



Glycosylation of the GLP-1 Receptor Is a Prerequisite for Regular Receptor Function

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GÖKE, R., R. JUST, B. LANKAT-BUTTGEREIT AND B. GÖKE. *Glycosylation of the GLP-1 receptor is a prerequisite for regular receptor function.* PEPTIDES 15(4) 675–681, 1994.—The GLP-1 receptor on RINm5F cells is a glycoprotein with a M_r of 63,000. Treatment of the receptor with glycopeptidase F generated a protein with a M_r of 51,000, indicating that the GLP-1 receptor contains *N*-linked glycans. Tunicamycin pretreatment concentration-dependently decreased GLP-1 binding to RINm5F cells due to a decreased receptor number without change of receptor affinity. Tunicamycin exerted no effect on the GLP-1 receptor mRNA expression. The stimulation of cAMP production was decreased in tunicamycin-treated cells. Our data show that glycosylation of the GLP-1 receptor is a precondition for regular receptor function.

GLP-1 Glycoprotein Receptor Tunicamycin RINm5F cells

THE preproglucagon-derived glucagon-like peptide-1(7–36)-amide (GLP-1) is a member of the glucagon/VIP/secretin family of peptides. GLP-1 has been identified in pancreatic A-cells and in the L-cells of the intestine (4). After food ingestion, GLP-1 is released into the circulation, stimulating insulin release. Its important role as an incretin hormone in the entero-insular axis has been described in several studies [for review see (11)].

Receptors for GLP-1 were demonstrated on different rat insulinoma cell lines (6,10), rat gastric glands (27), rat lung membranes (23), and in certain brain regions (28). Cross-linking studies with RINm5F cell membranes have revealed a molecular mass of the GLP-1 receptor of 63,000 Da and have shown that the receptor protein does not contain S-H bound subunits (9). The biological action of GLP-1 is mediated by an activation of the adenylate cyclase system and a rise of the intracellular cAMP level (12). Furthermore, GLP-1 was reported to potentiate glucose-stimulated insulin release by gating the voltage-dependent Ca^{2+} channels to increase intracellular $[Ca^{2+}]$ (19). Recently, using a rat islet cDNA library, a cDNA for the GLP-1 receptor was isolated (26). The receptor protein contains seven transmembrane regions. The GLP-1 receptor belongs to the family of G-protein-coupled receptors and shows sequence homology to the secretin receptor (13), the parathyroid hormone receptor (15), the calcitonin receptor (18), and the glucagon receptor (14,25). The *N*-terminal region of the GLP-1 receptor contains a hydrophilic domain of about 120 amino acids that exhibits three *N*-linked glycosylation sites (26). To date, no information about GLP-1 receptor glycosylation and its possible meaning for receptor function is available. The present study shows that the GLP-1 receptor in RINm5F cells is a functional glycoprotein

and that glycosylation is a prerequisite for normal receptor function.

Because of the limited availability of islet tissue, especially in amounts necessary for biochemical studies, in our experiments we used the RINm5F cell line. This cell line is derived from a radiation-induced, insulin-producing tumor (7). RINm5F cells show many of the differentiated functions characteristic for B-cells (29), and are, therefore, widely used as a model for studies on insulin secretory mechanisms.

METHOD

Reagents

Glucagon-like peptide-1(7–36)amide was purchased from Bissendorf Biochemicals (Hannover, Germany). Tunicamycin was from Calbiochem (Bad Soden, Germany). Stock solutions were made by dissolving tunicamycin in 0.01 N NaOH; they were stored frozen in aliquots. Disuccinimidyl suberate (DSS) was obtained from Pierce Europe B.V. (Oud-Beijerland, The Netherlands). [¹²⁵I]GLP-1(7–36)amide (specific activity approximately 2000 Ci/mmol) was prepared as described previously (10).

Cell Culture

RINm5F cells were grown in plastic culture bottles under conditions as described by Praz et al. (21). For experiments, cells at stationary growth were diluted with fresh medium and incubated at 37°C for 65 h with or without tunicamycin (0–1 µg/ml). Control cells received equivalent amounts of 0.01 N NaOH.

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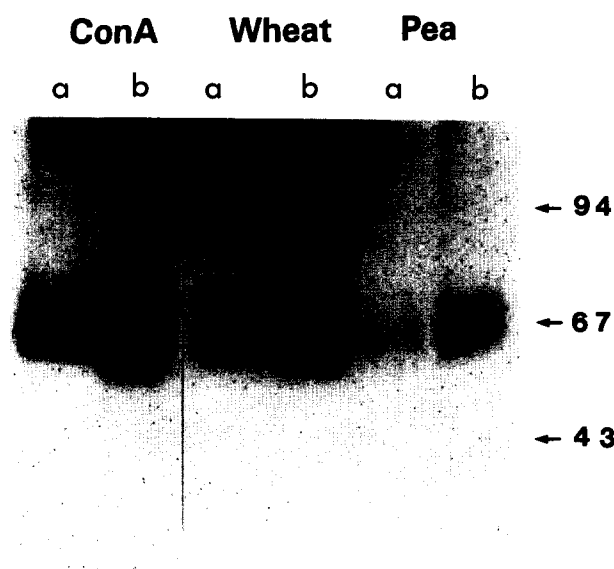


FIG. 1. Binding of radiolabeled GLP-1 receptor to different lectins. RINm5F cell plasma membranes were incubated with [^{125} I]GLP-1 for 30 min at 37°C and then treated with 0.1 mmol/l DSS. The labeled receptor was then applied to the lectin columns and recovered by washing the columns with an excess of the appropriate sugars.

