Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences

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Abstract Glucagon-like peptide-I(GLP-I), encoded by the glucagon gene and released from the gut in response to nutrients, is a potent stimulator of glucose-induced insulin secretion. In human subjects GLP-I exerts its physiological effect as an incretin. The incretin effect of GLP-I is preserved in patients with Type II diabetes mellitus (NIDDM), suggesting that GLP-I receptor agonist can be used therapeutically in this group of patients. In these studies we addressed the question of whether GLP-I has broader actions in human physiology. To investigate this issue we examined the tissue distribution of GLP-I receptor using RNAse protection assay in order to avoid the cross-reactivities with structurally related receptors and to increase the sensitivity of detection. The riboprobe was synthesized from the human pancreatic GLP-I receptor cDNA and used in hybridization experiments with total RNA isolated from different human tissues. In addition to the pancreas, we found expression of GLP-I receptor mRNA in lung, brain, kidney, stomach and heart. Peripheral tissues which are the major sites of glucose turnover, such as liver, skeletal muscle and adipose did not express the pancreatic form of the GLP-I receptor. We also cloned and sequenced GLP-I receptor cDNA from human brain and heart. The deduced amino acid sequences are the same as the sequence found in the pancreas. These results indicate that GLP-I might have effects beyond the pancreas, including the cardiovascular and central nervous systems where a receptor with the same ligand binding specificity is found.

Key words: Incretin; Type II diabetes mellitus; GLP-I receptor; RNase protection assay

1. Introduction

Glucagon-like peptide-I (GLP-I) is one of the most potent insulin secretagogues described to date. Its existence and structure were deduced from the nucleotide sequence of the glucagon gene [1–4]. There is a single mammalian gene and identical preproglucagons are expressed in the intestine and the pancreas [5,6]. The amino acid sequence of GLP-I shows structural homology to a number of metabolic peptides and neurotransmitters, such as glucagon, secretin, gastric inhibitory polypeptide (GIP), growth hormone releasing hormone (GHRH) and vasoactive intestinal polypeptide (VIP). At least three molecular forms of the peptide are released predominantly in the intestine following tissue specific posttranslational processing: a 37-amino acid peptide GLP-I(1–37), a 31-amino acid GLP-I (7–37) and a 30-amino acid peptide with an amidated carboxyl terminal termed GLP-I(7–36)amide [7]. No physiological function for the longer GLP-I(1–37) have yet been found. The shorter forms of the peptide, GLP-I(7–37) and GLP-I(7–36) amide, are indistinguishable in their ability to stimulate insulin secretion from the perfused rat pancreas [8], isolated islets maintained in cell culture [9,10], healthy human subjects and patients with Type II diabetes mellitus (NIDDM)[11–14]. The dose of GLP-I(7–37) and/or GLP-I(7–36)amide required to trigger insulin secretion is physiologic and glucose dependent [8]. In normal human subjects the shorter forms of GLP-I also suppressed circulating glucagon and somatostatin levels [12,13]. These results provided further experimental evidence for the postulated role of the enteroinsular axis in glucose homeostasis [15]. They also established the essential role of incretins [16], or substances secreted in the gut in response to nutrients, in the regulation of secretions of the pancreatic islet hormones. Administration of GLP-I(7–37) and GLP-I(7–36)amide together with a meal in patients with Type II diabetes mellitus (NIDDM) eliminated the postpandrial rise in blood glucose [11,12]. Most recently, it was found that the incretin effect of the shorter form of GLP-I was preserved in patients with Type II diabetes mellitus [13]. The results from the clinical studies taken together suggested that GLP-I(7–37) and/or GLP-I(7–36)amide receptor agonists might be therapeutically useful in this group of patients.

In addition to its metabolic effects on the pancreas, GLP-I(7–36)amide has other physiological functions. For example, it was recently reported that in rodent species GLP-I(7–36) amide can stimulate the secretions of macromolecules from lung airways and can produce relaxation of pulmonary artery [17]. The localization of GLP-I binding sites on airway gland cells, as well as on smooth muscle cells in the pulmonary artery is consistent with these observations [17]. The brain is another potential target tissue for GLP-I action. Both preproglucagon, the biosynthetic precursor of GLP-I peptides, and [^{125}I]GLP-I binding sites have been detected in various regions of mouse and rat brain, respectively [18–21]. On the basis of these observations it might be postulated that, like a number of other peptides secreted in the gut, GLP-I could also be considered to be a neurotransmitter. This hypothesis raises the question of whether the metabolic effects of GLP-I and its effects in the central nervous system are regulated by similar signal transduction pathways. To approach this question in a systematic manner we investigated the distribution of the GLP-I receptor in human tissues using the pancreatic form of the human GLP-I receptor cDNA as a molecular probe.

