1 sequence (proglucagon 72–108), most of the rest being accounted for by a fragment produced by removal of the first 6 amino acid residues of GLP-1, resulting in a truncated form, TGLP-1 (proglucagon 78–108) [5,7]. This peptide is released into the circulation postprandially [7], and has potent biological activities both in stimulating glucose-dependent insulin secretion [8] and in inhibiting pentagastrin-stimulated gastric acid secretion in man [9]. It is still uncertain to what extent, if any, the C-terminus is amidated, but the amidated and C-terminal glycine forms are approximately equipotent in the rat isolated pancreas [8]. Specific receptors for TGLP-1 have been described in rat insulinoma (RINm5F) cells [10], but not in gastric mucosa, although a stimulatory effect of TGLP-1 on cAMP production by rat isolated fundic glands and human gastric cancer cells has been reported [9]. We have therefore characterized the gastric receptor for TGLP-1 by means of direct binding studies in rat isolated fundic glands.

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2. MATERIALS AND METHODS

2.1. *Peptides and label*

Synthetic TGLP-1 in the amide form (proglucagon 78–107 amide), GLP-1 (proglucagon 72–107 amide) and human GLP-2 were from Peninsula Laboratories (Belmont, CA, USA). Pure pancreatic glucagon was from Eli Lilly and Co. (Indianapolis, IN, USA). TGLP-1 was trace-labelled with ^{125}I (Amersham International PLC, Amersham, Bucks, England) by the chloramine-T method [11], and mono-^{125}I-TGLP-1 was isolated by gel filtration on BioGel P-30 followed by reverse-phase HPLC on a C_{18} column using a shallow gradient of acetonitrile in water [10]. The specific activity of the label was calculated as 58 Bq/fmol.

2.2. *Tissue preparation*

Gastric mucosal glands were collected as described [12] from the fundic portion of everted stomachs of male Wistar rats (200–300 g) that had free access to commercial food pellets and water. EDTA (2.5 mM) in hypertonic saline (0.25 M NaCl) was used to separate the glands. The DNA content of aliquots was determined by Burton's method [13], with calf thymus DNA as standard.

2.3. *Incubations*

Glands were washed and suspended in Krebs-Ringer-phosphate buffer, pH 7.5. Incubations of glands (3–5 mg wet weight or 11–16 μg DNA/tube) with labelled TGLP-1 (40 pM) and/or unlabelled peptides (10^{-11}–10^{-6} M) were carried out in triplicate in the same buffer containing final concentrations of BSA 10 mg/ml and bacitracin 0.7 mg/ml, as described [14]. Glands were separated by rapid centrifuga-

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tion and washed once with incubation buffer for counting of bound radioactivity.

To determine tracer degradation, incubation supernatants were reincubated with fresh glands and the percentage of binding of radioactivity compared with that of controls incubated with the same concentration of fresh label under identical conditions. Tracer degradation was also determined from the increase in the percentage of non-precipitable radioactivity after treating incubation supernatants and control solutions of fresh label with trichloroacetic acid to a final concentration of 10%.

cAMP production was determined after 1 h incubation at 20°C in the presence of IBMX 0.5 mM. Incubation was terminated by the addition of trichloroacetic acid, and supernatants were extracted with diethyl ether for determination of cAMP by radioimmunoassay [15].

3. RESULTS

Binding of mono-^{125}I-TGLP-1 to rat fundic glands was time and temperature dependent (fig.1). As unlabelled TGLP-1 was almost as effective in inhibiting tracer binding at 10^{-8} M as at 10^{-6} M, the lower concentration of unlabelled peptide was used to demonstrate the difference between total and non-specific binding in these time-course studies. Specific binding reached a plateau in 2 h at 20°C, whereas at 37°C no approximation to equilibrium was achieved. Studies of tracer degradation by reincubation of supernatants with fresh glands showed that this could at least in part be attributed to rapid degradation of tracer, which was 30–34% (n = 3) degraded in 2 h at 37°C, compared with 12–18% at 20°C. Degradation of tracer as measured by trichloroacetic acid precipitation was also appreciably higher at 37°C than at 20°C (4.7% and 2.1% at 2 h, respectively). Specific binding at 20°C was linearly proportional to tissue concentration up to a concentration of 22 mg wet weight/ml (data not shown).

