0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 7
Printed in U.S.A.

Tissue Distribution of Messenger Ribonucleic Acid Encoding the Rat Glucagon-Like Peptide-1 Receptor

BRYANT P. BULLOCK*, R. SCOTT HELLER*, AND JOEL F. HABENER

Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02114

ABSTRACT

The incretin hormone glucagon-like peptide-1 (GLP-1) is an important regulator of postprandial insulin secretion. In addition to its insulinotropic actions on pancreatic β-cells, GLP-1 enhances glucose disposal by insulin-independent mechanisms, suggesting that GLP-1 receptors are located on extrapancreatic tissues. In this study, we examined the tissue distribution of GLP-1 receptor (GLP-1R) messenger RNA (mRNA) in rat by RNAse protection, RT-PCR, and *in situ* hybridization. We identified GLP-1R mRNA in the lung, pancreatic islets, stomach, and kidney by the RNAse protection assay. RT-PCR analysis also detected GLP-1R mRNA in the hypothalamus and heart. *In situ* hybridization experiments identified receptor mRNA in the gastric pits of the stomach, large nucleated cells in the lung, crypts of the duodenum, and pancreatic islets. No localized specific grains were found in kidney, skeletal muscle, heart, liver, or adipocytes. These results indicate that sequences corresponding to the cloned rat islet GLP-1 receptor are expressed in the pancreatic islets, lung, hypothalamus, stomach, heart, and kidney but not in adipose, liver, and skeletal muscle. Further, the GLP-1 receptor expressed in the kidney and heart may be structural variants of the known receptor. Therefore, the observed extrapancreatic actions of GLP-1 may not be strictly confined to interactions with the defined GLP-1 receptor. (*Endocrinology* **137:** 2968–2978, 1996)

THE INCRETIN hormone glucagon-like peptide-1 (GLP-1) is a major regulator in the entero-insular axis that is critical for controlling postprandial insulin secretion (1, 2). GLP-1 (7–36) amide, a posttranslational cleavage product of proglucagon, is secreted by the intestinal L-cells after oral ingestion of glucose or fat (3). GLP-1 acts through a G protein-coupled cell surface receptor (GLP-1R) and modulates insulin release from pancreatic β-cell in a glucose-dependent manner (4, 5). GLP-1 also markedly increases the biosynthetic levels of insulin in β-cells and thus is a potent insulinotropic hormone (6). GLP-1 stimulates insulin secretion and lowers postprandial hyperglycemia in diabetic subjects and therefore is a promising therapeutic agent for the treatment of noninsulin-dependent diabetes (7–9).

Evidence suggests that GLP-1 acting outside of the pancreas may be important for regulating glucose metabolism. Clinical studies have shown that GLP-1 (7–36) amide significantly reduces isoglycemic insulin requirements in both diabetic and normal humans (8). GLP-1 also has been shown to stimulate glucose disposal via an insulin-independent mechanism (10, 11). These results indicate that GLP-1 has important extrapancreatic effects on glucose utilization and may also be an effective therapeutic agent for insulin-dependent diabetes mellitus (8). Radioligand binding studies have localized GLP-1 receptors to a number of extrapancreatic tissues including brain (12–14), lung (14, 15), and gastric glands of the stomach (16). Interestingly, GLP-1 was shown to have physiological effects on glycogenesis in the liver (17), even though specific receptors could not be identified on isolated hepatocytes by radioligand binding (18). One potentially important site of GLP-1 action is the adipose tissue. GLP-1 has been shown to bind with high affinity to isolated rat adipocytes, activate cAMP production (19), and stimulate lipogenesis (20) or lypolysis (21). GLP-1 acting on receptors located within the skeletal muscle is another potentially important site for the regulation of glucose utilization because it is the predominant tissue for insulin resistance in diabetes mellitus (22, 23). GLP-1 has been shown to stimulate glycogen synthesis, glucose oxidation, and lactate formation in rat skeletal muscle (24). High affinity receptors were detected on skeletal muscle by the binding of radiolabeled GLP-1 (25). These experiments suggest that GLP-1 binding to its cognate receptor in extrapancreatic tissues, particularly adipose and skeletal muscle, may act as an important regulator of glucose metabolism.

The recent cloning of the rat and human GLP-1 (7–36) amide receptor cDNAs has greatly increased our knowledge of the mechanisms of action of GLP-1R (26, 27). The GLP-1 receptor cDNA encodes a 463-amino acid protein with a predicted mol wt of approximately 63K, consistent with the size determined by chemical cross-linking studies (28). It is a member of the seven transmembrane family of G protein-linked receptors, which includes the receptors for glucagon, secretin, calcitonin, vasoactive intestinal polypeptide (VIP), PTH, and pituitary adenylate cyclase activating peptide (PACAP) (29, 30). Recently, there have been numerous intriguing but conflicting reports describing the tissue-specific expression of the GLP-1R gene. GLP-1R messenger RNA (mRNA) was shown to be expressed in lung, stomach, and pancreatic islet cells; however, depending on reports, GLP-1R mRNA was also found in the brain, liver, skeletal muscle, adipose, and kidney (27, 31–33). Despite all of the

Received January 15, 1996.
Address all correspondence and requests for reprints to: Dr. Joel F. Habener, Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02114.
* The first two authors contributed equally.

detailed information known about the GLP-1R and the effect of GLP-1 on various cell lines, the actual tissue specific localization of the GLP-1R has yet to be analyzed fully.

The radioligand binding studies and the physiological effects of GLP-1 on adipose and skeletal muscle suggest that GLP-1 acts by binding to its cognate receptor, the cloned pancreatic GLP-1R, to elicit its effect. However, two other possibilities could occur to account for these reported effects: binding to an isotype of GLP-1 receptor, or cross-reactivity with a related receptor. Therefore, the purpose of this study is to determine the tissue distribution of the defined GLP-1R mRNA in rat by utilizing the sensitive and highly specific detection methods, RNAse protection, RT-PCR, and *in situ* hybridization. The search was focused to the tissues that would most likely be involved in GLP-1 regulated glucose utilization, *i.e.* liver, adipose, and muscle. Distribution of the GLP-1 receptor can provide information about the possible extrapancreatic role of GLP-1 on glucose metabolism and the physiological consequences of the use of GLP-1 as a therapeutic agent.

Materials and Methods

Preparation of RNA

Tissues were isolated from adult Spraque-Dawley rats (200–350 g), rinsed in PBS and frozen immediately in liquid nitrogen. Adipocytes and pancreatic islets were isolated from collagenase digested rat epididymal fat pads or cannulated rat pancreases, respectively (34, 35). The hypothalamus was dissected away from the whole brain by gently removing the hypothalamic wedge ventral to the optic nerves as described (36). Total RNA was isolated by the Li/Urea extraction method as previously described (37). Control cRNA was synthesized from the GLP-1R plasmid by transcribing with SP6 RNA polymerase after *Xba*I digestion as described below. RNA concentrations were determined by spectrophotometric analysis at OD 260/280 nm. RNA integrity was assessed by running 1 μg on a formaldehyde gel and staining with either ethidium bromide or Sybr Green II (Molecular Probes, Eugene, OR). The 18 and 28S ribosomal bands were visualized on the FluorImager 575 (Molecular Dynamics, Sunnyvale, CA).

RNAse protection assay

Tissue-specific RNA was hybridized with ^{32}P-labeled antisense GLP-1R riboprobes (200,000 cpm) overnight at 42 C in 30 μl hybridization buffer: 80% formamide, 40 mM piperazine-*N,N'*-bis[2-ethanesulfonic acid], pH 6.7, 0.4 M NaCl, 1 mM EDTA. Hybridized RNA was digested with nuclease P1 (20 μg/ml) and RNAse T1 (2 μg/ml) for 1 h at 25 C in 300 μl digestion buffer:10 mM Tris-HCL, pH 7.5, 5 mM EDTA, 0.3 M NaCl as previously described (37). Digestions were terminated by addition of 20 μl of 10% SDS and 50 μg proteinase K for 15 min at 37 C. After phenol/chloroform extraction and ethanol precipitation, the protected fragments were resolved on 8% sequencing gels and visualized by autoradiography. The autoradiograms were scanned with a computing densitometer and quantitated by the ImageQuant analysis program (Molecular Dynamics). The fragment sizes were determined from the known ^{32}P-labeled markers by linear regression analysis.

