Molecular cloning, functional expression, and signal transduction of the GIP-receptor cloned from a human insulinoma

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Abstract **Glucose-dependent insulinotropic polypeptide (GIP) plays an important role in the regulation of postprandial insulin secretion and proinsulin gene expression of pancreatic β-cells. This study demonstrates the molecular cloning of a cDNA for the GIP-receptor from a human insulinoma λgt11 cDNA library. The cloned cDNA encoded a seven transmembrane domain protein of 466 amino acids which showed high homology (41%) to the human glucagon-like peptide 1 (GLP-1) receptor. Homology to the GIP receptor from rat or hamster was 79% and 81%, respectively. When transfected 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 stimulation with human GIP-1–42 ($EC_{50} = 1.29 \times 10^{-13}$ M). The receptor accepted only human GIP 1–42 ($K_d = 1.93 \pm 0.2 \times 10^{-8}$ M) and porcine truncated GIP 1–30 ($K_d = 1.13 \pm 0.1 \times 10^{-8}$ M) as high affinity ligands. At 1 μM, exendin-4 and (9–39)amide weakly reduced GIP-binding (25%) whereas secretin, glucagon, glucagon-like peptide-1, vasoactive intestinal polypeptide, peptide histidine-isoleucine, and pituitary adenylyl cyclase activating peptide were without effect. In transfected CHL cells, GIP-1–42 did not increase intracellular calcium. Northern analysis revealed one transcript of human GIP receptor mRNA with an apparent size of 5.5 kb. The exact understanding of GIP receptor regulation and signal transduction will aid in the understanding of the incretin hormone's failure to exert its biological action at the pancreatic B-cell in type II diabetes mellitus.**

Key words: GIP receptor; Human insulinoma cDNA; CHL cell; cAMP; Calcium

1. Introduction

The postprandial release of gut hormones augments the insulin secretory response to a significant extent. We have learned that two peptide hormones are mainly responsible for this effect, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulin-releasing polypeptide (GIP) [1]. GLP-1 presently receives attention as potential therapeutic tool for the treament of type II diabetes mellitus since it is still effective in such patients [1,2]. Interestingly, there is data which indicates that GIP has only a grossly reduced effect in diabetes [2]. This clearly is not the consequence of deficient GIP release since most studies indicate normal or even an increased GIP secretion in diabetes mellitus [3]. Still, such circumstance may contribute to the impaired β-cell function in diabetics. Therefore, speculations about GIP receptor defects arose which find some support by a number of human diseases that can be attributed to point mutations in G protein-linked receptors [4,5,6]. Such defects alter either agonist binding, G protein–receptor interactions, or cause inproper membrane incorporation of the receptor [5,7]. In any case, cloning of the human GIP receptor is a prerequisite for its molecular characterization in diabetes. This was the objective of the present study.

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2. Experimental

2.1. Materials

All utilized peptides were purchased from Saxon Biochemicals (Hanover, Germany) or Peninsula (Frankfurt, Germany). ^{125}I-labeled GIP-1–30 was prepared as earlier described [8]. The specific activity of the tracer was approx. 1500 Ci/mmol. Formamide, formaldehyde, 50 × Denhardt's, and sonicated herring sperm DNA were obtained from Sigma, Deisenhofen, Germany. 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) at −80°C. Reverse transcriptase (Superscript Plus) and Taq-polymerase was obtained from Gibco BRL, Karlsruhe, Germany and from Boehringer, Mannheim (Germany), respectively. For subcloning, amplification and sequencing of DNA fragments the parental vector pGEM-3Z from Promega (Heidelberg, Germany) was used. For receptor binding studies the human GIP receptor cDNA was cloned into the expression vector pTEJ-8. pTEJ-8 [9] was kindly donated by Siv Hjort, Copenhagen (Denmark). Primer for reverse transcription (RT), polymerase chain reaction (PCR) and DNA sequencing were designed by us and manufactured by MWG-Biotech, Ebersberg, Germany. Sequenase was obtained from USB, Cleveland (USA).

2.2. Molecular cloning of the human insulinoma GIP receptor cDNA and RT-PCR

The human insulinoma random primed cDNA library [10] was screened with a radioactively labeled 1.1 kb *Bgl*II-fragment of the rat GIP receptor cDNA [11] according to established protocols. In addition the same cDNA library was screened with a 5′ specific fragment of the rat GIP receptor cDNA. This fragment (0.8 kb) was isolated from 1 μg total RNA of the rat insulinoma cell line RINm5F by RT-PCR using the primer 5′-AGCCAAGAAGCAGGTAGCAGC-3′ (nucleotide position 927–947) for reverse transcription and the primers 5′-TCCAGCCCAACTGCCTCGA-3′ (position 118–136) and 5′-GAGCGTCTCACAACCACC-3′ (position 902–919, sequences deduced from [11] for cDNA amplification, respectively. Inserts of plaque-purified clones were released by *Eco*RI digest, subloned into pGEM-3Z and sequenced by the dideoxy chain termination method utilizing Sequenase.

