Divergent regulation of human and rat proglucagon gene promoters in vivo

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Nian, Min, Daniel J. Drucker, and David Irwin. Divergent regulation of human and rat proglucagon gene promoters in vivo. *Am. J. Physiol.* 277 (*Gastrointest. Liver Physiol.* 40): G829–G837, 1999.—A single mammalian proglucagon gene is expressed in the brain, islets, and intestinal enteroendocrine cells, which gives rise to a unique profile of proglucagon-derived peptides (PGDPs) in each tissue. The biological importance of glucagon, glucagon-like peptide (GLP)-1, and GLP-2 has engendered considerable interest in the factors regulating the synthesis and secretion of the PGDPs in vivo. Although rat proglucagon gene transcription has been extensively studied, the factors important for control of human proglucagon gene expression have not been examined. We now report that, despite conservation of proximal promoter G1–G4 enhancer-like elements, human proglucagon reporter plasmids containing these elements are transcriptionally inactive in islet cell lines. Remarkably, larger human proglucagon promoter fragments, such as the 1604 hGLU-Luc, are expressed in GLUTag enteroendocrine cells but not in islet cell lines. A total of 5775 bases of human proglucagon promoter were required for expression in islet cell lines. Analysis of human proglucagon promoter expression in transgenic mice demonstrated that ~1.6 kb of human proglucagon gene sequences directs expression of a human growth hormone reporter gene to the brain and intestinal enteroendocrine cells but not islet cells in vivo. These findings provide the first evidence demonstrating divergence in the mechanisms utilized for tissue-specific regulation of the human and rodent proglucagon genes.

islets; glucagon-like peptide-1; glucagon-like peptide-2; intestine

THE MAMMALIAN PROGLUCAGON gene is expressed in the A cells of the pancreatic islets, the L cells of the small and large intestine, and selected neurons of the brain. In mammals, tissue-specific differences in the posttranslational processing of proglucagon result in the liberation of glucagon in the pancreas and two glucagon-like peptides, GLP-1 and GLP-2, in the intestine (7). The proglucagon-derived peptides (PGDPs) have diverse and essential roles in human physiology. Glucagon is an important regulator of carbohydrate, lipid, and amino acid metabolism and acts as a counterregulatory hormone to insulin in regulating levels of blood glucose levels. GLP-1 is an incretin hormone that potentiates insulin release from the pancreas and also plays a role in gastric emptying and feeding behavior (7). GLP-2 stimulates intestinal hexose transport and is trophic to the mucosal epithelium of the small and large intestine (6). In contrast, the biological activities and physiological relevance of oxyntomodulin and glicentin, PGDPs cosecreted with GLP-1 and GLP-2 from the gut, are less well established.

The finding that pancreatic and intestinal PGDPs have pleiotropic biological effects has stimulated considerable interest in understanding the regulation of PGDP biosynthesis. The majority of these studies have utilized rodent islet cell lines to identify the determinants regulating proglucagon gene expression in vitro. Transfection studies have identified three enhancer-like elements (designated G2, G3, and G4), and an islet-specific promoter element (G1), in the first 300 bp of the rat glucagon gene promoter (4, 32). Glucagon gene expression and secretion of the PGDPs are regulated by a cAMP-dependent pathway in both islets and intestinal cells, consistent with the functional localization of a cAMP response element in the rat proglucagon gene promoter (8, 24). Complementary studies using transgenic mice demonstrated that 1252 nt of rat glucagon promoter sequences are sufficient for targeting transgene expression to the A cells of the pancreatic islets, and transfection experiments have implicated a role for *cdx*-2/3, *isl*-1, *brn*4, *pax*6, and both hepatocyte nuclear factor (HNF)-3α and HNF-3β in the control of islet proglucagon gene transcription (17, 22, 23, 33, 36, 40).

The interest in gut-derived PGDPs such as GLP-1 and GLP-2 has stimulated interest in understanding the regulation of intestinal proglucagon gene expression. Studies using rodent intestinal cell lines demonstrate that peptide hormones such as gastrin-releasing peptide activate proglucagon gene transcription (27). Complementary experiments in rats indicate that enteral nutrition stimulates intestinal proglucagon gene expression, predominantly in the jejunum (16), whereas diets enriched in fiber or fatty acids increase proglucagon mRNA transcripts in the small and large bowel (35, 38). Intriguingly, although 1252 bp of rat proglucagon promoter sequences are sufficient for correct targeting of transgene expression to islet A cells (12), additional 5′-flanking sequences are required for specifying rat intestinal proglucagon gene transcription in vivo (25).

Despite the importance of human PGDPs in normal physiology and metabolic disorders such as diabetes, paradoxically little is known about the regulation of human proglucagon gene transcription. The structural organization of the human and rodent proglucagon cDNAs and genes appears similar (3, 15, 41), with significant nucleotide identity extending to key regulatory sequences such as the G1–G4 elements in the human and rat proglucagon gene 5′-flanking regions.