The 3′ specific GLP-1R riboprobe (Fig. 1) was produced by subcloning a *Xba*I-*Bam*HI fragment of the GLP-1R cDNA (27) into the *Xba*I-*Bam*HI sites of pGEM 7Z (Promega, Madison, WI). Transcription by T7 RNA polymerase after linearization by *Nci*I resulted in a probe of 616 nucleotides (nt) and protected a 555 nt fragment. Three other riboprobes spanning the GLP-1R mRNA were produced by PCR using the above GLP-1R cDNA as a template and standard techniques (see below). The E1 probe was produced using F32E (TCCTGAATTCCCCGCCATGGCCGTCACCCCCAG) and R150 (TCCGTGAGGAAACGTTGGCAC) as primers. After digestion with *Eco*RI, the PCR fragment was cloned into

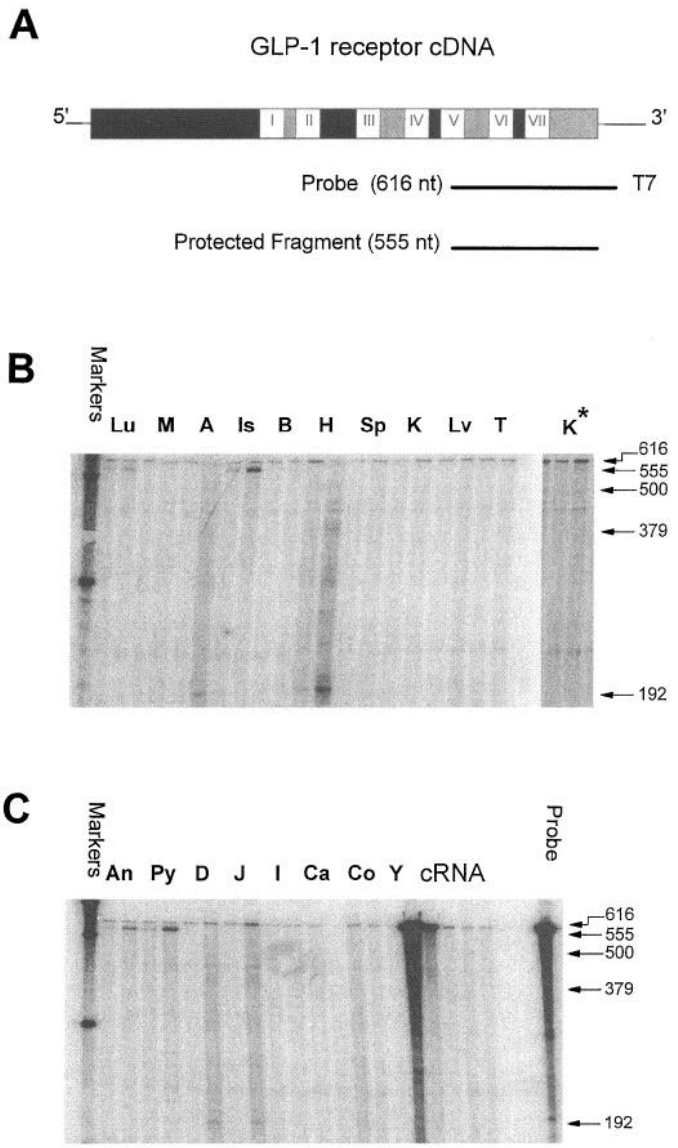

FIG. 1. Tissue distribution of GLP-1R mRNA as determined by RNAse protection. A, Schematic of the GLP-1R cDNA and 3′ riboprobe. The GLP-1R cDNA is shown with the regions that code for functional domains emphasized. *Filled boxes*, Extracellular domains. *Shaded boxes*, Intracellular domains. *Open boxes*, Transmembrane regions labeled in order from the 5′ (amino terminus) to the 3′ (carboxy terminus) ends. The 3′ riboprobe was synthesized by transcribing with T7 RNA polymerase to generate a 616-nt probe. The predicted protected fragment after hybridization and digestion would be 555 nt. B, Autoradiogram of a RNAse protection experiment in which 5 and 20 μg of total RNA, left and right lanes, respectively, from various rat tissues were hybridized with the 3′ GLP-1R riboprobe. Lu, Lung; M, skeletal muscle; A, adipose; Is, pancreatic islet; B, brain; H, heart; Sp, spleen; K, kidney; Lv, liver; and T, testes. The radiolabeled RNA markers shown are 518 and 277 nt. Autoradiograms were exposed for 10 days. The longer exposure (30 days) of the kidney lanes are shown as K*. C, Autoradiogram of a similar RNAse protection of various rat gastrointestinal tissues. Five and 20 μg of total RNA were tested for each tissue, left and right lanes, respectively, as described in Fig. 1B. An, Antrum of the stomach; Py, pylorus of the stomach; D, duodenum; J, jejunum; I, ileum, Ca, caecum; Co, colon; and Y, yeast, as control RNA. The control cRNA lanes contain 1000, 100, 10, 1, and 0.1 pg of GLP-1R cRNA (*left to right*). Undigested probe of 616 nt is indicated.

the *Eco*RI-*Sma*I sites of pGEM 7Z. Transcription by SP6 RNA polymerase after linearization by *Xba*I resulted in a probe of 241 nt and protected a fragment of 162 nt. The T2 probe was produced using two primers, F679E (GCGAATTCCTCTCTGGGCTGCCGACTG) and R972B (GCGGATCCACCCCGATTGCAAAGAGAA), whereas the TI2 probe was produced using F1130E (GCGAATTCCGAGCACGCCCGAGGAACC) and R1330B (CCGGATCCACTGCTGACGCTGCTGGTG) as primers. After digestion with *Eco*RI and *Bam*HI, both the T2 and TI2 fragments were cloned into the *Eco*RI-*Bam*HI sites of pGEM 7Z. Transcription by SP6 RNA polymerase after linearization by *Xba*I resulted in probes of 348 and 279 nt and protected 291 and 222 nt fragments for T2 and TI2, respectively.

RT-PCR

To produce cDNA for PCR analysis, 1 μg RNA was annealed in a 20 μl reaction containing 50 mM Tris-HCl, pH 8.3, 75 mM KCl, and 3 mM $MgCl_2$ with either 0.16 U random hexamers (Boehringer Mannheim, Indianapolis, IN) or 2 μg oligo(deoxythymidine)$_{16}$ primers at 95 C for 5 min and slow cooled to 37 C over 30 min. Five hundred Units of Superscript II RT (GIBCO BRL, Gaithersburg, MD) were added and incubated at 46 C for 60 min. The reaction contents were treated with 10 μg RNase A for 15 min at 37 C, phenol/cholorform extracted, ethanol precipitated and brought up to a final volume of 25 μl with distilled H_2O.

PCR was performed in a 50 μl volume containing 1 μl of tissue specific cDNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 2 mM deoxynucleotide triphosphates, 20 pmol of primers, 10% glycerol and 2.5 U AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The PCR conditions were: 95 C 5 min followed by 30 to 60 cycles of 94 C 1 min, 55 C 2 min, and 72 C for 3 min. Five microliters of PCR products were analyzed on 1.5% agarose gels and stained with Sybr Green I dye (Molecular Probes). All manipulations were performed in a Perkin and Elmer 9600 Thermocycler. The PCR products were transferred to Magna NT nylon membranes (Micron Separations Inc., Westboro, MA) and probed with three GLP-1R ^{32}P labeled oligonucleotides, either R1220 (CCTCATTGTTGACAAAGCAGT), R1330B (CCGGATCCACTGCTGACGCTGCTGGTG), or R1412X (GGACCATCTAGAAGCGCAGCACTG) by the technique of Southern (38).

Reverse transcribed GLP-1R mRNA was analyzed by using F1218 (GCTTTATGGTGGCTGTCTTGTACT) as the 3′ primers and R1728 (TTGCAGCTTTAACAGGAAGAAGC) as the 5′ primer resulting in a product of 553 bp. Control CREB mRNA was detected in the same cDNA samples by using F496 (GCTTTATGGTGGCTGTCTTGTACT) as the 3′ primer and R1118 (CGCGGATCCCAAATTAATCTGATT) as the 5′ primer, which produces a product of 627 bp. The PCR parameters were determined by the Oligo 4.0 analysis program (National Biosciences, Inc., Plymouth, MN).

In situ hybridization

Tissues were harvested from adult Spraque-Dawley rats and fixed at 4 C in 4% paraformaldehyde in 0.1 M PBS for 18 h or in Optiprobe tissue fixative (Oncor, Gaithersburg, MD) for 72 h. Tissues were rinsed in PBS and placed in 30% sucrose in PBS at 4 C for 2 h before OCT embedding (Miles Scientific, Naperville, IL) and freezing. Five to seven micron sections were cut on a cytostat, mounted on silanized glass slides, and stored at -80 C until use.

Adipocytes were isolated from epididymal fat pads from male Spraque-Dawley rats as described above and attached to microscope slides by using a slide centrifuge. Cells were immediately fixed in 4% paraformaldehyde for 30 min at room temperature, rinsed in diethylpyrocarbonate (DEPC)-treated PBS and DEPC-treated water, air dried, and stored at -80 C until use.

In situ hybridizations were performed as previously described (39). Briefly, slides were postfixed for 5 min in 4% paraformaldehyde on ice, rinsed in 0.1 M triethanolamine for 10 min followed by quick rinses in 0.25% acetic anhydride and 1 × PBS, dehydrated through 70, 90, and 100% ethanol series, and air dried. Slides were prehybridized by layering the slides with 100 μl of hybridization solution (50% formamide, 10% dextran sulfate, 0.3 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.2% BSA, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.5 mg/ml, and 10 mM DTT) and incubated for 3 h at 56 C, followed by 3 rinses in 2 × standard sodium citrate (SSC) and an alcohol dehydration series. One hundred microliters of hybridization solution were then added that contained 5 pmol of ^{33}P-riboprobes and then hybridized for 18–24 h at 56 C. After hybridization the slides were rinsed in 2 × SSC, 1 × SSC, 0.1 × SSC and 0.1 × SSC at 65 C for 1 h, ethanol dehydrated, air dried, and coated with photographic emulsion (Kodak NKB2, Eastman-Kodak, Rochester, NY). After exposing for 1–3 weeks the photographic emulsion was developed for 2 min in Kodak D-19 developer, rinsed in distilled water, and fixed for 5 min.

The GLP-1R antisense riboprobe used for *in situ* hybridization was the 3′ specific riboprobe described above. The sense GLP-1R riboprobe was produced by transcribing the same plasmid with SP6 RNA polymerase after digestion with *Xba*I. The GLUT-4 antisense riboprobe was synthesized by transcribing the mG4 plasmid (40) with T7 RNA polymerase after *Bam*HI digestion. Transcription of the rat insulin cDNA plasmid (41) with SP6 RNA polymerase after digestion with *Xba*I produced an antisense riboprobe specific for the rat insulin mRNA.