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Abbreviations: GLP-I, insulin releasing forms of the peptide (GLP-I(7–37) and GLP-I(7–36)amide); PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction.

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Here we report GLP-I receptor expression in human pancreas, lung, brain, stomach, kidney and heart. We did not detect the expression of GLP-I receptor in human liver, muscle and adipose tissues. We also show that GLP-I receptors expressed in pancreas, heart and brain have identical deduced amino acid sequences.

2. Experimental

2.1. Materials

Human total RNA's were obtained from commercial sources (Clontech, Palo Alto, CA) except for the human adipose tissue total RNA which was kindly provided by Dr. Susan Fried. Human glyceraldehyde 3-phosphate dehydrogenase (HGPDH) cDNA was purchased from Clontech and human glucose transporter (GLUT)4 cDNA was a generous gift of Dr. G. Bell.

2.2. Cloning of the human pancreatic GLP-I receptor

1.8×10^6 plaques from a human pancreatic cDNA library in λ-gt11 vector (Clontech, Palo Alto, CA) were screened with a full length GLP-I receptor cDNA cloned from the RIN 1046–38 cell line [22]. Positive clones with size greater than 1.5 kb were cloned into pBluescript II KS(−) vector (Stratagene, La Jolla, CA) and sequenced with Sequenase (U.S. Biochemical, Cleveland, OH). From this library we isolated a single positive clone that contained the entire sequence of the human GLP-I receptor cDNA (HPC10). The nucleotide sequence of HPC10 has been deposited in the Gene Bank (accession number U10037).

2.3. Synthesis of a GLP-I receptor RNA probe

RNA probes were synthesized as described [23]. Briefly, a 449 bp fragment was obtained from the 1.59 kb human GLP-I receptor cDNA (HPC10) by digestion with *Eco*RI and *Stu*I. This fragment contained 125 bp from the 5′-untranslated region and 324 bp of the 5′-coding sequence of GLP-I receptor cDNA. It was subcloned into pBluescript II KS(−) vector, and was further digested with *Bam*HI which cleaves at position 282. The resulting linear DNA template (1 μg) was then transcribed using standard published procedures [24].

2.4. Synthesis of HG3DH and GLUT4 RNA probes

Human glyceraldehyde 3-phosphate dehydrogenase (HG3DH) and human glucose transporter (GLUT4) RNA probes were prepared as positive controls for the RNase protection assay.

(i) HG3DH fragment was obtained by restriction enzyme digest at the *Hin*dIII site at positions 1 and 256 in the HG3DH cDNA [25], and it was subcloned into pBluescript KS(−) vector. Linear DNA was obtained by restriction enzyme digest with *Xba*I which cleaves at a site located in the vector at position immediately preceding the 5′-end of HG3DH.

(ii) A fragment of GLUT4 was obtained by restriction enzyme digest with *Apa*I which cleaves at positions 391 and 1485 of human GLUT4 cDNA [26]. This fragment was subcloned into pBluescript KS(−) vector and further digested with *Hin*dIII which cleaves at position 1141. The resulting DNA fragment was used as a template for the synthesis of a riboprobe.

The HG3DH and GLUT4 RNA probes were synthesized as described in [24].

2.5. RNase protection assay

In a typical experiment 15 μg of total RNA from lung, pancreas, brain, kidney, liver, stomach, muscle and adipose tissues (or yeast tRNA used as a control in every experiment) was mixed with 5×10^5 cpm of probe in a final volume of 40 μl containing 1 × PIPES (40 mM PIPES pH 6.7, 400 mM NaCl, 1 mM EDTA pH 8.0), and 50% formamide. The reaction mixture was heated at 80°C for 5 min and then hybridized at 55°C overnight. Following hybridization, the reaction mixture was incubated at 37°C for 15 min with 300 μl of 10 mM Tris (pH 7.5), 5 mM EDTA (pH 8.0), 300 mM NaCl, RNase (20 μg/ml) and RNase T1 (100 U/μl). 10% SDS (20 μl) and Proteinase K (50 μl of 1 mg/ml) were added for 15 min at 37°C. The solution was extracted with an equal volume of phenol/$CHCl_3$, and precipitated with ethanol (850 μl). The pellets were collected by centrifugation and lyophilized to dryness. They were dissolved in formamide gel dye (4 μl), heated at 100°C for 3 min and run on a standard sequencing gel. Results were visualized by exposing the gel to an X-ray film, first overnight and then for three weeks. The same protocol was used with 60 μg of total RNA from lung, pancreas, brain, kidney, liver, stomach and heart tissues. All the hybridizations were performed at least twice in different experiments using newly synthesized RNA probes.

