

Truncated glucagon-like peptide-1 (proglucagon 78–107 amide), an intestinal insulin-releasing peptide, has specific receptors on rat insulinoma cells (RIN 5AH)

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We studied binding of ^{125}I -labelled truncated-glucagon-like peptide-1 (proglucagon 78–107 amide) to a cloned rat insulin-producing cell line, RIN 5AH, in monolayer culture. Interaction of the peptide with pancreatic insulinoma cells was saturable and time dependent. Half-maximal binding was obtained when the cells were incubated in the presence of 3.3×10^{-9} mol/l unlabelled truncated-glucagon-like peptide-1 (proglucagon 78–107 amide). Neither glucagon, full-length glucagon-like peptide-1 (proglucagon 72–107 amide) nor gastric inhibitory peptide competed for binding in concentrations up to 10^{-6} mol/l.

Proglucagon; Enteroglucagon; Incretin; Insulinotropin

1. INTRODUCTION

Only one mRNA codes for proglucagon, the 160 amino acid precursor of glucagon [1,2]. The precursor, however, is processed differently in the pancreas, where the products are a glicentin-related pancreatic peptide (proglucagon 1–30), glucagon itself (proglucagon 33–61) and a large peptide, containing 2 glucagon-like sequences, and in the small intestine, where the precursor gives rise to glicentin (proglucagon 1–69) and two smaller peptides, glucagon-like peptide-1 and glucagon-like peptide-2 (GLP-1 and GLP-2) (fig.1). We recently isolated the natural glucagon-like peptide-1 from pig ileal mucosa and found that the peptide corresponded to proglucagon 78–107, or possibly 78–108 [3]. This peptide was designated truncated-GLP-1. Synthetic truncated-GLP-1 has a strong insulinotropic effect on the isolated pancreas of pig [3] and rat [4]. A similar effect on the pancreas could not be obtained when identical ex-

periments were carried out with synthetic full-length GLP-1 (proglucagon 72–107 amide), originally believed to be a naturally occurring proglucagon product because of its flanking basic dipeptides in proglucagon. In this study we investigated the binding of ^{125}I -labelled truncated-GLP-1 to cells of a cloned rat insulin-producing cell line, RIN 5AH, in monolayer culture, to demonstrate the possible presence of receptors for this peptide on rat insulinoma cells. We also investigated the interaction of the receptor with

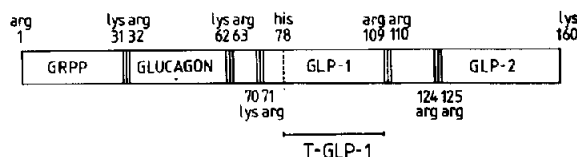


Fig.1. Diagrammatic representation of the structure of proglucagon. Proglucagon (160 amino acids) consists of glicentin-related pancreatic peptide (GRPP), corresponding to proglucagon 1–30, the glucagon sequence (proglucagon 33–61), and a sequence containing 2 glucagon-like sequences; glucagon-like peptide-1 (GLP-1) and glucagon-like-peptide-2 (GLP-2) (proglucagon 72–160). The structure of the naturally occurring peptide truncated-GLP-1 (proglucagon 78–107 amide) is indicated below (T-GLP-1).

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glucagon, full-length GLP-1 and gastric inhibitory peptide.

2. MATERIALS AND METHODS

2.1. Peptides

Both synthetic GLP-1 amide (code 7166, Peninsula, Merseyside, England) and synthetic truncated-GLP-1 amide obtained from Peninsula Laboratories, San Carlos, CA, by custom synthesis (lot no.008802) were gifts from Professor J. Holst (the Panum Institute, Copenhagen, Denmark). Peptide purity of synthetic truncated-GLP-1 was 72%. The peptide was further purified by isocratic high-pressure liquid chromatography in 44% ethanol (Merck, Darmstadt), containing in addition 0.1% trifluoroacetic acid (TFA; Pierce, Rockford, IL). Sequence determination confirmed that the peptide was proglucagon 78–107 amide. Highly purified porcine glucagon was obtained from the Novo Research Institute (Bagsværd, Denmark). Synthetic porcine gastric inhibitory peptide (GIP) (code 7192, Peninsula) was a gift from H. Kofod (Hagedorn Research Laboratory). Labelled peptides: ^{125}I -labelled, moniodinated, biologically active glucagon was from the Novo Research Institute. The specific activity of ^{125}I -labelled glucagon was 34 MBq/nmol. ^{125}I -labelled truncated-GLP-1 was prepared by the stoichiometric chloramine-T method as described [5] and purified by gel filtration on a Sephadex G 50, fine grade, column (Pharmacia, Uppsala, Sweden) in 0.04 M phosphate buffer, pH 7.4, containing in addition 0.1% human serum albumin (reinst, trocken, Behringwerke, Marburg, FRG) and immediately thereafter further purified on Sep-Pak^R, C-18 cartridges (Waters Associates, Milford, MA) eluted with a 0–100% gradient of ethanol in water, containing in addition 1% TFA. The Sep-Pak^R cartridges were activated according to the manufacturer's recommendations. The specific activity of ^{125}I -labelled truncated-GLP-1 was 35 MBq/nmol.