Graphical presentation

The images from Sybr green I stained agarose gels and scanned autoradiographs were processed using ImageQuant software (Molecular Dynamics) and converted to tagged image file format (TIFF). The images of the *in situ* hybridization experiments were directly accessed with an Optronics TEC-470 CCD camera (Optronics Engineering, Goleta, CA) attached to a Zeis Epifluorescence microscope (Carl Zeis, Inc., Oberkochen, Germany). After capturing the images using the IP Lab Spectrum analysis program (Signal Analytics Corp., Vienna, VA), the images were converted from color to 8 bit gray-scale and transferred as TIFF files. The TIFF images were further enhanced using the Aldus Photostyler 2.0 (Aldus Corp., Seattle, WA) and transferred to Lotus Freelance Graphics 2.0 for final documentation (Lotus Development Corp., Cambridge, MA). All the original images are available upon request.

Results

GLP-1R mRNA is detected in lung, islet, and stomach by RNAse protection

To examine the tissue distribution of GLP-1R mRNA, 5 and 20 μg of total RNA from a variety of rat tissues were analyzed by RNAse protection. A riboprobe was designed to hybridize specifically to the 3′ end of the receptor mRNA giving a 555-bp protected fragment (Fig. 1A). As shown in Fig. 1, B and C, the correct size protected fragment was found in lung, pancreatic islets, the antrum and pylorus of the stomach, and with a longer exposure also in the kidney (see Fig. 1B, *inset*). However, full length products were not found in any of the other tissues examined even after prolonged exposures. Because the detection limit of this assay is linear to 1 pg (see cRNA controls), GLP-1R mRNA would be expressed at extremely low levels in tissues lacking a signal (see *Discussion*). Interestingly, smaller fragments of 500, 379, and 192 nt were seen in skeletal muscle, heart, adipose, duodenum, jejunum, and colon. These extra fragments could be derived from alternatively spliced GLP-1R mRNA, a related yet unidentified gene product, or may be an artifact of the assay.

No alternative slice products of GLP-1R mRNA are detected by RNAse protection

To examine whether these smaller fragments represent true alternative splice forms, three additional riboprobes were designed (Fig. 2A) that span the extracellular domain (E1), transmembrane regions II-V (T2), and the transmembrane region VI to within the intracellular domain (TI2). We

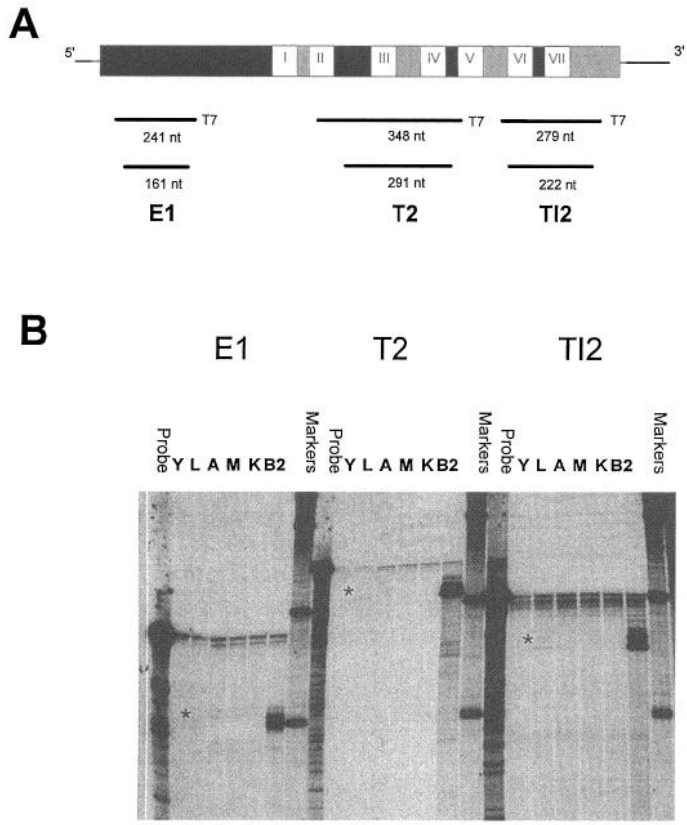

FIG. 2. Assessment of GLP-1R and alternative spliced mRNA by RNAse protection using three different riboprobes. A, Schematic of the GLP-1R cDNA and riboprobes. The GLP-1R cDNA is shown with the regions that code for functional domains emphasized (as described as in Fig. 1A). The E1 riboprobe, which encompasses sequences encoding the extracellular domain, was 241 nt in length and protects a 162-nt fragment. Similarly, the T2 riboprobe was 348 nt in length and protects a 291-nt fragment encompassing a region from transmembrane loop II to V. The TI2 riboprobe was 279 nucleotides in length, and it protects sequences of 222 nucleotides encompassing the transmembrane region VI to the intracellular domain. B, Autoradiograms of a RNAse protection experiment of total RNA from rat lung (L), isolated adipocytes (A), skeletal muscle (M), and kidney (K). Five micrograms of total RNA were tested for each tissue, along with 2 μg of RIN-B2 cell RNA (B2) as a positive control and 10 μg of yeast RNA (Y) as a negative control. The radiolabeled RNA markers shown are 518, 277, and 172 nt. This autoradiogram was exposed for 10 days. The predicted full length fragments in the lung lanes are indicated with an *. A protected product corresponding to the correct size (162 nt) was detected in lung RNA with the E1 probe after a 30-day exposure (data not shown).

reasoned that it was not likely that multiple alternative splicing events would occur simultaneously, therefore riboprobes spanning other regions of the GLP-1 receptor should produce the predicted protected fragments. As shown in Fig. 2B, the correct size protected fragments were only detected in lung and the somatostatin-secreting RIN-B2 cell line, and not in muscle, isolated adipocytes (epididymal), and skeletal muscle. On a longer exposure (30 days), the lung specific fragment is detected using the E1 probe. With the lung and RIN-B2 RNAs, all the riboprobes protected multiple full length products, which most likely represents incomplete digestion products. We concluded that there are no abundant alternative spliced forms of the GLP-1R transcript within any tissue that we examined and that the smaller fragments of 500, 379, and 192 nt initially described are either homologous sequences from a yet unidentified gene product or artifacts of the RNAse protection assay.

RT-PCR and Southern analysis identifies expression in multiple tissues

RT-PCR was performed on various rat tissues as a more sensitive assay for GLP-1R mRNA. After cDNA synthesis, the following primers were used in PCR (Fig. 3A): F1218, which lies within the coding region, and R1728, which lies within the 3′ untranslated region but not contained within the original GLP-1R cDNA clone (27). This strategy was chosen to ensure that products arising from the RT-PCR experiments were authentic and not due to contamination by plasmids existing within the laboratory. These primer pairs also would assess whether the alternative splice products, detected within the 3′ region of GLP-1R mRNA by the initial RNAse protection experiments, are found in those tissues. After the PCR reaction, the products were resolved on an agarose gel, stained with the fluorescent DNA stain Sybr Green I, and scanned on the Molecular Dynamics Fluoro-Imager. The fluorescent stain gives a 10-fold increase in sensitivity over ethidium bromide stained gels (data not shown). Figure 3B shows that the correct 553 bp PCR product is detected in hypothalamus, lung, pancreatic islets, and stomach. Other bands were also observed in heart, kidney, and skeletal muscle by Sybr Green, some of which had slightly lower molecular weight. In all cases, PCR products were not detected in the reverse transcription negative controls. CREB mRNA, a ubiquitously expressed transcription factor, was specifically amplified in all RT positive samples verifying the quality of the RNA and the validity of RT-PCR analysis (Fig. 3F). To confirm that these were GLP-1R specific PCR products, Southern blot analysis was performed using three different ^{32}P-labeled oligonucleotides spanning sequences of the GLP-1R cDNA containing the third extracellular loop, the seventh transmembrane region, and the carboxyl-terminal intracellular domain, R1220 (Fig. 3C), R1330B (Fig. 3D), or R1412X (Fig. 3E), respectively. The results of the Southern blot analysis revealed that only the hypothalamus, heart, islet, kidney, lung, and stomach cDNA expressed GLP-1R mRNA. Interestingly, only a single product of the predicted size of 553 bp was detected in the RNA from these tissues by Southern blot hybridization (despite the size variability shown by the Sybr Green gel) demonstrating that abundant alternative splice products are not produced within this region. GLP-1R specific products were not detected from adipocytes (epididymal), liver or skeletal muscle (gastrocnemius) total RNA (Fig. 3B), even in experiments in which the cDNA was amplified to up to 60 cycles (data not shown). We also did not detect GLP-1R specific transcripts by RT-PCR in brown fat, peritoneal fat, or 16-day-old white fat RNA (data not shown), indicating that the GLP-1R either does not exist in these subsets of adipose tissue or is below the level of detection of the assay. Densitometric scanning of the autoradiograms showed that the RT-PCR experiment is approximately 10-fold more sensitive in detecting GLP-1R mRNA levels than the RNAse protection assay (using the kidney

