GLP-1(7-36)AMIDE: CHARACTERIZATION OF ITS BINDING TO SPECIFIC RECEPTORS IN NORMAL AND TUMORAL RAT ISLET CELLS

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ABSTRACT

We have studied the binding of ^{125}I -GLP-1(7-36)amide to normal rat islet cells and rat insulinoma-derived RINm5F cells, and found it is time- and temperature-dependent, and directly proportional to cell concentration. In both cell types, the Scatchard plot demonstrates the presence of high- and low-affinity binding sites. The 50% inhibition of the maximal binding to 0.4 nM ^{125}I -GLP-1(7-36)amide was obtained when cells were incubated in the presence of about 3.0 nM of unlabelled peptide. Glucagon, oxyntomodulin and GLP-1(7-36)amide at high concentrations (10 μM) do not compete with the ^{125}I -GLP-1(7-36)amide binding. In pancreatic tumoral cells there seems to be a direct correlation between the maximal binding, the number of high-affinity binding sites and the amount of intracellular insulin.

The proglucagon structure of several mammalian, deduced from the genome sequence or the sequence of cloned DNA complementary to proglucagon mRNA, has been documented (1, 7); the mRNA that codes for proglucagon, the 160 amino acids glucagon precursor, is similar in all mammalian species studied. The proglucagon mRNA is identical in pancreas and intestine (12) but the pathway of post-translational processing of the primary transcript differs markedly in the two tissues (10). In the pancreas, the prohormone is processed to proglucagon 1-30, glucagon, proglucagon 64-69 and the COOH-terminal fragment (proglucagon 72-158); in the small intestine, the prohormone is processed to glicentin (proglucagon 1-69), GLP-1, proglucagon 111-123 and GLP-2 (10). The COOH-terminal residue of GLP-1 and of the intervening sequence, proglucagon 111-123, can be α -amidated (10, 13).

Characterization of GLP-1 from intestinal extracts has documented that this peptide is further processed to truncated forms GLP-1(7-37) and/or GLP-1(7-36)amide (11, 13); both, synthetic GLP-1(7-37) and GLP-1(7-36)amide have shown a

strong insulinotropic effect in isolated perfused pig (8) and rat (17) pancreas, while the full length GLP-1(1-37) molecule does not seem to equally affect the insulin secretion (17).

The presence of specific receptors for GLP-1(7-36)amide has been documented in two different cloned rat insulin-producing tumoral cells (6, 14); further studies demonstrate that the signal transmission after GLP-1(7-36)amide binding is facilitated by the adenylate cyclase system (5), yet, specific information is lacking on normal islet cells. The RINm5F cell line is derived from a radiation-induced insulin-producing rat pancreatic tumor (3) which retains many of the differentiated functions of pancreatic B cells (19). In this study, we have examined the specific binding of ^{125}I -GLP-1(7-36)amide to dispersed normal rat pancreatic islet cells as well as to tumoral cells of the RINm5F line.

MATERIALS AND METHODS

Cell Line

Tumoral islet cells of the RINm5F line were cultured, harvested and counted as previously de-

scribed (4). Except otherwise mentioned, groups of 1×10^6 cells were incubated for 30 min at 37°C in 0.1 ml of a buffered medium [25 mM Tris HCl, 120 mM NaCl, 1.2 mM MgSO_4 , 5 mM KCl, 1 mM EDTA, 10 mM glucose, 15 mM CH_3COONa , 1% bovine serum albumin and bacitracin (4 mg/ml), pH, 7.6] containing 0.4 nM ^{125}I -GLP-1(7-36)amide (Peninsula Laboratories) radiolabelled in our laboratory by the chloramine T method, and the mono- ^{125}I -GLP-1(7-36)amide, was separated by HPLC (16).

After incubation, cells were rapidly centrifuged (1 min at 1,500 rpm in a Beckman microfuge-12) and washed twice with 0.5 ml of iced non-radioactive medium; then, each cell pellet was examined for its gamma radioactive content. The non-specific binding was that obtained in the presence of 1 μM unlabelled GLP-1(7-36)amide.

