International Union of Pharmacology. XXXV. The Glucagon Receptor Family

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Abstract——**Peptide hormones within the secretin-glucagon family are expressed in endocrine cells of the pancreas and gastrointestinal epithelium and in specialized neurons in the brain, and subserve multiple biological functions, including regulation of growth, nutrient intake, and transit within the gut, and digestion, energy absorption, and energy assimilation. Glucagon, glucagon-like peptide-1, glucagon-like peptide-2, glucose-dependent insulinotropic peptide, growth hormone-releasing hormone and secretin are structurally related peptides that exert their actions through unique members of a structurally related G protein-coupled receptor class 2 family. This review discusses advances in our understanding of how these peptides exert their biological activities, with a focus on the biological actions and structural features of the cognate receptors. The receptors have been named after their parent and only physiologically relevant ligand, in line with the recommendations of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR).**

I. Introduction

Guanine nucleotide-binding protein (G protein)-coupled receptors have been subdivided into distinct subgroups, based upon shared structural identity and evolutionary origin (Josefsson, 1999). Receptors within the family B (or family 2) subgroup, exemplified by the secretin receptor, the original family B receptor member (Ishihara et al., 1991), exhibit less homology with other GPCR[1] subfamilies and consist of three distinct subgroups, with subfamily B1 containing multiple receptors for peptide hormones (Harmar, 2001). The genes encoding the structurally related peptides (Table 1 and Fig. 1) secretin, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, growth hormone releasing hormone, and glucose-dependent insulinotropic polypeptide are expressed in the gastrointestinal tract

and/or brain, and signal through G_s leading to activation of adenylate cyclase and increased levels of cyclic AMP. A single proglucagon gene in mammals (Irwin, 2001) encodes three distinct structurally related peptides, glucagon, GLP-1, and GLP-2, which exhibit unique biological actions mediated by separate receptors (Bataille, 1996a,b; Drucker, 2001c). In contrast, separate receptors for glicentin, oxyntomodulin, and miniglucagon, biologically active peptides derived from the identical proglucagon precursor, have not yet been identified (Drucker, 2001c, 2002). Several members of the secretin peptide family, including secretin, GLP-1(7–36amide) and growth hormone-releasing hormone (GHRH) are amidated at the carboxyl terminus, however amidation is not always an invariant requirement for biological activity, as the nonamidated GLP-1(7–37) is equipotent with GLP-1(7–36amide) (Orskov et al., 1993). Similarly, GHRH, GLP-1, glucose-dependent insulinotropic peptide (GIP), and GLP-2 are excellent substrates for the enzyme dipeptidyl peptidase IV, which inactivates these peptides following cleavage at the position 2 alanine or proline (De Meester et al., 1999).

Consistent with the structure of multiple G protein-coupled receptors within class 2, the secretin receptor family contains a disulfide bond linking the first and

[1]Abbreviations: GPCR, G protein-coupled receptor; GLP, glucagon-like peptide; GHRH, growth hormone-releasing hormone; GIP, glucose inhibitory polypeptide; VIP, vasoactive intestinal peptide; kb; kilobase(s); BHK, baby hamster kidney; RAMP, receptor activity modifying protein; RT-PCR, reverse transcription-polymer chain reaction; PACAP, pituitary adenylate cyclase-activating peptide; GLP-2R, glucagon-like peptide 2 receptor; PKA, protein kinase A; MAP kinase, mitogen-activated protein kinase; IGF, insulin-like growth factor; ERK, extracellular signal-regulated kinase.

TABLE 1
Prohormone precursors encoding peptide ligands for the glucagon receptor family

Prohormone		Swiss Prot	Peptide (AA)	Sequence
AA	M_r			
121	13,016	P09683	Secretin (27)	HSDGTFTSELSRLREGARLQRLLQGLV
180	20,909	P01275	Glucagon (29)	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
180	20,909	P01275	GLP-1 (30)	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR
180	20,909	P01275	GLP-2 (33)	HADGSFSDEMNTILDNLAARDFINWLIQTKITD
153	17,107	P09681	GIP (42)	YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQ
108	12,447	P01286	GHRH (44)	YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL
87	9,479	P26349	Exendin-4 (39)	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS

second extracellular loop domains (Asmann et al., 2000), a signal peptide, and a comparatively large extracellular domain important for ligand binding. The glucagon receptor has a region (FQG-hydr-hydr-VA*x*-hydr-YCF*x*-EVQ)—"hydr" being a hydrophobic and "*x*" any amino acid—that is highly conserved in all the members of the glucagon/secretin receptor subfamily. A highly conserved aspartic acid residue in the extracellular domain of several family B receptors has been shown to be critical for ligand binding, as exemplified by the *little* mouse mutation that encodes for a mutant GHRH receptor that fails to bind ligand due to replacement of the aspartic acid residue at position 7 in the extracellular domain with a glycine (Lin et al., 1993; Carruthers et al., 1994; Gaylinn et al., 1999). The nomenclature of the class 2 secretin family of receptors is comparatively straightforward, as each receptor is named for its principal and only physiologically relevant ligand, with no significant biologically meaningful cross-reactivity occurring across the spectrum of related peptide ligands and receptors (Tables 1 and 2).

II. Secretin Receptor

The secretin receptor is prototypic of the class II family of GPCRs, being the first member of this group of receptors to be cloned in 1991 (Ishihara et al., 1991). The concept of a circulating chemical messenger and even the introduction of the term "hormone" is related to the observation by Bayliss and Starling (1902) that a duodenal extract could stimulate pancreatic fluid secretion. That factor was subsequently purified to homogeneity and identified as a 27-residue linear polypeptide (Fig. 2) (Mutt et al., 1966) Extensive primary structure-activity studies of secretin have established that it has a diffuse pharmacophoric domain, spread throughout the length of the peptide (Ulrich et al., 1998) consistent with many of the currently recognized natural agonist ligands of the class II GPCRs.

Secretin is produced and secreted by secretory granule-containing endocrine S-cells that are scattered as single cells within the mucosal layer of the duodenum and proximal jejunum (Mutt, 1980). Major factors stimulating secretion of this hormone are luminal acid and fatty acids (Mutt, 1980), consistent with secretin actions on epithelial cells lining the pancreatic and biliary ducts, leading to the secretion of alkaline bicarbonate-rich fluid. This, in turn, helps to neutralize the acidic chyme emptied from the stomach, protecting the duodenal mucosa and providing an optimal pH for the action of bile acids and pancreatic zymogens. Secretin also slows gastric emptying to further protect the duodenum from being overwhelmed by excessively rapid delivery of acidic chyme.

A. Molecular Basis for Receptor Nomenclature

The secretin receptor cDNA was first identified and cloned in 1991 (Ishihara et al., 1991). Consistent with pharmacological studies performed in the precloning era, the recombinant receptor bound secretin with high affinity and bound vasoactive intestinal polypeptide (VIP) with low affinity (Gardner et al., 1976; Ulrich et al., 1993). Potencies for stimulation of biological responses of these peptides paralleled their binding affinities. Although secretin can also bind to and activate other class II family G protein-coupled receptors (such as the VIP receptor) at low affinity, to date there have been no other subtypes of the secretin receptor identified.

Similarly, highly selective agonists that are more stable or that exhibit enhanced potency, or nonpeptidic ligands have not yet been described for the secretin receptor. The most useful antagonist of secretin action is a peptide analog of secretin having a reduced peptide bond between residues four and five [(ψ4,5)secretin] (Haffar et al., 1991). This is consistent with primary structure-activity studies that have suggested that the selectivity of binding is most dependent on the NH_2-terminal portion of the diffuse pharmacophore, whereas the carboxyl-terminal portion further contributes to binding affinity and to biological action (Holtmann et al., 1995; Vilardaga et al., 1995).