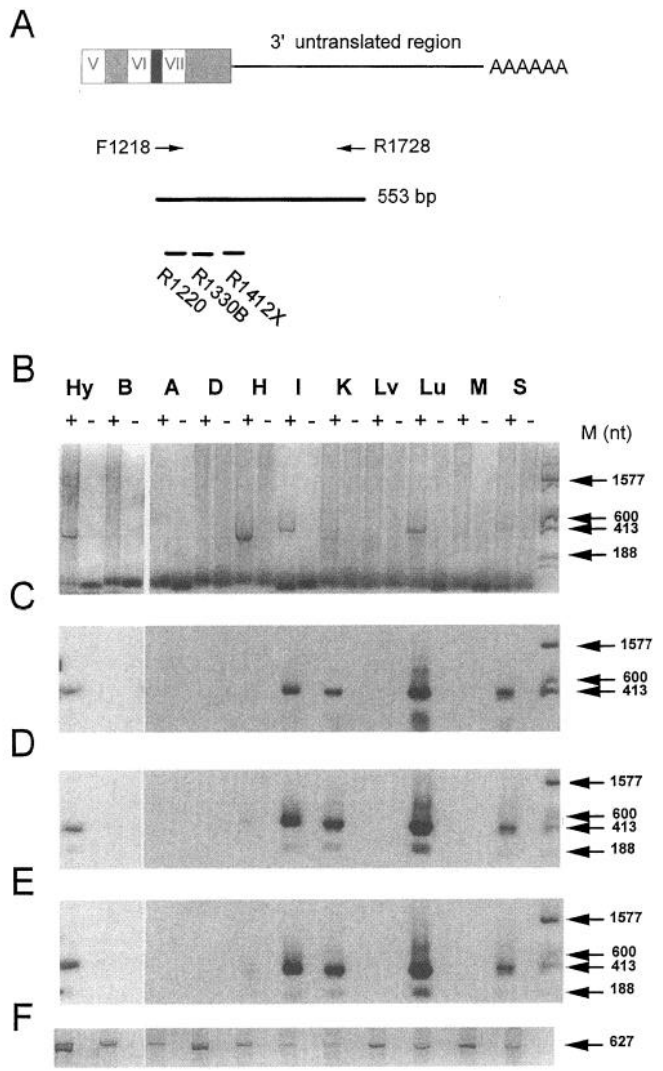

FIG. 3. Detection of GLP-1R mRNA in rat tissues by RT-PCR analysis. A, Schematic of the 3′ end of the GLP-1R cDNA from intron V to the polyadenylation site. The 5′ primer (F1218) and 3′ primer (R1728) used in the PCR reaction are shown along with the predicted product of 553 bp. The two oligonucleotides (R1330B and R1412X) used to probe the Southern blots are also shown. B, Picture of GLP-1R specific PCR products resolved on a 1.5% agarose gel and stained with Sybr Green I. PCR was performed on various rat tissue total RNA samples that were either incubated with (+) or without (−) RT. Hy, Hypothalamus; B, whole brain; A, adipose; D, duodenum; H, heart; I, pancreatic islet (I); K, kidney (K); Lv, liver (Lv); Lu, lung; M, skeletal muscle; and S, stomach. GLP-1R specific markers (right side) were produced by restriction digestion of the GLP-1R plasmid and are indicated by size (nt). The stained gels were scanned on the FluorImager 575 (Molecular Dynamics) with a 530 DF30 cutoff filter. C, Autoradiogram of a Southern blot of the above gel probed with ^{32}P-labeled R1220B oligonucleotide. The GLP-1R specific markers that hybridize to this probe are shown. The film was exposed for 24 h and scanned on a computing densitometer (Molecular Dynamics). D, Autoradiogram of the reprobe of the Southern blot shown in Fig. 3C with the ^{32}P-labeled R1330 oligonucleotide. E, Autoradiogram of the reprobe of the Southern blot shown in Fig. 3C with the ^{32}P-labeled R1412X oligonucleotide. F, Picture of a Sybr green I stained agarose gel of CREB control RT-PCR products from the identical cDNA samples as in Fig. 3B. The expected product size (627 nt) after PCR with CREB specific primers is indicated. The stained gels were scanned on the FluorImager 575 as described above. Note, brain RNA was loaded on three different bolts were probed with the GLP-1R specific probes.

band as the lower limit for the RNAse protection assay and the heart band as the lower limit for the RT-PCR). Therefore, we feel that the levels of GLP-1R mRNA, if they existed in tissues that showed negative results in the RT-PCR would be extremely low, assuming that the expression of the GLP-1R is not localized to discrete subsets of cells.

GLP-1R is localized to specific cell types by in situ hybridization

To identify which cells within specific tissues express GLP-1R mRNA, *in situ* hybridization was performed using antisense ^{33}P-labeled riboprobes, as well as sense riboprobes acting as negative controls and antisense riboprobes for insulin, and the glucose transporter 4 (GLUT 4). These experiments were aimed at determining whether GLP-1R mRNA is localized to a small population of cells within liver, skeletal muscle, and adipose tissues, which could account for the lack of signal in the RNAse protection and RT-PCR experiments. Figure 4, A and C, shows the results of hybridization of antisense GLP-1R riboprobes to pancreatic islet cells. The accumulation of specific grains, compared with sense controls (Fig. 4B), had a pattern very similar to those probed with the insulin-specific riboprobe (Fig. 4D), confirming the localization of GLP-1R mRNA to the endocrine cells of the pancreas. GLP-1R mRNA was also found in large nucleated cells present in the alveoli of the lung with a morphology suggestive of surfactant-secreting type II pneumocytes (Fig. 5, A and B). Similarly, the gastric pits of the stomach showed an increase in GLP-1R specific grains as compared with the sense probe (Fig. 5, C and D), probably representative of parietal cells, which have previously been shown to express the GLP-1 receptor (16). GLP-1R expressing cells were also diffusely scattered throughout the stomach. Even though we cannot positively identify these cells, GLP-1R expressing cells within the stomach may play an important role in glucose uptake within the stomach. These *in situ* hybridization results are in agreement with those obtained from the RNAse protection and RT-PCR experiments. However, we were unable to detect GLP-1R mRNA expression in skeletal muscle from rat gastrocnemius (Fig. 6, A and C) or isolated adipocytes (Fig. 6, B and D) even though GLUT-4 mRNA was readily detected in both tissues (Fig. 6, E and F). GLP-1R specific mRNA was also not detected in liver by *in situ* hybridization (data not shown). These results support the findings, by RNAse protection and RT-PCR, that the defined GLP-1 receptor is not expressed in the liver, adipose tissue, and skeletal muscle. Although a large number of sections were analyzed, we cannot rule out the possibility that some small region of cells within these tissues express the GLP-1R and was missed in our *in situ* hybridization screen. A specific pattern of GLP-1R riboprobe hybridization was not detected in the heart or kidney (Fig. 7, A and B, respectively) or in the darkfield images (data not shown), even though mRNA was detected by RT-PCR, indicating that GLP-1R expressing cells are not localized within isolated subsets of cells throughout the heart and kidney. Interestingly, specific GLP-1R mRNA containing cells were detected within the crypts of the duodenum (Fig. 7C), even though the samples were negative in the total RNA analysis (Figs. 1 and 3). This suggests that a

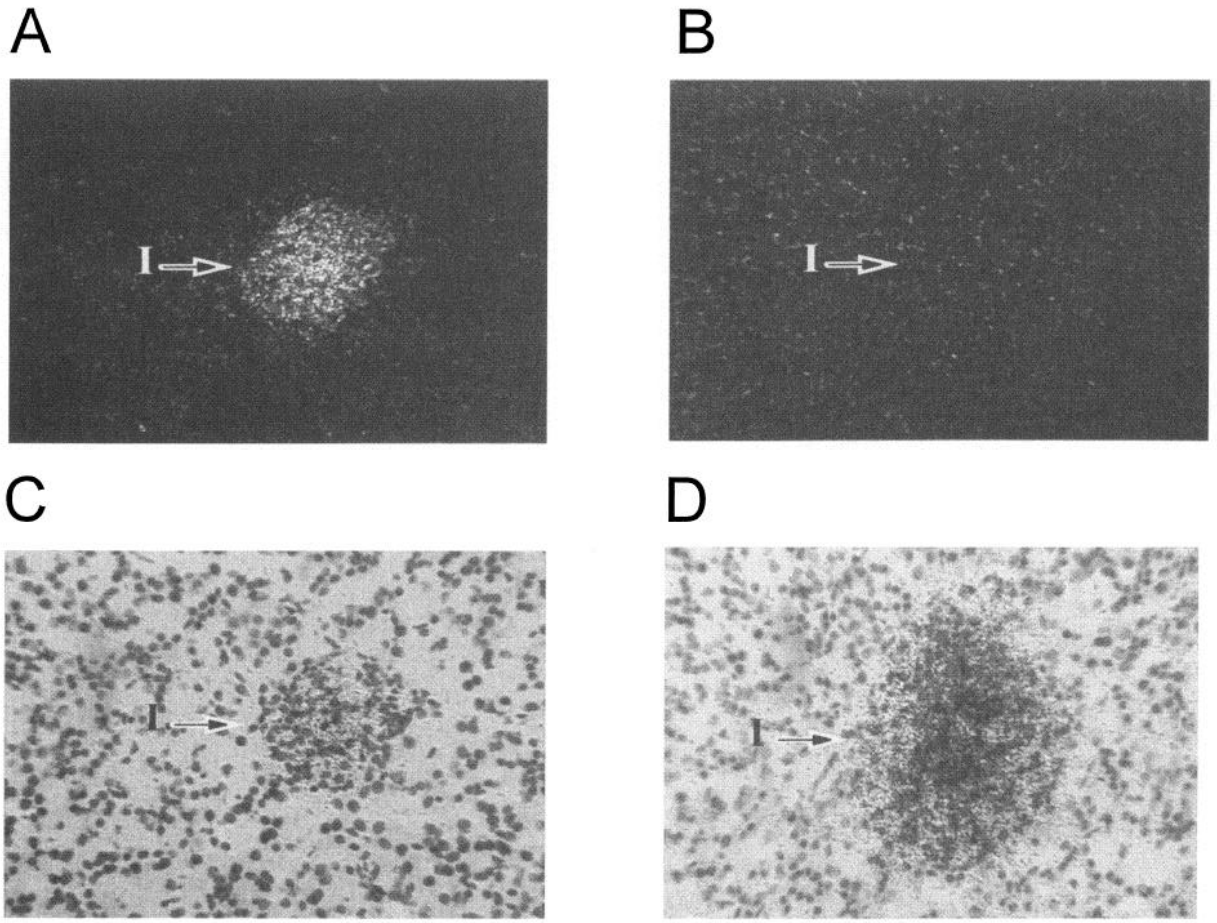

FIG. 4. GLP-1R mRNA expression in rat pancreas as examined by *in situ* hybridization. A, Sections of rat pancreas were hybridized with an antisense ^{33}P-GLP-1R riboprobe as described in *Materials and Methods*. The darkfield image is shown. B, A similar section was hybridized with a sense ^{33}P-GLP-1R riboprobe as a negative control. The darkfield image is shown. C, Brightfield image of panel A. D, A similar section was hybridized with an antisense ^{33}P-proinsulin riboprobe. The brightfield image is shown. Pancreatic islets are indicated (I). Magnification is 160×.