For some tissues different lots of the commercially obtained total RNA's were used. Adipose tissue total RNA was obtained from tissue samples excised from different anatomical sites. In all hybridizations experiments with the adipose tissue we used GLUT4 RNA probe as a positive control. Reproducible results were obtained in the multiple experiments with all tissues.

2.6. RT-PCR of the GLP-I receptor cDNA from human brain and heart

Sequence specific primers used to amplify human brain and heart GLP-I receptor cDNA were designed on the basis of the nucleotide sequence of the human pancreatic GLP-I receptor cDNA (clone HPC10). Primers HP126 (5′-CCATGGCCGGCGCCCCCGG-3′) and HP772 (5′-CATCCCACTGGTGCTGCT-3′) were used to amplify the 5′ region of the brain GLP-I receptor cDNA for the first round of PCR and HP126 and HP688 (5′-TGAAGGATGCAAACAGGTTCAG-3′) for the second round of PCR (Fig. 3, panel A(b and c)). From these reactions we obtained the sequence from position 126 to position 688 of the brain GLP-I receptor cDNA. To amplify the sequence of GLP-I receptor cDNA from position 266 to position 772 we used primers HP266 (5′-AGCGCTCCCTGACTGAGGAT-3′) (Fig. 3, panel A(d)) and HP772, and for the 3′-end from position 696 to 1568 we used primers HP696 (5′-AGCATTGTCCGTCTTCAT-3′) and HP1568 (5′-TTGGCCACCCGGCTGCAGCAA-3′) (Fig. 3, panel A(e)).

To amplify GLP-I receptor cDNA from human heart we used two sets of primers: (i) HP126 and HP772 (Fig. 3, panel A(b)) and (ii) HP696 and HP1568 (Fig. 3, panel A(e)).

RT-PCR was performed with 200 units of SuperScript II reverse transcriptase according to the specifications supplied by Life Technologies, Inc. (Gaithersburg, MD).

Thirty-six cycles of PCR were carried out at 94°C for 40 s, 56°C for 50 s and 72°C for 90 s. These conditions were applied for the amplification of the fragments of the brain (Fig. 3, panel A(d and e)) and heart (Fig. 3, panel A(b and e)) GLP-I receptor cDNA sequences.

To amplify the 5′-region of the human brain GLP-I receptor cDNA primers HP126 and HP772 (Fig. 3, panel A(b)) were first used for 36 cycles as described above. The amplified DNA was separated from free nucleotides and primers on a GlassMAX DNA isolation Spin Cartridge (Gibco-BRL, Gaithersburg, MD), and then reamplified by another nested set of primers HP126 and HP688 (Fig. 3, panel A(c)). PCR results were analyzed by electrophoresis through a 1% agarose gel. The amplified DNA was ligated into pCR vector (In Vitrogen, San Diego, CA), using T4 DNA ligase under conditions recommended by the manufacturer. They were sequenced on double-stranded DNA by the chain termination technique with Sequenase (U.S. Biochemical, Cleveland, OH).

3. Results

3.1. Tissue distribution of the human GLP-I receptor cDNA

RNase protection was used to identify tissues in which GLP-I receptor is expressed. We applied this method instead of Northern blot analysis in order to improve the sensitivity of detection, and to eliminate possible cross-reactivities with other structurally related receptors [23]. For the RNase protection assay we synthesized an RNA fragment designed to protect a 168 bp segment of the human GLP-I receptor mRNA transcript. This hybridization probe was derived from the region of the pancreatic human GLP-I receptor cDNA sequence that encodes the extracellular, putative ligand binding domain of the receptor, and contains the least structural homologies with the other related receptors. In the first round of experiments we included in addition to total RNA from human pancreas, total RNA's from human, lung, brain, stomach, kidney and liver. We used in the hybridization experiments 60 μg of total RNA