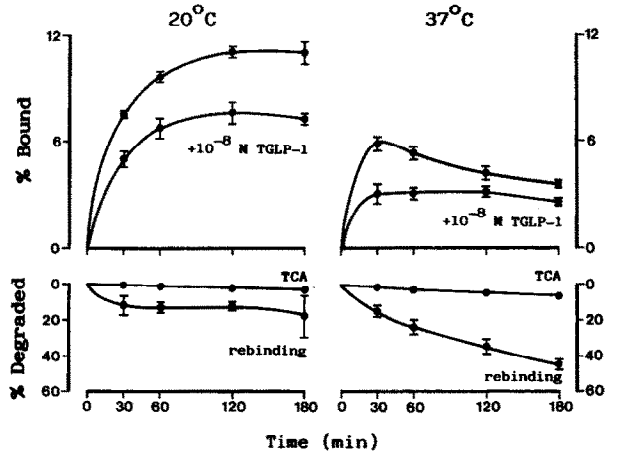

Fig.1. Binding and degradation of ^{125}I-TGLP-1 by rat fundic glands (mean ± SE, n = 3). Upper panels show time-course of binding in the presence and absence of unlabelled TGLP-1 10^{-8} M at 20°C and 37°C, and lower panels show the corresponding degradation of label as determined by rebinding studies and trichloroacetic acid precipitation.

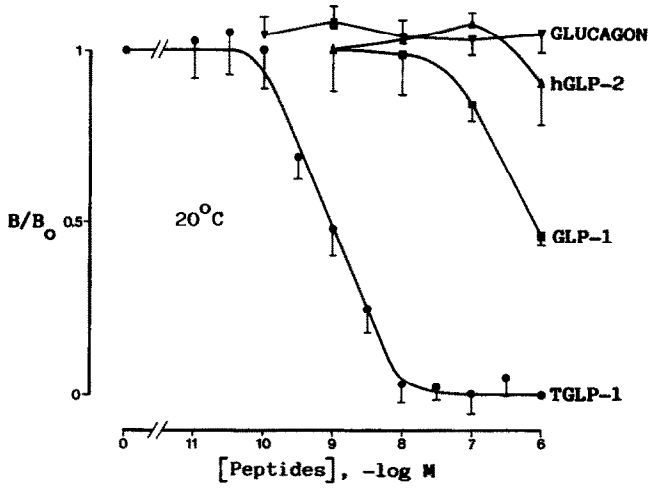

Fig.2. Inhibition of specific binding of ^{125}I-TGLP-1 to rat fundic glands by unlabelled TGLP-1 (●), whole GLP-1 (■), human GLP-2 (▲) and pancreatic glucagon (▼). Incubations were carried out for 2 h at 20°C. Data points show means ± SE, n = 4. B_0 is the specific binding of radioactivity in the absence of unlabelled peptides, and specific binding (B) is defined as the total binding of label minus the binding in the presence of unlabelled TGLP-1 10^{-6} M.

Specific tracer binding was inhibited in a concentration-dependent manner by unlabelled TGLP-1. Whole GLP-1 was approximately 700 times less potent in displacing the tracer, while human GLP-2 and pancreatic glucagon produced no significant displacement at concentrations up to 10^{-6} M (fig.2). Scatchard analysis (fig.3) was compatible with a single class of binding site for TGLP-1 of K_d (4.4 ± (SE) 0.8) × 10^{-10} M (n = 4), and a tissue concentration of binding sites of 1.0 ± (SE) 0.1 fmol/μg DNA.

TGLP-1 stimulated cAMP synthesis in the fundic glands with an EC_{50} of 1.6 × 10^{-9} M (fig.4).