B. Endogenous Agonist

The endogenous agonist for the secretin receptor is the linear 27-residue polypeptide, secretin (Fig. 1), secreted by endocrine S-cells in the upper small intestinal mucosa. This gastrointestinal hormone has now been isolated and sequenced in multiple animal species (Leiter et al., 1994). Minimal sequence differences are present in pig, cow, dog, rat, sheep, and human secretin,
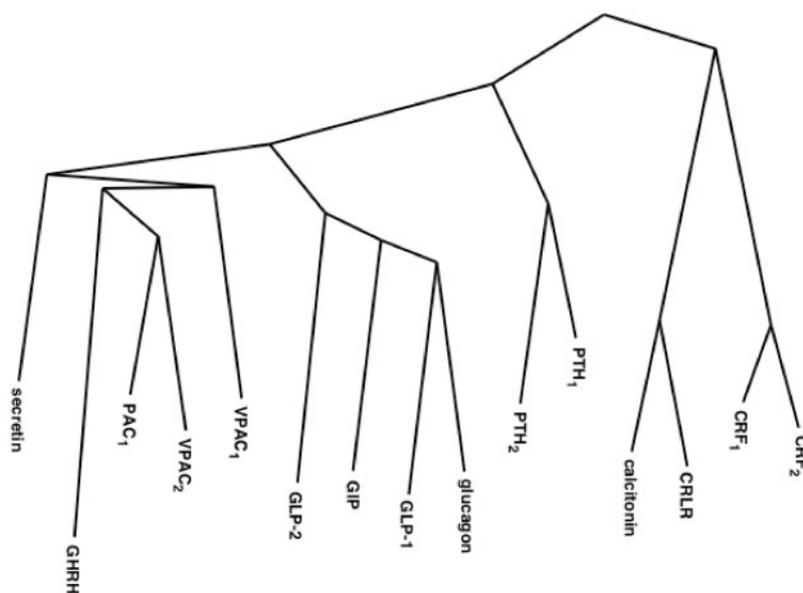
FIG. 1. Family tree of selected members of family B G protein-coupled receptors, as described by Harmar, 2001, and kindly provided by Dr. A. Harmar.

TABLE 2
Information shown is for the human receptors, followed below by receptors from other species

Receptor	Gene	Swiss Prot	S	AA	CL	Ligands	Principal Biological Actions
GHRH	GHRHR	Q02643	H	423	7p14/15	GHRH	GH secretion
	Ghrhr	P32082	M	423	6		
	GHRHR	P34999	P	423			
	GHRHR	Q02644	R	464			
GIP	GIPR	P48546	H	466	19q13.3	GIP	Insulin secretion
	GIPR	P43219	R	455			
	GIPR	P43218	SH	462			
Glucagon	GCGR	P47871	H	477	17q25	Glucagon	Fuel metabolism, glucoregulation
	Gcgr	Q61606	M	485	11		
	GCGR	P30082	R	485			
GLP-1	GLP1R	P43220	H	463	6p21	GLP-1, Exendin-4	Insulin and glucagon secretion, gastric emptying
	Glp1r	O35659	M	489	17		
	GLP1R	P32301	R	463			
GLP-2	GLP2R	O95838	H	553	17p13.3	GLP-2	Nutrient absorption, gut mucosal growth
	GLP2R	Q9Z0W0	R	550			
Secretin	SCTR	P47872	H	440	2q14.1	Secretin	Pancreatic secretion
	SCTR	P23811	R	449			
		O46502	RB	445			

S, species; CL, chromosomal localization; AA, amino acids; R, rat; M, mouse; RB, rabbit; SH, Syrian Golden hamster; P, pig.

with a fully conserved NH_2-terminal domain, and only residues in positions 14, 15, and 16 exhibiting species-specific changes. Secretin in mouse and rabbit differs at residues five and six. It is only when moving to a species as divergent as the chicken that more substantial sequence differences are present, although the NH_2 terminus of chicken secretin continues to be somewhat conserved. Of note, no single species has yet been described as having more than one normally occurring form of this hormone, and no molecular variants or mutant forms of secretin have yet been described.

Consistent with the species variation of the sequence of secretin, primary structure-activity studies using hormone fragments and peptide analogs have shown that the NH_2-terminal region of the natural hormone provides key determinants for receptor selectivity, whereas its carboxyl-terminal region provides determinants for high affinity binding and biological activity (Holtmann et al., 1995; Vilardaga et al., 1995). The carboxyl-terminal region of related peptides can be fused to the secretin NH_2 terminus resulting in a chimeric peptide that still retains high affinity binding and biological activity (Park et al., 2000).

C. Receptor Structure

The human secretin receptor is predicted to be 440 amino acids in length, having a 21-residue signal peptide that is cleaved during biosynthesis and a deduced mature protein of 419 residues (Chow, 1995). Secretin receptors from rat and rabbit have also been cloned (Ishihara et al., 1991; Svoboda et al., 1998). The rat receptor (P23811) is 449 amino acid residues, with a

22-residue signal peptide and a 27-residue mature protein. The rabbit receptor (AF025411) is 445 amino acid residues, with a 20-residue signal peptide and a 225-residue mature protein. These sequences are 78 to 83% identical, with the major variations residing in the signal peptides and in the carboxyl-terminal tail regions.

The secretin receptor is a seven transmembrane glycoprotein having five N-linked carbohydrate groups, with four of these in the NH_2 terminus and one in the second extracellular loop region. The typical heptahelical topology of this receptor has been established using epitope tags and photoaffinity labeling by cell impermeant hydrophilic peptide probes (Dong et al., 1999a,b, 2000; Holtmann et al., 1996a, 1995). This receptor has numerous sites of potential phosphorylation on serine and threonine residues within the NH_2-terminal tail and intracellular loop regions. Secretin receptor phosphorylation has been directly demonstrated (Ozcelebi et al., 1995; Holtmann et al., 1996b), although the precise sites of modification have not been defined. There are no sites of fatty acid acylation or predicted regions having kinase activity within this receptor. A variant secretin receptor in which the third exon was spliced out to eliminate residues 44–79 from the NH_2-terminal tail, has been identified in a gastrinoma, pancreatic cancer, and pancreatic cell lines. The variant receptor functions as a dominant-negative molecule and suppresses normal secretin receptor activity, likely through formation of a heterodimer with the wild-type receptor (Ding et al., 2002a).

D. Receptor Gene Structure

The secretin receptor gene is localized to human chromosome two (2q14.1) (Mark and Chow, 1995; Ho et al., 1999), spans more than 69 kb, and contains thirteen exons and twelve introns (Ho et al., 1999). The junctions between the exons interrupt at residues 24, 65, 101, 135, 168, 212, 264, 284, 307, 338, 380, and 394 (numbering of residues includes the signal peptide) (Fig. 3). The first four exons encode the critical NH_2 terminus of this receptor. Subsequent junctions are distributed throughout the loop and transmembrane regions. No alternatively spliced forms of the secretin receptor have been found to occur naturally. However, a misspliced form of this receptor that is missing exon three (leading to an in-frame deletion of residues 44 through 79 in the NH_2-terminal tail of the mature protein) was isolated from a gastrinoma in a patient with a false-negative provocative secretin stimulation test (Ding et al., 2002b). This variant form of the secretin receptor is unable to bind secretin or to signal in response to this hormone. Of particular interest, this mutant secretin receptor was shown to possess dominant-negative activity and inhibited the ability of secretin to bind and signal at a coexpressed wild-type secretin receptor (Ding et al., 2002b).

E. Molecular Basis of Receptor Binding and Action

The long and structurally complex NH_2 terminus is critical for binding and action of the natural peptide agonist ligand (Holtmann et al., 1995). This is an important signature region of class II G protein-coupled receptors that contains six conserved cysteine residues believed to be involved in three intradomain disulfide bonds critical for establishing functional receptor conformation (Asmann et al., 2000). The secretin receptor NH_2 terminus also contains a cysteine residue in a nonconserved position that is not involved in a disulfide bond (Asmann et al., 2000). The functional importance of this region has been established by truncation, site-directed mutagenesis, chimeric receptors, and photoaffinity labeling studies (Dong et al., 1999a,b, 2000, 2002; Holtmann et al., 1995, 1996a; Park et al., 2000). It is remarkable that photolabile residues situated throughout the pharmacophoric domain of secretin, in positions 6, 22, and 26 have all been shown to covalently label residues within the NH_2 terminus of the secretin receptor (Dong et al., 2002). Extracellular loop domains of the secretin receptor have also been shown to be important regions for secretin binding, acting as important complements to the NH_2-terminal region (Holtmann et al., 1996a). The precise mechanism of peptide binding and the roles of each domain have not yet been well defined.