The cells were detached from the surface of the bottles before experimentation using phosphate-buffered saline (NaCl, 136 mmol/l; KCl, 2.7 mmol/l; Na₂HPO₄, 8.1 mmol/l; KH₂PO₄, 1.5 mmol/l, pH 7.3) containing EDTA (0.7 mmol/l). Cell concentrations were determined by measurement of cellular DNA (2).

Binding Studies

Detached RINm5F cells were centrifuged (100 × *g*, 3 min) and resuspended in incubation buffer (Tris-HCl, 2.5 mmol/l; pH 7.4; NaCl, 120 mmol/l; MgSO₄, 1.2 mmol/l; KCl, 5 mmol/l; CH₃COONa, 15 mmol/l) containing 1% (w/v) human serum albumin, bacitracin (0.1%; w/v), and EDTA (1 mmol/l). Cells were incubated for 30 min at 37°C with [^{125} I]GLP-1 (approximately 20,000 cpm) in the absence or presence of a range of concentrations of unlabeled GLP-1. The reaction was stopped by centrifugation of aliquots of the cell suspension through an oil layer (10). The pellets were counted in a gamma-counter. Unspecific binding was defined as binding of tracer in the presence of 1 μmol/l unlabeled GLP-1.

Membrane Preparation

RINm5F plasma membranes were prepared as described previously (9). Briefly, RINm5F cells were suspended in ice-cold homogenization buffer (Tris-HCl, 10 mmol/l, pH 7.5; NaCl, 30 mmol/l; dithiothreitol, 1 mmol/l; phenylmethylsulfonyl fluoride 5 μmol/l) and disrupted in a glass-glass potter. The suspension was layered over a 41% (w/v) sucrose solution and centrifuged at 100,000 × *g* for 60 min at 4°C. The band of membranes at the interface of the layers was collected and diluted fourfold with homogenization buffer. Membranes were collected by centrifugation at 40,000 × *g* for 30 min at 4°C. The pellets were stored in liquid nitrogen. Protein was measured as described by Bradford (1).

Covalent Cross-Linking Experiments

[^{125} I]GLP-1 was cross-linked to RINm5F plasma membranes as described previously (9). In brief, plasma membranes (1 mg

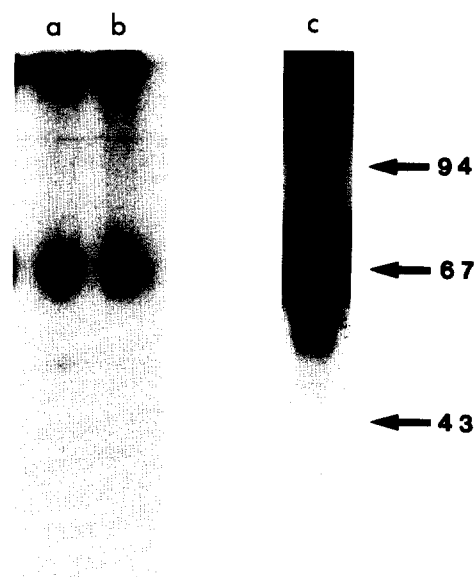


FIG. 2. Glycopeptidase F treatment of labeled GLP-1 receptor. Unpurified (a) and purified RINm5F cell plasma membranes were incubated with [^{125} I]GLP-1, treated with DSS, and then submitted to glycopeptidase F treatment as described in the Method section. Control samples (b) did not receive any enzymes. The labeled proteins were analyzed by SDS-PAGE. *M_r* standards are indicated on the right side by arrows.

protein/ml) were incubated with [^{125} I]GLP-1 (approximately 9×10^5 cpm) with or without unlabeled GLP-1 (1 μmol/l) in HEPES buffer (50 mmol/l, pH 7.5) containing 0.02% (w/v) human serum albumin for 30 min at 37°C. After centrifugation for 5 min at 10,000 × *g* and 4°C, pellets were resuspended in HEPES buffer (pH 9.0). A solution of DSS dissolved in dimethyl sulfoxide was added to give a final concentration of 0.1 mmol/l

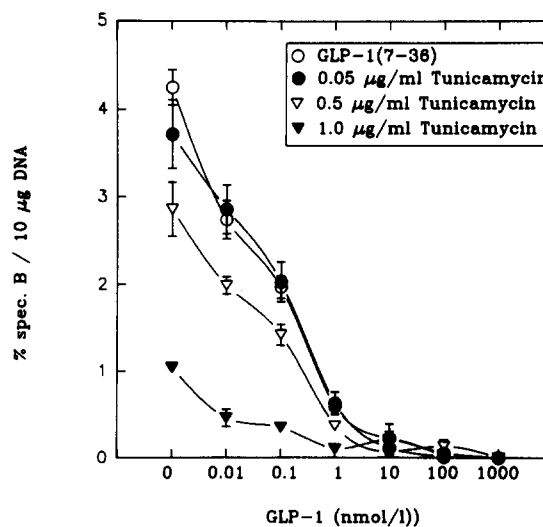


FIG. 3. Effect of tunicamycin on GLP-1 binding. RINm5F cells were incubated for 65 h at 37°C with a range of tunicamycin concentrations. Then cells were incubated for 30 min at 37°C with [^{125} I]GLP-1 in the presence or absence of unlabeled GLP-1 in the concentrations indicated. Data points show means ± SEM of six experiments.