2.2. Other materials

RPMI 1640 and fetal calf serum were provided by Flow Laboratories (Irvine, Scotland). Penicillin, streptomycin and trypsin were obtained from Gibco (Paisley, Scotland). Human serum albumin was from Behringwerke, Haemacel^R from Behring, Marburg, and aprotinin (Trasylol^R) from Bayer, Leverkusen. Cell culture flasks were from Nunc (Roskilde, Denmark) and incubation vials from BN plast (Copenhagen, Denmark).

2.3. Cell line

The RIN-5AH cell line was established from a serially transplantable, radiation-induced rat islet cell tumor [6]. The cells were grown in RPMI 1640 medium containing in addition 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin. The flasks were incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide. Culture medium was changed three times a week.

2.4. Receptor assay

The day before the assay, cells were trypsinized (as described in [7]) and placed in a spinner culture flask with RPMI 1640,

containing in addition 10 mM Hepes and 1% FCS at 37°C for 16 h to allow recovery of cell membrane proteins. After spinner culture, the cells were centrifuged for 5 min at $50 \times g$ and then counted and tested for viability. After washing in 10 mM Hepes buffer, pH 7.4, containing 130 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.4 mM MgSO_4 , 2.5 mM NaH_2PO_4 , 2 g/l human serum albumin, haemacel^R and 800000 KIU aprotinin/l, the cell suspension was diluted to contain approx. 2.5×10^6 cells per 800 µl. 800 µl cell suspension, 100 µl ^{125}I -labelled truncated-GLP-1 (approx. 50000 cpm) and 100 µl sample or buffer were placed in a 25 ml scintillation vial. The vials were incubated in a shaking water bath at either 4°C or 20°C for various periods of time. Two samples of 400 µl were then placed in 550 µl microfuge tubes containing 100 µl dibutyl phthalate and centrifuged for 1 min at $10000 \times g$. The tips of the tubes were cut through the oil phase and counted in a gamma counter.

2.5. Calculations

The specific binding was determined by subtraction from total cell-associated radioactivity, the radioactivity obtained in the presence of a surplus of unlabelled truncated-GLP-1 (6×10^{-7} mol/l). The latter, the non-specific binding (NSB), was 64% (± 3) in 10 experiments of the binding of the labelled peptide alone. In 10 experiments the binding of the labelled peptide alone was $3.0 \pm 0.4\%$ ($\pm$ SE) of the total added radioactivity. All incubations were made in duplicate and the mean values used for further calculations. The mean percent binding $\pm$ SE at each hormone concentration was calculated and plotted against the dose on a semilogarithmic scale.

3. RESULTS

The time course of total ^{125}I -labelled truncated-GLP-1 binding and non-specific binding to RIN-5AH cells was studied at 20°C and 4°C. At

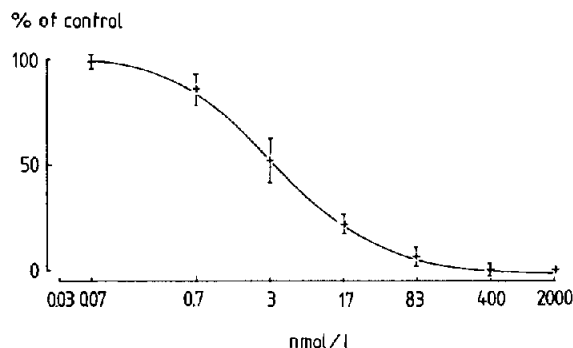


Fig.2. Displacement of binding of ^{125}I -labelled truncated-GLP-1 with unlabelled truncated-GLP-1 in RIN 5AH cells ($n = 10$). The mean binding of ^{125}I -labelled truncated-GLP-1 $\pm$ SE as % of control (= no addition of unlabelled peptide) is plotted against the concentrations of unlabelled peptide added.

the ^{125}I -labelled truncated-GLP-1 concentration used (approx. 0.1 pmol) the total binding at 20°C increased with time. A plateau was reached at 30 min. At 4°C, the binding increased for 4 h. The non-specific binding at the two different temperatures did not differ. The degradation of ^{125}I -labelled truncated-GLP-1 during incubation was tested by precipitation with 5% (final concentration) trichloroacetic acid of a sample of the supernatant from the incubation mixture after a 4 h incubation at 4°C. Compared to nonincubated labelled peptide, of which $94.7 \pm 2\%$ (mean $\pm$ SE of 4 experiments) of the radioactivity was precipitable in 5% trichloroacetic acid, $81.8 \pm 1.5\%$ (mean $\pm$ SE of 4 experiments) of the radioactivity was precipitable. The binding of ^{125}I -labelled truncated-GLP-1 was displaced by unlabelled truncated-GLP-1, and 50% displacement was obtained in the presence of $3.3 \pm 0.3 \times 10^{-9}$ mol/l (fig.2). Neither glucagon (10^{-6} mol/l), GLP-1 (6×10^{-7} mol/l) nor gastric inhibitory peptide (3×10^{-7} mol/l) were able to displace the binding of ^{125}I -labelled truncated-GLP-1 in five consecutive experiments (fig.3).