F. Receptor Distribution

The classical sites for secretin receptor localization are on epithelial cells within the pancreatic and biliary ducts (Ulrich et al., 1998). These are the sites of stimulation of bicarbonate-rich fluid that is important to neu-

Structural organization of the Secretin Gene
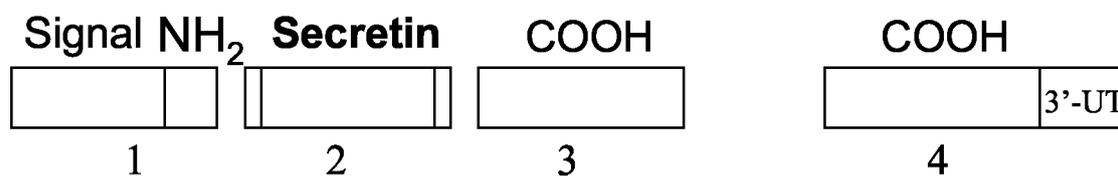
FIG. 2. Schematic representation of the secretin peptide gene structure. This gene contains four exons, with the first containing 5′-untranslated sequence, the signal peptide, and a portion of the amino-terminal peptide. The second exon includes the entire mature peptide coding region with a few residues of both amino-terminal and carboxyl-terminal peptides. The third and fourth exons include portions of the carboxyl-terminal peptide, with the latter also containing 3′-untranslated sequence. COOH, the secretin carboxyl-terminal peptide; NH2, the amino-terminal peptide as described by Kopin et al. (1990, 1991).

tralize gastric acid emptied into the duodenum. Additionally, secretin receptors are present on pancreatic acinar cells, gastric epithelial cells, intestinal epithelial cells, Brunner's glands, gastric and intestinal smooth muscle cells, and certain areas of the brain. Receptors at these sites contribute to pancreatic exocrine secretion and growth, stimulation of gastric pepsinogen secretion, inhibition of gastric acid secretion, and inhibition of gastric emptying and intestinal motility. As noted above, these actions all work in concert to protect the upper intestinal tract from the major stimulants of secretin secretion, acidic chyme in the lumen. Additionally, there have been potential roles of secretin proposed for islet cell, renal, and cardiac function, although these actions are not well defined or understood.

G. Secretin Receptor Signaling

Like most of the other receptors in the class II G protein-coupled receptor family, the secretin receptor has been shown to couple to G_s; the secretin receptor also couples to G_q. Receptor activation leads to increases in both cAMP and intracellular calcium (Trimble et al., 1987). Promiscuous coupling is typical of this receptor family and, like other members, the G_s coupling and cAMP signaling occur at the lowest concentrations of hormone and represents the physiological signaling pathway. The G_q coupling and intracellular calcium response occur in response to concentrations of secretin more than 100-fold higher than those stimulating the other pathway. Additional events along these signaling pathways have not been thoroughly examined or described.

H. Receptor Regulation

The secretin receptor is phosphorylated in response to agonist action, although the specific functional impact of this biochemical event is not well established (Ozcelebi et al., 1995; Holtmann et al., 1996b). The kinases implicated in secretin receptor phosphorylation include G protein-coupled receptor kinases and protein kinase C (Ozcelebi et al., 1995; Holtmann et al., 1996b; Shetzline et al., 1998). There is no current information regarding action of G protein-coupled receptor phosphatases that might be regulated and act on this receptor (Lutz et al., 1993). The secretin receptor has also been demonstrated to be internalized into the cell in response to agonist occupation (Holtmann et al., 1996b). This has been best studied in model cellular systems, and nothing is yet known about the behavior of this receptor as it naturally resides on various cellular populations.

I. Assay Systems

Recombinant secretin receptors expressed on Chinese hamster cell lines have been extremely useful for analysis of potential ligands and agonist activity (Ulrich et al., 1993). The rat pancreatic acinar cell is a natural site of secretin receptor expression that is a classical cell

biological model system. A problem with its use is the coexpression of VPAC1 receptors that bind and respond to low concentrations of VIP and high concentrations of secretin. These have contributed to the older physiologic literature that describes secretin-preferring and VIP-preferring receptors being expressed on these cells (Gardner et al., 1976).

J. Physiological Roles and Therapeutic Potential

The major physiological roles for secretin relate to establishing and maintaining an optimal intraluminal milieu in the duodenum and upper jejunum for digestion to take place (Mutt, 1980). Once gastric acid enters the duodenum, it can damage the mucosal cells, precipitate bile acids, and inactivate pancreatic enzymes. Secretin is secreted in response to the acid load and many of its actions involve the direct stimulation of alkaline bicarbonate-rich fluid or the slowing of gastric emptying or intestinal transit to minimize acid exposure and to provide optimal opportunity for neutralization.

Secretin administration has been used clinically as a provocative test for gastrin-secreting islet cell tumors (Isenberg et al., 1972). Although the normal islet cell does not express the secretin receptor, gastrinoma cells often express this receptor (Chiba et al., 1989). As noted above, one of the established causes for a false-negative provocative test in the gastrinoma syndrome is the missplicing of the secretin receptor (Ding et al., 2002b).

III. The Glucagon Receptor

Glucagon is a 29-amino acid peptide (Bromer et al., 1956) originally isolated from a side fraction of purified insulin (Kenny, 1955) as a hyperglycemic factor originating from the pancreas (Kimball and Murlin, 1923). Its primary structure is identical in most mammals including man, although some amino acid sequence changes are noted in glucagons from guinea pig or non-mammalian vertebrates (Irwin, 2001). Glucagon is synthesized mainly in the A-cells present at the periphery of the islets of Langerhans (Baum et al., 1962) and is also detected in specific cells in the stomach and intestine in some species (Baetens et al., 1976), as well as in specialized neurons of the central nervous system. Isolation of cDNAs encoding glucagon (Lund et al., 1982; Lopez et al., 1983) showed that the peptide is produced from a 160-amino acid precursor, proglucagon, which also contains two additional glucagon-like sequences at its carboxyl terminus (GLP-1 and GLP-2), which were subsequently shown to display specific biological activities (Drucker, 1998).

A. Precursor Processing

Tissue-specific post-translational processing of the NH_2-terminal portion of proglucagon (Fig. 3A) (Mojsov et al., 1986; reviewed in Bataille, 1996b) leads to production of glucagon in the pancreatic A-cells. In the

intestinal L-cells and central nervous system, the carboxyl-terminally extended forms, glicentin and oxyntomodulin (Bataille, 1996a), as well as GLP-1 and GLP-2 and two intervening peptides, IP-1 and IP-2 are produced. Further processing of glucagon may also produce the carboxyl-terminal undecapeptide miniglucagon, a powerful inhibitor of insulin secretion (Dalle et al., 1999, 2002).

B. Biological Activities

The biological activities of glucagon are directed mostly toward opposing insulin action in the liver in the control of glucose metabolism primarily via stimulation of glycogenolysis (Sutherland, 1950; reviewed in Stalmans, 1983) and gluconeogenesis from lactate, pyruvate, glycerol, and certain amino acids (Claus et al., 1983). Similarly, the ratio of circulating levels of insulin and glucagon can shift lipid metabolism from storage to release in specific tissues such as the liver (Steinberg et al., 1959; Eaton, 1977). Glucagon is also able to directly stimulate insulin release (Samols et al., 1966) through its own receptors expressed on pancreatic β-cells (Kawai et al., 1995; Moens et al., 1998). Although more likely pharmacological than physiological, glucagon administration produces positive inotropic and chronotropic effects on the heart (Farah, 1983), exerts spasmolytic effects on gastrointestinal smooth muscle (Diamant and Picazo, 1983) and growth hormone-releasing activities (Merimee, 1983).

