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Divergent Tissue-Specific and Developmental Expression of Receptors for Glucagon and Glucagon-Like Peptide-1 in the Mouse*

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ABSTRACT

Proglucagon mRNA transcripts are transcribed in the pancreas, bowel, and brain, after which posttranslational processing results in the liberation of a different profile of biologically active peptides in each tissue. The receptors for two of these peptides, glucagon and glucagon-like peptide-1 (GLP-1), have recently been identified, but only limited information is available concerning the tissue- and age-specific distribution of these receptors *in vivo*. We have investigated the expression of these receptors in the mouse using a combination of Northern blot analysis and reverse transcription-polymerase chain reaction. DNA sequence analysis of a partial mouse glucagon receptor cDNA demonstrated a high degree of sequence conservation across rodent species. Glucagon receptor mRNA transcripts were detectable by Northern blotting in poly(A)$^+$ RNA from liver and kidney. Reverse transcription-polymerase chain reaction also detected glucagon receptor mRNA transcripts in both fetal and adult pancreas and lung, jejunum, and ileum, but not in the large intestine. In contrast, mRNA transcripts for the GLP-1 receptor were detected in both small and large intestine as well as in pancreas, liver, lung, and kidney. Both glucagon and GLP-1 receptor mRNA transcripts were identified in different regions of the fetal and adult mouse brain, but the relative levels of GLP-1 receptor mRNA transcripts were much greater in the central nervous system. Furthermore, regulation of the GLP-1 receptor (but not the glucagon receptor) gene in the brain resembled the pattern of region-specific gene expression recently defined for the mouse proglucagon gene. Taken together, these studies define novel sites for both glucagon and GLP-1 receptor gene expression in the mouse and suggest that different regulatory mechanisms have evolved for tissue-specific and developmental control of receptor gene expression. (*Endocrinology* **134:** 2156–2164, 1994)

THE GENE encoding proglucagon is expressed in the A-cells of the endocrine pancreas, intestinal L-cells, and central nervous system (CNS) neurons (1–4). Tissue-specific elements have been identified that play important roles in the regulation of proglucagon gene transcription (5), and tissue-specific posttranslational processing results in a different profile of proglucagon-derived peptides liberated in these tissues (1, 6). Whereas the actions of glucagon in the control of hepatic carbohydrate, amino acid, and lipid metabolism have been clearly defined, considerably less is known about the role of glucagon in extrahepatic tissues (7). Similarly, despite the delineation of a specific role for glucagon-like peptide-1 (GLP-1) in the regulation of glucose-dependent insulin secretion (8–10), the biology of GLP-1 action in extrahepatic tissues is not well understood.

To understand the diverse actions of the proglucagon-derived peptides, recent efforts have focused on the identification and localization of receptors for the glucagon-like peptides. A combination of binding studies and autoradiographic analyses identified putative receptors for these peptides in both the CNS and peripheral tissues. Binding sites for GLP-1-(7–37) or GLP-1-(7–36) (tGLP-1) have been identified in rat fundic glands (11) and rat lung membranes (12, 13), although similar analyses of rat stomach and lung using ^{125}I-labeled GLP-1-(7–36) amide proved inconclusive (14). ^{125}I-Labeled monoiodoglucagon- and monoiodo-GLP-1-binding sites were found to be widely distributed in the rat brain, with higher concentrations of binding sites in the hypothalamus, pituitary, and medulla oblongata (12, 15–17). Further indirect evidence for GLP-1 action in the brain derives from experiments demonstrating GLP-1-mediated stimulation of adenylate cyclase activity in rat hypothalamic membrane preparations (12). Taken together, these studies provide indirect evidence of roles for glucagon and GLP-1 in extrahepatic and extrapancreatic tissues, respectively.

The elucidation of the nucleotide sequence of the GLP-1 receptor (18) has facilitated studies of GLP-1 receptor expression. Initial studies of GLP-1 receptor localization detected mRNA transcripts in rat islets, stomach, and lung, with no receptor mRNA transcripts observed in brain, liver, thymus, muscle, intestine, or colon (18). Subsequent studies of RNA also prepared from rat tissues confirmed the presence of GLP-1 receptor mRNA transcripts in islets as well as lung and kidney. With prolonged exposures, putative GLP-1 receptor mRNA transcripts were tentatively identified in brain, liver, and muscle (19). In contrast, Northern blot analysis of RNA isolated from human tissues by the same investigators detected a single GLP-1 mRNA transcript only