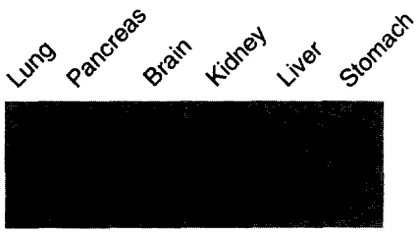

Fig. 1. RNase protection assay. A riboprobe synthesized from the human pancreatic GLP-I receptor cDNA was hybridized with total RNA (60 ug) isolated from human lung, pancreas, brain, kidney, stomach and liver. The molecular size of the RNA : RNA hybrids was determined by gel electrophoresis and visualized by autoradiography after exposure to an X-ray film for 21 days.

isolated from these tissues to establish limits of detection of the GLP-I receptor mRNA transcripts. As seen from Fig. 1, we detected the expected protected fragment in the samples of total RNA from human pancreas, as well as total RNA's from human lung, brain, stomach and kidney. There was no band in the sample of total RNA from human liver. The protected band appeared as a doublet which is a common artifact in RNase protection assays usually related to the redundancy at the ends of the probe [23]. In all the experiments we used a tRNA as a negative control (data not shown). The same protected fragment was also detected when 15 μg of total RNA isolated from these tissues was used in the hybridization experiments. As positive controls for the quality of the human brain, lung, stomach, kidney and liver total RNA's we used as a hybridization probe an antisense RNA fragment which was synthesized from human glyceraldehyde 3-phosphate dehydrogenase cDNAs (see section 2). All samples contained appropriate levels of HG3DH mRNA (data not shown).

The absence of GLP-I receptor mRNA transcripts from human liver is in agreement with our earlier experiments in which we demonstrated that GLP-I(7–37) did not have an effect on gluconeogenesis and cAMP levels in rat hepatocytes and did not antagonize the gluconeogenic action of glucagon [27].

On the basis of these findings we decided to extend our experiments and to determine whether other proposed target tissues for GLP-I action such as human adipose and muscle [12] also express GLP-I receptor mRNA. In these experiments we also included total RNA isolated from human heart. As positive controls in this set of experiments we used an RNA fragment synthesized from human glucose transporter 4 (GLUT4) cDNA [26] and total RNA from pancreas. Results shown in Fig. 2, panel A, demonstrate that only heart tissue was positive for GLP-I receptor expression, whereas GLUT 4 was detected in all three tissues (Fig. 2, panel B).

3.2. *Cloning and sequencing of the GLP-I receptor cDNA from human brain and heart*

Our initial intention was to amplify the full length GLP-I receptor cDNA, and for that purpose we used two primers: HP126 that corresponds to the sequence of the 5′-untranslated end and beginning of the coding sequence, and HP1568 that corresponds to the end of the coding sequence followed by the 3′ untranslated region of the pancreatic GLP-I receptor cDNA (Fig. 3, panel A(a)). However, we were unable to amplify any fragments using this set of primers (data not shown). These results are consistent with our earlier experiments (Y. Wei, and S. Mojsov, unpublished results) in which we could not amplify full length GLP-I receptor cDNA from the cDNA prepared from RIN 1046–38 cell line using primers corresponding to the sequence of the 5′ and 3′ ends of the rat islet GLP-I receptor cDNA. For these reasons we decided to use primers that will amplify smaller fragments of the brain and heart GLP-I receptor cDNA with overlapping sequences (Fig. 3, panel A(b) through (e)). Using these sets of primers we were able to amplify the expected fragments (0.56 kb, 0.57 kb and 0.87 kb, respectively) of GLP-I receptor cDNA from human brain (Fig. 3, panel B, lanes (C)–(E)) and heart (0.65 kb and 0.87 kb) as shown in Fig. 3, panel C, lanes (B) and (E).

The nucleotide sequence of the DNA fragments cloned from human brain corresponded to the sequence of the human pancreatic GLP-I receptor cDNA, with the exception of few single nucleotide substitutions in the following locations of the sequence: (i) 502, a change of G to A; (ii) 526, A to C; (iii) 780, A to C; (iv) 871, T to C and (v) 1270, C to T. These differences between the pancreatic and brain GLP-I receptor cDNA's found at the level of single nucleotides are most likely caused by copying errors of Taq polymerase [29] and do not represent actual differences. In a similar manner, the nucleotide sequence of GLP-I receptor cDNA from human heart contained a single nucleotide change in position 788 of T to A. These changes in the coding triplets are reflected in differences in the deduced amino acid sequences between the pancreatic form of GLP-I receptor and the its brain and heart forms (Fig. 4).