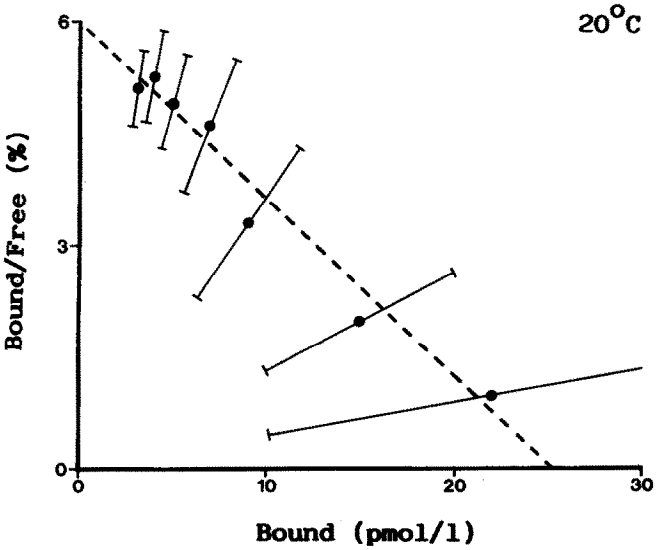

Fig.3. Example of Scatchard plot of TGLP-1 binding to rat fundic glands. Data points show mean ± SE of triplicates.

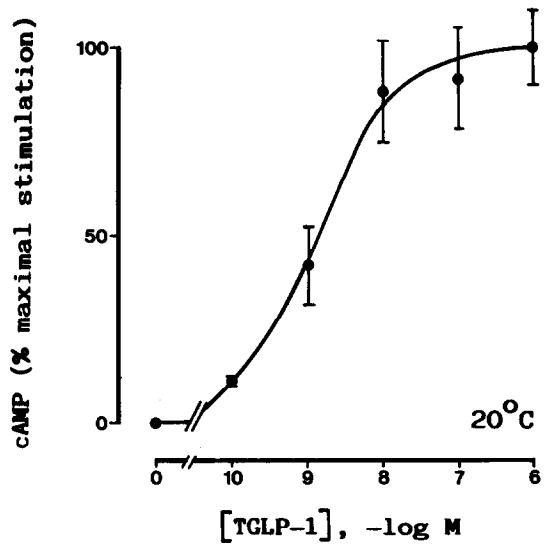

Fig.4. Stimulation by TGLP-1 of cAMP production in rat fundic glands. Cyclic AMP was determined after 1 h incubation at 20°C in the presence of IBMX 0.5 mM. Data points show mean ± SE of triplicates from one of 3 similar determinations.

4. DISCUSSION

These data demonstrate the presence in rat fundic glands of high-affinity receptors for TGLP-1, whose activation is linked to the stimulation of cAMP synthesis. The receptors are comparable to those described in rat insulinoma-derived RINm5F cells in showing a single class of binding site [10], but their affinities for both TGLP-1 and whole GLP-1 are slightly lower (K_d for TGLP-1 in RINm5F cells was 2×10^{-10} M [10]). Whether this is due to a difference in primary structure or to an environmentally determined influence on receptor conformation will become apparent when the receptor is isolated. The K_d reported here would nevertheless permit a degree of receptor activation at physiological postprandial plasma concentrations of TGLP-1, which may reach 10^{-10} M in human subjects [7].

It has been proposed that the effect of high doses of pancreatic glucagon to inhibit gastric acid secretion and stimulate cAMP synthesis in rat fundic glands may be mediated via the TGLP-1 receptor [9]. So far, our data do not support this view. The specificities of the receptor(s) that are activated by high concentrations of glucagon require further elucidation. It is a matter for speculation whether glucagon or other products of the glucagon precursor may exert a paracrine effect in the gastric mucosa of those species that have a significant number of glucagon-secreting cells in the stomach. It is at present not known whether these gastric A cells are capable of producing appreciable amounts of free TGLP-1.

As both the stimulatory effect of histamine and the inhibitory effect of TGLP-1 on gastric acid secretion appear to be associated with increased cAMP synthesis, it is apparent that the precise role of cAMP in the action of TGLP-1 in the gastric mucosa remains to be determined. It is not known which type of cell is responsible for the TGLP-1-induced cAMP production, but it has been suggested that it may be related to mucus secretion, while the inhibitory effect on gastric acid secretion may be mediated by other mechanisms [9].

The present results contribute to a picture of TGLP-1 as a postprandial hormone of considerable importance, not only in ensuring adequate insulin secretion as a part of the endocrine entero-insular axis [7], but also playing its part in switching off gastric acid secretion during the intestinal phase of digestion. It is thus a strong candidate for fulfilling the role of the classically described 'enterogastrone'.

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