FEBS Letters 348 (1994) 7–13

FEBS 14167

Signal transduction of the GLP-1-receptor cloned from a human insulinoma

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Received 14 May 1994

Abstract

GLP-1 (glucagon-like peptide 1 (7–36) amide) plays an important role in the regulation of insulin secretion and proinsulin gene expression of pancreatic β-cells. Patients with insulinoma tumors show uncontrolled insulin hypersecretion. This study demonstrates the molecular cloning of a cDNA for the GLP-1 receptor from a human insulinoma employing a λ-gt11 cDNA library. The cloned cDNA encoded a seven transmembrane domain protein of 463 amino acids which showed high homology to the GLP-1 receptor in normal human pancreas. Four amino acid exchanges were found in comparison to a receptor sequence obtained from regular pancreatic islets. When transfected transiently into COS-7 or stably into fibroblast CHL cells a high affinity receptor was expressed which coupled to the adenylate cyclase with normal basal cAMP and increasing intracellular cAMP levels under GLP-1 stimulation. The receptor accepted GLP-1 and the non-mammalian agonist exendin-4 as high affinity ligands. In transfected COS-7 cells, GLP-1 did not influence intracellular calcium, whereas in the stably transfected fibroblasts GLP-1 transiently increased intracellular calcium to a small extent. The understanding of GLP-1 receptor regulation and signal transduction will aid in the discovery of compounds that act as agonists of the GLP-1 receptor for potential use in the treatment of diabetes and will facilitate the understanding of its expression under normal and pathophysiological conditions.

Key words: Human insulinoma; GLP-1 receptor; COS-7 cell; CHL cell; Calcium; cAMP

1. Introduction

GLP-1 (glucagon-like peptide 1 (7–36) amide) arises as a post-translational product of preproglucagon processing in the mammalian intestine and is released in response to nutrient ingestion [1–3]. It is involved in the regulation of glucose-dependent insulin secretion and proinsulin gene expression [4]. Since GLP-1 has recently been under consideration as a new therapeutic agent in the treatment of diabetes mellitus [5,6], both peptide and corresponding receptor, have attracted significant attention. GLP-1 binding sites were found on human pancreatic β-cells [7], cell lines from endocrine pancreas [8,9], gastric cells [10], in the rat brain [11], and in lung [12,13]. The action of GLP-1 is mediated by coupling to a stimulatory G-protein [14–17] which is connected to the adenylate cyclase pathway [15]. Furthermore, it is known that the GLP-1 receptor, similar to the VIP receptor, undergoes important post-translational modifications, mainly glycosilation, which is important for its proper function [18,19].

The molecular characterization of the GLP-1 receptor is interesting for several reasons. A reduced effect of the stimulatory insulinotropic gut hormone GLP-1 may contribute to the impaired β-cell function in diabetics. Furthermore, the identification of a microsatellite in the 3′-untranslated region of the cDNA may, if polymorphic, be used for genetic studies aimed at linking a defect in this gene with disturbances of glucose homeostasis as was recently suggested [20,21]. Furthermore, in patients suffering from insulinoma tumors, uncontrolled insulin hypersecretion occurs hypothetically involving a mutated GLP-1 receptor. This is not unlikely since a number of human diseases can be attributed to point mutations in G protein-linked receptors [22–24] affecting either agonist binding, G protein–receptor interactions, or inproper membrane incorporation of the receptor [22,23,25].

In the present study, we have, therefore, cloned the GLP-1 receptor from human insulinoma tissue and characterized its signal transduction.

2. Experimental

2.1. Materials

GLP-1 (7–36) amide and the other utilized peptides (with the exception of exendin-4 and exendin (9–39) amide) were purchased from Saxon Biochemicals (Hannover, Germany). Exendin-4 and exendin (9–39) amide were synthesized as detailed before [26]. ^{125}I-Labeled GLP-1 was prepared as earlier described [9]. Formamide, formaldehyde, 50 × Denhardt's, and sonicated herring sperm DNA were obtained from Sigma, Deisenhofen, Germany. Nuctrap probe purification columns were purchased from Stratagene, La Jolla, USA. Multiprime labeling system, Hybond N membrane and [α^{32}P]dCTP (spec. act. 110 TBq/mmol) were from Amersham, Braunschweig, Germany. For autoradiography gels were exposed to X-OMAT AR X-ray film (Kodak)

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SSDI 0014-5793(94)00553-8

at −80°C. pTEJ-8 [27] was kindly donated by Siv Hjort, Copenhagen (Denmark).