Partial human GIP receptor cDNA sequences were also isolated from 1 μg total RNA of a human insulinoma [12] using RT-PCR. The primers used were 5′-AGAATGCCAAGAATGCGG-3′ (nucleotide position 945–962) for reverse transcription and the primers 5′-TCTCTCGCCACACTGCTGC-3′ (position 442–460) and 5′-CAAGATGGTCATGAGGATGG-3′ (position 906–924) for cDNA amplification, respectively. Reverse transcription was carried out according to the manufactures instructions. The cycling conditions for PCR were: 1 min, 94 °C, 1 min 60°C and 1.5 min 72°C.

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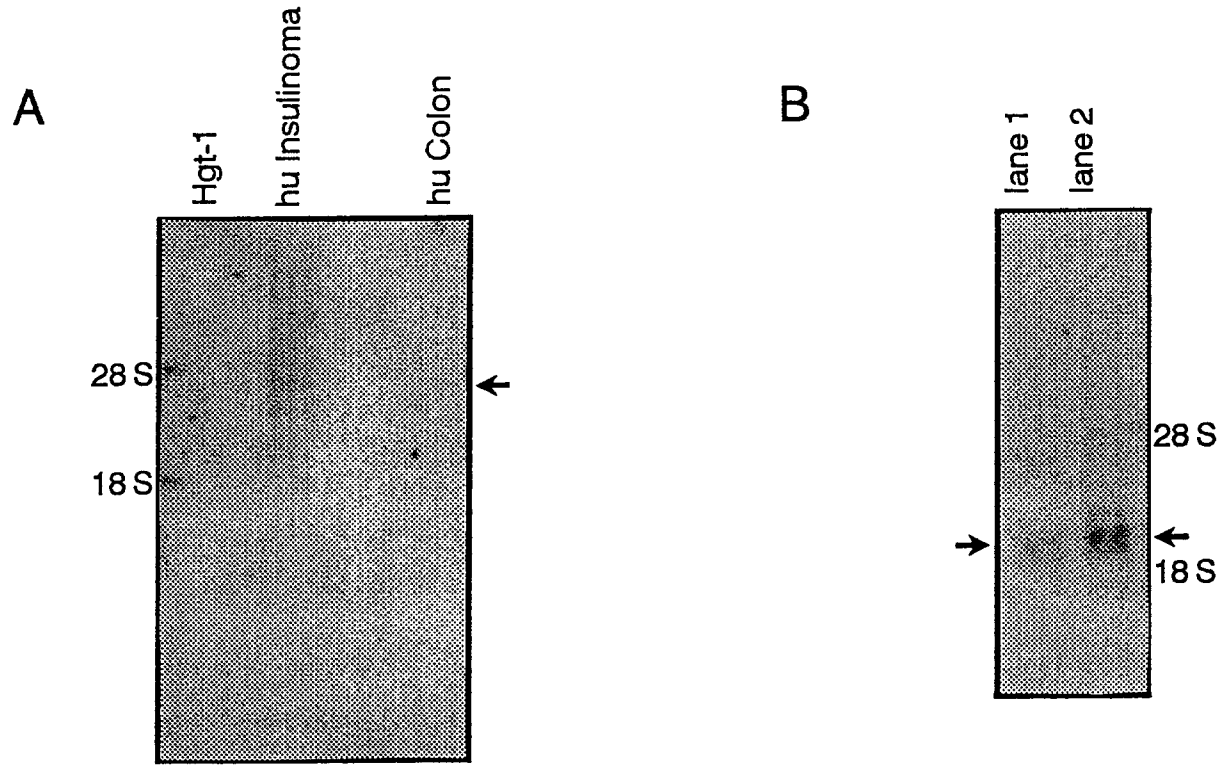


Fig. 1. Northern blot analysis of GIP receptor expression in (A) tissue of a human insulinoma, colon, stomach cancer HGT 1 cells, and (B) transfected CHL cells. A 1.0 kb fragment of the insulinoma-derived GIP receptor cDNA was utilized as probe. In (A) only total RNA from the insulinoma showed a transcript of 5.5 kb. In transfected CHL cells stably expressing the GIP receptor (B) the expected signal at 2.6 kb was detected (arrows) for both the intact and the cDNA containing the alternatively spliced IIΔ62 fragment.