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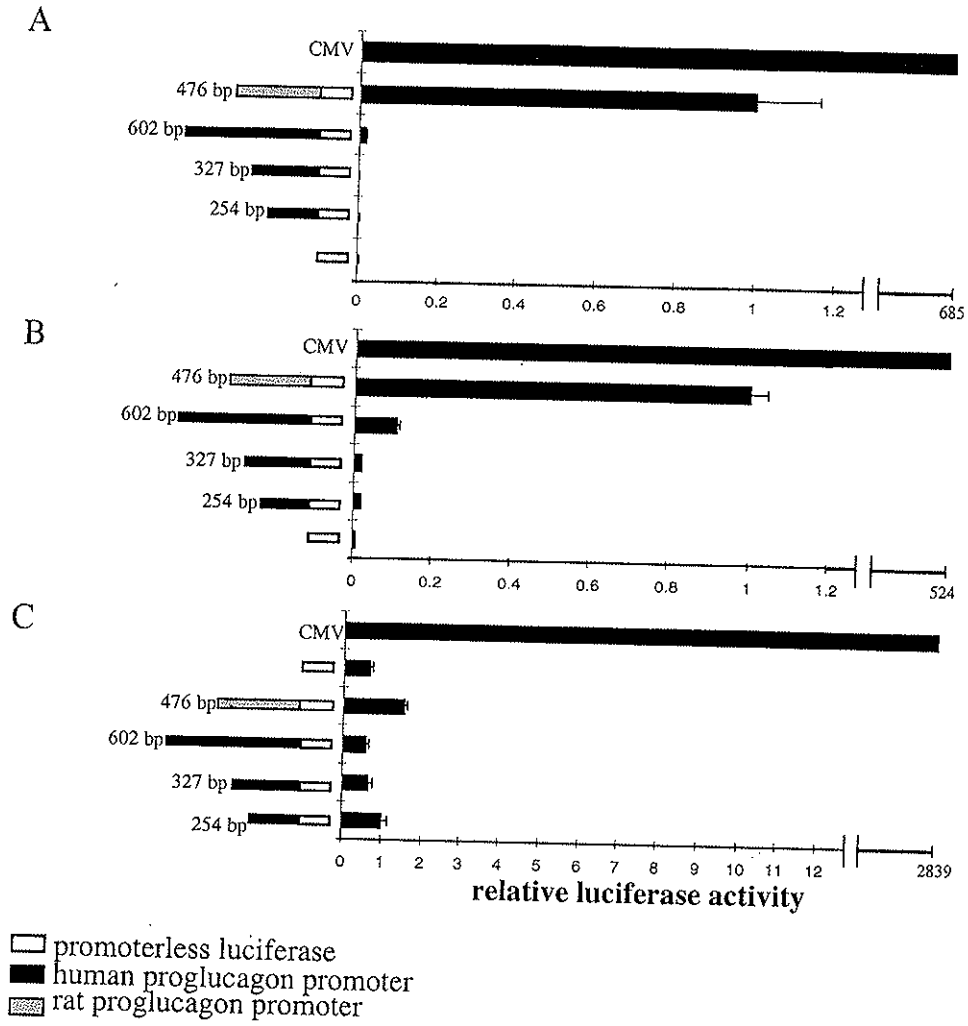

Fig. 1. Transcriptional activities of human proglucagon promoter plasmids. *A*: hamster pancreatic InR1-G9 cells. Ten micrograms of DNA were transfected by the calcium phosphate method, and cells were harvested for analysis of luciferase activity after 24 h. *B*: mouse intestinal GLUTag cells. Ten micrograms of DNA were transfected by the electroporation method, and cells were harvested for analysis of luciferase activity ~ 24 h later. *C*: baby hamster kidney fibroblast (BHK) cells. Ten micrograms of DNA were transfected by the calcium phosphate method, and cells were harvested for analysis of luciferase activity 24 h following transfection. For data in *A–C*, relative luciferase activity was normalized to the activity of the rat proglucagon promoter construct. CMV, CMV-Luc promoterless plasmid.

Although numerous studies have analyzed rat proglucagon gene transcription in both transgenic and transfection experiments, no reports have yet described the regulation of the human glucagon gene. In this study, we isolated human proglucagon gene regulatory sequences and analyzed human proglucagon gene transcription using transfected reporter genes and cell lines in vitro and transgenic mice in vivo. Our findings

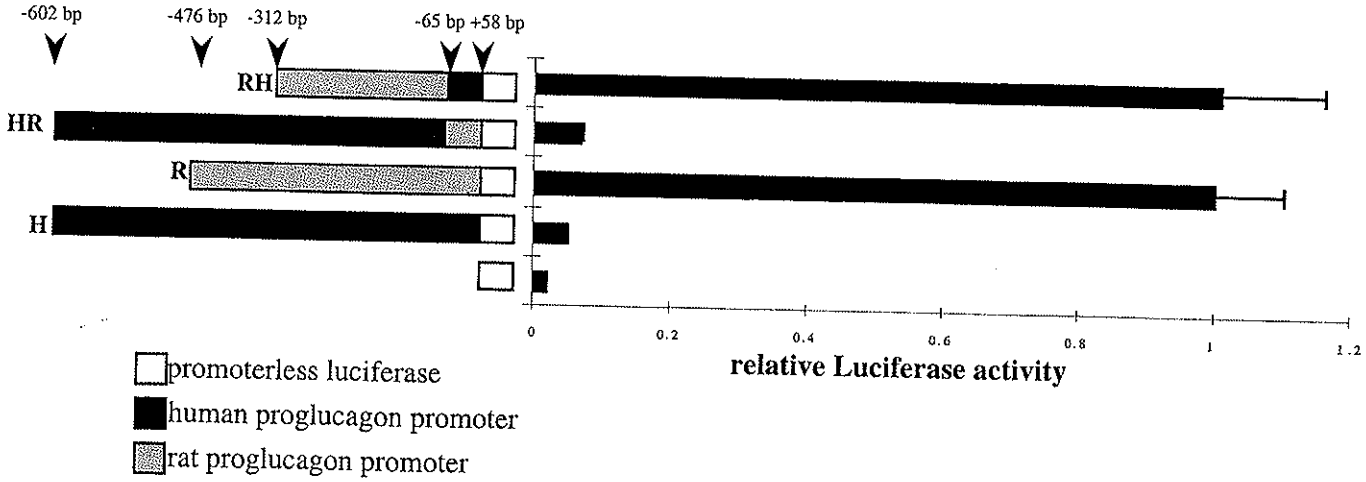

Fig. 2. Transcriptional activities of human (H)-rat (R) proglucagon promoter chimeras (see text for description of RH and HR). Hamster pancreatic InR1G9 islet cells were transfected with 10 μg of plasmid DNA, and relative luciferase activity was normalized to the activity of the wild-type rat promoter construct.

demonstrate that the human and rat proglucagon genes utilize different regulatory regions to achieve identical patterns of tissue-specific gene expression.