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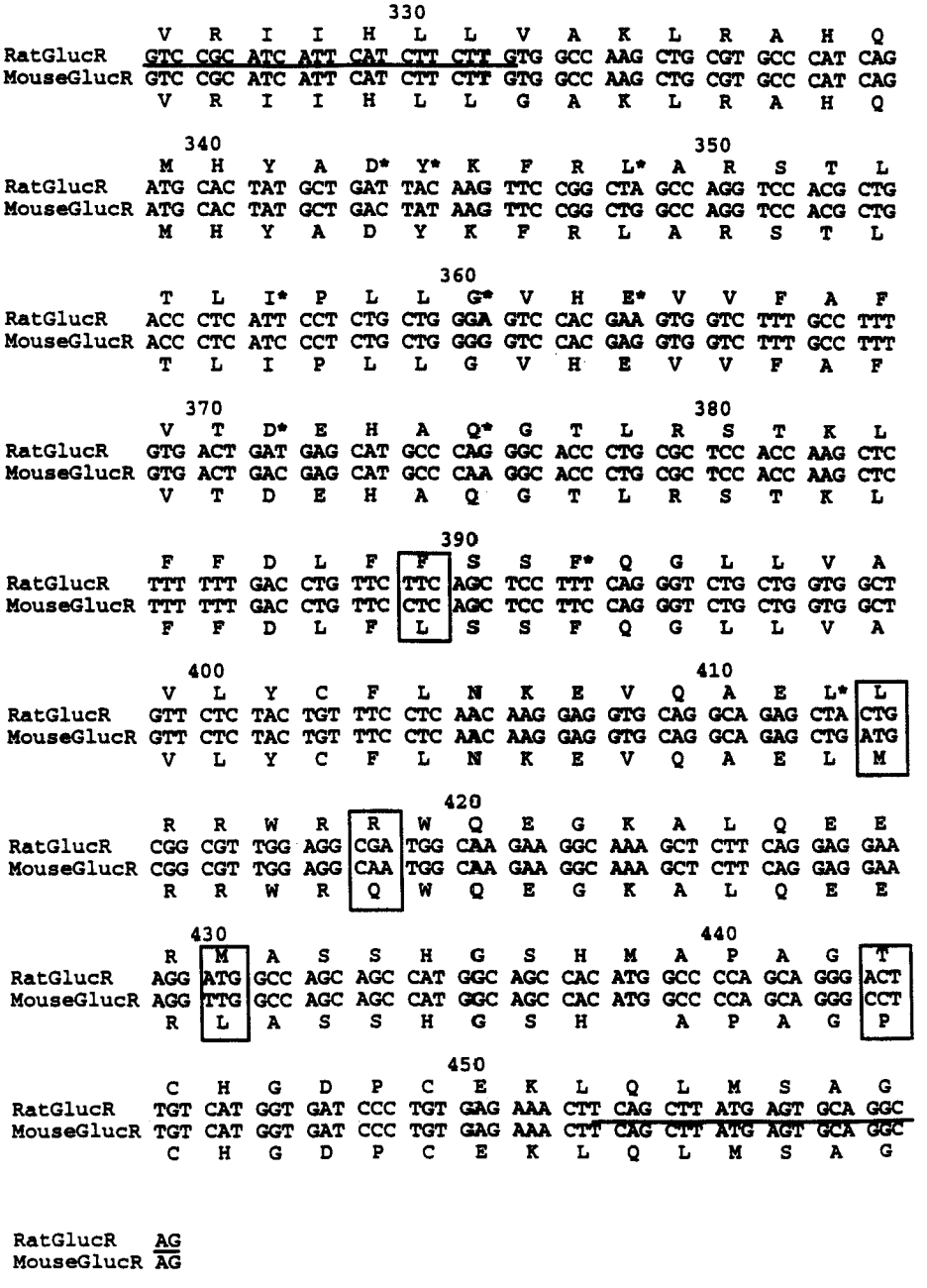

FIG. 1. Partial nucleotide sequences of the mouse and corresponding rat glucagon receptor cDNAs (positions 969–1375). Oligonucleotide primers (*underlined*) synthesized according to the sequence of the rat glucagon receptor (21) were used to amplify the mouse homolog from liver poly(A)$^+$ RNA. Silent nucleotide substitutions between the two sequences are marked with *asterisks*, whereas conservative (amino acids 389, 413, and 430) and replacement (amino acids 418 and 443) base changes are *boxed*. The predicted amino acid sequences encoded by this region are shown *above and below* the rat and mouse nucleotide sequences, respectively.

in the pancreas, with no mRNA transcripts detectable in RNA prepared from lung, kidney, liver, or brain (20). The explanation for this species-specific difference in GLP-1 receptor expression remains unclear.

More recently, a cDNA encoding the rat glucagon receptor has been isolated by expression cloning; however, the tissue distribution of glucagon receptor mRNA transcripts was not reported (21). To define the spectrum of tissues expressing both the glucagon and GLP-1 receptors in the mouse, we have studied the expression of these two murine receptors using reverse transcription-polymerase chain reaction (RT-PCR), a more sensitive technique for the analysis of low abundance mRNA transcripts. The results of our experiments delineate novel sites for glucagon and GLP-1 receptor expression and provide evidence for differential tissue-specific and developmental regulation of GLP-1 and glucagon receptor gene expression.

Materials and Methods

Reagents

All reagents and chemicals were purchased from Sigma (St. Louis, MO), BDH (Toronto, Canada), or ICN Biochemicals (St. Laurent, Canada). Restriction and modifying enzymes were obtained from Boehringer Mannheim (Toronto, Canada). [α-^{32}P]Deoxy-ATP was obtained from Amersham International (Toronto, Canada). Sequenase version 2.0

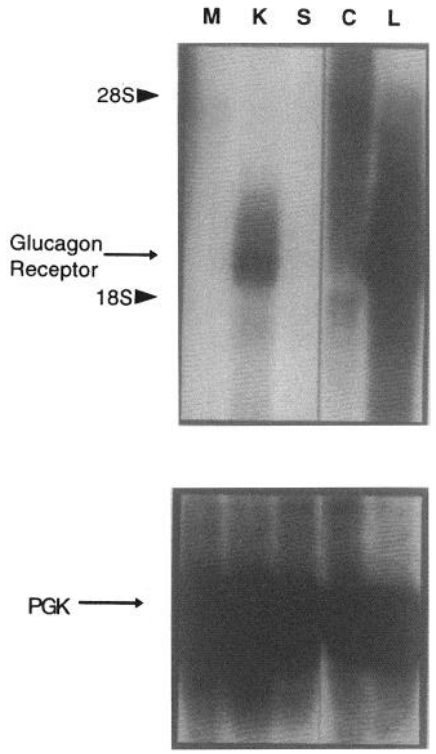

FIG. 2. Northern blot analysis of glucagon receptor mRNA transcripts in the mouse. Two micrograms of poly(A)$^+$ RNA (as assessed by quantitative dot blot analysis) from various adult mouse tissues were analyzed for glucagon receptor expression using a mouse glucagon cDNA fragment as probe. The blot was exposed for 9 h. The tissue sources of RNA were: M, muscle; K, kidney; S, stomach; C, cerebellum; and L, liver. The blot was reprobed with a cDNA for mouse PGK. The positions of the 28S and 18S ribosomal RNA bands are indicated with *arrowheads*.