very small population of GLP-1 responsive cells reside in the small intestine; however, their function is not clear. These *in situ* hybridization experiments have demonstrated that the GLP-1R resides within cells of the pancreatic islets, alveoli of the lung, gastric pits of the stomach, and crypts of the duodenum, whereas GLP-1R containing cells were not found in the liver, adipose tissues, or skeletal muscle.

Discussion

The actions of the insulinotropic incretin hormone GLP-1 on pancreatic β-cells to stimulate insulin secretion is an important mediator of postprandial glucose levels (1, 2). Clinical evidence has shown that in addition to its pancreatic effects, GLP-1 modulates circulating glucose levels via extrapancreatic actions as well (10, 11). Therefore, GLP-1 may prove to be useful as a therapeutic agent in controlling glucose levels in patients with both insulin-dependent and non-insulin-dependent diabetes mellitus. To determine which extrapancreatic tissues respond to GLP-1, we undertook experiments to detect mRNA encoding the characterized pancreatic GLP-1 receptor (27) in rat tissues by using sensitive and selective assays: RNAse protection, RT-PCR and *in situ* hybridization. We found that the tissues which express the highest level of GLP-1R mRNA are the pancreatic islets, lung, hypothalamus, and stomach. Lower levels of expression were detected in heart, kidney, and duodenum. Surprisingly, GLP-1R gene expression was not detected in the liver, adipocytes, or skeletal muscle, major sites involved in glucose metabolism. Because high affinity binding sites for and physiological responses to GLP-1 have been reported for liver, adipose, and skeletal muscle, we conclude that GLP-1 must not be acting via the pancreatic receptor in these tissues but is cross-reacting with related receptors.

The *in situ* hybridization analysis of the mRNAs in the rat pancreas showed a colocalization of cells expressing GLP-1R and the proinsulin gene. This indicates that GLP-1R receptors are produced in the pancreatic islets in agreement with the previously reported ligand-binding studies (42). However, our experiments could not determine which of the islet cell types expressed the GLP-1 receptor. Physiological studies have shown that GLP-1 not only has profound insulinotropic effects on pancreatic β-cells but also potently stimulates somatostatin release from the pancreatic δ-cells (43). GLP-1 also inhibits the secretion of glucagon, although whether this is due to a direct effect on pancreatic α-cells or the indirect paracrine actions of somatostatin and insulin has not been determined (3, 44). Studies using insulinoma cell-lines have

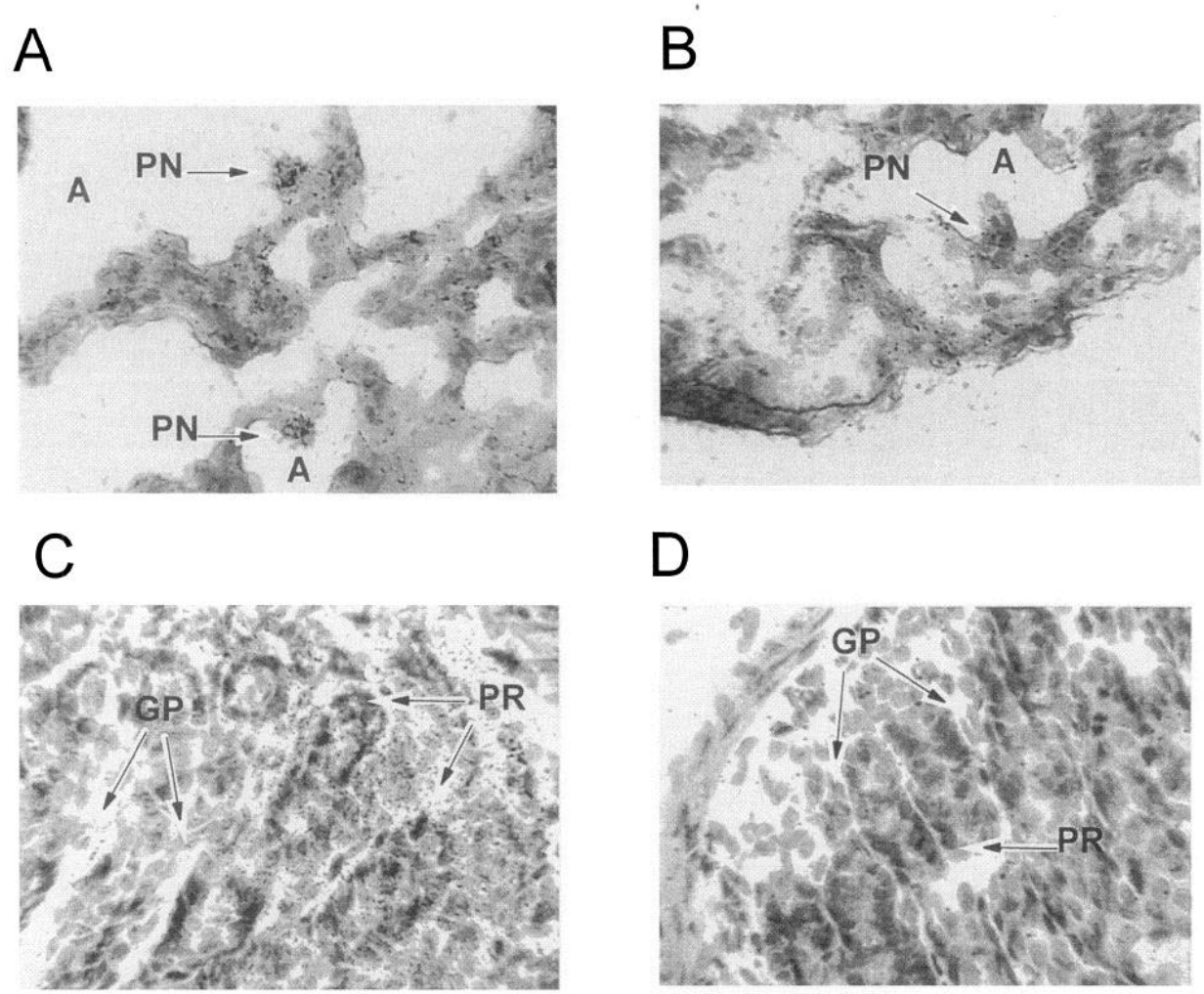

FIG. 5. *In situ* hybridization of GLP-1R mRNA in rat lung and stomach. A, Brightfield images of sections of rat lung hybridized with an antisense ^{33}P-GLP-1R riboprobe as described in *Materials and Methods*. B, Brightfield images of sections of rat lung hybridized with sense control. A, Alveoli; PN, type II pneumocytes. C, Brightfield images of sections of rat stomach hybridized with an antisense ^{32}P-GLP-1R riboprobe. D, Brightfield images of sections of rat stomach hybridized with a sense ^{32}P-GLP-1R riboprobe control. GP, Gastric pits; PR, parietal cells. Magnification for both sets of tissues is 400×.

demonstrated that the GLP-1R is found on β and δ cells but not on α- cells (45). However, ligand binding studies on whole pancreas and isolated islets indicate that GLP-1 can bind to all three cell types (42, 46).

GLP-1R mRNA was also detected at high levels in the rat lung. Richter and colleagues (47) have shown that GLP-1 binds to receptors on the submucosal glands of the trachea and the smooth muscle of the pulmonary arteries, causing a significant increase in mucous secretion and pulmonary smooth muscle relaxation. Our *in situ* hybridization experiments, using the GLP-1R specific riboprobe, have identified GLP-1R mRNA within large nucleated cells within the alveoli of the lung. The morphology of these cells is reminiscent of the type II pneumocytes, the major surfactant secreting cells of the lung, which is consistent with the known effect of GLP-1 on mucous secretion. The potential effect of GLP-1 on the regulation of surfactant secretion may provide new insight into the physiology of the lung. High levels of GLP-1R mRNA are also detected in total stomach RNA. The majority of GLP-1R mRNA containing cells detected by *in situ* hybridization are diffusely localized within the gastric pits of the stomach, reinforcing previous studies demonstrating an effect of GLP-1 on acid secretion from parietal cells (16, 48). These studies also showed that GLP-1R mRNA is highly concentrated to specific cells scattered throughout the stomach, which may represent somatostatin-secreting cells (49). Finally, GLP-1R mRNA was detected the hypothalamus and agrees with other reports (14, 50). These results are intriguing considering the reported effect of GLP-1 on appetite control (51). The lack of signal in the whole brain is most likely due to the dilution of GLP-1R specific mRNA to undetectable levels in the total RNA preparation. Further experiments are required to specifically localize GLP-1R mRNA within the brain.