C. Glucagon Receptors

Rodbell and coworkers (Rodbell et al., 1971a,b) established that the glucagon receptor is involved in the activation of adenylate cyclase and that intracellular signaling is achieved through GTP-binding heterotrimeric G proteins of the G_s type. Besides this universally ac-
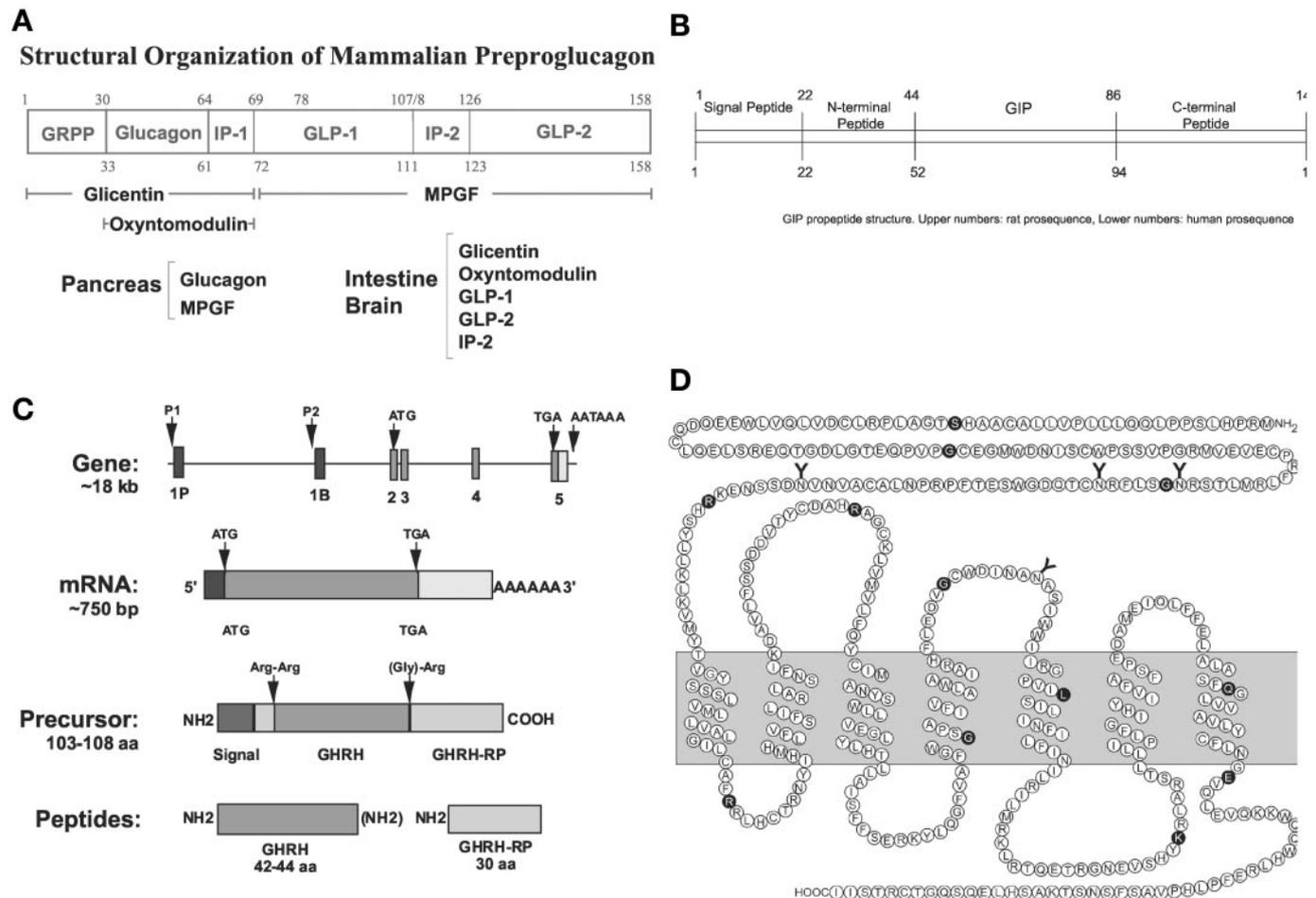
FIG. 3. A, structure of the mammalian proglucagon gene and the proglucagon-derived peptides liberated in distinct tissues. GRPP, glicentin-related pancreatic polypeptide; MPGF, major proglucagon-derived fragment; IP, intervening peptide. B, structural organization of the GIP precursor, as described by Tseng et al. (1993). The upper numbers represent amino acids in the rat pro-GIP sequence; lower numbers correspond to the human proGIP sequence. C, biosynthesis of GHRH. The GHRH gene includes 5 exons, and alternative untranslated exons 1 are used in different tissues (1P, placenta; 1B, hypothalamus). The GHRH mRNA encodes a prepro-GHRH precursor protein that is 103–108 amino acids, depending on the species. This is processed to generate two major peptides. GHRH is 42–44 amino acids, depending on the species, and is generally carboxyl-terminally amidated, except in rodents. A 30-amino acid GHRH-related peptide has been characterized from rat and mouse. Further processing to generate as many as five peptide forms has been reported (Nillni et al., 1999) although GHRH and GHRH-RP are the only well characterized products of the precursor. D, schematic structure of the secretin receptor. Shown is the predicted amino acid sequence and membrane topology of the human secretin receptor. Highlighted in white lettering on a black background are residues at the junctions of the exons, based on the genomic structure.

cepted mode of action, which was later shown to be shared by many other peptides and neurotransmitters, glucagon may also, at very low doses, activate the phospholipase C/inositol phosphate pathway leading to Ca^{2+} release from intracellular stores in a physiological context. The signal transduction pathways activated by the glucagon receptor coupled to calcium release include stimulation of adenylate cyclase via G_s, and experiments using BHK fibroblasts implicate a role for both G_s- and G_i-activated pathways in the Ca^{2+} response to glucagon (Wakelam et al., 1986; Hansen et al., 1998). Using the affinity-labeling approach, the hepatic glucagon receptor was isolated as a 62-kDa polypeptide that contained four N-linked oligosaccharide chains (Iyengar and Herberg, 1984).

D. Gene Structure and Expression

Cloning of the rat glucagon receptor cDNA was achieved via two parallel and simultaneous approaches: expression cloning (Jelinek et al., 1993) and polymerase chain reaction-based cloning (Svoboda et al., 1993). Cloning of the human (Lok et al., 1994; MacNeil et al., 1994; Menzel et al., 1994) and mouse receptor genes (Burcelin et al., 1995) followed. The human glucagon receptor gene is localized to chromosome 17, at 17q25 (Lok et al., 1994; Menzel et al., 1994). The rat and mouse glucagon receptors are 485-amino acid seven transmembrane domain proteins with four N-linked glycosylation sites and a sequence motif in the third intracellular loop (RLAR) known to be required for G protein activation. The human receptor is shorter, with 477 amino acids and contains a similar (RLAK) G protein-coupling motif as well as four N-linked glycosylation sites. Mouse and rat receptors are very similar (93% identity in amino acid sequence), whereas the human receptor is only 80% identical to the mouse receptor (Sivarajah et al., 2001). The glucagon receptor has a region (FQG-hydr-hydr-VAx-hydr-YCFxEVQ)—hydr being a hydrophobic and x any amino acid—that is highly conserved in all the members of the glucagon/secretin receptor subfamily. A cDNA encoding an amphibian glucagon receptor was obtained from *Rana tigrina rugulosa*, the first nonmammalian glucagon receptor characterized (Ngan et al., 1999). More recently, the structures of the receptors from two more amphibians (*Xenopus laevis* and *Rana pipiens*) were determined (Sivarajah et al., 2001).