2.2. Molecular cloning of the human insulinoma GLP-1 receptor cDNA

The human insulinoma cDNA library in λ-gt11 vector (prepared according to standard procedures in cooperation with Stratagene, La Jolla, USA) was screened with a radioactively labeled full-length rat GLP-1 receptor cDNA probe (gift from B. Thorens, Lausanne, Switzerland [28]) according to established protocols. Inserts of plaque-purified clones were released by *Eco*RI digest and subcloned into pBluescript SK +/− phagemid (Stratagene, La Jolla, USA) using Ready to Go Ligase (Pharmacia, Uppsala, Sweden). Nucleotide sequences were determined by the dideoxy chain termination method utilizing Sequenase (USB, Cleveland, USA) according to the supplier's protocol, with primers for T3 and T7 promoters and oligonucleotides designed according to the determined sequence (MWG-Biotech, Ebersberg, Germany).

2.3. RNA Isolation and Northern blot analysis

Total RNA from human insulinoma tissue was isolated [29] and poly(A)$^+$ RNA enriched by oligo-dT column (Poly-A Quick Kit; Stratagene, Heidelberg, Germany) according to the supplier's protocol. A 1,000 bp fragment of cDNA coding for human insulinoma GLP-1 (7–36) amide receptor was radioactively labeled. RNA was fractionated on 1% agarose gels containing 2.2 M formaldehyde, transferred to Hybond N membranes and immobilized by UV cross-linking. RNA quantity was verified by reversibly staining membranes with methylene blue prior to hybridization. Hybridization was performed as described previously [30] and migration positions of the signals were calculated by comparison to RNA markers. Exposure time varied from 12 h (CHL cells) to 14 days (RNA from human islets). Final washing was in 0.1 × SSC at 55°C.

2.4. Cell transfections

For transient expression, COS-7 cells were seeded in 100 mm coated dishes (Greiner, Germany) and cultured in DMEM medium supplemented with 10% fetal calf serum. Cells were transfected with 10 μg of p72 or Δ9, respectively, using chloroquine and $CaCl_2$. 24 h after transfection, the cells were detached from dishes using PBS with 1 mM EDTA, collected by centrifugation, reseeded into dishes or 24-well plates according to need for determinations of [^{125}I]GLP-1 binding, cAMP or Ca^{2+} levels, and incubated for another 24 h at 37°C.

Stable cell clones which selectively expressed the insulinoma GLP-1 receptor were established in Chinese hamster lymphoblast cells (CHL) by transfection using the calcium phosphate precipitation method (CellPhect Transfection Kit; Pharmacia, Germany). Together with the receptor cDNA a resistence against geneticin (expression vector pTEJ-8; [27] and, consequently, treated cells were screened and selected for by their ability to resist geneticin treatment (final concentration 0.8 mg/ml) and by their ability to specifically bind radiolabeled GLP-1 with high affinity.

2.5. Receptor binding studies [9,16,17]

Transfected cells were incubated for 30 min at 37°C with radiolabeled tracer (20,000 cpm) and unlabeled peptides in a final volume of 330 μl. COS cells were utilized 48 h after transfection. After incubation, the cell suspensions were centrifuged at 12,000 × *g*; the radioactivity retained in the pellet was determined in a γ-counter. Binding in the presence of an excess of unlabelled GLP-1 (7–36) amide was subtracted in each experiment.

2.6. Measurement of cAMP [9]

COS-7 cells 24 h after transfection and stably transfected CHL cells were passaged into 24-well plates and cultured for an additional 24 h to confluency. 10^6 cells were collected in 500 μl of KRB buffer; after a preincubation with 50 μl of IBMX for 20 min, GLP-1 was added. Following 10 min of incubation, cells were washed with ice-cold buffer, and TCA (4%) was added to stop the reaction. After diethylether extraction, supernatants were used for the determination of cAMP levels by radioimmunoassay (Immunotech, Marseille, France).