Lower levels of GLP-1R gene expression were detected in kidney, heart, and duodenum. GLP-1R mRNA was barely detectable in the heart by RT-PCR; thus, the GLP-1 receptor seems to be synthesized at low levels in cardiac tissue. Even though the presence of the GLP-1R mRNA within the heart is supported by findings reported earlier (31), we failed to detect any specific signals in the heart by *in situ* hybridization, indicating that GLP-1R is not localized to a specific

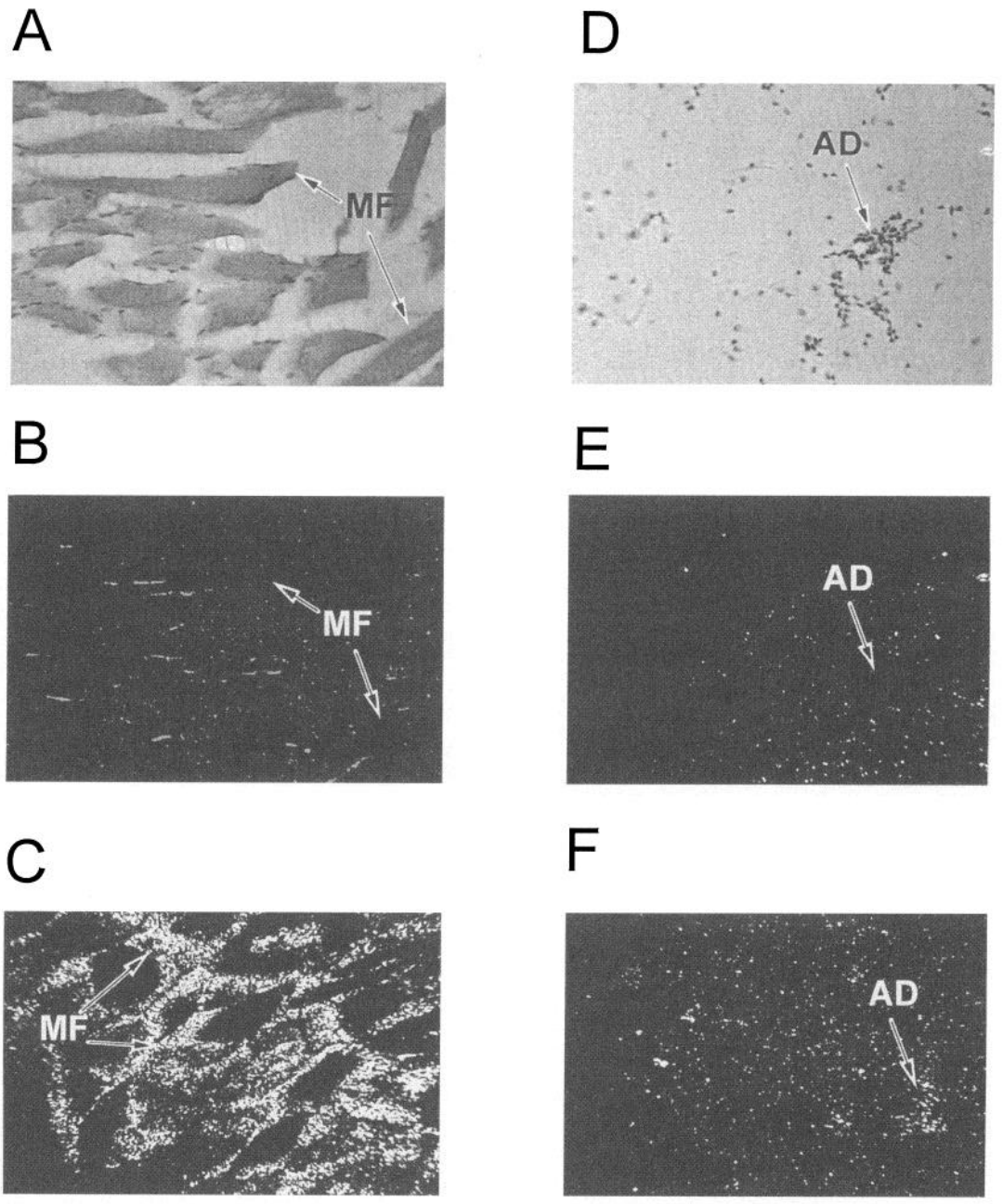

FIG. 6. *In situ* hybridization of GLP-1R mRNA in rat skeletal muscle and isolated adipocytes. A, Brightfield image of sections of skeletal muscle from the rat gastrocnemius hybridized with an antisense ^{33}P-GLP-1R riboprobe as described in *Materials and Methods*. B, Darkfield image of panel A. C, Similar section of rat gastrocnemius hybridized to ^{33}P-GLUT-4 riboprobe. MF, Muscle fibers. D, Brightfield image of isolated adipocytes from the rat epididymal fat pads hybridized with an antisense ^{33}P-GLP-1R riboprobe. E, Darkfield image of panel D. F, Similar adipocytes hybridized to ^{33}P-GLUT-4 riboprobe. AD, Isolated adipocytes. Magnification for both sets of tissues is 160×.

subset of cells within the heart. The low levels of expression of the GLP-1R in the heart suggest that GLP-1 signaling through the pancreatic GLP-1R may not be physiologically significant and that the reported actions of GLP-1 on changes in arterial blood pressure and heart rate (52) may be due to alternative mechanisms. Detection of GLP-1R specific mRNA in rat kidney by RT-PCR and RNAse protection indicates that the GLP-1R gene in the kidney is also expressed at low levels. GLP-1R specific signals were not detected in kidney slices by *in situ* hybridization, suggesting that, similar to the heart, these receptors may be diffusely localized throughout the kidney. Even though glucagon has been shown to have effects on the kidney (53), there has been no report of any responses to GLP-1. Interestingly, Campos and colleagues (32) have shown that expression of the GLP-1R gene in mouse kidney is greatest shortly after birth (week 2) and diminishes to low levels by week 12. This suggests that GLP-1 plays an important role during the development of the kidney, whereas any physiological effect in adult kidneys remains unknown. Surprisingly, GLP-1R specific signals

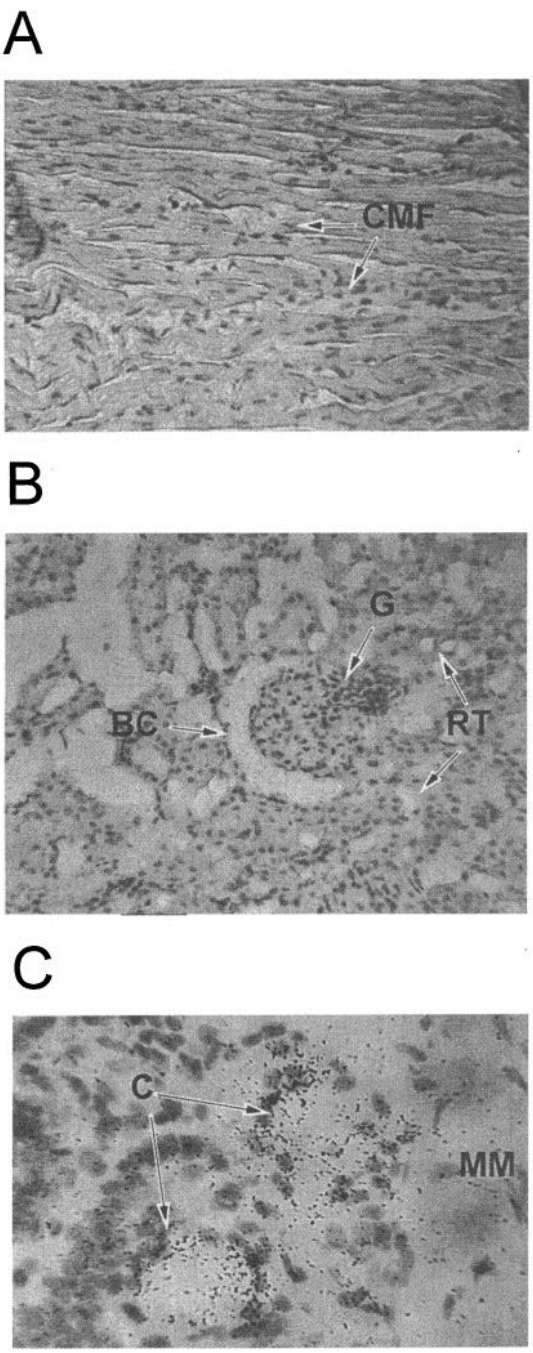

FIG. 7. *In situ* hybridization of GLP-1R mRNA in rat heart, kidney, and small intestine. Panel A, Brightfield images of sections of rat ventricle hybridized with an antisense ^{32}P-GLP-1R riboprobe as described in *Materials and Methods*. CMF, Cardiac muscle fibers. Panel B, Brightfield images of sections of rat kidney hybridized with an antisense ^{32}P-GLP-1R riboprobe. BC, Bowman's capsule; RT, renal tubules; G, glomerulus. Panel C, Brightfield images of sections of rat duodenum hybridized with an antisense ^{32}P-GLP-1R riboprobe. C, Crypts; MM, muscularis mucosae. Magnification for heart is 160× and for kidney and small intestine is 400×.

were detected in the crypts of the duodenum by *in situ* hybridization, even though GLP-1R mRNA was not detected by either RNAse protection or RT-PCR. Further analysis of the effects of GLP-1 within the gastrointestinal tract may provide valuable information about the regulation of glucose-dependent peptide hormone secretion and its overall effect on the postprandial insulin response.