Glucagon receptor expression in islet cells is up-regulated by glucose, and glucose-responsive sequences have been identified in the glucagon receptor gene promoter (Abrahamsen and Nishimura, 1995; Yamato et al., 1997; Portois et al., 1999). Agents such as forskolin and 3-isobutyl-1-methylxanthine, which increase levels of cAMP, and the glucocorticoid dexamethasone, inhibit glucagon receptor expression in rat islets, whereas somatostatin, which reduces cAMP, increases levels of islet glucagon receptor RNA transcripts (Abrahamsen and Nishimura, 1995). Similarly, glucose increases and

agents that augment levels of cAMP decrease glucagon receptor expression in primary cultures of rat hepatocytes (Abrahamsen et al., 1995). Furthermore, levels of hepatic glucagon receptor mRNA transcripts are down-regulated following exposure to glucagon in a dose-dependent manner (Abrahamsen et al., 1995).

E. Structure-Activity Relationships

Extensive analysis of glucagon receptor sequences has identified specific amino acids essential for ligand binding and signal transduction (Unson and Merrifield, 1994; Christophe, 1996; Buggy et al., 1997; Cypess et al., 1999; Unson et al., 2000). The importance of an aspartic acid residue in the extracellular domain for ligand binding was demonstrated (Carruthers et al., 1994). Structure-function studies (Unson et al., 1995) resulted in the conclusions that 1) all seven transmembrane helices are required for the proper folding and processing of the receptor; 2) glycosylation does not play an essential role in its membrane localization or its activity; 3) the amino-terminal extracellular portion is required for ligand binding; and 4) most of the distal carboxyl-terminal tail is not necessary either for ligand binding or for coupling to adenylate cyclase. The 206–219 segment of the first extracellular loop is important for both glucagon binding and receptor activation (Unson et al., 2002). Deletion of residues 252–259 corresponding to the second intracellular loop appears to lock the protein in the conformation promoted by divalent cations and prevents the protein from normal coupling to G_s, and the intracellular i2 and i3 loops play a role in glucagon receptor signaling, consistent with recent models for the mechanism of activation of G protein-coupled receptors (Cypess et al., 1999). The human glucagon receptor has been shown to interact with receptor activity modifying proteins (RAMPs), specifically RAMP2, in transfected fibroblasts (Christopoulos et al., 2002); however, the potential biological significance of this interaction for glucagon receptor expression and activity in vivo has not yet been determined.

F. Tissue Distribution

The distribution of the glucagon receptor was studied in the rat and found to be expressed mainly in liver and kidney and, to a lesser extent, in heart, adipose tissue, spleen, thymus, adrenal glands, pancreas, cerebral cortex, and throughout the gastrointestinal tract (Svoboda et al., 1994; Dunphy et al., 1998). Ligand binding studies have identified glucagon binding sites in rat kidney tubules including the thick ascending limb of Henle's loop, the distal convoluted tubule, and the collecting tubule (Butlen and Morel, 1985). In the rat brain, radioligand binding studies detected glucagon binding sites in the olfactory tubercle, hippocampus, anterior pituitary, amygdala, septum, medulla, thalamus, olfactory bulb, and hypothalamus (Hoosein and Gurd, 1984b).

G. Mutant or Polymorphic Receptors

No spontaneous mutations leading to constitutively active glucagon receptors have been found in humans. A missense mutation leading to a Gly^{40} to Ser substitution has been associated with type 2 diabetes mellitus in French and Sardinian subjects (Hager et al., 1995) potentially associated with decreased glucagon-dependent insulin secretion. Alternatively, this mutation may be in linkage disequilibrium with another gene located in the same region, as another polymorphism found in intron 8 of the receptor cosegregates with the Gly^{40}Ser mutation in all individuals tested. A recent study indicates that there is no linkage between this mutation and type 2 diabetes in Brazilian patients (Shiota et al., 2002). It has also been suggested that this missense mutation may be associated, in some individuals, with essential hypertension (Morris and Chambers, 1996).

H. Glucagon Receptors and the Islets of Langerhans

Authentic glucagon receptors, distinct from receptors for GLP-1 that may also recognize high concentrations of glucagon within the islet (Moens et al., 1998), have been detected in insulin-secreting β-cells by several approaches (Kawai et al., 1995; Dalle et al., 1999; Huypens et al., 2000) including use of specific functional antagonists and identification of the mRNA encoding the glucagon receptor by Northern blotting and reverse transcription-polymerase chain reaction (RT-PCR) experiments. Islet glucagon receptors are coupled to adenylate cyclase and trigger insulin secretion. This observation is intriguing and paradoxical in that glucagon, which exhibits glycogenolytic and gluconeogenic effects in the setting of hypoglycemia, is also able to release insulin. Recent observations have demonstrated that miniglucagon, the carboxyl-terminal glucagon fragment also present in mature secretory granules of the A-cells, is released together with native glucagon (Dalle et al., 2002). Because of the huge difference in affinity between the two peptides for their respective receptors (3 to 4 orders of magnitude higher for the smaller peptide), miniglucagon, a very efficient inhibitor of insulin release that acts by closure of the β-cell voltage-gated calcium channels consecutive to membrane repolarization (Dalle et al., 1999), completely blocks any possible insulinotropic effect of glucagon. Consistent with these findings, although exogenous glucagon stimulates insulin secretion, endogenously released glucagon has no effect on the magnitude of glucose-induced insulin secretion (Moens et al., 2002).

I. Competitive Antagonists

Peptide antagonists of the glucagon receptor have been described, most of which lacked the amino-terminal histidine residue and contained a modified amino acid at position 9 such as des-His^1-[Nle^9-Ala^{11}-Ala^{16}] glucagon (Unson et al., 1991, 1993). A nonpeptidic competitive antagonist of the glucagon receptor (NNC 92-1687) has also been described (Madsen et al., 1998). There remains active interest in the search for nonpeptide glucagon receptor antagonists, which may be useful for the treatment of type 2 diabetes (Ling et al., 2001a, 2002; Petersen and Sullivan, 2001; Ladouceur et al., 2002).

J. Lessons from Transgenic Models

Mice with a targeted disruption of the glucagon receptor, leading to a complete ineffectiveness of glucagon on its target tissues, have α cell hyperplasia near normal levels of insulin, mild fasting hypoglycemia, normal levels of fasting cholesterol and triglycerides, and improved glucose tolerance despite very high levels of circulating glucagon (Parker et al., 2002; Gelling et al., 2003).

K. Receptors for Other Glucagon Sequence-Containing Peptides

Nothing is known about the putative molecular structure of receptors for the related proglucagon-derived peptides oxyntomodulin (glucagon plus a carboxyl-terminal extension), glicentin (glucagon plus both an NH_2- and carboxyl-terminal extension), or miniglucagon (the carboxyl-terminal [19–29] glucagon sequence) (Fig. 3). Whether these peptides act through known members of the secretin receptor family or exert their actions through distinct novel receptors, remains unclear. However, it must be noted that

1. Oxyntomodulin and glicentin, secreted from the same intestinal L-cells as GLP-1, display specific biological activities directed toward regulation of gastric acid secretion and gut motility (Bataille, 1996a), and these peptides appear to act via receptors linked to both the inositol phosphate and cyclic AMP pathways (Rodier et al., 1999). Intracerebroventricular administration of oxyntomodulin inhibits food intake in rats, and this effect is blocked by the GLP-1 receptor antagonist exendin-(9–39); however, the specific receptor that mediates this effect has not yet been identified (Dakin et al., 2001). Administration of glicentin to rats or mice produces small bowel growth, although not to the same extent as the more potent intestinotrophic peptide GLP-2 (Drucker et al., 1996).

2. Miniglucagon, produced from circulating glucagon in target-tissues (Bataille, 1996b) and present in glucagon-containing secretory granules (Dalle et al., 2002), activates receptors coupled to the plasma membrane calcium pump (Mallat et al., 1987) via a G_s or G_s-like GTP-binding protein (Lotersztajn et al., 1990) in liver and to potassium channels via a G_i or G_o GTP-binding protein in pancreatic β-cells (Dalle et al., 1999). Studies examining the biological actions of glicentin, oxyntomodulin, or miniglucagon in mice with targeted disruption of the re-

lated glucagon or GLP-1 receptors have not yet been reported.