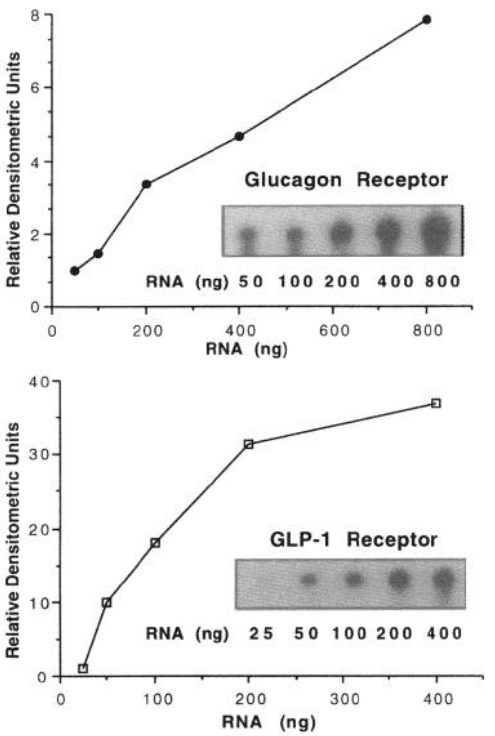

FIG. 3. RT-PCR analysis of glucagon and GLP-1 receptor mRNA transcripts. Known amounts of total cellular RNA from 2-week-old mouse liver (glucagon receptor) and lung (GLP-1 receptor) were reverse transcribed and amplified by PCR using gene-specific primer pairs to generate standard curves for glucagon and GLP-1 receptor mRNAs. After agarose gel electrophoresis and transfer to a nylon membrane, the blots were hybridized with either mouse glucagon or GLP-1 receptor cDNA probes (labeled to identical specific activities) and exposed for 45 min and 16 h, respectively. Autoradiograms were scanned with a laser densitometer, and the relative densitometric units were quantitated by assigning a value of 1 to the signal generated by the lowest standard of each curve. For Figs. 5 and 9, the relative values depicted for glucagon receptor mRNA have been multiplied by 1000 for ease of comparison.

DNA sequencing kit was purchased from U.S. Biochemical (Cleveland, OH). The glucagon receptor cDNA was generated by PCR, ligated into a Bluescript (Strategene, La Jolla, CA) vector, and double stranded DNA sequencing was performed using T7 and T3 primers.

Animals

All animals were handled in accordance with ethical guidelines set forth by the Toronto General Hospital Animal Care Committee. Wild-type CD1 mice used in this study were obtained from Charles River Canada (Toronto, Canada) at 2, 5, and 12 weeks of age, and pregnant females on approximately day 19 of gestation. The transgenic CD1 mice studied here (GLUTag-B line) harbored a glucagon promoter-simian virus-40 (SV40) T-antigen fusion gene (5, 22). Only heterozygotes were examined, as all homozygotes became ill and died of neuroendocrine carcinoma of the bowel at 2–8 weeks of age. Age- and sex-matched nontransgenic littermates were used as wild-type controls in studies of receptor expression in the brain.

RNA isolation and Northern blot analysis

Total cellular RNA was isolated by the acid-guanidinium isothiocyanate method (23). For CNS studies, brain regions were carefully dissected to eliminate the possibility of cross-contamination. Hypothalami from day 19 fetuses could not be accurately removed without small amounts of contaminating adjacent tissue and, hence, were not analyzed at this time point. Brain regions from wild-type or transgenic animals were separately pooled (n = 3–8 for each region for each time point) before isolation of total cellular RNA. The results presented here are from one experiment that is representative of analyses of at least three different litters for each age group. The peripheral tissues collected were lung, jejunum, distal ileum, colon, pancreas, liver, and kidney. In day 19 embryos, total intestinal tissue was analyzed, as precise segregation of bowel regions could not be accomplished accurately and quickly enough to prevent RNA degradation. Peripheral tissues from day 19 wild-type and transgenic embryos were pooled separately before preparation of total cellular RNA. At least two samples for each time point were analyzed for receptor mRNA expression, and RNA was treated with DNAse-1 before analysis by RT-PCR.

Poly(A)$^+$ RNA was isolated from total cellular RNA using the Poly AT Tract mRNA Isolation System III from Promega (Madison, WI). Two micrograms of poly(A)$^+$ RNA from muscle, kidney, stomach, cerebellum, and liver were electrophoresed on a 1% (wt/vol) agarose-formaldehyde gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH) by vacuum blotting (Pharmacia LKB Biotechnology, Uppsala, Sweden). The RNAs were fixed to the membrane by exposure to UV light, prehybridized, and hybridized as previously described (24). A mouse glucagon receptor cDNA fragment was generated by PCR from poly(A)$^+$ liver RNA, radiolabeled with [α-^{32}P]deoxy-ATP by the random priming method, and used for hybridization at a concentration of 1×10^6 cpm/ml for 16 h at 42 C. Final washing conditions were 0.2 × SSC-