2.3. RNA Isolation and Northern blot analysis

Total RNA from human insulinoma tissue, stomach carcinoma HGT 1-cells, and colon was isolated [12]. A 1.0 kb fragment of cDNA coding for human insulinoma GIP receptor was radioactively labeled. RNA was fractionated on 1% agarose gels containing 2.2 M formaldehyde, transfered 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 [10,13] and migration positions of the signals were calculated as compared to RNA markers. Final washing was in 0.1 × SSC at 65°C.

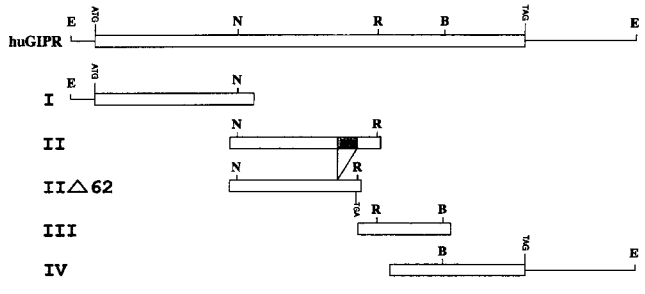


Fig. 2. Construction of the full-length clone (huGIPr) of the human GIP receptor. Alignment of overlapping partial cDNA clones. The cDNA fragments I, III and IV were obtained by screening of the human insulinoma cDNA library. The cDNA fragments II and IIΔ62 were amplified by RT-PCR from human insulinoma RNA. The full-length clone was constructed using the common restriction sites N (*Nhe*II), R (*Rca*I) and B (*Bgl*III). The hatched box indicates the 62 bp exon absent in the IIΔ62 alternative splice product coding for a truncated GIP receptor protein. ATG and TAG (IIΔ62) indicate translation initiation and termination respectively. E = *Eco*RI.

2.4. Cell transfections

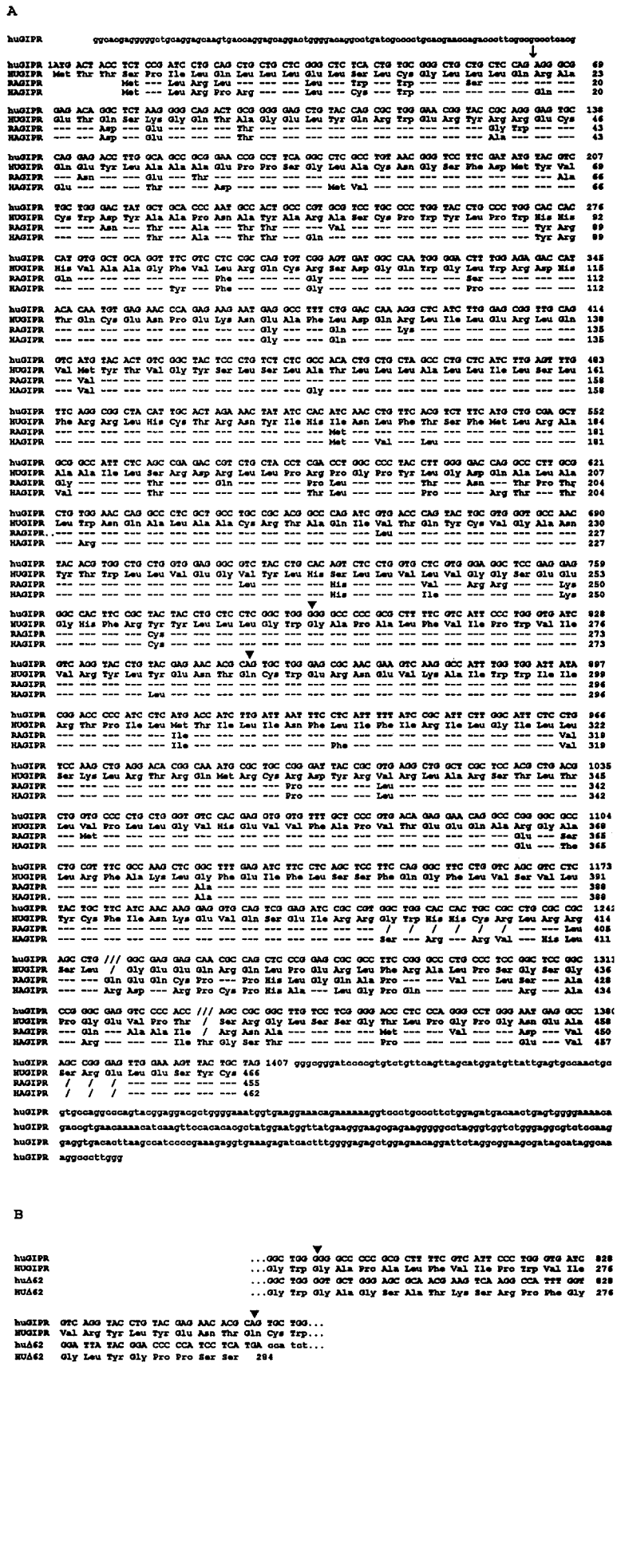
Recognition sequences of restriction enzymes present in overlapping sequences (Fig. 2) were utilized to combine the cDNA clones I, II, III and IV. For expression of the GIP receptor protein the full-length cDNA was cloned into the expression vector pTEJ-8. Stable cell clones which selectively expressed the insulinoma GIP receptor were established in Chinese Hamster Lymphoblast (CHL) cells 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) was introduced and, consequently, treated cells were screened and selected by their ability to resist against a geneticin treatment (final concentration 0.8 mg/ml) and by their ability to specifically bind radiolabeled porcine GIP-1-30 with high affinity.