Islet Cells

Dispersed islet cells were prepared from pancreatic islets (15), previously isolated by the collagenase technique, from fed albino rats (200–250 g). Prior to the experiment, islet cells were cultured overnight in the same conditions as the RINm5F cells (4).

For binding studies, groups of 0.25×10^6 cells were treated following the same procedure as described for RINm5F cells.

Calculations

The specific binding was determined by subtracting, from the total cell-associated radioactivity, the non-specific binding obtained in the presence of 1 μM unlabelled GLP-1(7-36)amide, which represented 1.68 ± 0.28 ($n=15$), and 1.72 ± 0.13 ($n=46$) percent of the total radioactivity for 0.25×10^6 normal cells and 1.0×10^6 RINm5F cells, respectively. Results are expressed as the mean value ($\pm$ SEM), and the number of individual determinations (n) is stated.

RESULTS

RINm5F Cells

The specific binding of ^{125}I -GLP-1(7-36)amide (0.4 nM) to tumoral islet cells was time- and temperature-dependent, reaching the maximal at 30 min and 37°C (Fig. 1A).

When cells were first exposed for 30 min to ^{125}I -

Fig. 1 A: Relative specific binding of ^{125}I -GLP-1(7-36)amide (0.4 nM) to rat RINm5F cells (1×10^6 cells/100 μl) at 25°C and 37°C as function of time. B: Dissociation of the 30 min 37°C, ^{125}I -GLP-1(7-36)amide binding to rat RINm5F cells (1×10^6 cells/100 μl) at 4°C and 37°C. Data are shown as mean $\pm$ SEM ($n=6-12$ for A and $n=3-10$ for B).

GLP-1(7-36)amide (0.4 nM), washed once, and then incubated for 1 to 20 min at 37°C in the sole presence of unlabelled GLP-1(7-36)amide (1 μM), 50% of the cell-associated radioactivity was released within the first 7 min (Fig. 1B). In these experiments, the non-specific binding was measured in cells previously incubated in 1 μM GLP-1(7-36)amide, and then treated in the same manner. Results were identical when dissociation was carried out with no addition of unlabelled GLP-1(7-36)amide in the second incubation (data not shown).

Incubation of increasing RINm5F cell concentrations with ^{125}I -GLP-1(7-36)amide, showed a proportional specific binding in a cell range of 0.25 to 0.75×10^6 cells/100 μl , whereas higher cell concen-

Table 1 Specific Binding of ^{125}I -GLP-1(7-36)Amide (0.4 nM) to 1×10^6 High ($n=6$) and Low ($n=33$) Insulin Content RINm5F Cells

N	Insulin content ng/ 10^6 cells	^{125}I -GLP-1(7-36)amide (0.4 nM) % bound
6	236.4 ± 6.10	4.05 ± 0.60
33	$1.9 \pm 0.58^*$	$1.11 \pm 0.20^*$

Data are expressed as mean $\pm$ SEM. * $P < 0.001$

tration (1×10^6 cells/ $100 \mu\text{l}$) was out of line.

Although the protein content of RINm5F cells remains fairly stable in distinct sub-lines, the insulin content displays wide variations (18). The specific maximal binding [$0.4 \text{ nM } ^{125}\text{I}$ -GLP-1(7-36)amide] was higher (1.62 ± 0.24 , $n=6$, fmol bound/ 10^6 cells) for cells with high insulin content ($236.4 \pm 6.1 \text{ ng}/10^6$ cells) than for those with low insulin content ($1.9 \pm 0.58 \text{ ng}/10^6$ cells) in which, the amount of GLP-1(7-36)amide bound was 0.44 ± 0.07 , $n=33$, fmol/ 10^6 cells (Table 1).