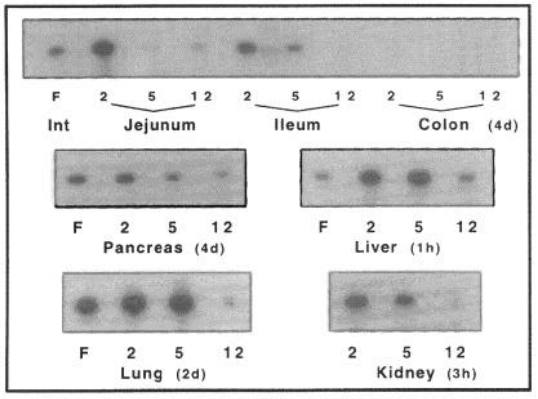

FIG. 4. Glucagon receptor mRNA transcripts in different tissues of the mouse. Wild-type CD1 mice were analyzed on fetal day 19 and at 2, 5, and 12 weeks of age. Total cellular RNA was prepared from various peripheral tissues and subjected to RT-PCR, followed by Southern blot hybridization of the PCR products with a mouse glucagon receptor cDNA probe. The exposure times required for each tissue blot are indicated in *parentheses*. F, Fetal; Int, whole intestine; 2, 5, and 12, ages of mice in weeks.

0.2% (wt/vol) sodium dodecyl sulfate at 55 C. Autoradiography was performed using Kodak X-Omat film (Eastman Kodak, Rochester, NY) at −70 C.

RT-PCR

RT-PCR was performed to determine the relative levels of glucagon and GLP-1 receptor mRNA transcripts in a semiquantitative manner. The RT-PCR methodology for these studies has been previously described (22). Primer pairs used for PCR were based on the reported sequences for rat glucagon (Genebank Accession no. M96674) and GLP-1 receptor cDNAs (18). Amplification of glucagon receptor cDNA was performed at an annealing temperature of 68 C for 28 cycles, resulting in the generation of a 407-basepair (bp) product, whereas GLP-1 receptor cDNA amplification was performed at an annealing temperature of 58 C for 32 cycles, producing a 251-bp fragment. The sequences of the glucagon and GLP-1 receptor primer pairs were 1) glucagon receptor, 5′-GTCCGCATCATTCATCTTCTTG-3′ and 5′-CTGCCTGCACTCATAAGCTGA-3′; and 2) GLP-1 receptor, 5′-AGGAACCCTACGCTTCGTCAAG-3′ and 5′-TTTGGCAGGTGGCTGCATACAC-3′. To control for differences in initial RNA levels and tube to tube variation in RT-PCR, a primer pair for phosphoglycerate kinase (PGK) that gives rise to a 191-bp cDNA product (22) was included in each PCR amplification. Analysis of PCR products was performed by gel electrophoresis and Southern blotting using cDNA probes labeled to identical specific activities, as previously described (22). Final washing conditions for the RT-PCR Southern blots were 0.2 × SSC-0.2% (wt/vol) sodium dodecyl sulfate at 65 C.

Densitometry

Autoradiograms were scanned with a Hewlett-Packard laser scanner (Palo Alto, CA), and the signal densities were quantitated using the NIH Image 1.42 software. The scanned signals were derived from short exposures of most films to ensure that densitometry was carried out within the linear range of the film. The autoradiograms presented in the figures usually illustrate longer exposures of the scanned images to

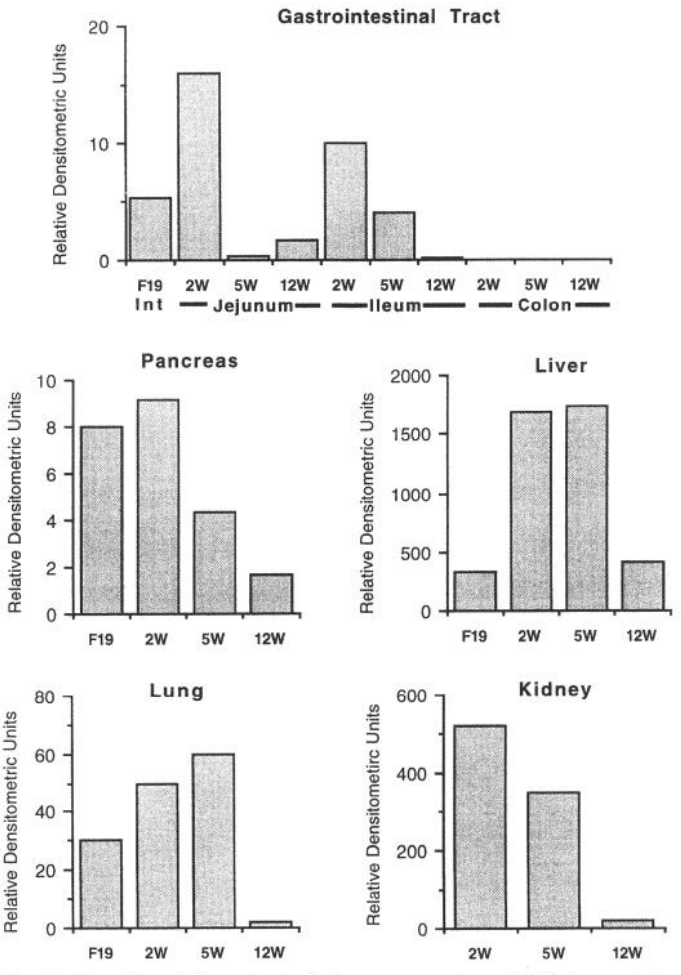

FIG. 5. Densitometric analysis of glucagon receptor mRNA transcripts in peripheral tissues. Different exposures of the blots shown in Fig. 4 were scanned by computerized densitometry. The relative densitometric units represent values derived from standard curves of the RT-PCR reactions (see Fig. 3). F, Fetal; w, weeks; Int, intestine.