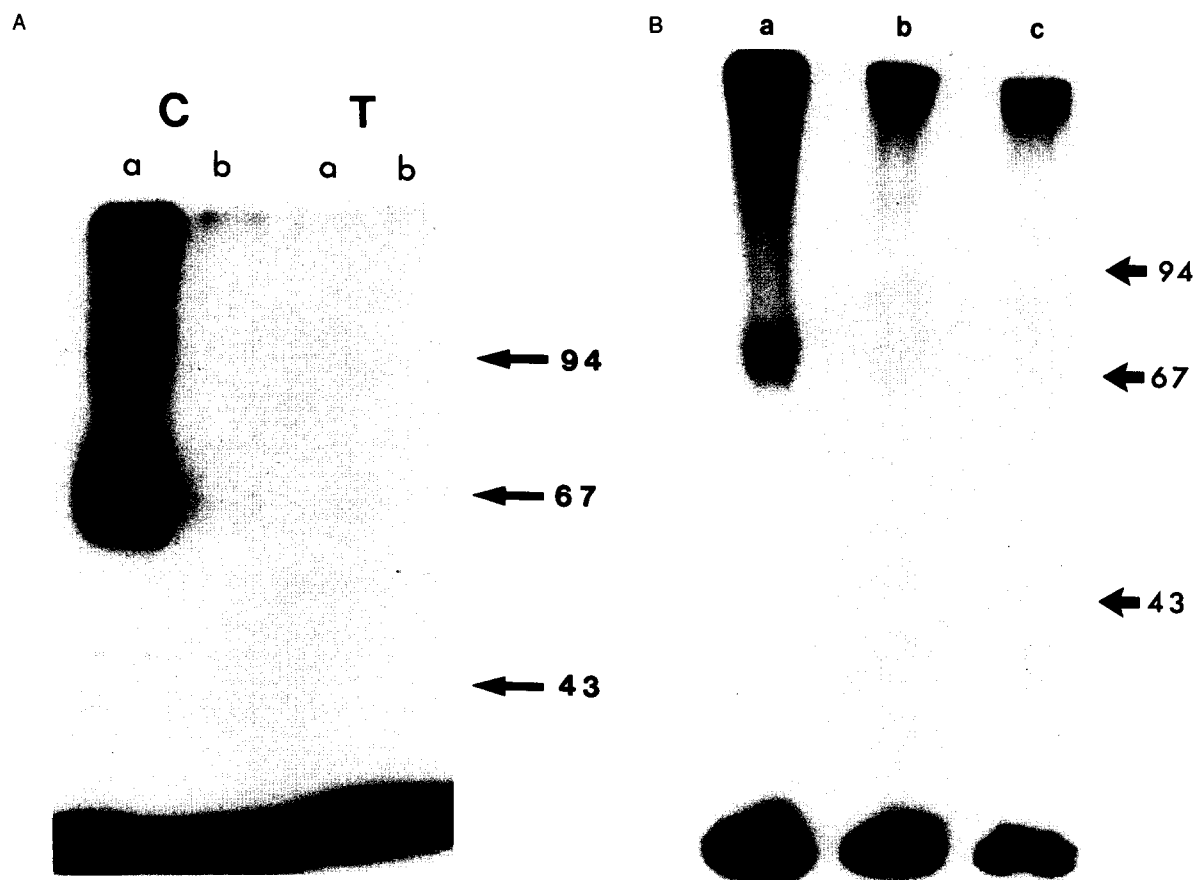


FIG. 4. (A) Cross-linking of [^{125} I]GLP-1 to plasma membranes of untreated or tunicamycin-treated RINm5F cells. Cells were incubated for 65 h at 37°C without (C) or with (T) tunicamycin (1.0 μ g/ml). After incubation with [^{125} I]GLP-1 in the absence (a) or presence (b) of 1 μ mol/l unlabeled GLP-1 for 30 min at 37°C cells were treated with DSS. The labeled proteins were analyzed by SDS-PAGE. M_r standards are indicated on the right side by arrows. (B) Cross-linking of [^{125} I]GLP-1 to plasma membranes of untreated and tunicamycin-treated RINm5F cells. Cells were incubated for 65 h at 37°C without (a) or with 0.5 μ g/ml (b) or 1.0 μ g/ml (c) tunicamycin. After incubation with [^{125} I]GLP-1 for 30 min at 37°C, cells were treated with DSS. The labeled proteins were analyzed by SDS-PAGE. M_r standards are indicated on the right side by arrows.