2.7. Measurement of the cytosolic Ca^{2+} concentration [31]

Loading of cells (48 h after transfection) with the fluorescent calcium indicator fura-2 was performed as described [32]. Measurements of the $[Ca^{2+}]_{cyt}$ concentration and calibration of the data were performed essentially as described previously [32,33], using a modified Krebs-Ringer bicarbonate buffer containing 4 mM glucose and 1.5 mM $CaCl_2$. The measurements were performed at 37°C. Since CHL cells displayed dye efflux during the measurements, we corrected the data as described previously [31] using either addition of manganese and the manganese chelator diethylenetrianminepenta acetic acid (DTPA) in the beginning and at the end of each measurement, or performing the measurements in the presence of 250 μM sulfinpyrazone and adding manganese and DTPA.

3. Results

3.1. RNA Isolation and Northern blot analysis

Utilizing isolated total RNA from three human insulinomas and a 1,000 bp *Eco*RI fragment of the cDNA encoding for the human GLP-1 (7–36) amide receptor for Northern blot analysis, major hybridization signals were found at about 6 and 7 kb and less prominently at 4.1 kb (Fig. 1). Identically sized transcripts were detected with RNA from normal human islets, although much

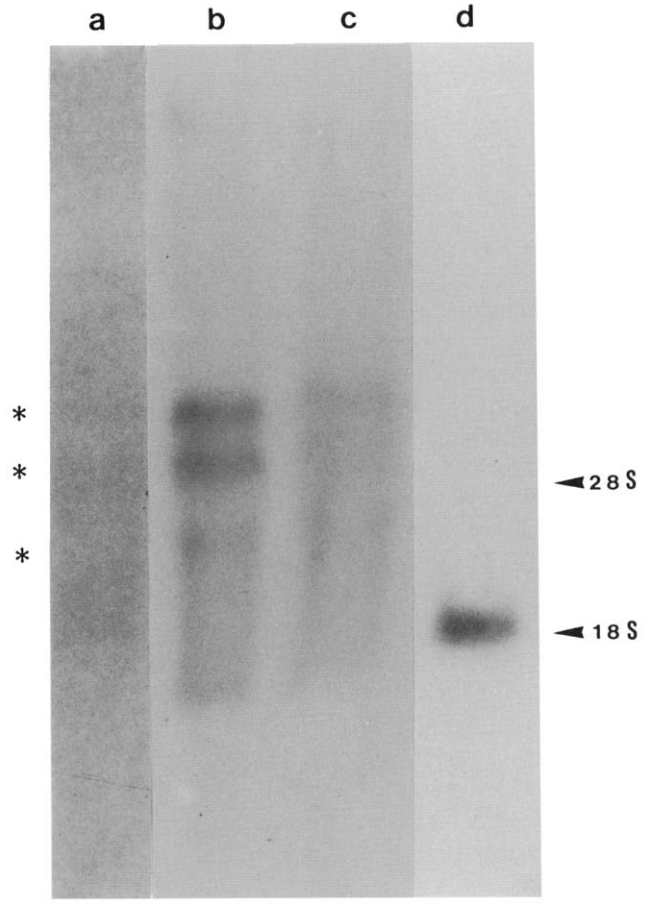

Fig. 1. Northern blot analysis of GLP-1 receptor expression in insulinomas, regular islet cells, and transfected CHL cells. A 1,000 bp cDNA fragment of the insulinoma-derived GLP-1 receptor cDNA was utilized as probe. Total RNA from two out of three studied insulinomas (lanes b and c) and human islets (a) show the main transcripts of 6 and 7 kb and a minor signal at 4.1 kb. In transfected CHL cells stably expressing the GLP-1 receptor (d) the expected signal at 2 kb was detected. Arrows indicate migration positions of 18 and 28 S rRNA. Asterisks mark the weak bands of RNA from islet cells.

fainter signals were obtained due to the small amount of available RNA. Furthermore, RNA from stably transfected CHL cells was isolated and analyzed by Northern hybridization. A strong transcript signal of the expected size of approximately 2 kb was detected (Fig. 1).