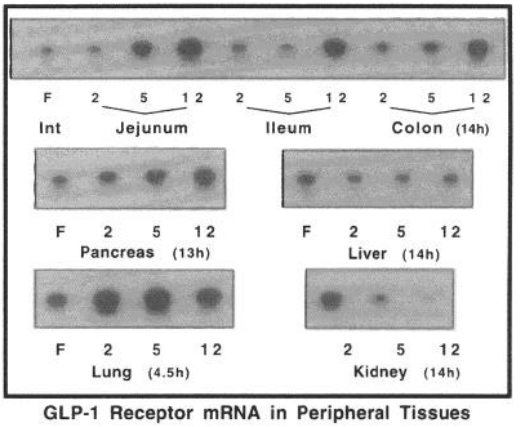

FIG. 6. RT-PCR analysis of GLP-1 receptor expression in mouse tissues. F, Fetal; Int, intestine. Exposure times for the various blots are indicated in *parentheses*.

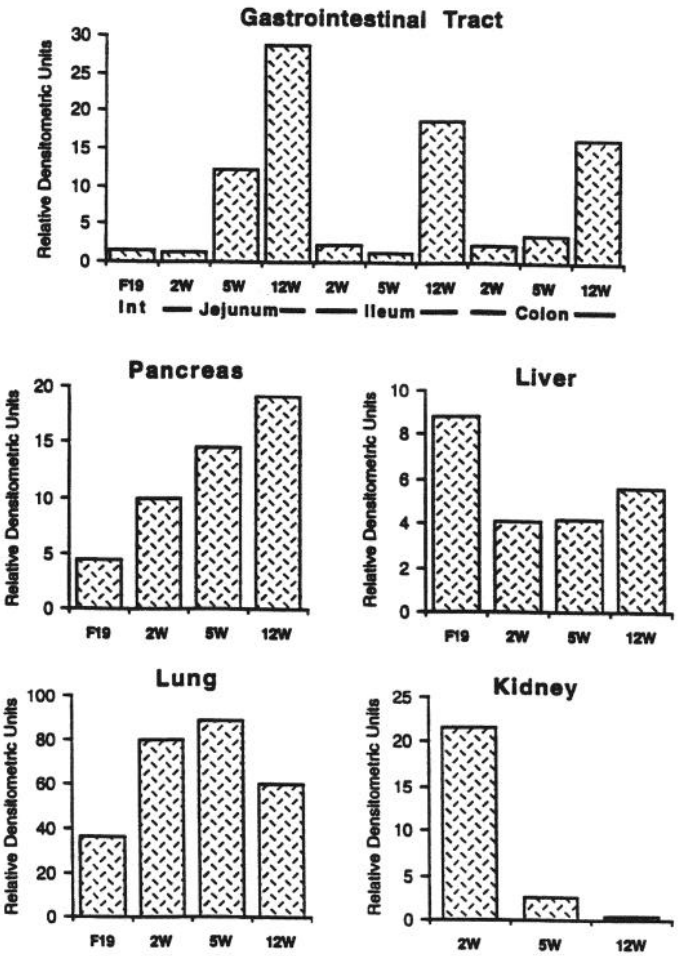

FIG. 7. Densitometric analysis of GLP-1 receptor expression in mouse tissues. F, Fetal (day 19); Int, intestine; w, weeks. The values are plotted as relative densitometric units (see Fig. 3).

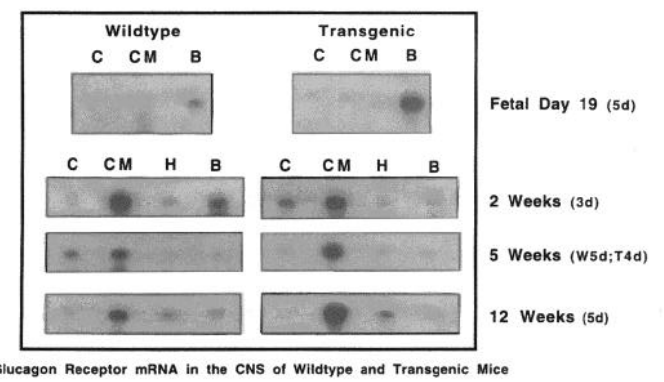

FIG. 8. Glucagon receptor mRNA transcripts in wild-type and transgenic mouse brain on day 19 of gestation and at 2, 5, and 12 weeks of age. Total cellular RNA was prepared from various brain regions and subjected to RT-PCR, followed by Southern blot hybridization with a mouse glucagon receptor cDNA. C, Cortex; CM, cerebellum; H, hypothalamus; B, brain stem. The exposure times for each blot are shown in *parentheses*.

better demonstrate low abundance transcripts. To quantitate the relative densitometric units for each experiment, a relative value of 1 was assigned to the lowest signal from the standard curves (50 ng RNA from 2-week-old liver for glucagon receptor; 25 ng RNA from 2-week-old

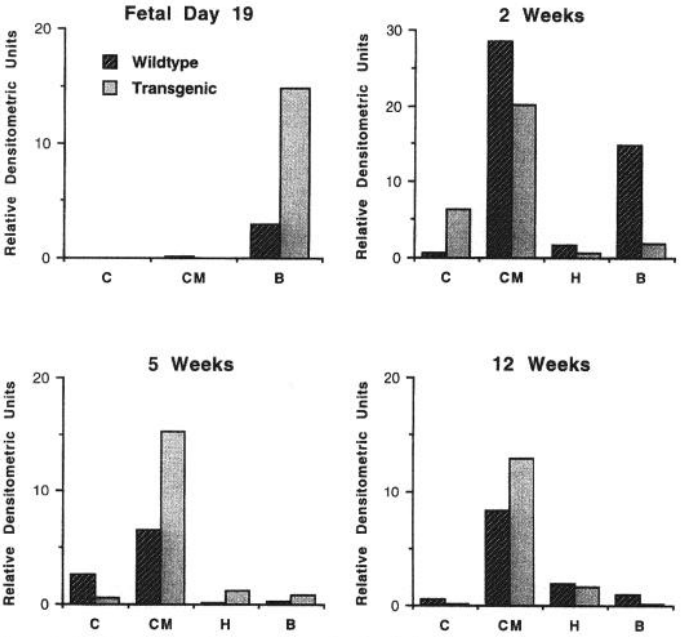

FIG. 9. Relative densitometric analysis of glucagon receptor expression in the brain. The blots shown in Fig. 8 were scanned by laser densitometry, and the relative densitometric units were assigned as described in Fig. 3. C, Cortex; CM, cerebellum; H, hypothalamus; B, brain stem.