IV. The Glucagon-Like Peptide-1 Receptor

Peptidergic signals derived from the intestine augment the insulin response induced by nutrients ("the incretin effect") (Dupre and Beck, 1966; Fehmann et al., 1995a). This functional connection between the intestine and the islets of Langerhans was termed the "incretin axis" or "entero-insular-axis" (Unger and Eisentraut, 1969; Fehmann et al., 1995a). The gut-derived peptides GLP-1 and GIP are important mediators in this axis (Fehmann et al., 1995a; Drucker, 1998; Kieffer and Habener, 1999).

GLP-1, together with GLP-2, oxyntomodulin, and glicentin, is derived from post-translational processing of proglucagon in the intestinal L-cells of the small and large intestine (Mojsov et al., 1986) (Fig. 3). GLP-1 stimulates insulin secretion in a glucose-dependent manner via a specific receptor expressed on islet β-cells. GLP-1 also increases proinsulin gene transcription and insulin production and suppresses glucagon secretion from islet α-cells (Fehmann and Habener, 1992; Fehmann et al., 1995a; Drucker, 1998). Whether the effect of GLP-1 on inhibition of glucagon secretion is direct, via α-cell expression of the GLP-1 receptor, or indirect, perhaps via stimulation of insulin or somatostatin secretion, remains unclear (Moens et al., 1996; Heller et al., 1997). GLP-1 has central nervous system effects resulting in delayed gastric emptying (Schirra et al., 1997) and appetite regulation (Turton et al., 1996; Gutzwiller et al., 1999; Verdich et al., 2001), and the circulating peptide may gain access to the brain from the periphery by simple diffusion (Kastin et al., 2002). Systemic administration of GLP-1 in rodents activates the sympathetic nervous system leading to increased tyrosine hydroxylase gene transcription, enhanced sympathetic outflow, and increased heart rate and blood pressure (Barragan et al., 1999; Yamamoto et al., 2002).

A. Identification of the Glucagon-Like Peptide-1 Receptor

GLP-1 receptors were first identified by a combination of radioligand binding experiments and measurements of cyclic AMP accumulation using rat insulinoma-derived RIN1046-38 cells (Drucker et al., 1987; Goke and Conlon, 1988) followed by localization on additional rodent insulinoma cell lines (Fehmann et al., 1995a) as well as on rat (Moens et al., 1996) and human (Fehmann et al., 1995b) pancreatic islet β-cells and somatostatin-secreting cells (Fehmann and Habener, 1991; Gros et al., 1992). GLP-1 binding sites have also been identified on isolated rat gastric parietal cells (Uttenthal and Blazquez, 1990; Schmidtler et al., 1994), human gastric cancer cells (HGT-1) (Hansen et al., 1988), solubilized membranes of rat epididymal adipose tissue (Valverde

et al., 1993), 3T3-L1 adipocytes (Montrose-Rafizadeh et al., 1997), membranes from the rodent thyrotrope cell line α-TSH (Beak et al., 1996), and in rat lung (Richter et al., 1990, 1991) and brain (Shimizu et al., 1987; Uttenthal and Blazquez, 1990; Goke et al., 1995; Wei and Mojsov, 1995).

Analysis of data obtained from binding experiments with RINm5F cells revealed that GLP-1 binds to a single class of binding sites (Goke and Conlon, 1988). Cross-linking studies with ^{125}I-GLP-1 demonstrate a single band with an apparent molecular mass of 63,000 (Goke et al., 1989a, 1992). The GLP-1 receptor protein is glycosylated and glycosylation may modulate receptor function (Goke et al., 1994). Agonists at the receptor include GLP-1(7–37), GLP-1(7–36)amide, the *Heloderma suspectum* peptides exendin-3 and exendin-4 (Goke et al., 1993; Thorens et al., 1993), and labeled ligands such as fluorescein-Trp25-exendin-4 (Chicchi et al., 1997) ^{125}I-GLP-1, and Tyr39-exendin-4. In contrast, structurally related members of the glucagon family such as GLP-2, glucagon, and GIP do not exhibit cross-reactivity at the GLP-1 receptor at physiologically relevant concentrations.

Agonist potencies at the receptor exhibit a K_d of 0.3 nM for GLP-1(7–37)/(7–36)amide and a K_d of 0.1 nM for the naturally occurring Gila monster peptide exendin-4 (Goke et al., 1993). The truncated lizard peptide GLP-1 receptor antagonist exendin-(9–39) (Goke et al., 1993; Thorens et al., 1993) exhibits a K_d of 2.9 nM (Goke et al., 1993; Thorens et al., 1993). This compound has been successfully utilized for in vitro (Goke et al., 1993; Thorens et al., 1993) and in vivo studies (Kolligs et al., 1995; Schirra et al., 1998) for elucidation of the physiological importance of the GLP-1 receptor. A small nonpeptide ligand (T-0632) for the GLP-1 receptor has been described that binds to the amino-terminal hormone binding domain with micromolar affinity (Tibaduiza et al., 2001). Interestingly, this (non)peptide antagonist exhibits ~100-fold selectivity for the human versus the highly homologous rat GLP-1 receptor, due to the presence of Trp versus Ser at position 33 in the human versus rat receptors, respectively (Tibaduiza et al., 2001). Hence it may be feasible to develop nonpeptide orally bioavailable small molecule GLP-1 receptor modulators for therapeutic purposes.

B. Molecular Characterization of the Glucagon-Like Peptide-1 Receptor

Molecular characterization of the GLP-1 receptor was achieved by cloning the rat and human β-cell GLP-1 receptor cDNAs (Thorens, 1992; Dillon et al., 1993; Graziano et al., 1993; Thorens et al., 1993; Van Eyll et al., 1994) followed by isolation of cDNAs encoding the rat lung and the brain GLP-1 receptor (Lankat-Buttgereit et al., 1994; Wei and Mojsov, 1995). The human receptor protein consists of 463 amino acids (Van Eyll et al., 1994). The rat and human GLP-1 receptors exhibit 90%

sequence identity at the amino acid level. The receptor sequence contains a large hydrophilic, extracellular domain preceded by a short leader sequence required for receptor translocation across the endoplasmic reticulum during biosynthesis, and seven hydrophobic membrane-spanning domains that are linked by hydrophilic intra- and extracellular loops (Thorens and Widmann, 1996). Distinct amino acids within the amino-terminal domain of the receptor are crucial for ligand binding (Parker et al., 1998; Tibaduiza et al., 2001; Wilmen et al., 1997), and the region encompassing transmembrane domains 1 to 3 is also involved in ligand binding (Xiao et al., 2000b). Different domains in the third intracellular loop of the GLP-1 receptor are responsible for specific G protein-coupling (and $G\alpha_s$, G_i, and G_o activation) (Hallbrink et al., 2001), and in the best studied cellular model, islet β-cells, GLP-1 receptor signaling acts predominantly via G_s to increase cAMP accumulation; however, activation of downstream signaling pathways may occur in a protein kinase A-independent manner (Bode et al., 1999; Holz et al., 1999). GLP-1-mediated closure of ATP-sensitive potassium (K_{ATP}) channels, and the differential effect of ADP levels on K_{ATP} channel closure may provide a cellular mechanism for the glucose-sensitivity of GLP-1 action in β-cells (Light et al., 2002).

C. The Glucagon-Like Peptide-1 Receptor Gene

The human GLP-1 receptor gene was localized to the long arm of chromosome 6 (hchr 6p21) (Stoffel et al., 1993). The GLP-1 receptor gene spans 40 kb and consists of at least 7 exons. The 5′-flanking and promoter region of the human GLP-1 receptor gene has been cloned and functionally characterized (Lankat-Buttgereit and Goke, 1997). The cell- and tissue-specific transcriptional regulation of GLP-1 receptor expression has been studied in cell transfection experiments and is mainly achieved by selective utilization of positive and negative control sequences and silencing elements, the latter located between −574 and −2921 (Galehshahi et al., 1998; Wildhage et al., 1999).