3.1. Cloning of a human insulinoma GLP-1 receptor cDNA

Screening of a human insulinoma λ-gt11 cDNA library was performed with the rat GLP-1 receptor cDNA [28]. Four different clones encoding partly overlapping regions were isolated. One cDNA, h10, was 1,700 bp in length and lacked approximately 27 nucleotides at the 3′ end of the receptor coding region. Another cDNA clone, h1, was 900 bp in length and showed 99% homology to the human islet GLP-1 receptor from nucleotide 560–1,460. To obtain a full-length human insulinoma GLP-1 receptor cdNA, both a *Hin*dIII–*Sac*I fragment of h10 (coding for the 5′ end) and a *Sac*I–*Eco*RI fragment of h1 (containing the 3′ end) were subcloned into

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  1 MAGAPGPLRLAVLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSL  50
    ----------L-------------------------------------
    ----------L-------------------------------------

 51 TEDPPPATDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHV 100
    ----------------------------------------------------
    ----------------------------------------------------
    ----------------------------------------------------

101 YRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSREEQLLFLYIIYTV 150
    ------------------------------------P-------------
    ----------------------------------WG-------------
    ------------------------------------P-------------

151 GYALSFSALVIASAILLGFRHLHCTRNYIHLNLFASFILRALSVFIKDAA 200
    ----------------------------------------------------
    A---------------------------------------------------
    ----------------------------------------------------

201 LKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVY 250
    ----------------------------------------------------
    ----------------------------------------------------
    ----------------------------------------------------

251 LYTLLAFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRN 300
    ----------------------------------------------------
    ------------------------------------I---------------
    ---------L------------------------------------------

301 SNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLA 350
    ----------------------------VI--------------------
    ----------------------------------------------------
    ----------------------------------------------------

351 KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAI 400
    ----------------------------------------------------
    ----------------------------------------------------
    ----------------------------------------------------

401 LYCFVNNEVQLEFRKSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAG 450
    ----------------------------------------------------
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451 SSMYTATCQASCS 463
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Fig. 2. Comparison of the deduced amino acid sequences of human GLP-1 (7–36) amide receptors. Top line, human insulinoma (present data); second line, human islets [34], third line, human islets [21], fourth line, human gastric tumor [35]. The predicted transmembrane segments are underlined.

POS.	INSULINOMA	ISLET (Dillon)	ISLET (Thorens)	GASTR. TUMOR	RAT
3	G	G	G	G	V
4	A	A	A	A	T
6	G	G	G	G	S
7	P	P	P	P	L
12	V	L	V	L	L
17	M	M	M	M	A
33	W	W	W	W	S
44	R	R	R	R	H
49	S	S	S	S	F
53	D	D	D	D	A
55	P	P	P	P	L
56	P	P	P	P	L
59	E	E	E	E	D
68	E	E	E	E	D
76	E	E	E	E	P
96	P	P	P	P	L
109	L	L	L	L	I
112	Q	Q	Q	Q	H
131	R	R	R	R	Q
135	S	S	S	S	N
136	S	S	W	S	S
137	R	P	G	P	P
143	F	F	F	F	S
151	G	G	A	G	G
167	L	L	L	L	V
168	G	G	G	G	S
225	S	S	S	S	G
260	F	F	F	L	F
264	W	W	W	W	R
267	R	R	R	R	K
270	V	V	V	V	L
282	V	V	V	V	I
289	Y	Y	I	Y	Y
323	I	I	I	I	V
325	V	V	V	V	I
330	I	V	I	I	I
331	V	I	V	V	V
332	V	V	V	V	I
333	S	S	S	S	A
382	I	I	I	I	V
396	L	L	L	L	F
400	I	I	I	I	V
411	L	L	L	L	M
424	H	H	H	H	R
426	H	H	H	H	N
443	L	L	L	L	V
449	A	A	A	A	V
453	M	M	M	M	V
455	T	T	T	T	A
460	A	A	A	A	N

Fig. 3. Variations of amino acid composition of human GLP-1 receptors in comparison to the rat GLP-1 receptor [28,44]. Boxes indicate variations between the different human sequences. The more prominent boxes indicate the four amino acid exchanges in human insulinoma as compared to [21]. All positions showing variations between the rat sequence and at least one of the human sequences are presented.

*Hin*dIII/EcoRI-cut pTEJ-8 expression vector [27]. The plasmid was designated p72. The product was restriction mapped and sequenced to confirm that it corresponded to human insulinoma GLP-1 receptor sequence. Similarly, the h1 insert, coding for a C-terminally truncated receptor, was subcloned into pTEJ-8. It was utilized for the expression of a receptor devoid of the 9 amino acids at the regular C-terminus and was termed Δ9.