The response to GLP-1 in liver, skeletal muscle, and adipose tissue, the major extrapancreatic sites for the regulation of glucose metabolism, has been of interest because of its insulin-independent actions on glucose metabolism (8). However, the effects of GLP-1 on the physiological responses in liver, muscle, and adipose tissues are contradictory. In isolated rat hepatocytes, it was shown that GLP-1 induced an increase in glycogen synthesis and a decrease in cAMP formation (17), although in other reports GLP-1 did not have any effect on cAMP, Ca^{++} levels, or carbohydrate metabolism in isolated hepatocytes or glycogenolysis/ketogenesis in perfused rat liver (18, 54, 55). Even though a GLP-1R specific fragment was detected by RT-PCR analysis in total mouse liver RNA (32), GLP-1 specific receptors could not be detected in rat liver membranes by ligand binding experiment (54). Because in our studies GLP-1 specific mRNA was not detected in rat liver by either RNAse protection, RT-PCR or *in situ* hybridization, we conclude that GLP-1 is not acting on the liver through the pancreatic receptor.

GLP-1 increases both lipolysis and cAMP formation in isolated rat adipocytes and had a similar effect on fatty acid synthesis in explants of rat adipose tissue (20, 21). Both high and low affinity GLP-1 receptors have been described on solubilized rat adipocytes membranes, although these results have not been confirmed by others (19). In another report, a GLP-1R specific PCR fragment was detected after 30 cycles of rat fat pad cDNA, although the levels seem to be extremely low (33). Interestingly, GLP-1 enhances insulin-stimulated glucose metabolism in the 3T3-L1 adipocyte cell line, which also produced a specific RT-PCR product supporting the idea that GLP-1 interacts with the characterized pancreatic receptor on adipocytes (33). However, we failed to detect any GLP-1R mRNA in adipocytes isolated from rat epididimyl fat pads even though the GLUT-4 control was readily detected. We also did not detect any GLP-1R in abdominal fat, brown intrascapular fat, or 16-day white fat indicating that GLP-1 does not act via the characterized pancreatic GLP-1R in adipose tissue.

Two conflicting reports have addressed the effect of GLP-1 on glycogenesis in skeletal muscle, one showing a potent effect (24), whereas in the other, no effect was demonstrated (56). GLP-1 binding sites were also detected on skeletal muscle, even though there seems to be a lack of specificity (25). PCR analysis of reverse transcribed mRNA from rat skeletal muscle also indicates that GLP-1 receptors are localized to the rat skeletal muscle (33). However, as in the case for the liver and adipose tissue, we did not detect any GLP-1R mRNA in skeletal muscle from rat gastrocnemius. Therefore, we feel that the observed physiological responses to GLP-1 on skeletal muscle may not occur through an interaction with the pancreatic GLP-1 receptor. Our findings suggest that the peripheral effects of GLP-1 in liver, skeletal muscle, and adipose tissues are due either to binding to yet unidentified GLP-1 related receptors or through other hormone receptors such as VIP or PACAP.

The absence of specific fragments from the RNAse protection and RT-PCR analysis of total rat tissue RNA strongly suggests that the GLP-1R gene is not expressed in the liver,

skeletal muscle, or adipose tissue. However, it is possible that significant levels of GLP-1R mRNA are localized to a distinct subset of cells within these tissues that were not detected by the *in situ* experiments and is diluted to below detectable levels in preparation of total tissue RNA. It is also possible that these assays are not sensitive enough to detect physiologically significant levels of GLP-1R mRNA in a total RNA population. However, the RNAse protection assay can detect GLP-1R specific mRNA down to 1 pg, equivalent to one copy of mRNA per cell. This calculation is based on two assumptions; mRNA comprises approximately 3% of total RNA (57), which would equate to 0.6 μg out of the 20 μg of the RNA which was assayed, and there are at the high end, approximately 500,000 molecules of mRNA per cell (58). Therefore, one copy per cell of a specific mRNA species would produce 1.2 pg of specific mRNA from the 20 μgs of assayed. Because we demonstrated in Fig. 3 that the RT-PCR analysis is at least 10 times more sensitive than the RNAse protection assay, any tissue expressing the GLP-1R gene should be detected by this assay. One final possibility is that GLP-1R mRNA is unusually stable in these tissues, in which case very low levels of mRNA might be sufficient to synthesize significant levels of receptor. Although a detailed study of the stability of GLP-1R mRNA in rat tissues has not been done, it has been reported that the half-life of the GLP-1R mRNA in RINm5f cells is between 3 and 6 h, not particularly stable considering that the average turnover rate for all mRNAs within a cell is approximately 6 h (57, 59). Therefore, we conclude by using these sensitive RNA detection methods that the characterized pancreatic GLP-1R gene is not expressed in rat liver, skeletal muscle, and adipose tissues.

The physiological studies show that GLP-1 has effects in liver, adipose tissues, and muscle even though we have determined that the cloned pancreatic GLP-1 receptor is not synthesized in these tissues. One likely explanation for this is that GLP-1 binds to different but related receptors:.; either isotypes of the pancreatic receptor or homologous receptors coded by different genes. The most interesting possibility for generation of different isotypes of the GLP-1 receptor would involve alternative splicing of the GLP-1R gene product. Analysis of the PTH and glucagon receptor genes indicates that the genomic structure is complex within the members of this receptor family. Thus, the likelihood for alternative splicing is high; for instance, the glucagon gene contains eleven introns (60). As an example, alternative splicing of the PACAP receptor mRNA generates receptors that either contain or lack specific amino acids within the third transmembrane loop (61). Even though each of these variants can bind PACAP with equal affinity, they differ greatly in their ability to activate either adenylate cyclase or phospholipase C. Alternative splice variants have also been described for the calcitonin and glucagon receptor (60, 62). In light of this, we were surprised that after an extensive search using both RNAse protection and RT-PCR assays, alternative spliced variants of the GLP-1 receptor were not detected. It is also possible that unidentified GLP-1 receptor is coded from a different gene. This is the case for the intronless somatostatin receptor, for which five different isotypes are each expressed from five different genes (63). Experiments are in progress to determine whether another GLP-1R exists in the peripheral tissues. The other alternative is that GLP-1 is interacting with related receptors to elicit the observed physiological responses. In preliminary RT-PCR experiments, we have identified glucagon, VIP, and PACAP receptor mRNA within the rat skeletal muscle and adipose tissues. Binding of GLP-1 to one or more of these related receptors in liver, adipose tissues, and skeletal muscle could conceivably explain the observed physiological responses of GLP-1 on these tissues.

References

1. **Orskov C** 1992 Glucagon-like peptide-1, a new hormone of the entero-insular axis. Diabetologia 35:701–711
2. **Fehmann H-C, Habener JF** 1992 Insulinotropic glucagonlike peptide-I (7–37)/(7–36) amide: a new incretin hormone. Trends Endocrinol Metab 3:158–163
3. **Kreymann B, Williams G, Ghatei MA, Bloom SR** 1987 Glucagon-like peptide-1 7–36: a physiological incretin in man. Lancet 2:1300–1304
4. **Fehmann H-C, Göke R, Göke B** 1995 Cell and molecular biology of the incretin hormones glucagon-like peptide-1 and glucose-dependent insulin releasing polypeptide. Endocr Rev 16:390–410
5. **Holz GG, Habener JF** 1992 Signal transduction crosstalk in the endocrine system: pancreatic β -cells and the glucose competence concept. Trends Biol Sci 17:388–393
6. **Fehmann H-C, Habener JF** 1992 Insulinotropic hormone glucagon-like peptide-1 (7–37) stimulation of proinsulin biosynthesis in insulinoma β TC-1 cells. Endocrinology 130:159–166
7. **Amiel SA** 1994 Glucagon-like peptide: a therapeutic glimmer. Lancet 343:4–5
8. **Gutniak M, Ørskov C, Holst JJ, Ahrén B, Efendíc S** 1992 Antidiabetogenic effect of glucagon-like peptide-1 (7–36) amide in normal subject and patients with diabetes mellitus. N Engl J Med 326:1312–1322
9. **Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF** 1992 Insulinotropic action of glucagon-like peptide-1-(7–37) in diabetic and nondiabetic subjects. Diabetes Care 15:270–276
10. **D'Alessio DA, Kahn SE, Leusner C, Ensick J** 1994 Glucagon-like peptide I(7–36 NH2) enhances glucose tolerance both by stimulation of insulin release and by increasing insulin independent glucose disposal. J Clin Invest 93:2263–2266
11. **D'Alessio DA, Prigeon RL, Ensinck JW** 1995 Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes: a physiological role of glucagon-like peptide I. Diabetes 44:1433–1437
12. **Uttenthal LO, Toledano A, Blázquez E** 1992 Autoradiographic localization of receptors for glucagon-like peptide-1 (7–37) amide in rat brain. Neuropeptides 21:143–146
13. **Shimizu I, Hirota M, Ohboshi C, Shima K** 1987 Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. Endocrinology 121:1076–1082
14. **Kanse SM, Kreymann B, Ghatei MA, Bloom SR** 1988 Identification and characterization of glucagon-like peptide-1 7–36 amide-binding sites in the rat brain and lung. FEBS Lett 241:209–212
15. **Richter G, Göke R, Göke B, Arnold R** 1990 Characterization of receptors for glucagon-like peptide-1(7–37) amide on rat lung membranes. FEBS Lett 267:78–80
16. **Uttenthal LO, Blázquez E** 1990 Characterization of high-affinity receptors for truncated glucagon-like peptide-1 in rat gastric glands. FEBS Lett 262:139–141
17. **Valverde I, Morales M, Clemente F, López-Delgado M, Delgado E, Perea A, Villanueva-Peñacarrillo M** 1994 Glucagon-like peptide 1: a potent glycogenic hormone. FEBS Lett 349:313–316
18. **Blackmore PF, Mojsov S, Exton JH, Habener JF** 1991 Absence of insulinotropic glucagon-like peptide-I (7–37) receptors on isolated rat liver hepatocytes. FEBS Lett 283:7–10
19. **Valverde I, Mérida E, Delgado E, Trapote MA, Villanueva-Peñacarrillo ML** 1993 Presence and characterization of glucagon-like peptide-1 (7–36) amide receptors in solubilized membranes of rat adipose tissue. Endocrinology 132:75–79