1. After incubation for 10 min at 2°C, the reaction was stopped by addition of ammonium acetate (final concentration 10 mmol/l). Membranes were centrifuged at $10,000 \times g$ and 4°C for 5 min and washed twice with ice-cold HEPES buffer (pH 7.5). Finally, pellets were resuspended in sodium phosphate buffer (10 mmol/l, pH 7.5) containing 2% sodium dodecylsulphate (SDS). Samples were boiled for 5 min. Electrophoresis was carried out as described by Laemmli (16). After drying gels were exposed to Kodak type X-Omat AR film for up to 2 weeks at -80°C using an image-intensifying screen.

Binding to Lectin-Linked Sepharose

Before cross-linking with labeled GLP-1(7-36)amide, aliquots of the solubilized receptor were loaded onto lectin columns (8) (first column, 0.5 ml concanavalin A, 10-15 mg lectin/ml gel attached to Sepharose 4B; second column, 0.5 ml wheat germ, 5 mg lectin/ml gel insolubilized on Sepharose 6MB; and third column, 0.5 ml peanut agglutinin, 10 mg lectin/ml gel attached to Sepharose 4B, samples were loaded with/without previous sialidase treatment to expose putative binding disaccharide). After washing the columns with 50 mM HEPES buffer (pH 7.4) containing 150 mM NaCl, 10 mM $MgSO_4$, and 0.1% (w/v) digitonin,

the receptors were eluted with the aforementioned HEPES buffer containing the appropriate sugar [con A, 0.3 M α -D-mannopyranoside; wheat germ, 0.3 M *N*-acetyl- β -D-glucosamin; and pea, 0.3 M galactose (β 1-3)-galNAc]. Samples were further analyzed by gel electrophoresis followed by autoradiography as described.

Glycopeptidase F (PNGase F) Treatment

After cross-linking, labeled receptors were solubilized in PNGase F buffer as has been detailed before (5). PNGase F (20 units/ml reaction mixture) was added and the mixture was incubated overnight at 37°C. Controls did not receive any enzyme. Samples were then analyzed by gel electrophoresis and autoradiography.

Preparation of RINm5F Cytosol

RINm5F cell cytosol was prepared after harvesting the cells and sonification of the cell pellets (10 s) in HEPES buffer with 0.1 mM PMSF and 1 mM benzamidinium followed by ultracentrifugation (4°C, 40,000 rpm). The supernatant was then saved and subjected to the cross-linking procedure.