Sequence alignment revealed that the isolated full-length cDNA encoded a protein of 463 amino acids showing > 90% homology to recently cloned human islet GLP-1 receptors from islets [21,34] and from the gastric tumor cell line HGT-1 [35] (Fig. 2). The presently reported insulinoma receptor contains seven potential transmembrane spanning domains and three N-linked glycosylation sites in the putative extracellular domains.

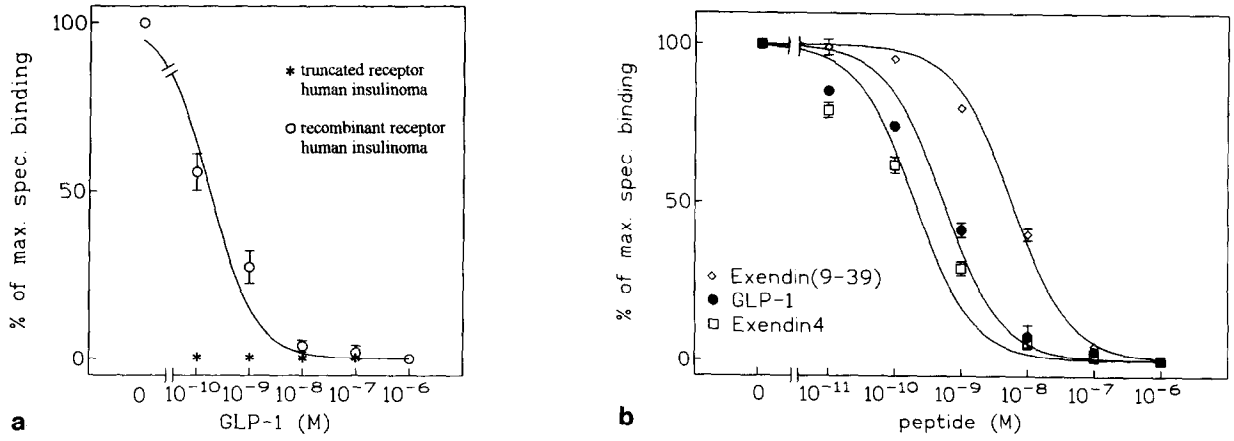

Fig. 4. (a) Displacement of [^{125}I]GLP-1 (7–36) amide binding to transiently transfected COS-7 cells by unlabeled GLP-1. COS-7 cells were transfected with human insulinoma GLP-1 receptor full-length cDNA in pTEJ-8. (b) Displacement of [^{125}I]GLP-1 (7–36) amide binding to stably transfected CHL cells by unlabeled GLP-1 and the endoxins. CHL cells were transfected with human insulinoma GLP-1 receptor full-length cDNA in pTEJ-8. Data shown are means ± S.E.M. (n = 8), respectively.

The human insulinoma GLP-1 receptor was not identical to the human GLP-1 receptors of normal pancreas. In fact, from comparing recent data [21], four amino acid exchanges were found (Fig. 3): at sequence position 136 W (normal) was substituted by S (tumor); at 137 G by R; at 289 I by Y; at position 151, in the putative first transmembrane domain a conservative amino acid exchange occurred (A by G). The amino acid exchanges were verified by sequence analysis of two or three independent clones, respectively.

3.2. *Specific binding of GLP-1 (7–36) amide to transfected cells*

To confirm that the amplified receptor cDNA was appropriately expressed in COS-7 cells, binding analysis was performed with GLP-1 (7–36) amide, and VIP. Expression of p72 conferred specific binding of ^{125}I-labelled GLP-1 (7–36) amide upon COS-7 cells which was inhibited in a concentration-dependent manner by GLP-1. Analysis of the data by the Scatchard method indicated the presence of a single class of binding sites with a K_d of 1.5×10^{-10} M (n = 8) (Fig. 4a). This value lies within the range of reported binding properties of rat and human GLP-1 receptors in RIN- and COS-7 cells [36]. Vasoactive intestinal peptide (VIP) did not displace ^{125}I-labelled GLP-1 (7–36) amide binding. Transfection experiments with the truncated receptor mutant Δ9 did not reveal specific binding of GLP-1 (Fig. 4a).