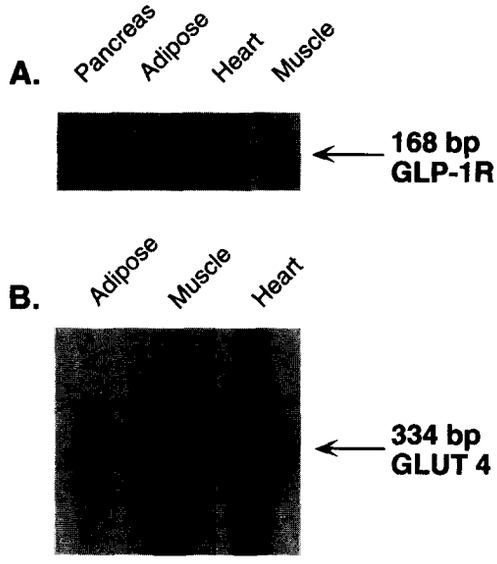

Fig. 2. RNase protection assay. Panel (A) A riboprobe synthesized from human pancreatic GLP-I receptor cDNA was hybridized with total RNA isolated from human pancreas, muscle, adipose (15 μg) and heart (60 μg). Panel (B) A riboprobe synthesized from human GLUT4 cDNA [25] was hybridized with total RNA prepared from human adipose, muscle and heart (15 μg). The experimental conditions of the assay were the same as described in legend to Fig. 1 and in section 2. The arrows indicate the expected molecular size of the protected fragments of human GLP-I receptor (panel A) and human GLUT4 mRNA transcripts (panel B).