20. **Oben J, Morgan L, Fletcher J, Marks V** 1991 Effect of the entero-pancreatic hormones, gastric inhibitory polypeptide and glucagon-like polypeptide-1 (7–37) amide, on fatty acid synthesis in explants of rat adipose tissues. J Endocrinol 130:267–272
21. **Ruiz-Grande C, Alarcón C, Mérida E, Valverde I** 1992 Lipolytic action of glucagon-like peptides in isolated rat adipocytes. Peptides 13:13–16
22. **Baron AD, Laakso M, Brechtel G, Edelman SV** 1991 Reduced capacity and affinity of skeletal muscle for insulin-mediated glucose uptake in noninsulin-dependent diabetic subjects. J Clin Invest 87:1186–1194
23. **DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J** 1985 Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin dependent (type II) diabetes mellitus. J Clin Invest 76:149–155
24. **Villanueva-Peñacarrillo ML, Alcántara AI, Clemente F, Delgado E, Valverde I** 1994 Potent glycogenic effect of GLP-1 (7–36) amide in rat skeletal muscle. Diabetologia 37:1163–1166
25. **Delgado E, Luque MA, Alcántara A, Trapote MA, Clemente F, Valverde I, Villanueva-Peñacarrillo ML** 1995 Glucagon-like peptide-1 binding to rat skeletal muscle. Peptides 16:225–229
26. **Dillion JS, Tanizawa Y, Wheeler MB, Leng X-H, Ligon BB, Rabin DU, Yoo-Warren H, Permutt MA, Boyd III AE** 1993 Cloning and functional expression of the human glucagon-like peptide-1 (GPL-1) receptor. Endocrinology 133:1907–1910
27. **Thorens B** 1992 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
28. **Göke R, Cole T, Conlon JM** 1989 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
29. **Inagaki N, Hidehiko Y, Mizuta M, Mizuno N, Fujii Y, Gonoi T, Miyazaki J-I, Seino S** 1994 Cloning and functional characterization of a third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. Proc Natl Acad Sci USA 91:2679–2683
30. **Segre GV, Goldring SR** 1993 Receptors for secretin, calcitonin, parathyroid hormone (PTH)/PTH-related peptide, vasoactive intestinal peptide, glucagon-lie peptide-1, growth hormone-releasing hormone and glucagon belong to a newly discovered G-protein linked receptor family. Trends Endocrinol Metab 4:309–314
31. **Wei Y, Mojsov S** 1995 Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart, and pancreatic forms have the same deduced amino acid sequences. FEBS Lett 358:219–224
32. **Campos RV, Lee YC, Drucker DJ** 1994 Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. Endocrinology 134:2156–2164
33. **Egan JM, Montrose-Rafizadeh C, Yihong W, Bernier M, Roth J** 1994 Glucagon-like peptide-1 (7–36)amide (GPL-1) enhances insulin-stimulated glucose metabolism in 3T3–L1 adipocytes: one of several potential extrapancreatic sites of GLP-1 action. Endocrinology 135:2070–2075
34. **Rodbell M** 1964 Metabolism of isolated fat cells. J Biol Chem 239:375–380
35. **Lacy PE, Kostianovsky M** 1967 Method for the isolation of intact pancreatic islets from the rat pancreas. Diabetes 16:35–39
36. **Palkovits M** 1973 Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res 59:449–450
37. **Bullock BP, McNeil GP, Dobner PR** 1994 Synergistic induction of neurotensin gene transcription in PC12 cells parallels changes in AP-1 activity. Mol Brain Res 27:232–242
38. **Southern E** 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503
39. **Alexander MJ, Miller MA, Dorsa DM, Bullock BP, Melloni RH, Dobner PR, Leeman SE** 1989 Distribution of neurotensin/neuromedin N mRNA in rat forebrain: unexpected abundance in hippocampus and subiculum. Proc Natl Acad Sci USA 86:5202–5206
40. **Kaestner KH, Christy RJ, McLenithan JC, Braiterman LT, Cornelius P, Pekala PH, Lane MD** 1989 Sequence, tissue distribution, and differential expression of mRNA for a putative insulin-responsive glucose transporter in mouse 3T3–L1 adipocytes. Proc Natl Acad Sci USA 86:3150–3154
41. **Giddings SJ, Carnaghi L** 1989 Rat insulin II gene expression by extraplacental membranes: a non-pancreatic source for fetal insulin. J Biol Chem 264:9462–9469
42. **Orskov C, Poulsen SS** 1991 Glucagonlike peptide-1-(7–36)-amide receptors only in islets of Langerhans. Diabetes 40:1292–1296
43. **Schauder P, McIntosh C, Arends G, Arnold R, Frerichs H, Creutzfeldt W** 1976 Dynamics of somatostatin release from isolated rat pancreatic islets. FEBS Lett 68:225–227
44. **Holst JJ, Ørskov C** 1994 Glucagon and other pro-glucagon-derived peptides. In: Walsh JH, Dockery GJ (eds) Gut Peptides: Biochemistry and Physiology, Raven, New York 305–340
45. **Fehmann H-C, Habener JF** 1991 Functional receptors for the insulinotropic hormone glucagon-like peptide-1(7–37) on a somatostatin secreting cell line. FEBS Lett 279:335–340
46. **Heller RS, Aponte GW** 1995 Intra islet regulation of hormone secretion by glucagon-like peptide-1(7–37) amide. Am J Physiol 269: G852–G860
47. **Richter G, Feddersen O, Wagner U, Barth P, Göke R, Göke B** 1993 GLP-1 stimulates secretion of macromolecules from airways and relaxes pulmonary artery. Am J Physiol 265: L374–L381
48. **Schmidtler J, Dehne K, Offermanns S, Rosenthal W, Classen M, Schepp W** 1994 Stimulation of rat parietal cell function by histamine and GLP-1-(7–37) amide is mediated by Gsα. Am J Physiol 29:G775–G782
49. **Eissele R, Koop H, Arnold R** 1990 Effect of glucagon-like peptide-1 on gastric somatostatin and gastrin release in the rat. Scand J Gastroenterol 25:449–454
50. **Kreymann B, Ghatei MA, Burnet P, Williams G, Kanse S, Diani AR, Bloom SR** 1989 Characterization of glucagon-like peptide-1-(7–36)amide in the hypothalamus. Brain Res 502:325–331
51. **Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Health MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR** 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72
52. **Barragán JM, Rodríguez RE, Blázquez E** 1994 Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7–37) amide in rats. Am J Physiol 266: E459–E466
53. **Kolanowski J** 1983 Influence of glucagon on water and electrolyte metabolism. In: Lefèbvre PJ (ed) Glucagon II, Springer-Verlag, Berlin 525–536
54. **Ghiglione M, Blázquez E, Uttenthal LO, de Diego JG, Alvarez E, George SK, Bloom SR** 1985 Glucagon-like peptide-I does not have a role in hepatic carbohydrate metabolism. Diabetologia 28:920–921
55. **Murayama Y, Kawai K, Suzuki S, Ohashi S, Yamashita K** 1990 Glucagon-like peptide-1 (7–37) does not stimulate either hepatic glycogenolysis or ketogenesis. Endocrinology (Jpn) 37:293–297
56. **Fürnsinn C, Ebner K, W W** 1995 Failure of GLP-1 (7–36) amide to affect glycogenesis in rat skeletal muscle. Diabetologia 38:864–867
57. **Lewin B** (ed) 1985 Genes II, ed. 2. John Wiley and Sons, New York, pp 304–311
58. **Young BD, Birnie GD, Paul J** 1976 Complexity and specificity of polysomal ploy(A)$^+$ RNA in mouse tissues. Biochemistry 15:2823–2828
59. **Fehmann H-C, Jiang J, Pitt D, Schweinfurth J, Göke B,** Regulated expression, function of the insulinotropic GLP-1 receptor in insulin-secreting RINm5f cells. Program of the 77th Annual Meeting of The Endocrine Society. Washington DC, 1995 (Abstract PZ-380), p 385
60. **Magat B, Tastenoy M, Svoboda M** 1994 Sequencing of eleven introns in genomic DNA encoding rat glucagon receptor and multiple alternative splicing of its mRNA. FEBS Lett 351:271–275
61. **Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg P, Journot L** 1993 Differential signal transduction by five splice variants of the PACAP receptor. Nature 365:170–175
62. **Moore EE, Kuestner RE, Stroop SD, Grant FJ, Matthewes SL, Brady CL, Sexton PM, Findlay DM** 1995 Functionally different isoforms of the human calcitonin receptor result from alternative splicing of the gene transcript. Mol Endocrinol 9:959–968
63. **Reisine T, Bell GI** 1995 Molecular biology of somatostatin receptors. Endocr Rev 16:427–442