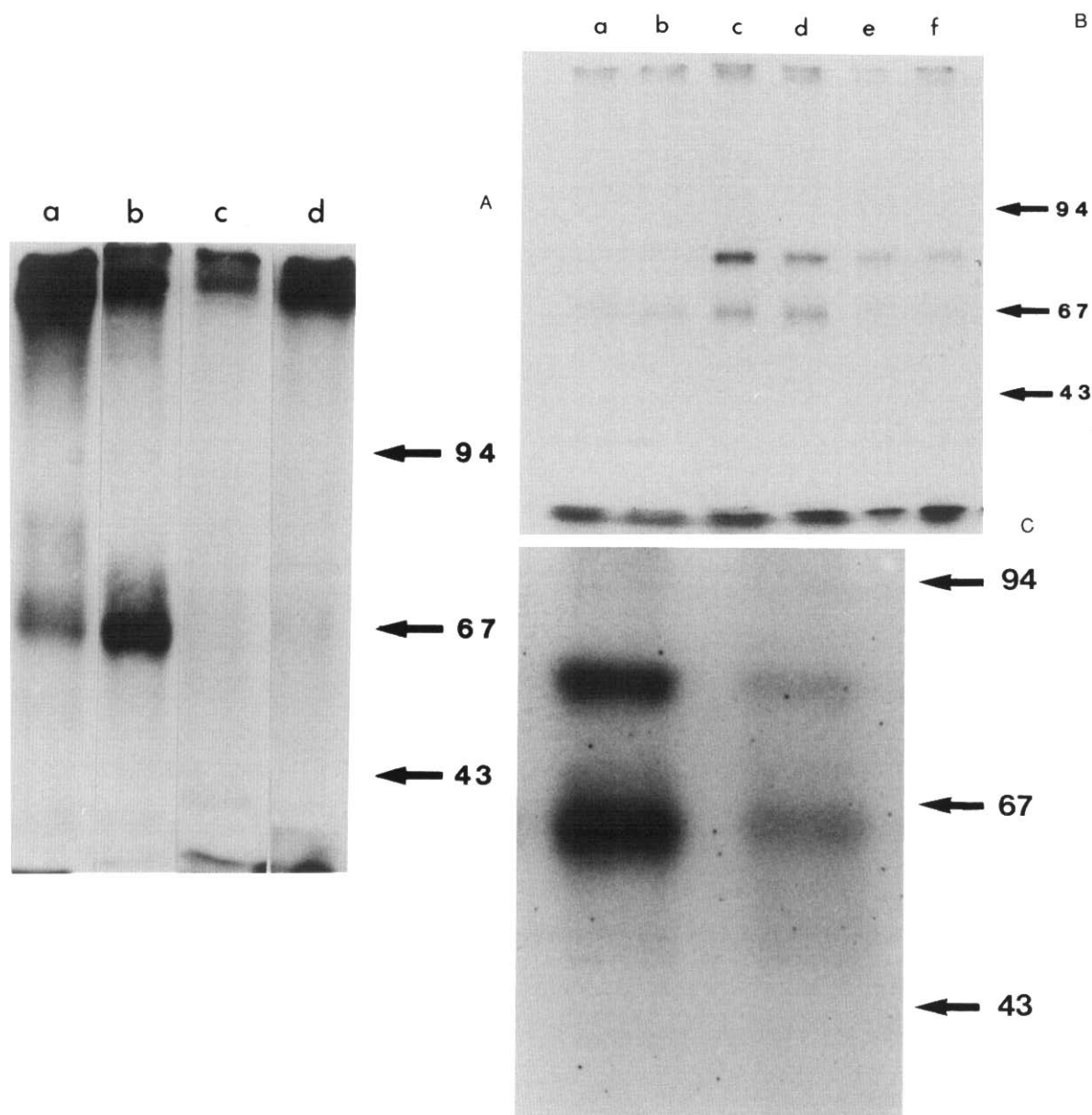


FIG. 5. (A) Cross-linking of [^{125} I]GLP-1 to plasma membranes and cytosol of RINm5F cells. Plasma membranes (a) and cytosol (b,c) of RINm5F cells were incubated for 30 min at 37°C with [^{125} I]GLP-1 without (a,b) or with (c) 1 μ M unlabeled GLP-1 and then treated with DSS. As a control, cytosol of receptor-negative cells was incubated for 30 min at 37°C with [^{125} I]GLP-1 before treatment with DSS. The labeled proteins were analyzed by SDS-PAGE. M_r standards are indicated on the right side by arrows. (B) Cross-linking of [^{125} I]GLP-1 to receptors in cytosol from untreated or tunicamycin-treated RINm5F cells. Cytosol from untreated (a,b) or tunicamycin-treated [0.5 μ g/ml (c,d); 1.0 μ g/ml (e,f)] cells was incubated with [^{125} I]GLP-1 for 30 min at 37°C and then treated with DSS. The labeled proteins were analyzed by SDS. M_r standards are indicated on the right side by arrows. (C) Cross-linking of [^{125} I]GLP-1 to receptors in cytosol from tunicamycin-treated RINm5F cells in the absence (left lane) or presence of an excess of unlabeled GLP-1 (1 μ M; right lane).

Preparation of GLP-1 Receptor mRNA From RINm5F Cells

Total RNA was isolated from cells according to Chomczynski and Sacchi utilizing RNA isolation kits (3).

Northern Blot

A 1500 bp Eco RI fragment of the cDNA coding for rat GLP-1(7–36)amide receptor (gift of Dr. Thorens, Lausanne) (26) was

radioactively labeled with [α^{32} P]dCTP, utilizing a random priming procedure (Amersham) according to the supplier's protocol and was purified with NucleoTrap Columns. RNA was fractionated on 1% agarose gels containing 2.2 M formaldehyde (28), transferred to Hybond N membranes, and immobilized by UV cross-linking. Hybridizations were performed in 50% formamide, 5 $\times$ Denhardt's, 5 $\times$ SSC, and 0.1 mg/ml sonicated herring sperm DNA at 42°C using 2–3 $\times 10^6$ cpm of the labeled

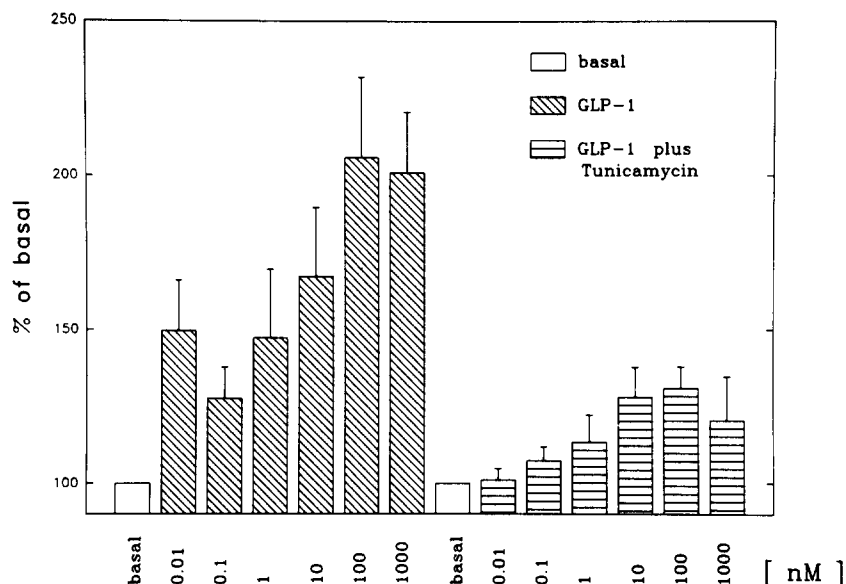


FIG. 6. Effect of GLP-1 on the cAMP generation in untreated or tunicamycin-treated RINm5F cells. Cells were treated without or with 1.0 μ g/ml tunicamycin for 65 h at 37°C. Then cells were incubated with a range of GLP-1 concentrations for 3 min at 37°C. cAMP levels were determined as described in the Method section. Data shown are means $\pm$ SEM of six experiments.

cDNA fragment. Final washings were performed in $0.1 \times$ SSC at 42°C. RNA quantity and integrity was verified by reversibly staining the Hybond N membranes with methylene blue prior to hybridization. Migration positions of the signals were calculated as compared to RNA markers.