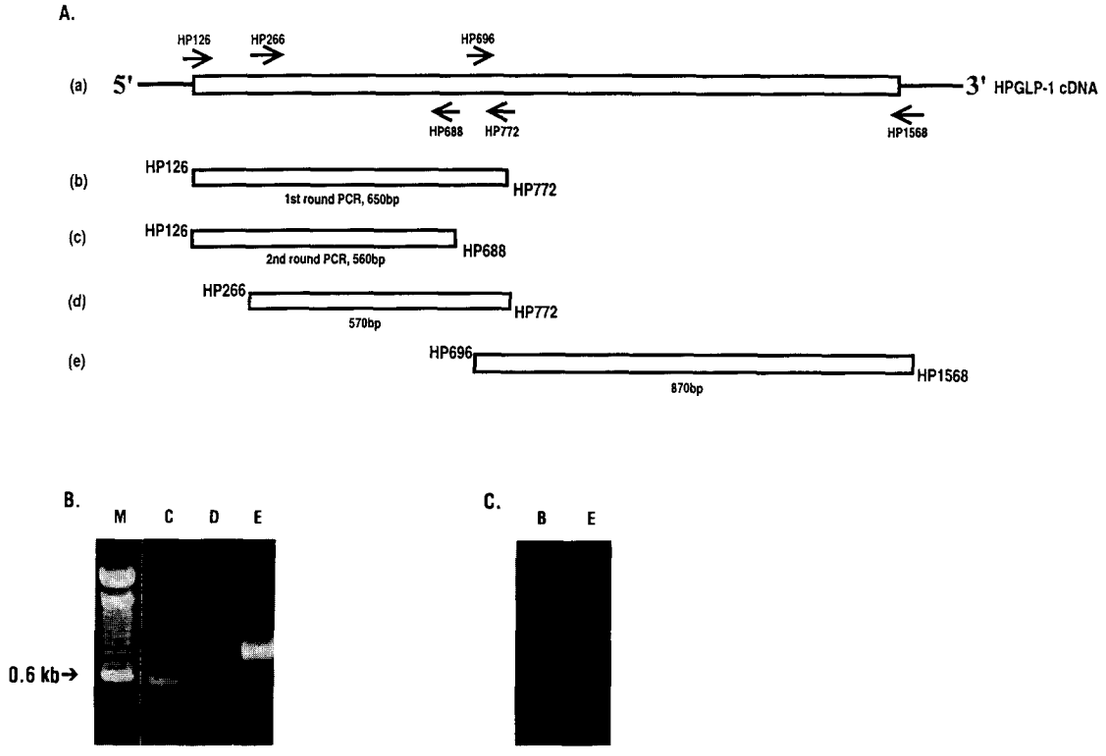

Fig. 3. RT-PCR analysis of GLP-I receptor expression in human brain and heart. Panel A, schematic representation of (a) human pancreatic GLP-I receptor cDNA sequence (clone HPC10) and the position of the oligonucleotide primers used for the PCR amplification of the brain and heart GLP-I receptor cDNA; (b) through (e) expected size of the amplified fragments of the brain and heart GLP-I receptor cDNA. Open box represents the coding sequence. Numbers on top and bottom of the arrows represent the nucleotide position in the sequence of the pancreatic human GLP-I receptor cDNA (clone HPC10). Panels B and C, detection of the amplified fragments of brain and heart GLP-I receptor cDNA by agarose gel electrophoresis. Panel B, lane M, markers, lanes (C)–(E), the molecular weight of the amplified fragments of brain GLP-I receptor cDNA. Panel C, lanes (B) and (E) the molecular weight of the amplified fragments of heart GLP-I receptor cDNA. Detected fragments (C)–(E) in Panel B and (B) and (E) in panel C correspond to the expected fragments represented in (c)–(e) in Panel A.

4. Discussion

In our studies we utilized the RNase protection assay in order to increase the sensitivity of detection of GLP-I receptor mRNA transcripts and to eliminate the cross-reactivities with structurally related receptors. The results show that GLP-I receptor is expressed not only in human pancreas, but also in lung, brain, stomach, kidney and heart. They extend the previous findings which detected by Northern blot analysis the expression of GLP-I receptor mRNA transcripts only in human pancreas [30]. Our results are also in agreement with the findings of GLP-I receptor expression in rat lung, brain, stomach and kidney [28,31] and further indicate that GLP-I in human and rodent species has similar if not identical biological effects. The detection of GLP-I receptor in human heart is a novel observation and its physiological function in this organ needs to be elucidated.

Our findings suggest that in humans the shorter forms of GLP-I might have more diverse physiological functions than previously described, and are of immediate interest in view of the ongoing efforts to develop therapeutic agents based on GLP-I structure for treatment of disorders of glucose metabolism manifested in patients with Type II diabetes mellitus.

In our experiments we were able to detect GLP-I receptor mRNA transcripts in total RNA from human pancreas, where islet cell population that expresses GLP-I receptor is present in about 1–2% of the tissue. The increased sensitivity of RNase protection assay utilized in our studies was also confirmed in experiments in which we were not able to detect GLP-I receptor mRNA transcripts in human heart by Northern blot analysis (data not shown). Nonetheless, we did not find expression of the GLP-I receptor in tissues which are the major sites of glucose turnover, such as liver, skeletal muscle, and adipose. In contrast to these findings, other investigators have reported metabolic effects of GLP-I in rat adipocytes and rat hepatocytes [32–34]. In addition, pharmacological doses of GLP-I(7–36)amide increased glucose utilization in both healthy human subjects [35] and patients with insulin dependent diabetes mellitus (IDDM) [12]. These differences can be a result of one or more factors. The first one, is that the effects of GLP-I in peripheral tissues are mediated through a GLP-I receptor subtype which is different from the pancreatic form of the receptor. The second, and not mutually exclusive one, is that GLP-I can stimulate other gut derived substances. This could be accomplished through an enteroendocrine loop in the intestine in a similar manner as described recently for GIP stimulation of GLP-I secretions in rodent species [36]. Finally, we can not exclude the possibility that we were unable to detect the expression of GLP-I receptor in specialized compartments that make up less than 1% of the cell population in human liver, muscle