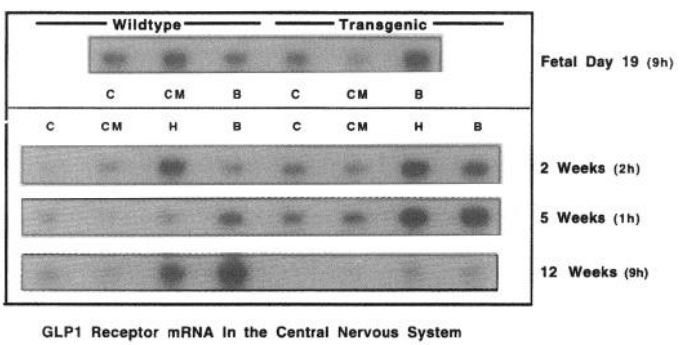

FIG. 10. RT-PCR analysis of GLP-1 receptor expression in wild-type and transgenic mouse brain on day 19 of gestation and at 2, 5, and 12 weeks of age. Total cellular RNA was prepared from various brain regions and subjected to RT-PCR, followed by Southern blot hybridization with a mouse GLP-1 receptor cDNA. C, Cortex; CM, cerebellum; H, hypothalamus; B, brain stem. The exposure times for each blot are shown in *parentheses*.

lung for GLP-1 receptor). Differences in autoradiogram exposure times were incorporated into the calculation of relative densitometric values. For comparative purposes to illustrate low abundance transcripts, relative densitometric values obtained for glucagon receptor mRNAs were multiplied by 1000.

Results

Although GLP-1 receptor mRNA transcripts have been detected by Northern blot analysis in various rat and human tissues, no information is yet available concerning the tissue-specific distribution of glucagon receptor gene expression. Furthermore, although the mouse is commonly used for transgenic studies and experiments using homologous recombination, expression of the murine GLP-1 and glucagon

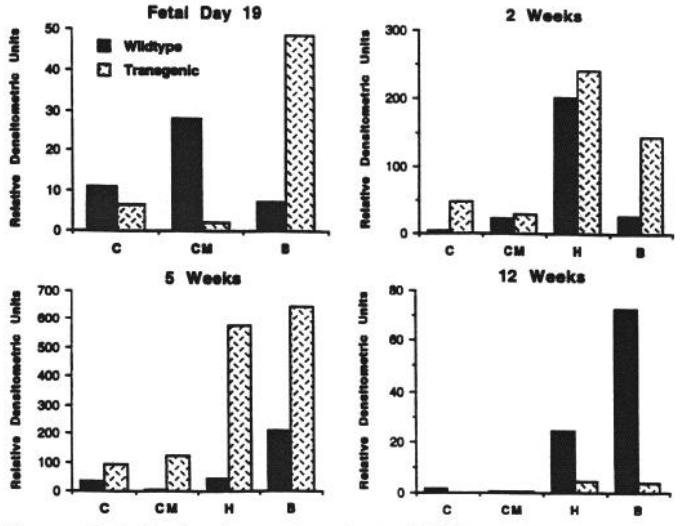

FIG. 11. Relative densitometric analysis of GLP-1 receptor expression in the brain. The blots shown in Fig. 10 were scanned by laser densitometry, and the relative densitometric units were assigned as described in Fig. 3. C, Cortex; CM, cerebellum; H, hypothalamus; B, brain stem.

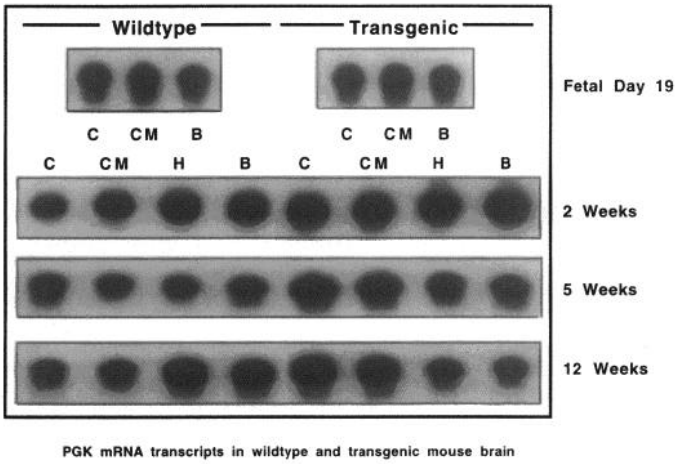

FIG. 12. RT-PCR analysis of PGK mRNA transcripts in wild-type and transgenic mouse brain. The PCR products were analyzed by gel electrophoresis and transferred to a nylon membrane, and the blot was hybridized with a mouse PGK cDNA probe. C, Cortex; CM, cerebellum; H, hypothalamus; B, brain stem.

receptors has not yet been reported. To examine the expression of the glucagon receptor in the mouse, we designed oligonucleotide primers complementary to the rat sequence (21) and used these primers to generate a cDNA of the expected size from mouse liver RNA. To ensure that the amplified cDNA fragment represented the mouse homolog of the rat glucagon receptor, the cDNA was ligated into pBluescript and sequenced on both strands. The nucleotide sequence of the partial mouse glucagon receptor cDNA, as well as the corresponding rat glucagon receptor sequence (21) are shown in Fig. 1. The mouse glucagon receptor cDNA contained 10 silent, 3 conservative, and 2 replacement nucleotide substitutions within the 407-bp sequence. The predicted amino acid sequences encoded by this mouse cDNA are 96% identical to the sequence reported for the rat glucagon receptor. Taken together, this evidence strongly supports the likelihood that we have isolated a PCR product corresponding to the mouse glucagon receptor cDNA. Furthermore, it appears that the amino acid sequence of these two rodent receptors is highly conserved.