2.5. Receptor binding studies

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. 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 unlabeled GIP (human GIP-1–42 or truncated porcine GIP-1–30) or other peptides (as indicated) was substracted in each experiment [10].

→

Fig. 3. (A) Nucleotide sequence of the human GIP receptor cDNA (huGIPR). The deduced amino acid sequence (HUGIPR) is compared with the amino acid sequences of the GIP receptor cDNA from rat: RAGIPR [11] and hamster: HAGIPR [17]. Identical amino acids are indicated by (---) and gaps (/) were introduced to allow optimal alignment. The 62 bp exon absent in one splice variant is indicated by ▼. (B) Comparison of the nucleotid sequence and the deduced amino acid sequence of the human GIP receptor cDNA and the truncated splice variant Δ62. The potential restiction site of the signal peptide is indicated by ↓.



2.6. Measurement of cAMP

Transfected CHL cells in KRB-buffer (10^5/300 μl) were incubated at 37°C for 10 min without or with various GIP-1–42 concentrations. After centrifugation for 2 min at 6000 × *g*, aliquots of the supernatants were used for the determination of cAMP levels by radioimmunoassay (Immunotech, Marseille, France).

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

Loading of cells (48 h after transfection) with the fluorescent calcium indicator fura-2 was performed as described [14,15]. Measurements of the $[Ca^{2+}]_{cyt}$ concentration and calibration of the data were performed essentially as described previously [14,15], 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 [14] 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

Northern blot analysis of total RNA from human insulinoma tissue, human stomach carcinoma HGT-1 cells, and human colon was performed with a 1.0 kb cDNA probe (position −94 to 924). GIP receptor mRNA with a calculated transcript size of 5.5 kb was detectable only in the insulinoma sample (Fig. 1A). Furthermore, RNA from cells transfected with either GIP receptor- or truncated GIP receptor- (lacking a 62 bp exon; Fig. 1B) cDNA was hybridized with the same probe. Transscript signals of expected sizes for both constructs were detected, i.e. approximately 2.6 kb for both.

3.2. Cloning of a human insulinoma GIP receptor cDNA

A human insulinoma λgt 11 cDNA library [10] was screened with a 0.8 kb PCR fragment located in the 5′region and a 1.1 kb *Bgl*III fragment located in the 3′ region of the rat GIP receptor cDNA [11]. Five different clones were isolated. Three of those (I, III, IV) were sequenced and used for the cloning of the functional human GIP receptor (Fig. 2). Two clones contained incompletely spliced cDNA sequences from the middle portion of the human GIP receptor gene (data not shown). For determination of the completely spliced cDNA a RT-PCR fragment spanning this region was isolated from human insulinoma total RNA (Fig. 2). The DNA sequence of this PCR fragment was identical to the coding region of the two incompletely spliced cDNA clones. Interestingly, an additional PCR product lacking a 62 bp exon was obtained (Fig. 2; IIΔ62). In this alternatively spliced product the predicted reading frame was shifted within the fourth transmembrane domain leading to a truncated GIP receptor protein (Fig. 3B).