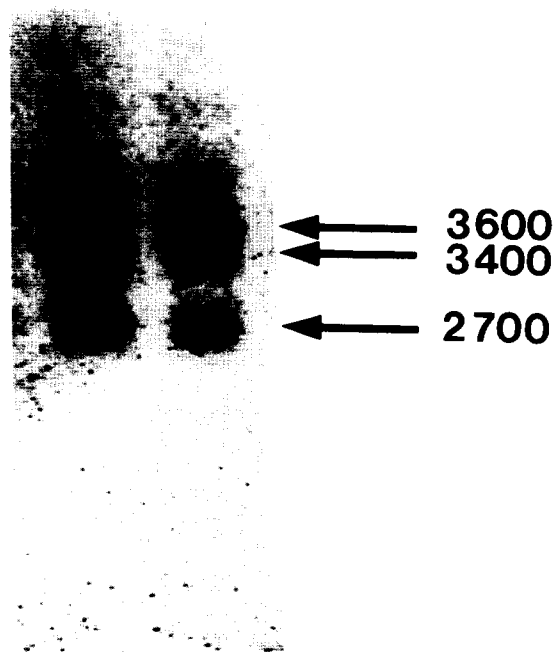


FIG. 7. Northern blot analysis of GLP-1 receptor expression in RINm5F cells with (right lane) or without (left lane) tunicamycin pretreatment (1 μ g/ml). A 1500 bp Eco RI cDNA fragment coding for the rat GLP-1 receptor was used as probe.

Assay for cAMP

Cells in 500 μ l buffer (HEPES, 10 mmol/l, pH 7.4; NaCl, 113 mmol/l; KCl, 4.7 mmol/l; KH_2PO_4 , 1.2 mmol/l; CaCl_2 , 2.5 mmol/l; MgSO_4 , 1.2 mmol/l) containing 1% (w/v) human serum albumin were incubated for 3 min at 37°C with or without peptide. The reaction was stopped by addition of 200 μ l ice-cold trichloroacetic acid (12%; w/v), sonication for 15 s at 50-W power (Labsonic 2000, Braun, Melsungen, Germany), and centrifugation for 5 min at $11,500 \times g$ at 4°C. After extraction with diethyl ether, samples were freeze dried and redissolved in 200 μ l sodium acetate buffer (50 mmol/l, pH 5.8). cAMP concentrations were determined using a test kit (Amersham-Buchler, Braunschweig, Germany) according to the manufacturer's instructions.

RESULTS

To determine whether the GLP-1 receptor is a glycoprotein, we initially investigated the ability of the cross-linked receptor protein to bind to immobilized WGA, Con A, or Pea. For this, the cross-linked receptor was solubilized and applied to the corresponding lectin. Approximately 5% of the radiolabeled GLP-1-receptor complex passed unbound WGA and approximately 25% Con A (Fig. 1). The remainder was bound to WGA and Con A and was readily eluted by an excess of *N*-acetylglucosamine and α -methylmannoside, respectively. Only a small amount of the peptide-receptor complex bound to Pea, irrespective of whether the receptors were pretreated with sialidase or not.

To examine whether the receptor protein contains *N*-linked carbohydrates, plasma membranes were cross-linked with [^{125} I]GLP-1 and then treated with glycopeptidase F. As shown in Fig. 2, treatment with glycopeptidase F resulted in an altered electrophoretic mobility of the peptide-receptor complex. The major complex with a molecular mass of $63,000 \pm 1000$ Da was partially converted to a smaller complex with a molecular mass

of $51,000 \pm 2000$ Da. To study whether *N*-linked oligosaccharides are important for receptor function, RINm5F cells were treated with tunicamycin. Tunicamycin is an antibiotic that inhibits asparagine-linked glycosylation by preventing the transfer of the first *N*-acetylglucosamine residue to dolichol phosphate (17). Incubation of cells with a range of tunicamycin concentrations decreased binding of GLP-1 concentration dependently (Fig. 3). Further analysis of the binding data using the PC program InPlot (Graph Pad Software, San Diego, CA) revealed that the decrease of binding was not related to a change in the affinity of the receptor but to a reduced number of binding sites. This data was confirmed by studies in which RINm5F cells, after incubation with or without tunicamycin for 65 h, were cross-linked with [^{125} I]GLP-1: in experiments with control cells, a single ligand-binding protein complex could be detected. The intensity of this band was reduced when incubations were carried out in the presence of $1 \mu\text{mol/l}$ unlabeled GLP-1. In experiments with cells incubated with tunicamycin, no radiolabeled protein band was detectable (Fig. 4(A,B)). In cross-linking studies with RINm5F cell cytosol, a single ligand-binding protein complex was detectable with the same molecular mass as the plasma membrane-associated receptor [Fig. 5(A)], which was not detectable if incubations were carried out in the presence of $1 \mu\text{mol/l}$ unlabeled GLP-1. As a control, we investigated cells (INR1G9 glucagonoma cells) that do not exhibit GLP-1 receptors on their plasma membranes. In these cells, no GLP-1 binding proteins were detectable in the cytosol [Fig. 5(A)]. In cytosol of RINm5F cells that had been treated with tunicamycin, an additional band with a molecular mass of $79,000 \pm 1000$ appeared [Fig. 5(B)], which could not be identified if incubations were carried out with $1 \mu\text{mol/l}$ unlabeled GLP-1 [Fig. 5(C)].