In tumoral cells, the Scatchard plot of data collected at increasing concentrations of added GLP-1(7-36)amide (10^{-10} to 10^{-7} M) demonstrated the presence of high- and low-affinity binding sites. The apparent K_d of the high affinity binding sites was 1.0 nM and 4.0 nM for the low and high insulin content cells, respectively, and the K_d of the low-affinity binding sites was 170 nM and 26 nM for the low and high insulin content cells, respectively (Fig. 2); these data, represented by displacement curves, are shown in Fig. 3. The 50% binding inhibition was close to 10^{-9} M GLP-1(7-36)amide in low insulin content tumoral cells, and close to 10^{-8} M in those with high insulin content; nevertheless, this difference was not statistically significant.

Crystalline porcine glucagon (Lilly Research Laboratories) and synthetic GLP-1(1-36)amide and oxyntomodulin with Lys in position 33, both from Peninsula Laboratories, at high concentrations ($0.1 \mu\text{M}$), did not compete with the ^{125}I -GLP-1(7-36)amide binding.

Normal Islet Cells

In normal rat dispersed pancreatic cells, the specific binding at 30 min incubation at 37°C in the presence of $0.4 \text{ nM } ^{125}\text{I}$ -GLP-1(7-36)amide, averaged $0.45 \pm 0.15 \text{ fmol GLP-1(7-36)amide bound}/10^6$ cells.

Fig. 2 Scatchard plots of the specific binding of ^{125}I -GLP-1(7-36)amide to high (A) and low (B) insulin content rat RINm5F cells (1×10^6 cells/ $100 \mu\text{l}$). Data are shown as mean $\pm$ SEM ($n=6$ for A and $n=15-33$ for B).

The Scatchard plot of data collected at increasing concentrations of added GLP-1(7-36)amide (10^{-10} to 10^{-7} M) demonstrated the presence of high- and low-affinity binding sites (K_d 1.8 nM and 460 nM , respectively) (Fig. 4B). The 50% inhibition was seen to occur at 10^{-9} M GLP-1(7-36)amide when the same data were represented as a displacement curve (Fig. 4A).

DISCUSSION

Our results demonstrate that normal insulin-producing cells contain specific receptors for GLP-1(7-36)amide, and indicate also that both, normal and tumoral cells, present two different binding sites, a high- and a low-affinity, being the high-affinity one similar to that described by Göke and Conlon in RINm5F line (6).

As the GLP-1(7-36)amide concentration re-

Fig. 3 Displacement of the specific binding of ^{125}I -GLP-1(7-36)amide to rat high (A) and low (B) insulin content RINm5F cells (1×10^6 cells/ $100 \mu\text{l}$) by increasing concentration of GLP-1(7-36)amide (0 – 10^{-7} M). Data are shown as mean $\pm$ SEM ($n=6$ for A and $n=15$ – 33 for B).

quired for half maximal binding, in normal rat islet cells, was close to 10^{-9} mol/l, an insulinotropic dose (17) not much higher than the stimulated postprandial plasma levels (9), our findings support the suggested role of GLP-1(7-36)amide in the physiological regulation of insulin release (8, 9).

The data obtained from the two tumoral cell sublines, which differed in their insulin content, suggest a direct correlation between the maximal binding and the amount of intracellular insulin, associated with the high-affinity binding sites.

Glucagon is known to stimulate insulin release and the presence of receptors for glucagon has been demonstrated on insulinoma (RINm5F) cells (2); yet, glucagon failed to displace the ^{125}I -GLP-1(7-36)amide binding, suggesting that GLP-1(7-36)amide and glucagon have separated receptors.

Fig. 4 A: Displacement of the specific binding of ^{125}I -GLP-1(7-36)amide to normal rat islet cells (0.25×10^6 cells/ml) by increasing concentrations of GLP-1(7-36)amide (0 – 10^{-7} M). Data are shown as mean $\pm$ SEM ($n=2$ – 5). B: Scatchard plot of the specific binding of ^{125}I -GLP-1(7-36)amide to normal rat islet cells (1×10^6 cells/ $100 \mu\text{l}$).

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