The sequence of the human GIP receptor cDNA (Fig. 3A) revealed a 1389 bp open reading frame encoding for a 466

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                                                                  HUPTHR  MMGTARIAPGLALLLCCPVLSSGYALVDADDVMTKEE   38

HUGIPR  M---TTSPILQ-LLLGLSLCGLLLQRAETGSKGQTAGELYQRWERYRRECQETLAAAEPP-SGLACNGSFDMYV-CWDYAAPNATARASCPWYLPWHH  94
HUGLP1R MAGAPGPLRLAVLLLGMVG----RAGPRPQGATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEYA-CWPDGEPGSFVNVSCPWYLPWAS  95
HUGLUR  M--PPCQPQRPLLLL----LLLLACQPQVP-SAQVMDFLFEKWKLY-GDQCHHNLSLLPPPTELVCNRTFDKYS-CWPDTPANTTANISCPWYLPWHH  91
HUSECR  M-RPHLSPPLQQLLLPVLLACAAHSTGALPRLCDVLQVLWEEQDQCLQELSREQTGDLGTEQPVPGCEGMWDNISCWPSSVPGRMVEVECPRFLRMLT  98
HUPTHR  QIFLLHRAQAQCEKRLKEVLQRPASIMESDKGWTSASTSGKPRKDKASGKLYPESEEDKEAPTGSRYRGRPCLPEWDHILCWPLGAPGEVVAVPCPDY  136

                                                                                  I                    II
HUGIPR  HVAAGFVLRQCRSDGQW-------GLWRDHTQCENPEKNEAFLDQRLILER-LQVMYTVGYSLSLATLLLALLILSLFRRLHCTRNYIHINLFTSFML  184
HUGLP1R SVPQGHVYRFCTAEGLWLQKCNSSLPWRDLSECEESKRGERSSREEQLLF--LYIIYTVGYALSFSALVIASAILLGFRHLHCTRNYIHLNLFASFIL  191
HUGLUR  KVQHRFVFKRCGPDGQW-VRGPRGQPWRDASQCQMDGEEIEVNKEVAKMYSSFQVMYTVGYSLSLGALLLALAILGGLSKLHCTRNAIHANLFASFVL  188
HUSECR  SRNGSLFRNCTQ-DGWSETFPRPNLACGVNVNDSSNEKRHSYLLK-------LKVMYTVGYSSSLVMLLVALGILCAFRRLHCTRNYIHMHLFVSFIL  189
HUPTHR  IYDFNHKGHYRRCDRNGSWELVPGHNRTWANYSECVKFLTNETREREVFDR-LGMIYTVGYSVSLASLTVAVLILAYFRRLHCTRNYIHMHLFLSFML  234

        _______                                                  III                    IV
HUGIPR  RAAAILSRDRLLPRPGPYLGDQAL---------------ALWNQALAACRTAQIVTQYCVGANYTWLLVEGVYLHSLLVLVGGSE-EGHFRYYLLLGW  266
HUGLP1R RALSVFIKDAALKWMYSTAAQQHQWDG------------LLSYQGSLSCRLVFLLMQYCVAANYYWLLVEGVYLYTLLAFSVFSE-QWIFRLYVSIGW  276
HUGLUR  KASSVLVIDGLLRTRYSQKIGDD-----------LSVSTWLSDGAVAGCRVAAVFMQYGIVANYCWLLVEGLYLHNLLGLATLPE-RSFFSLYLGIGW  274
HUSECR  RALSNFIKDAVL------FSSDD---------------VTYCDAHRAGCKLVMVLFQYCIMANYSWLLVEGLYLHTLLAISFFSERKYLQGSFVAFGW  280
HUPTHR  RAVSIFVKDAVLYSGATLDEAERLTEEELRAIAQAPPPPATAAAGYAGCRVAVTFFLYFLATNYYWILVEGLYLHSLIFMAFFSEKKYLWGFTV-FGW  331

        _______                                  V                                                  VI
HUGIPR  GAPALFVIPWVIVRYLYENTQCWERNEVKAIWWIIRTPILMTILINFLIFIRILGILLSKLRTRQMR---CRDYRVRLARSTLTLVPLLGVHEVVFAP  361
HUGLP1R GVPLLFVVPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMC---KTDIKCRLAKSTLTLIPLLGTHEVIFAF  371
HUGLUR  GAPMLFVVPWAVVKCLFENVQCWTSNDNMGFWWILRFPVFLAILINFFIFVRIVQLLVAKLRARQMH---HTDYKFRLAKSTLTLIPLLGVHEVVFAF  369
HUSECR  GSPAIFVALWAIARHFLEDVGGWDINANASIWWIIRGPVILSILFNFILFINILRILMRKLRTQETR-GNQVSHYKRLARSTLLLIPLFGIHYIVFAF  377
HUPTHR  GLPAVFVAVWVSVRATLANTGCWD-LSSGNKKWIIQVPILASIVLNFILFINIVRVLATKLRETNAGRCDTRQQYRKLLKSTLVLMPLFGVHYIVFMA  428