In further experiments we examined whether or not the GLP-1 receptor from tunicamycin-treated cells is a functional protein. For this, the effect of GLP-1 on cAMP generation in RINm5F cells incubated with or without tunicamycin was studied. As shown in Fig. 6, the stimulating effect of GLP-1 on cAMP production was drastically reduced after tunicamycin treatment.

To exclude any effect of tunicamycin on the GLP-1 messenger RNA expression, RINm5F cells were subjected to the same tunicamycin treatment protocol as described above before the GLP-1 mRNA was isolated. In Northern blots, three transcripts corresponding to 3600, 3400, and 2700 bp were identified (Fig. 7). The intensity of the bands was not different between tunicamycin-treated or untreated cells.

DISCUSSION

Our data show that the GLP-1 receptor is a glycoprotein with a molecular mass of 63,000 Da containing *N*-linked carbohydrates. Treatment of the GLP-1 receptor with glycopeptidase F resulted in the generation of a binding protein with a molecular mass of 51,000 Da. This decrease of the molecular mass might not quantitatively correspond to a loss of 20% from the native protein but, nevertheless, indicates that the *N*-linked carbohydrates are largely involved in the structure of the GLP-1 receptor. Therefore, we investigated the role of the *N*-linked glycans on the binding properties and function of the GLP-1 receptor. For this, we used the antibiotic tunicamycin, which specifically inhibits *N*-glycosylation (17). Tunicamycin concentration-dependently decreased GLP-1 binding to RINm5F cells due to a decreased number of receptors with no change

in the receptor affinity. Similar results have been shown for receptors for epidermal growth factor (EGF) and insulin, which, if unglycosylated, are incapable of binding their ligands (20,24). The results of our cross-linking experiments suggest that the unglycosylated GLP-1 receptor is not exposed on the cell surface of the RINm5F cells. In previous studies with receptors for transferrin (22), EGF (24) and prostaglandin E_1 (30), it was shown that the unglycosylated form of these receptors is not transported to the cell surface. These observations are explained by the concept that *N*-linked carbohydrate chains are thought to play a crucial role for intracellular routing of glycoproteins towards the plasma membrane. Thus, it can be speculated that the unglycosylated GLP-1 receptor is unable to enter the plasma membrane and, therefore, is not detectable in cross-linking studies. On the other hand, it cannot be excluded that the GLP-1 receptor is exposed in the plasma membrane but is unable to bind the ligand. However, in the cross-linking studies analyzing RINm5F cytosol, a single, specifically labeled band was identified with a molecular mass identical to the membrane-bound GLP-1 receptor. This band probably represents a pool of intracellular GLP-1 receptors. These might be either newly synthesized and not yet transported to the plasma membrane or have been internalized to be further metabolized or recycled into the plasma membrane.

Interestingly, in tunicamycin-treated cells, an additional ligand-protein complex with a molecular mass of 79,000 Da was detectable, which showed the typical binding competition behavior when unlabeled peptide was employed to compete with labeled peptide. Because *N*-linked glycans may play a role in intracellular routing, this complex could represent a nascent GLP-1 binding protein that is unglycosylated and, therefore, cannot be transported to another intracellular compartment to be further processed. This would support the above-mentioned assumption that the unglycosylated GLP-1 receptor is able to bind GLP-1 but cannot be detected in plasma membranes because it is unable to enter the cell surface. This would then make it unlikely that there are unglycosylated GLP-1 receptors in the plasma membrane that are unable to bind GLP-1. The observed 79,000 Da ligand-receptor complex might be coupled to an intracellular factor (chaperone?) that plays a role in the sorting process of proteins and that is not able to exert its function being associated with unglycosylated proteins.

Our failure to detect GLP-1 receptors in plasma membranes of tunicamycin-treated RINm5F cells was not caused by tunicamycin suppressing the generation of the corresponding receptor mRNAs. The present experiments clearly show that cells treated with tunicamycin generated as much GLP-1 receptor mRNA as untreated cells.

In RINm5F cells, the biological effect of GLP-1 is mediated by the adenylate cyclase system (12). Treatment of RINm5F cells with tunicamycin resulted in a reduced cAMP generation in response to GLP-1. This effect is most likely a consequence of the decreased number of GLP-1 receptors in the plasma membrane following tunicamycin treatment.

In essence, our data show that the glycosylation of the GLP-1 receptor is mandatory for regular receptor function.