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                .         .         .         .         .
HPGLP-1    1  MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSL 50
HBGLP-1    1  MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSL 50
HHGLP-1    1  MAGAPGPLRLALLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSL 50
                .         .         .         .         .
HPGLP-1   51  TEDPPPATDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHV 100
HBGLP-1   51  TEDPPPATDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHV 100
HHGLP-1   51  TEDPPPATDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHV 100
                .         .         .         .      ______
HPGLP-1  101  YRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTV 150
HBGLP-1  101  YRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTV 150
HHGLP-1  101  YRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLYIIYTV 150
              ____I_____________  .      __________II__________  .
HPGLP-1  151  GYALSFSALVIASAILLGFRHLHCTRNYIHLNLFASFILRALSVFIKDAA 200
HBGLP-1  151  GYALSFSALVIASAILLSFRHLHCTRNYIHLNLFASFILRALSVFIKDAA 200
HHGLP-1  151  GYALSFSALVIASAILLGFRHLHCTRNYIHLNLFASFILRALSVFIKDAA 200
                .         .      _____________III__________
HPGLP-1  201  LKWMYSTAAQQHQWDGLLSYLDSLSCRLVFLLMQYCVAANYYWLLVEGVY 250
HBGLP-1  201  LKWMYSTAAQQHQWDGLLSYLDSLSCRLVFLLMQYCVAANYYWLLVEGVY 250
HHGLP-1  201  LKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVY 250
              __              ___________IV___________  .      .
HPGLP-1  251  LYTLLAFSVLSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRN 300
HBGLP-1  251  LYTLLAFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLHEDEGCWTRN 300
HHGLP-1  251  LYTLLAFSVLSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRN 300
                ___________V_____________         .      .
HPGLP-1  301  SNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLA 350
HBGLP-1  301  SNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLA 350
HHGLP-1  301  SNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLA 350
               ________VI____________      .      ___________VII
HPGLP-1  351  KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAI 400
HBGLP-1  351  KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAI 400
HHGLP-1  351  KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAI 400
              _________  .         .         .         .         .
HPGLP-1  401  LYCFVNNEVQLEFRKSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAG 450
HBGLP-1  401  LYCFVNNEVQLEFRKSWERWRLEYLHIQRDSSMKPLKCPTSSLSSGATAG 450
HHGLP-1  401  LYCFVNNEVQLEFRKSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAG 450
                .
HPGLP-1  451  SSMYTATCQASCS                                      464
HBGLP-1  451  SSMYTATCQASCS                                      464
HHGLP-1  451  SSMYTATCQASCS                                      464
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Fig. 4. Composite deduced amino acid sequence of the human brain (HBGLP-I) and heart (HHGLP-I) GLP-I receptor and comparison with deduced amino acid sequence of the human pancreatic GLP-I receptor (HPGLP-I), clone HPC10. The lines above the sequence designated I through VII represent the putative membrane spanning domains. The amino acid differences between the pancreatic, brain and heart forms of the GLP-I receptors are indicated by the bold letters. They are most likely caused by errors in nucleotide incorporation of Taq polymerase during PCR amplification (see section 3 and ref. 27).

and adipose tissues. However, it is highly unlikely that the peripheral effects of GLP-I would be mediated through a GLP-I receptor which is present only in a very small subgroup of cells in this tissues.

The sequence of the GLP-I receptor is homologous to the sequences of the newly identified family of G-protein coupled receptors for several endocrine peptides, such as glucagon, secretin, growth hormone releasing hormone (GHRH), vasoactive intestinal peptide (VIP), calcitonin and parathyroid hormone (PTH) [37]. The highest sequence similarities are found in the second and sixth membrane spanning domains, while there is very little sequence conservation in the their extracellular domains. For these reason it has been postulated that they represents the ligand binding domains. The riboprobe used in our studies was specific for the putative ligand binding domain of the GLP-I receptor and protected the same fragment of the receptor in all tissues. Thus, we can postulate that different human tissues express GLP-I receptor with similar if not identical ligand binding specificity. To gain further experimental evidence in support of this conclusion we cloned and sequenced the GLP-I receptor cDNA from human brain and heart. The deduced amino acid sequence of the brain GLP-I receptor contained four amino acids that were different from the pancreatic form of the receptor, while in the heart the difference was in a single amino acid. However, these changes, most likely, do not represent actual differences in the pancreatic, brain and heart GLP-I receptor sequences, and are produced during the PCR amplification of the brain and heart GLP-I receptor cDNA. The recent cloning of GLP-I receptor cDNA from rat lung which is identical to the rat pancreatic GLP-I receptor cDNA [38] provides additional evidence in support of our conclusions.

Our finding that GLP-I receptor in human pancreas, heart and brain have the same amino acid sequences will provide a basis for further investigation of the mechanism of interaction of the GLP-I receptor with distinct signal transduction path-

ways present in these tissues. This knowledge is essential for our understanding of the physiological role of GLP-I peptides in such diverse organs as the human heart and brain.

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