D. Tissue Distribution of the Glucagon-Like Peptide-1 Receptor

Studies investigating the distribution of rat and human GLP-1 receptor mRNA by RNase protection and RT-PCR detected GLP-1 receptor mRNA transcripts in pancreatic islets, lung, brain, stomach, heart, and kidney but not in liver, skeletal muscle or adipose tissue of most species (Wei and Mojsov, 1995; Bullock et al., 1996). In contrast, GLP-1 receptor transcripts have been identified in canine muscle and adipose tissue (Sandhu et al., 1999). Although quantitative comparative analyses of the levels of GLP-1 receptor expression in distinct isolated cell types are not yet available, Northern blot and RNase protection analyses demonstrates comparatively greater levels of GLP-1 receptor mRNA transcripts in heart and lung compared with other tissues

(Thorens, 1992; Wei and Mojsov, 1995; Bullock et al., 1996). In rat brain, GLP-1 receptors have been found in the lateral septum, subfornical organ, thalamus, hypothalamus, interpeduncular nucleus, posterodorsal tegmental nucleus, area postrema, inferior olive, and nucleus of the solitary tract (Goke et al., 1995; Shughrue et al., 1996). Activation of brain GLP-1 receptors likely occurs via GLP-1 produced in the brainstem, which then is transported to distant regions of the central nervous system (Drucker and Asa, 1988; Jin et al., 1988; Larsen et al., 1997; Merchenthaler et al., 1999) and via activation of GLP-1 receptors in the area postrema that then activate brainstem GLP-1+ neurons (Kastin et al., 2002; Yamamoto et al., 2002).

E. Signal Transduction of the Glucagon-Like Peptide-1 Receptor

The GLP-1 receptor is functionally coupled to adenylate cyclase (Drucker et al., 1987) via the stimulatory G protein G_s. GLP-1-binding at pancreatic β-cells increases free cytosolic calcium concentrations after cell depolarization in some but not all cell types (Goke et al., 1989b; Lu et al., 1993; Yada et al., 1993; Holz et al., 1995, 1999; Bode et al., 1999). GLP-1-dependent stimulation of intracellular calcium may occur via a ryanodine-sensitive pathway (Holz et al., 1999), and in a cAMP-dependent, protein kinase A-independent manner through small G proteins distinct from G_s (Kang et al., 2001; Kashima et al., 2001).

F. Functional Regulation and Biological Significance of the Glucagon-Like Peptide-1 Receptor

GLP-1 receptor function has been studied using various approaches. Radioligand assays were used to characterize binding at the endogenous receptor expressed in rat insulinoma cell lines (Goke and Conlon, 1988; Fehmann et al., 1995a), recombinant receptors expressed in transfected COS cells (Thorens, 1992; Thorens et al., 1993) or transfected Chinese hamster lung fibroblast (rCHL) cells (Van Eyll et al., 1994). Homologous desensitization and internalization of the GLP-1 receptor is strictly dependent on the phosphorylation of three serine doublets within the cytoplasmic tail (Widmann et al., 1997). Experiments with mutant GLP-1 receptors revealed that the number of phosphorylation sites correlated with the extent of desensitization and internalization. However, the two processes showed a different quantitative impairment in single versus double mutants suggesting different molecular mechanisms controlling desensitization and internalization (Widmann et al., 1997). The specific identity of the protein kinases regulating GLP-1 receptor phosphorylation and receptor desensitization remain unclear.

$Glp1r^{-/-}$ mice with a targeted genetic disruption of the GLP-1 receptor gene demonstrate modest glucose intolerance and fasting hyperglycemia with defective glucose-stimulated insulin secretion (Scrocchi et al., 1996).

$Glp1r^{-/-}$ mice also exhibit subtle abnormalities in the hypothalamic-pituitary-adrenal axis, specifically, an abnormal corticosterone response to stress (MacLusky et al., 2000). Despite the putative importance of GLP-1 as a satiety factor, even combined disruption of leptin and GLP-1 action as exemplified by generation and analysis of a double mutant ob/ob:$Glp1r^{-/-}$ mouse, did not modify weight gain or feeding behavior beyond that observed in the control ob/ob mouse alone (Scrocchi et al., 2000). Similarly, although exogenous administration of GLP-1 receptor ligands stimulates islet neogenesis and proliferation (Stoffers et al., 2000), complete disruption of $Glp1r^{-/-}$ signaling produces only modest defects in islet formation and topography (Ling et al., 2001b) and does not impair up-regulation of insulin gene expression or development of islet hyperplasia in the setting of leptin deficiency (Scrocchi et al., 2000). These findings illustrate the complexity of inferring the physiological importance of receptor function from studies of knockout mice in vivo (Seeley et al., 2000).

G. The Glucagon-Like Peptide-1 Receptor as a Therapeutic Target

GLP-1 receptor agonists are being evaluated for clinical use as antidiabetic agents (Byrne and Goke, 1996; Drucker, 2001a; Zander et al., 2002). Since the half-life of the naturally occurring peptide in plasma is too short for optimal clinical use, long-acting degradation resistant GLP-1 analogs have now been developed (Deacon et al., 1998; Ritzel et al., 1998; Burcelin et al., 1999a; Siegel et al., 1999; Doyle et al., 2001; Xiao et al., 2001), and these analogs, together with the lizard peptide exendin-4, are being assessed in studies of patients with type 2 diabetes (Agerso et al., 2002; Egan et al., 2002; Juhl et al., 2002).

The overexpression of the GLP-1 receptor in insulin-releasing INS-1 cells increases the potency and efficacy of D-glucose on insulin gene transcription by a putative autocrine signaling mechanism (Chepurny and Holz, 2002). This observation affirms the idea that β-cell lines could be engineered for efficient glucose-dependent insulin synthesis and secretion by overexpression of the GLP-1 receptor. Alternatively, genetic engineering of cells for expression of GLP-1 receptor ligands has also been proposed (Burcelin et al., 1999b).

Activation of GLP-1 receptor signaling has been proposed as a therapeutic strategy for treatment of peripheral diabetic neuropathy and other neurodegenerative processes. GLP-1, and its longer-acting analog exendin-4, completely protected cultured rat hippocampal neurons against glutamate-induced apoptosis (Perry et al., 2002a), and GLP-1 promotes nerve growth factor-mediated differentiation in PC12 cells (Perry et al., 2002b). Activation of GLP-1 receptor signaling also promotes proliferative and anti-apoptotic actions in the endocrine pancreas, providing a potential opportunity for interventions directed at expanding β-cell mass in subjects with diabetes (Li et al., 2003; Drucker, 2003).

V. The Glucagon-Like Peptide-2 Receptor

GLP-2 was first identified as a novel peptide encoded within the mammalian proglucagon cDNA sequence (Fig. 3) carboxyl-terminal to GLP-1 (Bell et al., 1983a,b), and subsequent isolation and characterization of the peptide from porcine and human small bowel confirmed the synthesis and liberation of full-length GLP-2(1–33) (Buhl et al., 1988). The GLP-2 amino acid sequence is flanked by pairs of dibasic residues characteristic of prohormone cleavage sites. GLP-2 is cosecreted along with GLP-1, oxyntomodulin, and glicentin from intestinal endocrine cells (Mojsov et al., 1986; Orskov et al., 1986). The principal role of GLP-2 appears to be the maintenance of growth and absorptive function of the intestinal mucosal villus epithelium (Drucker et al., 1996). GLP-2 administration to rodents enhances villus growth and increases small bowel mass, with weaker but detectable trophic effects observed in the large bowel and stomach (Drucker et al., 1997a,b; Tsai et al., 1997a,b). GLP-2 also rapidly up-regulates hexose transport and nutrient absorption (Cheeseman and Tsang, 1996; Brubaker et al., 1997) and enhances sugar absorption and intestinal adaptation in rats following major small bowel resection (Scott et al., 1998). GLP-2 reduces intestinal permeability in rodents within hours of peptide administration in vivo but has no effect on mucosal permeability when administered in vitro (Benjamin et al., 2000), consistent with the established indirect actions of GLP-2 (Drucker, 2001b).