The binding of ^{125}I -glucagon was displaced by glucagon. 50% displacement was obtained in the presence of 2.9×10^{-9} mol/l (not shown). Truncated-GLP-1, 6×10^{-7} mol/l, did not displace the binding of ^{125}I -labelled glucagon (fig.4).

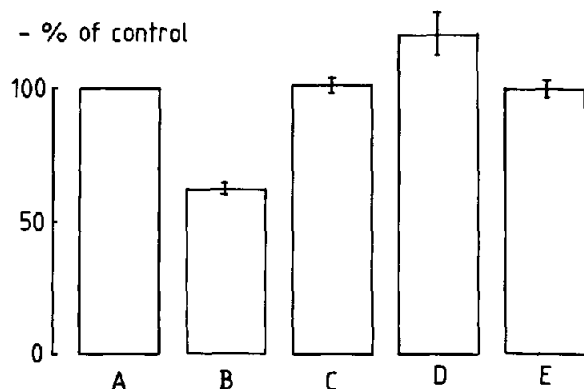


Fig.3. Displacement of binding of ^{125}I -labelled truncated-GLP-1 by: B, truncated-GLP-1 (6×10^{-7} mol/l) ($n = 10$); C, GLP-1 (6×10^{-7} mol/l) ($n = 4$); D, glucagon (10^{-6} mol/l) ($n = 6$); and E, gastric inhibitory peptide (3×10^{-7} mol/l) ($n = 3$) as % of control (A, no addition of unlabelled peptide).

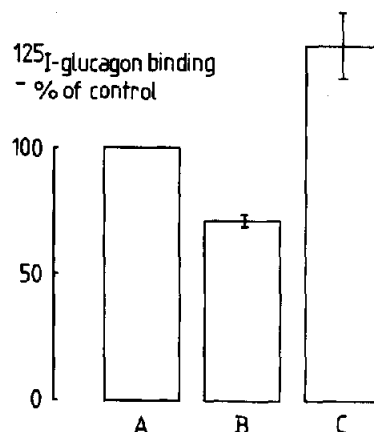


Fig.4. Displacement of binding of ^{125}I -labelled glucagon by: B, glucagon (10^{-6} mol/l) ($n = 4$); and C, truncated-GLP-1 (6×10^{-7} mol/l) ($n = 4$) as % of control (A, no addition of unlabelled peptide).

4. DISCUSSION

Our results indicate that the rat insulinoma cell line RIN 5AH has specific receptors for truncated-GLP-1. The non-specific binding found in this study is rather high. The identical NSBs found at 20°C and 4°C, where internalization does not occur [8], rule out that the rather high NSB is due to internalization. It is more likely that the extensive degradation of the labelled peptide during incubations, demonstrated by precipitation of labelled peptide in 5% trichloroacetic acid, can account for the high NSB. Possibly, low molecular mass radioactive degradation products may enter the cells during the incubations.

In agreement with our findings, Drucker et al. [9] recently described an effect on insulin gene expression of this peptide on a similar cell line, RIN 1046-38. These results are also in agreement with the strong insulinotrophic effect of truncated-GLP-1 on pig pancreas, earlier reported by our laboratory [3] and on rat pancreas reported by Mojsov et al. [4].

Glucagon (proglucagon 33–61) is known as a potent stimulus for insulin release [10], and receptors for glucagon have been demonstrated on insulinoma cells from Syrian hamsters [11] and on rat insulinoma (RIN-m) cells [12]. We also found saturable and specific binding of glucagon to the present cell line. However, glucagon did not displace the binding of truncated-GLP-1 and

truncated-GLP-1 did not displace the binding of glucagon. Therefore, truncated-GLP-1 and glucagon have separate receptors. The mean binding of ^{125}I -labelled truncated-GLP-1 to RIN 5AH cells was slightly higher in the presence of glucagon than alone (fig.3). This finding was not significant. In all four experiments the binding of ^{125}I -labelled glucagon in the presence of truncated-GLP-1 was higher than the binding of glucagon alone (fig.4). We do not have any explanation for this finding. The related peptide gastric inhibitory peptide, also known as a potent insulin releasing peptide, which also possesses specific receptors on beta cells [13], was without effect on the binding of truncated-GLP-1 suggesting that the GIP receptor is not responsible for the binding of truncated-GLP-1.

The concentration of truncated-GLP-1 required for half-maximum binding was 3×10^{-9} mol/l. This is in agreement with the pronounced effect on insulin secretion of this concentration of the peptide in the isolated perfused porcine pancreas [3]. A similar value was found for the interaction of glucagon with its receptor. Thus our results provide further support for the theory that truncated-GLP-1, secreted from the lower small intestine, may play a physiological role in the control of insulin secretion.

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