To determine the distribution of glucagon receptor mRNA transcripts in the mouse, poly(A)$^+$ RNA was prepared from various tissues and examined for receptor expression by Northern blot analysis (Fig. 2). A single glucagon receptor mRNA transcript, approximately 2.3–2.5 kilobases in size, was detected in poly(A)$^+$-enriched RNA from mouse kidney and liver. The blot was rehybridized (to assess loading and integrity of RNA) with a cDNA probe for mouse PGK (Fig. 2). As lower levels of receptor mRNA transcripts may not be detected by Northern blot analysis, we examined both glucagon and GLP-1 receptor expression using the more sensitive RT-PCR method.

We initially defined the PCR parameters for reproducible and semiquantitative analysis of glucagon and GLP-1 receptor mRNA abundance. Varying amounts of total cellular RNA from 2-week-old mouse liver and lung were reverse transcribed, and the resultant cDNAs were subjected to PCR to generate standard curves for detection of glucagon and GLP-1 receptor mRNA transcripts (Fig. 3). The specific parameters, including cycle length (see *Materials and Methods*), for each primer pair were chosen to permit reproducible semiquantitiative estimation of mRNA transcript abundance. The linearity of signal intensities achieved over a range of RNA concentrations for both receptors illustrated the utility of the chosen conditions for semiquantitative detection of both glucagon and GLP-1 receptor mRNA transcripts.

The expression of both the glucagon and GLP-1 receptor mRNA transcripts was analyzed by RT-PCR using total cellular RNA isolated from mouse intestine, pancreas, liver, lung, and kidney of CD1 wild-type mice on fetal day 19 and 2, 5, and 12 weeks after birth. Glucagon receptor mRNA was most abundant in mouse liver at all time points studied (Figs. 4 and 5). Glucagon receptor mRNA transcripts were consistently less abundant in kidney (compared to liver), followed by lung and pancreas. Analysis of the relative distribution of glucagon receptor mRNA transcripts across different regions of the small and large intestine revealed that although both the jejunum and ileum expressed the glucagon receptor, no transcripts were detectable in mouse colon at any of the ages studied (Figs. 4 and 5).

In contrast, GLP-1 receptor expression was clearly detectable and relatively abundant in mouse colon at 2, 5, and 12 weeks of age (Figs. 6 and 7). GLP-1 receptor mRNA transcripts were also detectable in pancreas, lung, liver, and kidney at all ages studied. Furthermore, the relative levels of GLP-1 receptor mRNA transcripts were consistently greater than the levels of glucagon receptor mRNA for all tissues studied (except liver and kidney). Analysis of the developmental profiles of receptor expression demonstrated tissue-specific differences in the developmental control of receptor expression. The relative abundance of GLP-1 receptor mRNA transcripts increased with age in RNA from mouse jejunum, ileum, and colon. In contrast, glucagon receptor mRNA

transcripts were much less abundant in mouse small bowel at 12 weeks. Similarly, although the relative levels of GLP-1 receptor expression increased slightly in the pancreases of older mice, analysis of the identical RNA preparations revealed a slight decrease in the levels of glucagon receptor mRNA transcripts. A marked decrease in glucagon receptor (but not GLP-1 receptor) gene expression was also observed in 12-week-old lung. In contrast, the levels of both GLP-1 and glucagon receptor mRNAs were relatively lower in RNA isolated from 12-week-old mouse kidney than those at earlier time points.

The results of previous analyses have shown that the proglucagon gene is expressed in different brain regions of both fetal and adult mice (22). These observations suggest that the receptor(s) for one or more of the proglucagon-derived peptides are probably expressed in the CNS. Glucagon and GLP-1 receptor expression was examined in various brain regions of both wild-type mice and mice harboring a glucagon promoter-SV40Tag transgene (5). The GLUTag mice were included in this study because they have previously been shown to exhibit both region- and age-specific differences in CNS proglucagon gene expression (22). Glucagon receptor mRNA was detected in the brain stem, but not the cortex or cerebellum of both wild-type and GLUTag transgenic mice on embryonic day 19 (Figs. 8 and 9). In contrast, the highest levels of glucagon receptor mRNA transcripts (in the brains of either wild-type or trangsenic mice) were consistently observed in RNA from mouse cerebellum at 2, 5, and 12 weeks. Although glucagon receptor mRNA transcripts were slightly more abundant in wild-type compared to transgenic brain stem at 2 weeks, no marked differences in the levels of glucagon receptor mRNA transcripts were detected in wild-type *vs.* transgenic mice.