MATERIALS AND METHODS

Reagents. Reagents and chemicals were purchased from Bioshop Canada (Burlington, ON), Caledon (Georgetown, ON), Difco (Detroit, MI), Pharmacia (Baie d'Urfe, PQ), Sigma Chemical (St. Louis, MO), and Canadian Life Technologies (Burlington, ON). Restriction endonucleases and DNA modifying enzymes were from New England Biolabs (Mississauga, ON). *Taq* DNA polymerase, 10× PCR buffer, and deoxynucleotide triphosphates were obtained from Boehringer Mannheim (Laval, PQ). Oligonucleotide primers were synthesized by ACGT (Toronto, ON). T7 sequencing kit was purchased from Pharmacia (Montreal, PQ). Radioactive deoxynucleotide labels were obtained from Amersham (Oakville, ON).

Plasmids. A human P1 DNA clone encoding glucagon gene sequences was isolated, using a PCR-generated probe, by Genome Systems (St. Louis, MO). Human proglucagon promoter sequences were isolated, characterized by restriction mapping and DNA sequencing, and subcloned into the promoterless plasmid SK-Luc (21) immediately adjacent to the coding sequences of the firefly luciferase reporter gene.

Cell culture and transfections. The hamster islet cell line InR1-G9 (11) and baby hamster kidney fibroblasts (BHK cells) were grown in DMEM (4.5 g glucose/l) supplemented with 5% calf serum and 10% penicillin and streptomycin. The mouse enteroendocrine cell line GLUTag (9) was grown in DMEM supplemented with 10% fetal bovine serum. InR1-G9 and BHK cells were transfected with 10 μg of plasmid DNA/5-cm plate by the calcium phosphate method. GLUTag cells were transfected with 10 μg of plasmid DNA/5-cm plate by electroporation, and cells were harvested 20–36 h after transfection. Each set of transfections was carried out on three separate occasions in triplicate for each plasmid. The promoterless plasmid SK-Luc and CMV-Luc were used as negative and positive controls in each transfection. Luciferase activities were analyzed using a Lumat LB 9501 (EG&G Berthold, Wellesley, MA).

A fragment of the human proglucagon gene 5′-flanking region from an *Xba* I site (−1602) to +120 in exon 1 was subcloned into the promoterless plasmid P0GH (37) immediately adjacent to a 2.1-kb *Bam*H I-*Eco*R I human growth