CHL cells stably transfected with p72 showed specific competition for binding of GLP-1, exendin-4, and exendin (9–39) amide. The order of potency of displacement by the unlabelled peptides was exendin-4 > GLP-1 > exendin (9–39) amide (Fig. 4b). K_d values were: exendin-4, 2.0×10^{-10}; GLP-1, 5.4×10^{-10}; exendin (9–39) amide, 5.8×10^{-9} M.

3.3. *Effect of GLP-1 (7–36) amide on cyclic AMP concentration in transfected cells*

To verify that the cloned receptor signaled through the adenylyl cyclase system, the effects of GLP-1 on cAMP levels in extracts of transfected COS-7 cells were examined. GLP-1 increased the cAMP content in p72 transfected cells. The cAMP level rose to 20% above basal levels by stimulation with 10^{-10} M GLP-1, and to 42% above basal levels with 10^{-9} M GLP-1. There was no increase in cAMP in mock transfected COS-7 cells.

In stably transfected CHL cells, intracellular generation of cAMP was stimulated in a dose-dependent manner (Fig. 5). The cAMP level rose to 60% above basal levels by stimulation with 10^{-12} M GLP-1, and to 150% above basal levels with 10^{-11} M GLP-1 (Fig. 5). The EC_{50} was approximately 5×10^{-12} M. Concentrations of GLP-1 (7–36) amide higher than those maximally stimulating

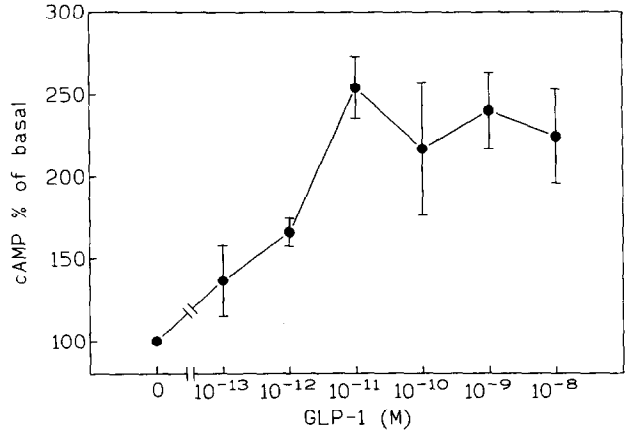

Fig. 5. GLP-1 induced cAMP production in CHL cells transfected with the human insulinoma GLP-1 receptor. Data shown are means ± S.E.M. (n = 6).

cAMP production showed a saturation of the stimulating effect. There was no stimulation in CHL control cells (not transfected).

3.4. Effects of GLP-1 (7–36) amide on cytosolic Ca^{2+} concentration in transfected cells

In COS-7 cells transiently transfected with the insulinoma GLP-1 receptor, addition of GLP-1 in concentrations from 10^{-10} to 10^{-6} M did not influence $[Ca^{2+}]_{cyt}$ (not shown). A set of experiments was performed to confirm the integrity of the principle calcium mobilization and influx mechanisms in the transfected COS-7 cells. Addition of 1 μM of the intracellular calcium pump inhibitor thapsigargin induced a rapid rise of cytosolic calcium, followed by a decline to an elevated plateau (not shown). This demonstrated filling of intracellular calcium stores and presence of the capacitative calcium entry pathway [37] in the transfected cells. Similarly to thapsigargin, addition of 100 μM ATP caused a sharp peak of cytosolic calcium followed by a sustained elevation (not shown). ATP increases cytosolic calcium via binding to purinergic receptors, with subsequent opening of receptor-activated calcium channels and activation of the phospholipase C pathway [38]. The effect of ATP showed that the cytosolic calcium homeostasis in the transfected COS-7 cells was coupled to receptor-mediated signal transduction.