ACKNOWLEDGEMENTS

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REFERENCES

- Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* 72:248-254; 1976.
- Burton, K. A study of the conditions and mechanisms of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* 62:315-323; 1956.
- Chomczynski, P.; Sacchi, N. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159; 1987.
- Eissele, R.; Göke, R.; Willemer, S.; et al. Glucagon-like peptide 1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur. J. Clin. Invest.* 22:283-291; 1992.
- Fabre, C.; Battari, A.; Karamanos, Y.; et al. Glycosylation of VIP receptors: A molecular basis for receptor heterogeneity. *Peptides* 14: 483-489; 1993.
- Fehmann, H. C.; Habener, J. F. Insulinotropic hormone glucagon-like peptide 1 (7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma β TC-1 cells. *Endocrinology* 130:159-166; 1992.
- Gazdar, A. F.; Chick, W. L.; Oie, H. K.; et al. Continuous, clonal insulin- and somatostatin-secreting cell line established from a transplantable rat islet cell tumor. *Proc. Natl. Acad. Sci. USA* 77: 3519-3523; 1980.
- Gerard, C. Purification of glycoproteins. *Methods Enzymol.* 182: 529-539; 1990.
- Göke, R.; Cole, T.; Conlon, J. M. Characterization of the receptor for glucagon-like peptide-1(7-36)amide on plasma membranes from rat-insulinoma-derived cells by covalent cross-linking. *J. Mol. Endocrinol.* 2:93-98; 1989.
- Göke, R.; Conlon, J. M. Receptors for glucagon-like peptide-1(7-36)amide on rat insulinoma-derived cells. *J. Endocrinol.* 116:357-362; 1988.
- Göke, R.; Fehmann, H. C.; Göke, B. Glucagon-like peptide-1(7-36)amide is a new incretin/enterogastrone candidate. *Eur. J. Clin. Invest.* 21:135-144; 1991.
- Göke, R.; Trautmann, M. E.; Haus, E.; et al. Signal transmission after GLP-1(7-36)amide binding in RINm5F cells. *Am. J. Physiol.* 257:G397-G401; 1989.
- Ishihara, T.; Nakamura, S.; Kaziro, Y.; Takahashi, T.; Takahashi, K.; Nagata, S. Molecular cloning and expression of a cDNA encoding the secretin receptor. *EMBO J.* 10:1635-1641; 1991.
- Jelinek, L. J.; Lok, S.; Rosenberg, G. B.; et al. Expression cloning and signaling properties of the rat glucagon receptor. *Science* 259: 1614-1616; 1993.
- Jüppner, H.; Abou-Samra, A. B.; Freeman, M.; et al. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. *Science* 254:1024-1025; 1991.
- Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685; 1970.
- Lehle, L.; Tanner, W. The specific site of tunicamycin inhibition in the formation of dolichol-bound N-acetylglucosamin derivatives. *FEBS Lett.* 71:167-170; 1976.
- Lin, H. Y.; Harris, T. L.; Flannery, M. S.; et al. Expression cloning of an adenylate cyclase-coupled calcitonin receptor. *Science* 254: 1022-1024; 1991.
- Lu, M.; Wheeler, M. B.; Leng, X. H.; Boyd, A. E. The role of the free cytosolic calcium level in the B-cell signal transduction by gastric inhibitory polypeptide and glucagon-like peptide-1(7-37). *Endocrinology* 132:94-100; 1993.
- Olson, T.; Lane, M. Post-translational acquisition of insulin binding activity by the insulin proreceptor. *J. Biol. Chem.* 262:6816-6822; 1987.
- Praz, G. A.; Halban, P. A.; Wollheim, C. B.; Blondel, B.; Strauss, J. A.; Reynold, A. E. Regulation of immunoreactive insulin release from a rat cell line (RINm5F). *Biochem. J.* 210:345-352; 1983.
- Reckhow, C. L.; Enns, C. A. Characterization of the transferrin receptor in tunicamycin-treated A431 cells. *J. Biol. Chem.* 263:7297-7301; 1988.
- Richter, G.; Göke, R.; Göke, B.; Arnold, R. Characterization of receptors for glucagon-like peptide-1(7-36)amide on rat lung membranes. *FEBS Lett.* 267:78-80; 1990.
- Soderquist, A. M.; Carpenter, G. Glycosylation of the epidermal growth factor receptor in A-431 cells. The contribution of carbohydrate to receptor function. *J. Biol. Chem.* 259:12586-12594; 1984.
- Svoboda, M.; Ciccarelli, E.; Tastenoy, M.; Robberecht, P.; Christophe, J. A cDNA construct allowing the expression of rat hepatic glucagon receptors. *Biochem. Biophys. Res. Commun.* 192:135-142; 1993.
- Thorens, B. Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide-1. *Proc. Natl. Acad. Sci. USA* 89:8641-8645; 1992.
- Uttenthal, L. O.; Blázquez, E. Characterization of the gastric receptor for truncated glucagon-like peptide-1 in the rat. *FEBS Lett.* 262: 139-141; 1990.
- Uttenthal, L. O.; Toledano, A.; Blázquez, E. Autoradiographic localization of receptors for glucagon-like peptide-1(7-36)amide in rat brain. *Neuropeptides* 21:143-146; 1992.
- Wollheim, C. B.; Pozzan, T. Correlation between cytosolic free Ca^{2+} and insulin release in an insulin secreting cell line. *J. Biol. Chem.* 259:2262-2267; 1984.
- Yatsunami, K.; Fujisawa, J.; Hashimoto, H.; Kimura, K.; Takahashi, S.; Ichikawa, A. Effect of tunicamycin on function of PGE_1 receptors from mouse mastocytoma P-815 cells. *Biochim. Biophys. Acta* 1051: 94-99; 1990.