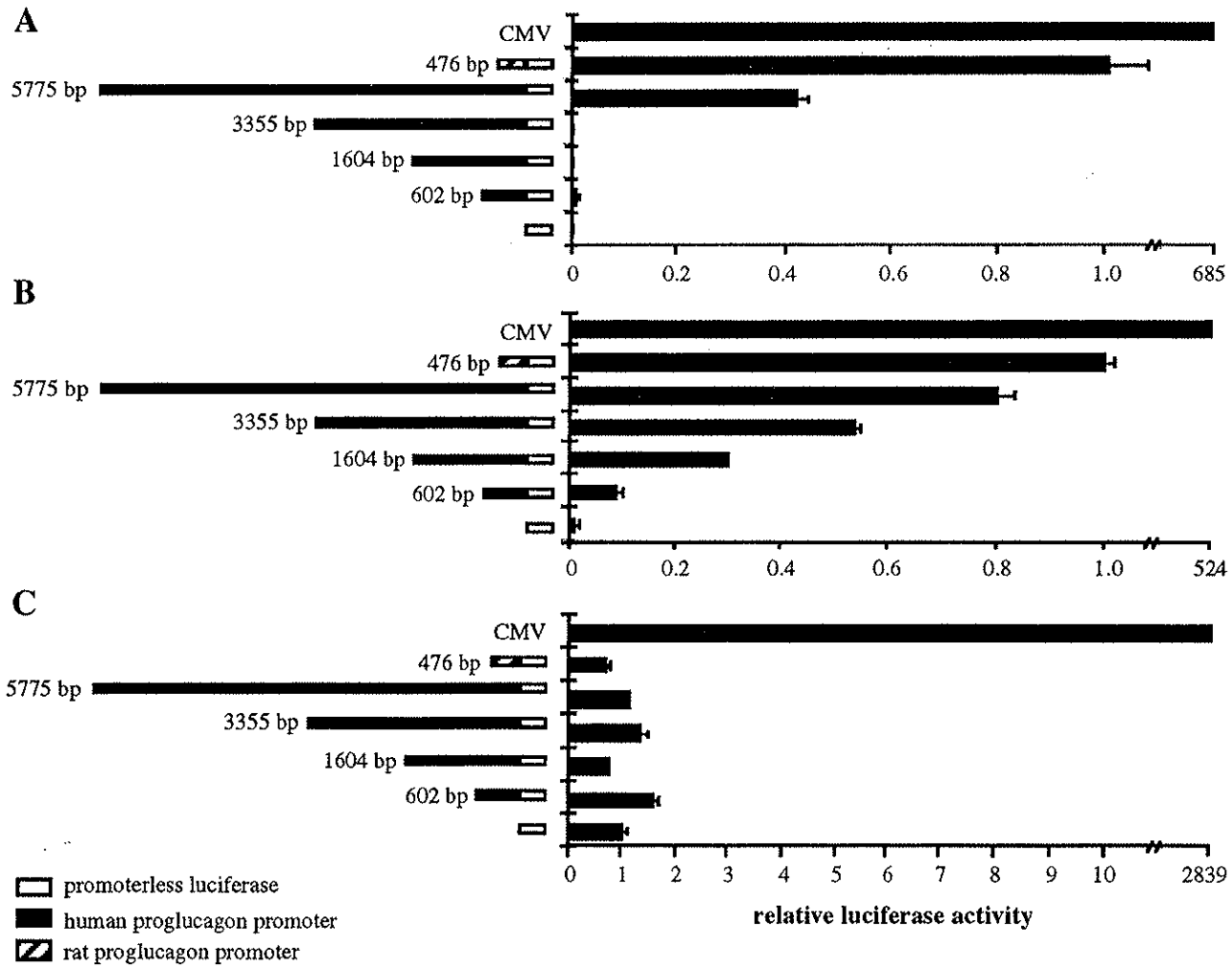

Fig. 3. Transcriptional activity of the human proglucagon promoter. *A*: hamster pancreatic InR1-G9 cells. Total amount of DNA in each transfection was kept constant at 10 μg by adding appropriate amount of Bluescript vector DNA. Relative luciferase activity was normalized to the rat promoter construct. *B*: mouse intestinal GLUTag cells. GLUTag cells were transfected as described in MATERIALS AND METHODS and Fig 1. *C*: BHK cells. BHK cells were transfected as described in MATERIALS AND METHODS and Fig 1.

hormone (hGH) gene fragment that contains hGH sequences from the start of the transcription (*Bam*H I) site to 526 bp past the poly(A)$^+$ addition site. This plasmid was designated as 1.6hGLU-GH.

Generation of transgenic mice. The purified 3.8-kb human proglucagon promoter/hGH chimeric gene was used to generate transgenic founders (Chrysalis DNX Transgenics, Princeton, NJ), and mice were characterized by Southern blot analysis of tail DNA as previously described (25). Nylon membranes were exposed on the phosphorimage screen overnight and analyzed using a STORM 840 (Molecular Dynamics, Sunnyvale, CA).

RNA isolation and analysis. Total cellular RNA was isolated from various tissues using TRIzol reagent (Life Technologies). cDNA was synthesized by reverse transcription using a cDNA synthesis kit (Amersham Pharmacia Biotech, Toronto, ON). Primers for the hGH coding sequence were 5′-GAAGAAGCCTATATCCCAAAG-3′ and 5′-GAGTAGTGCGTCATCGTTGTG-3′, generating a 378-bp cDNA. The PCR reaction was carried out for 35–40 cycles (94°C for 1 min and then annealing at 55°C for 1 min and extension at 72°C for 1 min), and amplification products were analyzed on 2% agarose gels stained with ethidium bromide. mRNA was isolated from 400 μg of total RNA using Oligotex mRNA Mini kit (Qiagen, Chatsworth, CA). RNA hybridization and washing were carried out as described, using [α-^{32}P]dATP randomly labeled hGH and mouse glyceraldehyde-3-phosphate dehydrogenase cDNA probes (8).

Immunohistochemical analysis. Tissues from 6- to 8-wk-old mice were fixed in buffered neutral formalin for 24 h. Tissues were processed for immunohistochemistry and/or intestinal morphometry as previously described (1, 2). Formalin-fixed, paraffin-embedded tissue was sectioned at 4 μm for imunohistochemistry using the streptavidin-biotin-peroxidase complex technique. Primary antisera and antibodies were directed against the following antigens and were used at the specified dilutions: GLP-1 (polyclonal antiserum prepared by D. J. Drucker) at 1:2,500 for 30 min and hGH at 1:1,000 (DAKO, Toronto, ON). The reactions were visualized using 3,3′-diaminobenzidine and hydrogen peroxide. Appropriate positive and negative controls were performed in each case.

RESULTS

Regulation of human proglucagon gene expression in transfected cell lines. Because a 300-bp fragment of rat proglucagon promoter sequences is sufficient for directing reporter gene expression in pancreatic islet cell lines (10), we initially surmised that similarly sized fragments of the proximal human proglucagon gene promoter would also activate luciferase gene expression in islet cells. Three plasmids containing 254, 327, or 602 bp of human proglucagon gene 5′-flanking sequences ligated upstream of the luciferase-coding sequence were transfected into islet InR1-G9 cells, mouse GLUTag intestinal cells, and BHK fibroblasts. Surprisingly, no significant luciferase activity was detected after transfecting the hGLU-Luc fusion genes into hamster islet InR1-G9 cells (Fig. 1*A*). The 602-bp hGLU-Luc plasmid generated <5% of the luciferase activity generated using 476 bp of the rat proglucagon promoter. Similarly, the hGLU-Luc plasmids were significantly less transcriptionally active in GLUTag enteroendocrine cells compared with −476 rat GLU-Luc (Fig. 1*B*). Neither the human nor rat GLU-Luc plasmids induced significant luciferase activity in BHK fibroblasts, with luciferase activities <1% of those obtained with CMV-Luc.