In stably transfected CHL cells, GLP-1 produced a very small and transient rise of $[Ca^{2+}]$cyt. 10^{-8} M GLP-1 produced a maximal effect and raised $[Ca^{2+}]_{cyt}$ by 7.5 ± 3.3 nM (n = 4), from a basal value of 121.8 ± 5.4 nM. This $[Ca^{2+}]_{cyt}$ elevation peaked at approximately 2 min after GLP-1 addition, and $[Ca^{2+}]_{cyt}$ returned to the previous basal value within 5 min of GLP-1 addition. In comparison, depletion of intracellular calcium stores by 1 μM thapsigargin transiently raised $[Ca^{2+}]_{cyt}$ to 248 ± 20 nM (n = 4). After chelation of extracellular calcium, a GLP-1-induced rise of $[Ca^{2+}]_{cyt}$ could no longer be detected. Since the thapsigargin-induced $[Ca^{2+}]_{cyt}$ elevation was also reduced considerably, any GLP-1-associated $[Ca^{2+}]_{cyt}$ elevation probably would have been too low for reliable detection. Therefore, it was not possible to determine whether the GLP-1-mediated $[Ca^{2+}]_{cyt}$ elevation was predominantly generated by calcium release from intracellular stores or whether it depended on influx of extracellular calcium.

4. Discussion

It is generally believed that an exact characterization of GLP-1 receptors will facilitate the understanding of the regulated expression of the receptor under normal and pathophysiological conditions. The present study evaluates the molecular and functional properties of a GLP-1 receptor from a human insulinoma.

During the past years, more than 100 G protein-coupled receptor subtypes have been cloned and sequenced, based largely on the conservation of the primary structure among G protein-coupled receptors, particularly within families, allowing the isolation of new cDNA and genomic clones by cross-hybridization. Very recently, a new subfamily of G protein-linked receptors was discovered, which, among others, comprises the receptors of secretin [39], vasoactive intestinal polypeptide [40], pituitary adenylyl cyclase-activating polypeptide [41], and glucagon [42]. This family of receptors contains seven potential transmembrane domains, but, interestingly, shows no sequence similarity with other reported G protein-coupled receptors like the adrenergic receptors [43]. By transient expression of a whole rat pancreatic islet cDNA library into COS cells, it was recently possible to clone a cDNA of another member of this receptor family encoding for the pancreatic islet GLP-1 receptor [28]. This cDNA served as a template for several studies which revealed the molecular features of GLP-1 receptors in lung [44], the gastric tumor cell line HGT-1 [35], normal human islets [21,34], and now in human insulinoma tissue.

At first sight it was questionable whether human insulinomas still have the capability of expressing GLP-1 receptors since we know that insulinoma cells show a variety of significant characteristics which make them different from normal islet β-cells, like the occurance of stimulus-uncoupled insulin secretion and the neo-expression of proteins [45–47]. Some data suggest that insulinoma-derived β-cells even show a closer relationship to neuroendocrine cells than to regular β-cells. Therefore, before we decided to generate a cDNA library from insulinoma RNA we prepared membrane material from an insulinoma which showed high tissue preservation when morphologically studied. These membranes were chemically cross-linked with labeled GLP-1 to reveal the existence of specific GLP-1 binding sites in this tumor [7]. We then cloned and sequenced the GLP-1 receptor utilizing a cDNA library derived from the above-mentioned insulinoma.

Previously, analysis of the effects of deletion mutants, chimeric receptors, effects of peptides corresponding to portions of the receptor sequence, and antibodies directed against different cytoplasmatic regions, have been used to map potential sites of interaction between receptor and G protein or to analyze generally the function of the ligand–receptor complex [25,48,49]. The expression of receptors in diseased tissue provides another approach to correlate the structure of receptors with cell function. Actually, discrete mutations in several diseases, sporadic pituitary and thyroid tumors, McCune-Albright syndrome, in adrenal and ovarian tumors result in increased signal transduction [25,50].

Analysis of the deduced amino acid sequence and comparison with recently published sequences from

human receptors revealed a high homology between GLP-1 receptors in normal and insulinoma tissue. Still, several amino acid exchanges were found and it was an open question whether the variations in the sequences found could result into functional consequences, for example permanent activation of a signal transduction pathway contributing to the clinically observed hyperinsulinemia in our insulinoma patient. Previous data have shown that substitution of amino acid residues in the C-terminal segment of the third intracellular loop of seven transmembrane domain receptors produced marked agonist-independent stimulation of cellular phosphatidylinositol turnover [23]. Although no amino acid exchanges were found in this specific region, we functionally expressed the insulinoma GLP-1 receptor in COS and CHL cells to study its signal transduction properties.