                                  VII
HUGIPR  VTEEQARGALRFAKLGFEIFLSSFQGFLVSVLYCFINKEVQSEIRRGWHHCRLRRSLGEEQRQLPERLFRALPSGSGPGEVPTSRGLSSGTLPGPGNE  459
HUGLP1R VMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFRKSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAGSSMYTATCQASCS      463
HUGLUR  VTDEHAQGTLRSAKLFFDLFLSSFQGLLVAVLYCFLNKEVQSELRRRWHRWRLGKVLWEERNTSNHRASSSPGHGPPSKELQFGRGGGSQDSSAETPL  467
HUSECR  SPEDAMEIQ-----LFFELALGSFQGLVVAVLYCFLNGEVQLEVQKKWQQWHLREFPLHPVASFSNSTKASHLEQSQGTCRTSII              455
HUPTHR  TPYTEVSGTLWQVQMHYEMLFNSFQGFFVAIIYCFCNGEVQAEINKCWSRWTLALDFKRKARSGSSSYSYGPMVSHTSVTNVGPRVGLGLPLSPRLLP  526

HUGIPR  ASRELESYC                                                                                    466
HUGLUR  AGGLPRLAESPF                                                                                  477
HUPTHR  TATTNGHPQLPGHAKPGTPALETLETTPPAMAAPKDDGFLNGSCSGLDEEASGPERPPALLQEEWETVM                         593
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Fig. 4. Comparison of the deduced amino acid sequences of the human GIP receptor, human GLP-I receptor [10], human glucagon receptor [28], human secretin receptor [29] and the human parathormone receptor [30]. The predicted transmembrane domains are indicated by roman numbers. Amino acids which are conserved in more than two receptors are indicated with bold letters.

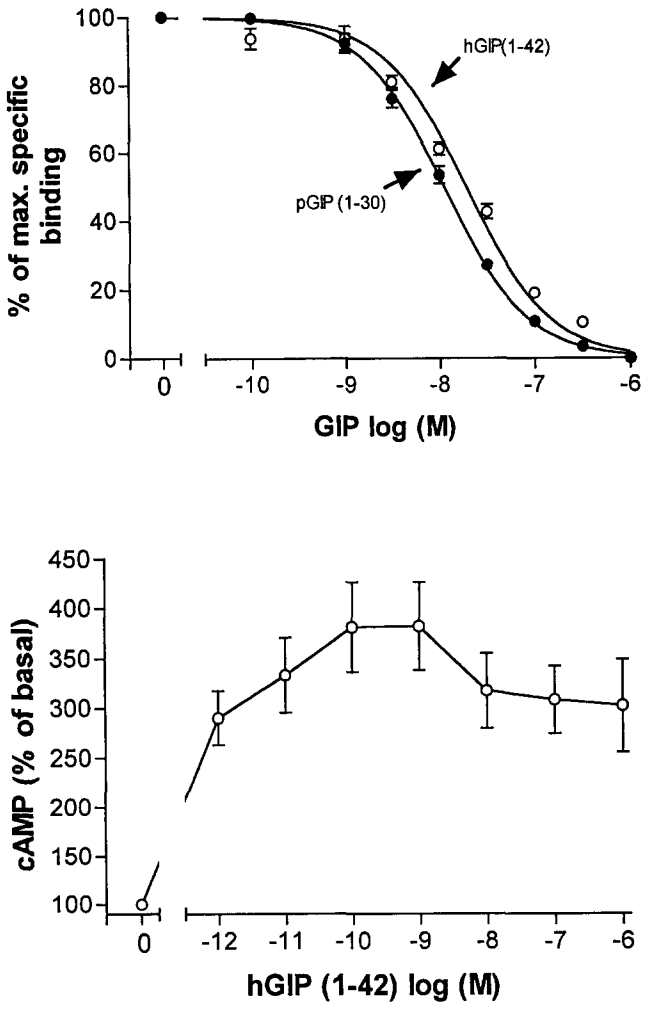

Fig. 5. (Upper panel) Displacement of [^{125}I]GIP-1–30 binding to stably transfected CHL cells by unlabeled human GIP-1–42 (hGIP-1–42) and porcine truncated GIP-1–30 (pGIP-1–30). CHL cells were transfected with human GIP receptor full-length cDNA in pTEJ-8. Data shown are means ± S.E.M. (n = 6), respectively. (Lower panel) hGIP-1–42 induced cAMP production in CHL cells transfected with the human insulinoma GIP receptor. Data shown are means ± S.E.M. (n = 6).