To determine whether sequence differences in the proximal human glucagon promoter explained the lack of human proglucagon promoter activity in islet and intestinal cells, we constructed two hybrid human/rat promoter-luciferase fusion genes. The HR hybrid promoter contains human sequences from −602 to −66 bp fused to sequences from −65 to +58 bp of the rat proglucagon gene. The RH hybrid promoter contains sequences from −312 to −66 bp from the rat proglucagon promoter fused to −65 to +58 bp of the human proglucagon gene. Transfection of the RH plasmid into islet cells revealed that the tandemly linked rat upstream enhancers G2, G3, and G4 were sufficient for transcriptional activation of the more proximal human promoter sequences. In contrast, the equivalent human proglucagon gene sequences containing G2, G3, and G4

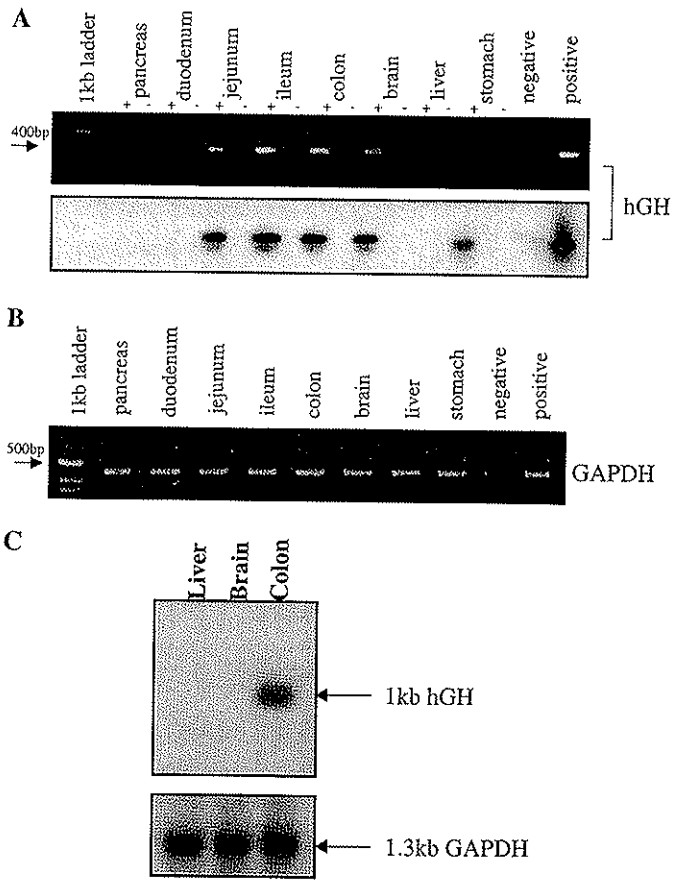

Fig. 4. Tissue-specific expression of the human growth hormone (hGH) transgene in 1.6hGLU-GH transgenic mice. *A*: RT-PCR of various tissues from a 1.6hGLU-GH transgenic mouse. + and −, Presence or absence, respectively, of RT in the preparation of the cDNA template for the PCR. RT-PCR DNA was transferred to a nylon membrane and hybridized with a [γ-^{32}P]dATP end-labeled internal hGH oligonucleotide DNA probe. *B*: quality of the cDNA for tissue-specific expression was assessed by RT-PCR using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers as described in MATERIALS AND METHODS. *C*: Northern blot analysis of transgene expression in 1.6hGLU-GH transgenic mice. Poly(A)$^+$ RNA (4 μg) was loaded in each lane, and blot was hybridized with a [α-^{32}P]dATP-labeled hGH cDNA probe. Blot was then rehybridized with an [α-^{32}P]dATP-labeled GAPDH cDNA probe.

elements did not activate transcription from the proximal rat promoter (Fig. 2).

The failure of human proglucagon proximal promoter sequences to support transcriptional activity in transfected islet and intestinal cells suggested that additional elements, possibly 5′ to −600, were required for human proglucagon gene transcriptional activity. To test this hypothesis, we isolated additional human glucagon gene genomic sequences for construction of larger hGLU-Luc reporter genes. Plasmids containing 1604, 3355, and 5775 bp of human proglucagon gene 5′-flanking sequences were transfected into InR1G9, GLUTag, and BHK cell lines. Only the plasmid containing 5775 bp of human proglucagon 5′-flanking sequences was transcriptionally active (Fig. 3) in the InR1G9 islet cell line. In contrast, hGLU-Luc plasmids containing 1604 or 3355 bp of human proglucagon promoter sequences were transcriptionally active in GLUTag enteroendocrine cells. However, none of the human or rat proglucagon promoter-luciferase plasmids generated significant activity in BHK fibroblasts (<1% activity obtained with CMV-Luc).

Regulation of human proglucagon gene expression transgenic mice. The results of the cell transfection studies suggested that transcription of the human proglucagon gene is differentially regulated in both islet and intestinal cell lines, since different promoter sequences are required for human vs. rat proglucagon gene transcription in islet and intestinal endocrine cells. To exclude the possibility that these results reflected species-specific differences in the ability of immortalized rodent cell lines to support reporter gene expression and not true biological differences in promoter regulation, we introduced a human proglucagon promoter-reporter transgene, 1.6hGLU-GH (Fig. 3) into mice. This transgene was predicted, on the basis of cell line experiments, to support transcriptional activation in the intestine, but not the pancreas, of transgenic mice. The transgene contains 1604 bases of human proglucagon promoter linked to the hGH gene. Six transgenic founders were obtained, and germ line transmission was observed in two founder lines as assessed by Southern blot analysis (data not shown).