The recombinantly expressed insulinoma GLP-1 receptor was coupled to the adenylate cyclase system which was true for both transfected COS and CHL cells. Such coupling has been shown similarly for the GLP-1 receptor in lung [17], and rat insulinoma cells [15]. An abnormally increased basal cAMP level was not found. Instead we found a clear increase in cAMP in response to GLP-1 stimulation. This reflects the physiological situation where cAMP potentiates glucose-stimulated insulin secretion [2].

In contrast to findings from rat insulinoma RINm5F cells [15] others have demonstrated in hamster insulinoma HIT-15 cells that GLP-1 stimulates an increase in intracellular calcium through voltage-dependent calcium channels not associated with phospholipase C activation [51]. The same laboratory has proposed that calcium, not cAMP, is the true 'second messenger' for GLP-1 stimulated insulin secretion [34,51]. This proposal was based upon transfection studies with the cDNA of the normal islet GLP-1 receptor into COS cells [34]. These data suggested that a single recombinant human receptor species signalled through both activation of phospholipase C to release calcium from an intracellular pool, and activation of adenyl cyclase. Such coupling of a peptide receptor to two different second messenger systems has recently been shown for neuropeptide Y [52] and the cholecystokinin receptor [53]. However, our present data contrast with those findings. Addition of GLP-1 did not produce any elevation of the cytosolic calcium ion concentration ($[Ca^{2+}]_{cyt}$) in COS-7 cells transiently transfected with the insulinoma GLP-1 receptor, and it generated only a very small and transient rise in $[Ca^{2+}]_{cyt}$ in stably transfected CHL cells. These results suggest that, in contrast to the evident coupling to adenylate cyclase, there was no relevant interaction of the insulinoma GLP-1 receptor with the phospholipase C system. Due to its minimal size, the GLP-1 induced $[Ca^{2+}]_{cyt}$ rise in stably transfected CHL cells was not amenable to further, detailed analysis. It was not possible to assess, for example, whether it involved calcium release from intracellular stores. Our results are in accordance with previous observations concerning GLP-1-associated signal transduction in endocrine pancreatic β-cells and insulin-secreting cell lines. GLP-1 did not elevate $[Ca^{2+}]_{cyt}$ at all in RINm5F rat insulinoma cells [15], and it raises $[Ca^{2+}]_{cyt}$ in rat islet β-cells [54] and in HIT hamster insulinoma cells [51] only via an indirect, glucose-dependent action, probably involving cAMP-mediated activation of L-type voltage-dependent calcium channels. In contrast to these findings and our results, GLP-1 appeared to activate both adenylate cyclase and phospholipase C in COS-7 cells transiently transfected with the rat GLP-1 receptor [32]. However, it has to be considered that activation of signal transduction pathways not normally known to be associated with a particular receptor in vivo can be observed when receptors are over-expressed in a heterologous system [25]. This might also apply to the rat GLP-1 receptor- transfected COS-7 cells, especially in light of the lack of effect of GLP-1 on $[Ca^{2+}]_{cyt}$ in the rat cell line RINm5F [15]. Therefore, the pronounced phospholipase C-mediated elevation of $[Ca^{2+}]_{cyt}$ by GLP-1 in COS-7 cells transfected with the rat GLP-1 receptor [32] probably does not represent a true fundamental difference in signal transduction between that receptor and the human insulinoma receptor described here.

The recombinant insulinoma GLP-1 receptor showed specific binding characteristics. High affinity binding was only found for GLP-1 and the GLP-1 agonist exendin-4 and the antagonist exendin (9–39) amide [26], whereas vasoactive intestinal polypeptide was ineffective. Therefore, the insulinoma GLP-1 receptor showed the characteristics of regular GLP-1 receptors. These functional data are, furthermore, supported by results from computer hydrophobicity plots of insulinoma and normal islet GLP-1 receptor amino acid sequences (Lasergene, DNA; London, UK) which showed only minor variations in the calculated hydrophobicity.

While cloning the receptor we isolated an additional receptor mutant lacking the last nine cytoplasmatic amino acids. When this truncated receptor was expressed in COS cells a drastic reduction in binding ocurred which might suggest that the integrity of the intracellular C-terminal domain is important for proper binding. However, these studies clearly need more work.

Considering the expected future therapeutic use of GLP-1 in diabetes the need for detailed knowledge of the binding properties and the coupling to second messenger pathways of the GLP-1 receptors in the different target tissues is important.

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