amino acid comprising protein with a predicted start codon surrounded by an almost perfect translation initiation side (10 bp identical to the 13 bp-consensus sequence; [16]). The human GIP receptor shows 79% and 81% homology to the rat receptor [11] and the hamster receptor protein [17], respectively. The potential cleavage site between Gln_{21} and Arg_{22} [18] of the apparent signal peptide is conserved among the rat and human GIP receptor species (Fig. 3A). A comparison of the human GIP receptor with other members of the secretin/VIP/glucagon-receptor family showed that the seven predicted transmembrane domains are highly conserved (Fig. 4). Based upon sequence comparison the GIP receptor is closest related to the glucagon receptor (46%) and the GLP-1 receptor (41% homology).

3.3. Specific binding of GIP to transfected cells

To confirm that the amplified receptor cDNA was appropriately expressed in CHL cells, binding analysis was performed with hGIP-1–42 and truncated pGIP-1–30. Furthermore, exendin-4, exendin-(9–39) amide [19], and several members of the glucagon-secretin-VIP peptide family were utilized for competition experiments at concentrations of 1 μM. Expression of the GIP receptor conferred specific binding of ^{125}I-labeled GIP-1–30 upon CHL cells which was inhibited in a concentration-dependent manner by both, GIP-1–30 (K_d = 1.13 ± 0.1 × 10^{-8} M) and GIP-1–42 (K_d = 1.93 ± 0.2 × 10^{-8} M) (Fig. 5). Analysis of the data by the Scatchard method indicated the presence of a single class of binding sites. Vasoactive intestinal peptide (VIP), GLP-1, glucagon, pituitary adenylyl cyclase activating peptide (PACAP) -27 and -38, peptide histidine-isoleucine (PHI), and secretin (all at 1 μM) did not displace ^{125}I-labeled GIP-1–30 binding (Fig. 6). Only the exendins (-4 and -(9–39) amide) had a small but significant effect (approximately 25% reduction of labeled GIP-(1–30) binding; $P < 0.05$ for both exendins; t-test). Transfection of the alternatively spliced cDNA product IIΔ62 did not reveal any binding of GIP to the cells (data not shown) although a specific transcript was detected (Fig. 1B).

3.4. Effect of GIP on cyclic AMP concentration in transfected cells

To verify that the cloned receptor signaled through the adenylyl cyclase system, the effects of human GIP-1–42 on cAMP levels in transfected CHL cells were examined. GIP increased in a dose-dependent manner cAMP content in the transfected cells (Fig. 5). The cAMP level rose to 280% above basal level by stimulation with 10^{-12} M GIP, and to 380% above basal level with 10^{-10} M GIP. The EC_{50} was approximately 1.29 ± 0.29 × 10^{-13} M. Concentrations of GIP > 10^{-9} M, higher than those maximally stimulating cAMP production, showed a certain saturation of stimulation accompanied with a slight reduction of the effect. At the supramaximal concentration of 10^{-6} M GIP, cAMP was still increased 300% above basal. There was no increase of cAMP in mock transfected cells.

3.4. Effects of GIP on cytosolic Ca^{2+} concentration in transfected cells

In the stably transfected CHL cells, human GIP-1–42 (at concentrations of 10^{-10} to 10^{-8} M) exerted no effect on $[Ca^{2+}]_{cyt}$ concentration. In control experiments, depletion of intracellular calcium stores by 1 μM thapsigargin clearly raised $[Ca^{2+}]_{cyt}$ concentration (Fig. 7).

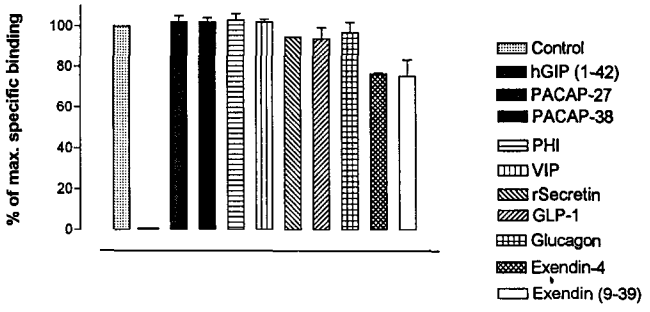

Fig. 6. Ligand screen of the cloned GIP receptor stably expressed in CHL cells. Peptides were utilized at 1 μM to compete with [^{125}I]GIP-1–30 binding. Each peptide was tested 6 times. As control, hGIP-1–42 abolished tracer binding. Both exendins had a significant effect ($P < 0.05$; 25% reduction of tracer binding). For abbreviations see section 2.