Transgene expression was assessed using a combination of RT-PCR, Northern blot analyses, and immunocytochemistry. hGH mRNA transcripts were detected in the stomach, jejunum, ileum, colon, and brain but not in the pancreas or liver of two lines of 1.6hGLU-GH transgenic mice (Fig. 4*A*). Northern blot analysis demonstrated an ~1-kb hGH mRNA transcript in colon mRNA, consistent with the predicted size of the transgene transcript if expression initiated correctly from the human proglucagon promoter sequences. To determine if the 1.6-kb human proglucagon promoter contained the necessary elements required for directing transgene expression to enteroendocrine cells, hGH expression was localized by immunocytochemistry. hGH-immunoreactive cells were detected in the stomach, jejunum, ileum, and colon (Fig. 5). Furthermore, staining of adjacent sections for hGH and GLP-1 demonstrated that the same population of enteroendocrine cells contained both hGH and GLP-1 immunopositivity (data not shown). Similar colocalization of GLP-1 and hGH immunopositivity was observed in occassional brainstem neurons, consistent with the known localization of central nervous system (CNS) proglucagon gene expression. In contrast, no transgene expression was detected, as assessed by either RT-PCR or immunocytochemistry, in the pancreas of 1.6hGLU-GH transgenic mice.

DISCUSSION

The rodent and human proglucagon genes share an identical exon/intron organization, and studies of tissue-specific processing of the PGDPs demonstrate an identi-

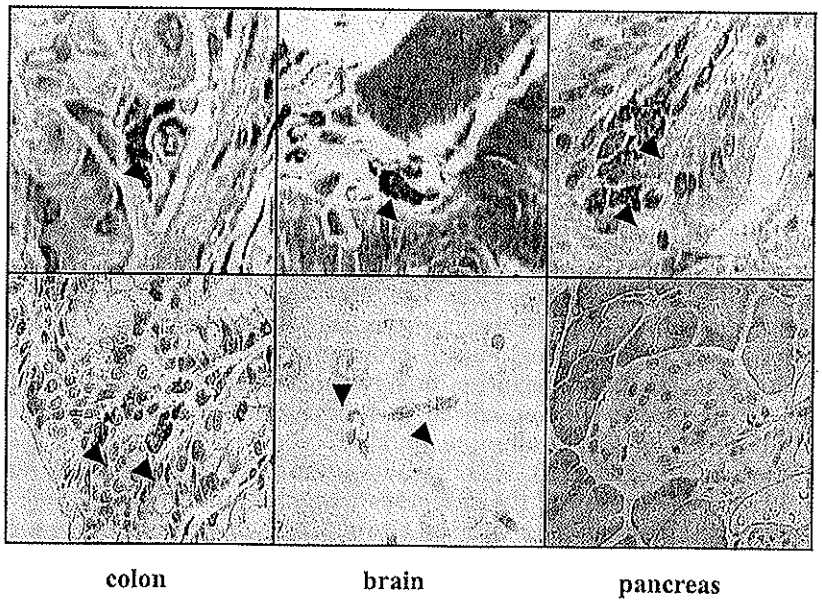

Fig. 5. Immunocytochemical localization of growth hormone-immunopositive cells in 1.6hGLU-GH transgenic mice. Tissues from a 6-wk-old 1.6GLU-GH mouse were stained with antisera against hGH antibody and rat glucagon-like peptide-1 (not shown). Arrows indicate the location of the growth hormone-immunopositive cells. Magnification = ×400.

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Human          -1603bp   TCTAGACCGACCTA----AGGCTTGACAC   -1580bp
Rat                      ...-.C.A..G..GGATGGA...G.....

Human CTTTCTCTGCTCTTTCACCCCCTCTGCTGGTAGAGTGCCAAGAAGCAGGT   -1530bp
Rat   ....................A.....C..-.TC-........-..T.CC

Human CTCAGATCAAGACACCAGCAGTACCAATGAGGAGATAAAGTGAGGCAGAT   -1480bp
Rat   ..G.................C.........-----.......ATT....

Human ATTTTATGCAGCTGTG-CTTCT--CAAATTT--TAATATGAACACAGATT   -1435bp
Rat   :A.....A........A....AGC.T...C.GC...---.-.-.---..

Human TCCTTAGGATTTTGTTAAAAT--ACA----GATTC--T------GA--T-   -1402bp
Rat   -...GG..GCCG..A......GC...TTCT.....CA.AAACCA..CC.G

Human AGGCACGAGAT-TCTGCATTTCTAGCA-AGCTCC-CAGATGATGCTGGT    -1356bp
Rat   ..C..A.....G.-.C.....T..--.T..GA..T..TGGA.-....AT

Human CCTTAGACC-ACACTTT-GCAAAGCAAGGCTTTCTAGCACCCAATAATCA   -1308bp
Rat   ..C.G..G.T.-...C.A.GT.C-..G......A..T..T.-...G.-..

Human CC-AGCAC-AAAACCCCTCAATGATGTCTTCCAGTCTGACTTG-AAAGTG   -1261bp
Rat   A.T.A.G.G.......-...G.A.G....C...A-.....AG.G......

Human ATCTC-TGTGGCCTTCAA-GTAGCTCTACAT-TCAAAC-GTTGGTCTCA-   -1216bp
Rat   G.-..C.A.TT.T.--..T.A.....--...G..C.C.A...A..-...A

Human TGCACAAAT-TGAACA-GTTAAGTTCATTTGGAATATTTCTCCAGTTGCC   -1168bp
Rat   ......T-.C....G.A..A..-......CA..G..C.C.........TT

Human TCAATGA--T-TTACCTCCATACAAGTCATCAGTGACATCATGCG-GTTG   -1122bp
Rat   ......GGC.A..---C.....A....T....A.T.T.C.....-GA....

Human GTTATA-TTAGA-ATATTGGAATCTAGTTAGGAAATGTTGTGATGAGT     -1076bp
Rat   A.-.C.C..G..C.CCCA.--..T...C.G.......C....G...-.

Human -TTG-CAATATGGAATAAAATTTTTATTTTAGAAAAGACATTTAC-CA      -1031bp
Rat   C...T..-...A.....TG.G...C....A.AC...TCG-.....A..