In contrast to the relatively low levels of glucagon receptor gene expression in the brain, GLP-1 receptor mRNA transcripts were generally more abundant in different brain regions of both fetal and adult mice (Figs. 10 and 11). Furthermore, GLP-1 receptor mRNA transcripts were detected in fetal cortex and cerebellum; they were not restricted to the brain stem. In contrast to the relatively comparable expression patterns of glucagon receptor mRNA in wild-type and transgenic mice, GLP-1 receptor mRNA transcripts were clearly increased in different regions of transgenic mouse brain at 5 weeks and markedly decreased at 12 weeks. To control for potential differences in RNA preparation, handling/analysis, and RT-PCR reactions, all samples were also analyzed (by RT-PCR) for the expression of PGK mRNA transcripts. A representative control experiment is shown in Fig. 12. No marked sample to sample variation was detected for the analysis of PGK mRNA transcripts in different tissues.

Discussion

The cloning of the rat GLP-1 and glucagon receptor cDNAs has opened new avenues into the investigation of the biology of the proglucagon-derived peptides. Although proglucagon gene transcription is restricted to pancreas, intestine, and brain, the widespread distribution of glucagon and GLP-1 receptor mRNA transcripts supports an endocrine role for the proglucagon-derived peptides in various tissues, aside from the liver and pancreas. Our results for GLP-1 receptor gene expression are in good agreement with the distribution of mRNA transcripts recently reported in the rat (19). Furthermore, we have demonstrated that the GLP-1 receptor gene is widely expressed in different regions of the small and large intestine; the significance of GLP-1 receptor expression in bowel remains to be defined, however, elevations in the intestinal levels of proglucagon-derived peptides concentrations have implicated these hormones in the mediation of intestinal growth observed after small bowel resection (25–27). GLP-1 receptor expression was also detected in liver at relatively high levels. The expression of the GLP-1 receptor in liver supports the hypothesis that GLP-1 may also modulate glucose disposal, possibly at the level of the hepatocyte (9).

No studies to date have reported the tissue distribution of glucagon receptor mRNA transcripts. Northern blot analysis of poly(A^+) RNA detected glucagon receptor mRNA transcripts in liver and kidney; in contrast, the more sensitive RT-PCR analyses detected glucagon receptor mRNA transcripts in pancreas, lung, brain, and small intestine. Whereas GLP-1 receptor binding has been localized to the islets of Langerhan's, the cellular localization (endocrine *vs.* exocrine) of glucagon receptor expression in the pancreas is not known.

The results of the RT-PCR analyses demonstrate that mRNA transcripts for the glucagon and GLP-1 receptors are widely distributed in the CNS and appear to be regulated in a developmental and region-specific manner. Although glucagon receptor mRNA transcripts were detected in the brain in relatively low abundance in mice of different ages, glucagon receptor expression in fetal brain was restricted to the brain stem on embryonic day 19. Low but detectable levels of glucagon receptor mRNA transcripts were detected in cortex, cerebellum, hypothalamus, and brain stem of mice 2–12 weeks of age. These observations together with the data demonstrating proglucagon gene expression in comparable regions of mouse brain (22) support a biological (possibly paracrine) function for glucagon in the CNS. These anatomical observations are supported by indirect evidence that intracerebroventricular administration of glucagon induced hyperglycemia in mice (28) and rats (29).

Whereas glucagon receptor mRNA transcripts were not detected in fetal cortex or cerebellum, GLP-1 receptor mRNA was readily detectable in these same regions of day 19 fetal mouse brain. Furthermore, the region- and age-specific pattern of GLP-1 receptor expression in the brain more closely resembled the data reported for mouse proglucagon gene expression (22). For example, both proglucagon as well as GLP-1 receptor mRNA transcripts are initially comparatively lower in the brain stem of 2-week-old mice, whereas a marked induction of both proglucagon and GLP-1 receptor mRNA transcripts is detected in the brain stem of 5- and 12-week-old animals. These observations suggest a possible link between the region- and age-specific expression of a proglucagon-derived peptide and its receptor, indicating coordinate regulation of ligand/receptor gene expression. Alternatively, the presence of GLP-1 in the brain stem may be associated

with the induction of the mRNA for its receptor, a hypothesis that may be testable in future experiments.

The striking induction of GLP-1 receptor mRNA transcripts in the hypothalamus and brain stem of 2- and 5-week-old GLUTag transgenic mice, followed by the marked suppression of GLP-1 receptor gene expression in the hypothalamus and brain stem at 12 weeks, differs from the temporal changes in CNS proglucagon gene expression previously described in GLUTag transgenic mice. Whereas proglucagon mRNA transcripts were induced in the hypothalamus and brain stem of GLUTag mice at 2 weeks, a marked suppression of proglucagon and SV40Tag mRNA transcripts in the brain was observed at 5 weeks (22). The reason for these differences in proglucagon/GLP-1 receptor expression are not clear, but strongly point to divergent control mechanisms for the regulation of the proglucagon and GLP-1 receptor genes in GLUTag mice.

Recent studies of GLP-1 action have shown that GLP-1 is a potent glucose-dependent activator of insulin gene expression and insulin secretion from the endocrine pancreas (8–10, 30). These observations have rekindled an interest in the biology of the proglucagon-derived peptides, and the efficacy of GLP-1 in human studies has led to the suggestion that GLP-1 may be a novel therapeutic agent for the treatment of diabetes mellitus (9). The results of the experiments described here extend our concepts of the tissue distribution of the GLP-1 and glucagon receptors. The differential tissue-specific localization of receptor expression suggests that the two receptor genes have evolved distinct transcriptional control mechanisms for the regulation of receptor expression. Furthermore, the demonstration of receptor expression in multiple fetal tissues suggests a possible role for the proglucagon-derived peptides during fetal development. The identification of receptor mRNA transcripts in tissues such as kidney and lung as well as the spatial restriction of glucagon, but not GLP-1, receptor expression in the intestine suggest that novel physiological actions mediated by the proglucagon-derived peptides and their cognate receptors in different tissues remain to be elucidated.

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