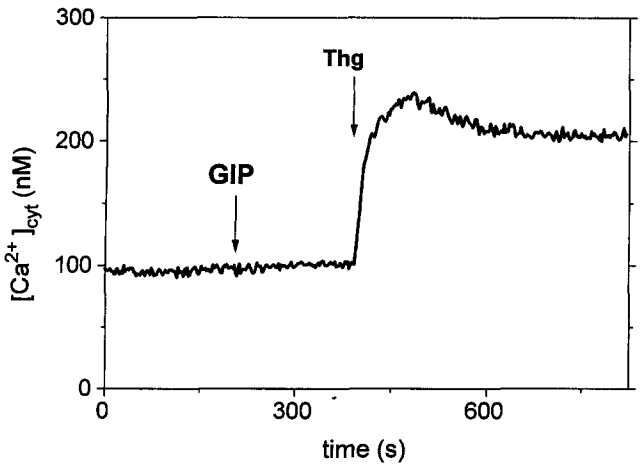

Fig. 7. hGIP-1–42 (at concentrations of 10^{-10} to 10^{-8} M) had no effect on $[Ca^{2+}]_{cyt}$ concentration in the stably transfected CHL cells. Shown is a representative experiment at 10^{-9} M GIP ($n = 6$). Depletion of intracellular calcium stores by 1 μM thapsigargin clearly raised $[Ca^{2+}]_{cyt}$ concentration.

4. Discussion

A cDNA encoding the human GIP receptor was isolated from a insulinoma cDNA library. Previously, we have utilized this library to clone the human GLP-1 receptor [10]. Both receptors belong to a subfamily of the 7 transmembrane domain receptors, also comprising the receptors for glucagon, parathormon, secretin, and vasoactive intestinal polypeptide, among others. Previously, Usdin et al. isolated a rat cDNA encoding the GIP receptor [11]. This cDNA served as template for our studies which revealed the molecular features of a GIP receptor expressed in human insulinoma tissue. Analysis of the deduced amino acid sequence and comparison with the recently published sequences from rat [11] and hamster [17] receptors revealed high homology between GIP receptors in various species.

The recombinantly expressed insulinoma-derived human GIP receptor is coupled to the adenylate cyclase system. We found a clear increase of cAMP in response to GIP stimulation. This reflects the physiological situation where cAMP potentiates glucose-stimulated insulin secretion [1].

Addition of GIP induced no increase of $[Ca^{2+}]_{cyt}$ 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 GIP receptor with the phospholipase C system. Our results are in accordance with previous observations concerning the signal transduction of the other insulinotropic gut hormone, GLP-1, in endocrine pancreatic beta cells and insulin-secreting cell lines. GLP-1 did not elevate $[Ca^{2+}]_{cyt}$ at all in RINm5F rat insulinoma cells [20], and it raised $[Ca^{2+}]_{cyt}$ in rat islet β cells [21] and in HIT hamster insulinoma cells [22] only via an indirect, glucose-dependent action, probably involving cAMP-mediated activation of L-type voltage-dependent calcium channels. A similar scenario is probably in effect for the closely related GIP receptor.

The recombinant insulinoma GIP receptor showed specific binding characteristics. High affinity binding was only found for human GIP-1–42 and truncated porcine GIP-1–30. This is of interest since both peptides were very recently characterized as stimulators of proinsulin gene transcription [23]. Interestingly, exendin-4 and exendin-(9–39) amide, both considered potent ligands at the GLP-1 receptor [19], bound weakly at the GIP receptor whereas secretin, glucagon, vasoactive intestinal polypeptide, peptide histidin-isoleucine, and pituitary adenylyl cyclase activating peptide were ineffective. This could reflect the close relationship of the GIP and the GLP-1 receptors as demonstrated by the 41% amino acid sequence homology of the binding proteins. However, GLP-1 was not effective in competing with labeled GIP for binding. Furthermore, in the in vivo situation an effect of the exendins at the GIP receptor is highly unlikely to occur [24].

With the sequence of the human GIP receptor known and the cDNA available, more work needs to be undertaken to evaluate whether GIP receptor mutations may contribute to the development of diabetes mellitus. The expression of receptors in diseased tissue provides an approach to correlate the structure of receptors with cell function [7,25,26]. Actually, discrete mutations in several diseases, sporadic pituitary and thyroid tumors, McCune-Albright syndrome, in adrenal and ovarian tumors result into an altered signal transduction [7,27]. In any case, such studies are now easily to perform and may expand our knowledge about the pathophysiology of type II diabetes.

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