Human ACCCCTCTCAT--TT-T-ACAAATAAGGAGGCAGAGACACAAAGAGAATG   -985bp
Rat   .A---..A-..GC..C.C......C..C.--.....T.C......C--..

Human CGTGACTTGACCAAGGTCACACAGC-TGGTCA--ATAACAGGCAAAACTA   -938bp
Rat   T...G....G-..-....T..T...C.AT.GTG....G....G..G.--.

Human AAATCCAAATTAT---CCCAAC--ACTACTTCTTATGCTGTCT-T-T-C-   -896bp
Rat   .....T.C-....GAT...--.GG.G.--.....--.......C.G.T.A

Human CAGAT------TATTTACCATTTTTCTATTTCTCTACAGT-GA-----CC   -858bp
Rat   ..C..CCTGGT.....C...A.....--.....-..T.T..T..AATGG..

Human AGAATCAT-T-TCTGCTAAATGTCACAGATCATTA--AAG--CCTGTGTG   -814bp
Rat   .....--.G.A.......T..---..T-..A....GT...GA...A.A.A

Human TCCAGT-CACAAAACT-CAGGAAACGTGAAAAT-ATGCATCTCAT-CTCA   -768bp
Rat   -.--..G.TGG..GT.A...-...-..-..C.GC..........GA.-..
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Fig. 6. Nucleotide sequence alignment of the human and rat (20) proglucagon gene 5′-flanking regions. Nucleotide sequences shown in bold correspond to putative transcription factor binding sites. Conserved nucleotides are indicated by dots. Gaps (–) were introduced into the nucleotide sequences to maximize alignment. G1–G4 enhancer and promoter regions are underlined; +1 refers to the transcription start site, as previously defined for the rat promoter (26).

cal profile of peptide processing in human vs. rodent tissues (29, 30). Remarkably, despite the strong conservation of proximal promoter elements known to be important for islet cell-specific rat proglucagon gene transcription, human proglucagon reporter genes containing either 254, 327, or 602 bp of 5′-flanking sequences were transcriptionally inactive in pancreatic islet InR1-G9 cells. Comparison of the rat and human proximal proglucagon promoter sequences (Fig. 6) show that, although they are indeed highly related, several differences in nucleotide sequence are apparent. The G1 promoter element is almost perfectly conserved, as are the binding sites for *isl*-1, *cdx*-2/3, and *Brn*-4 (17, 21, 40). In contrast to G1, the upstream G2–G4 enhancer sequences are less well conserved (Fig. 6). Nucleotide changes are observed between the rat and human proglucagon promoters at the binding sites of four transcription factors that interact with upstream promoter sequences, including cAMP response element binding protein, *pax*6, HNF-3α and HNF-3β (9, 23, 33, 36) (Fig. 6). Whether changes in the interaction of one or more of these factors with the human proglucagon promoter is sufficient to extinguish expression in human islets seems unlikely, given our findings that

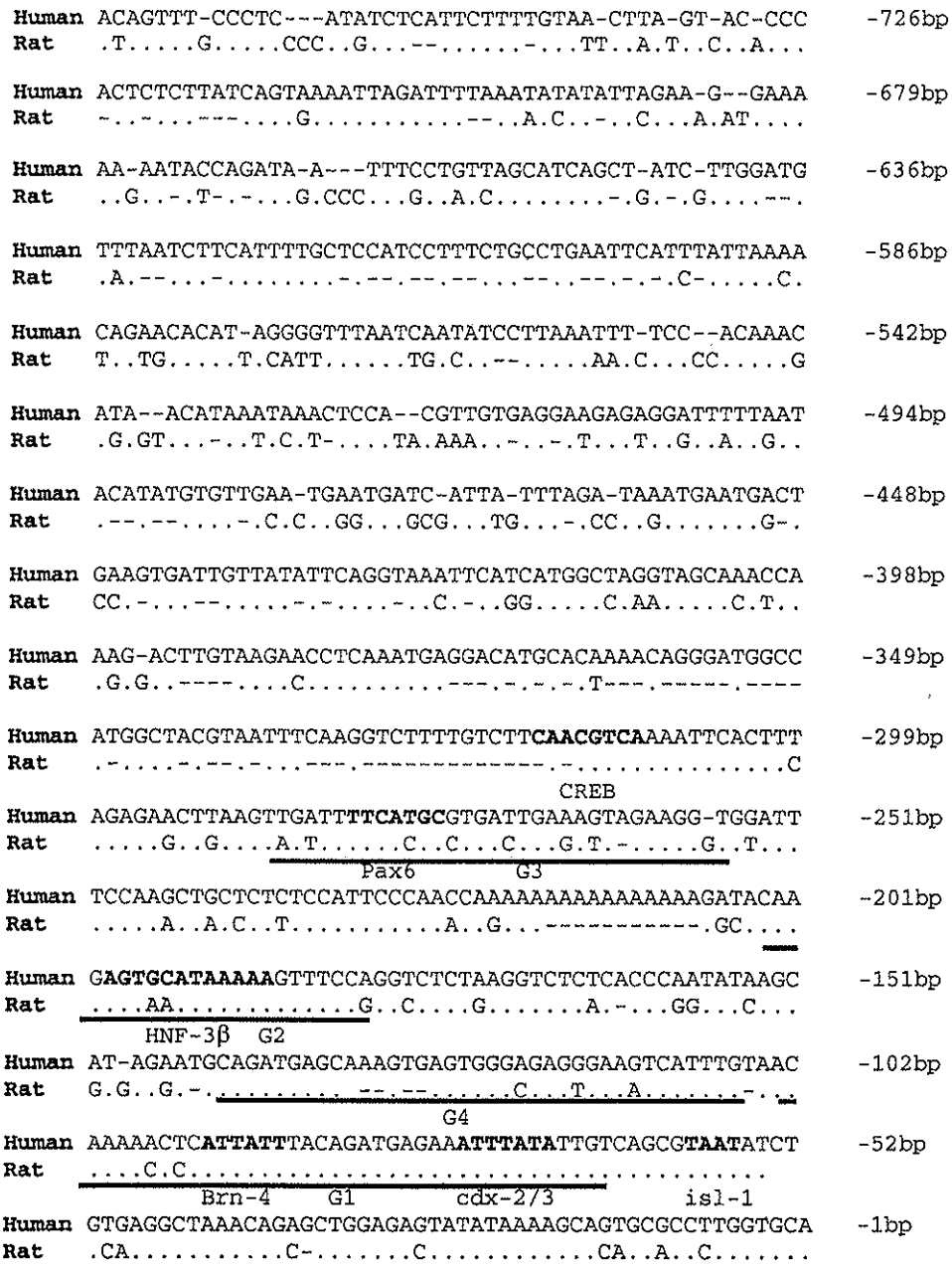

Fig. 6.—*Continued.*

addition of more distal 5′-flanking sequences restores expression in islet cell lines.

The results obtained from analysis of the hybrid HR and RH proximal promoter plasmids demonstrate that, whereas the rat upstream G2–G4 enhancers can effectively activate expression of the more proximal human promoter, the comparable human sequences homologous to the G2–G4 enhancers are not sufficient to activate reporter expression from the proximal rat promoter in islet and intestinal cells (Fig. 2). These results strongly suggest that changes in sequence between −66 and −300 bp of human proglucagon promoter (i.e., G2, G3, and G4) contribute to the lack of expression of the human proglucagon promoter in islet cell lines. To compensate, it seems likely that the human proglucagon gene has evolved one or more additional upstream regions that direct expression in islet cells. The available evidence from our transfection studies suggests that a pancreatic islet enhancer is likely located between −3355 and −5775 in the human proglucagon gene promoter, a hypothesis that should be further explored in future experiments.

The results of previous studies examining rat proglucagon gene expression in cell lines and transgenic mice demonstrated that, whereas 1252 bp of rat proglucagon 5′-flanking sequences directed transgene expression to the brain and islets of transgenic mice, no expression was observed in enteroendocrine cells of the small or large intestine (12). Addition of upstream 5′-flanking sequences extending to −2252 was required for expression of the rat proglucagon promoter in intestinal enteroendocrine cells (25). In contrast, our results from

both cell lines and transgenic mice clearly demonstrate that, although ~1600 bp of human proglucagon gene sequences are sufficient for expression in brain and intestine, additional regulatory sequences are required for human islet cell expression.

These findings suggest that, although the mechanisms specifying CNS and enteroendocrine proglucagon gene transcription are likely highly conserved across species, the DNA sequences and transcription factors regulating human and rat proglucagon expression in the islet have clearly diverged. Previous studies of rat proglucagon promoter expression using transgenic mice and cell transfections identified a modular glucagon gene intestinal enhancer, designated GUE (glucagon upstream enhancer), between −2292 and −1253 in the rat proglucagon gene 5′-flanking region (20). A smaller 45-bp subdomain of the GUE was identified, between −1431 and −1387, that exhibited intestinal enhancer-like activity in enteroendocrine cells (20). The results of our human proglucagon gene transgenic and transfection studies demonstrate that intestine-specific expression is mediated by sequences contained within the first ~1600 bp of the human proglucagon gene 5′-flanking region. Hence, it seems reasonable to infer, based on the available rat and human data, that sequences comprising a functional GUE likely reside between −1252 and −1600 bp. Within this region, there are conserved potential binding motifs for GATA, basic helix-loop-helix, and *cdx*-2/3 transcription factors.

The reason(s) for utilization of different regulatory sequences in the human and rat proglucagon genes is not apparent. Studies of the human and rat insulin promoter in transgenic mice have not revealed major differences in the sequences required for β-cell-specific expression in vivo (14). In humans, glucagon secretion appears regulated primarily by nutrients, especially glucose, insulin, and the autonomic nervous system. To date, the results of experiments in rats and mice suggest that the regulation of rodent glucagon secretion is comparable to that described in human studies (7). No studies have examined the regulation of human proglucagon gene expression in human islet cells; hence, whether additional differences exist in the regulation of human vs. rodent proglucagon gene expression remains to be determined.

Although rodents are excellent models for studying gene expression and physiology, there are several instances in which significant differences exist between rodent and human physiology and regulation of gene expression. For example, the human albumin promoter has tissue-specific enhancers immediately adjacent (within 500 bases) to its promoter (13), whereas the rat albumin gene enhancers are located far upstream (8–10 kb to its promoter) (34). Similarly, the murine and human P-selectin gene promoters exhibit several structural nucleotide differences that correlate with differential transcription factor binding and species-specific differences in gene regulation (31). Moreover, species-specific responses to fibrate administration have been mapped to differences in promoter elements of the rat vs. human apolipoprotein A-I gene promoters (39). The results of our experiments provide an additional example of species-specific differences in the control of tissue-specific gene transcription. Previous studies have demonstrated that gene duplication, tissue-specific mRNA splicing, and tissue-specific posttranslational processing contribute to diversity of PGDP expression in different species (5, 18, 19, 28, 29). Our findings extend these results by demonstrating that diversity in tissue-specific proglucagon gene expression in mammals is also achieved via utilization of different promoter elements. Future studies directed at identifying the precise molecular basis for and physiological relevance of these findings are clearly warranted.

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