A. Biological Activity

The trophic and proabsorptive actions of GLP-2 have prompted studies of whether pharmacological GLP-2 administration may produce beneficial effects in rodent models of intestinal disease. GLP-2 treatment ameliorates the severity of small bowel enteritis and facilitates adaptive small bowel mucosal repair following surgical or chemical injury in both rats and mice (Chance et al., 1997; Scott et al., 1998; Boushey et al., 1999; Alavi et al., 2000; Prasad et al., 2000, 2001). A GLP-2 analog also reduces weight loss and facilitates mucosal healing in mice with experimental colitis (Drucker et al., 1999b). A pilot study of GLP-2 administration for 4 weeks to human subjects with short bowel syndrome produced significant improvement in lean body mass, intestinal histology, and energy retention (Jeppesen et al., 2001). Although the CNS actions of GLP-2 remain poorly understood, pharmacological administration of intracerebroventricular GLP-2 modestly and transiently reduces food intake in rats and mice (Tang-Christensen et al., 2000; Lovshin et al., 2001).

B. Receptor Structure and Localization

Although GLP-2 binding sites have not yet been reported on cell lines expressing an endogenous GLP-2 receptor, GLP-2 activates adenylate cyclase in rat hypo-

thalamic and pituitary membranes (Hoosein and Gurd, 1984a), and administration of radiolabeled GLP-2 to rats results in detectable radioligand binding to intestinal epithelial cells along the crypt to villus axis (Thulesen et al., 2000). A cDNA encoding a GLP-2 receptor was isolated from hypothalamic and intestinal cDNA libraries using a combined PCR expression cloning approach (Munroe et al., 1999). The GLP-2 receptor cDNA encodes an open reading frame of 550 amino acids that gives rise to a structurally related member of the class 2 glucagon-secretin receptor family (Munroe et al., 1999). The GLP-2 receptor gene was localized to human chromosome 17p13.3 and has not yet been linked to specific human diseases. The GLP-2 receptor is expressed in a tissue-specific manner in the stomach, small and large intestine, central nervous system, and lung (Munroe et al., 1999; Yusta et al., 2000b). GLP-2 receptor expression in the human gut epithelium has been localized by immunohistochemistry to subsets of enteroendocrine cells in the stomach, and both small and large intestine using a polyclonal antiserum (Yusta et al., 2000b). Although the majority of human enteroendocrine cells do not express the GLP-2 receptor, all GLP-2 receptor-immunoreactive cells identified to date express one or more gut endocrine markers, including GIP, peptide YY, serotonin, chromogranin, and GLP-1 (Yusta et al., 2000b). In contrast, the same antisera did not identify GLP-2 receptor-immunopositive endocrine cells in rodents, where GLP-2 receptor RNA transcripts have been localized by in situ hybridization to subsets of enteric neurons (Bjerknes and Cheng, 2001). These findings imply an indirect model for GLP-2 action whereby GLP-2 released from enteroendocrine L-cells or rodent neurons stimulates the release of downstream mediators of GLP-2 action (Fig. 4). The downstream mediators are responsible for the proliferative, anti-apoptotic, and pro-absorptive effects of GLP-2; however, the identity of these GLP-2-dependent factors has not yet been established.

C. Hormone Binding Activity

Consistent with the original description of GLP-2 action in the brain (stimulation of adenylate cyclase activity) in hypothalamic and pituitary membranes (Hoosein and Gurd, 1984a), GLP-2 increases intracellular cAMP in fibroblasts transfected with the rat or human GLP-2 receptor cDNA with an EC_{50} of 0.58 nM, and binding studies demonstrate a K_d of 0.57 nM (Munroe et al., 1999; Yusta et al., 1999; DaCambra et al., 2000). In contrast, structurally related members of the glucagon peptide family such as glucagon, GLP-1, GIP, secretin, growth hormone-releasing factor, pituitary adenylate cyclase-activating peptide (PACAP), and VIP do not activate the transfected rat or human GLP-2 receptor at concentrations of 10 nM in vitro (Munroe et al., 1999; DaCambra et al., 2000). Structure-function analyses of GLP-2 ligand-receptor interactions demonstrate that both GLP-2(1–33) and GLP-2(1–34) are biologically active, and the ability of amino-terminally truncated or carboxyl-terminally extended GLP-2 derivatives to stimulate GLP-2 receptor-dependent cAMP accumulation in vitro correlates with the intestinotrophic properties of these peptides in a murine bioassay in vivo (Munroe et al., 1999).

The structure-function relationships for GLP-2 receptor activation have been examined through a combination of alanine scanning and position 2 substitution experiments using the human GLP-2 peptide sequence as a starting peptide and baby hamster kidney fibroblasts transfected with the rat GLP-2 receptor (BHK-GLP-2R cells) (DaCambra et al., 2000). The majority of position 2 h[GLP-2] substitutions exhibit normal to enhanced GLP-2R binding; in contrast, position 2 substitutions were less well tolerated for receptor activation as only Gly, Ile, Pro, α-aminobutyric acid, D-Ala, or nor-Val substitutions enhanced GLP-2 receptor activation (DaCambra et al., 2000). Alanine-scanning mutational analyses revealed that alanine replacement at positions 5, 6, 17, 20, 22, 23, 25, 26, 30, and 31 led to diminished GLP-2R binding (DaCambra et al., 2000). Position 2 residue substitutions containing Asp, Leu, Lys, Met, Phe, Trp, and Tyr, and Ala substitutions at positions 12 and 21, which all exhibited normal to enhanced GLP-2 receptor binding but greater than 75% reduction in receptor activation (DaCambra et al., 2000). Circular dichroism analysis indicated that the enhanced activity of these GLP-2 analogs was independent of the α-helical content of the peptide. GLP-2(3–33), the amino-terminally truncated product generated following dipeptidyl peptidase IV-mediated cleavage of GLP-2(1–33) (Drucker et al., 1997b), functions as a weak GLP-2 antagonist and partial agonist at the rodent GLP-2 receptor (Thulesen et al., 2002). Although studies in the rat brain suggest that the GLP-1 receptor antagonist exendin-(9–39) blocks the effects of pharmacological GLP-2 administration on food intake (Tang-Christensen et al., 2000), exendin-(9–39) does not function as an antagonist at the cloned GLP-2 receptor in vitro (Lovshin et al., 2001), and GLP-2 exhibits enhanced anorexic action following intracerebroventricular administration in mice with complete disruption of GLP-1 receptor signaling (Lovshin et al., 2001).

D. Intracellular Signaling

GLP-2 activates cAMP production in rodent and human cells transfected with the rat or human GLP-2 receptors (Munroe et al., 1999; Yusta et al., 1999, 2000a; DaCambra et al., 2000). Although GLP-2 promotes significant enlargement of the gut mucosa following administration to rodents in vivo, pharmacological concentrations of GLP-2 promote a very weak direct proliferative response in fibroblasts transfected with the rat GLP-2 receptor (Yusta et al., 1999). Activation of GLP-2 receptor signaling inhibits cycloheximide-induced apoptosis in BHK-GLP-2R cells, with reduced DNA fragmentation

and improved cell survival, in association with reduced activation of caspase-3 and decreased poly(ADP-ribose) polymerase cleavage and reduced caspase-8- and caspase-9-like activities (Yusta et al., 2000a). Both GLP-2 and forskolin reduced mitochondrial cytochrome *c* release and decreased the cycloheximide-induced cleavage of caspase-3 in the presence or absence of a dominant-negative PKA subunit, or the PKA inhibitor H-89 (Yusta et al., 2000a). Similarly, GLP-2 increased cell survival following cycloheximide in the presence of the MAP kinase inhibitor PD98054 and phosphatidyl-inositol 3-kinase inhibitor LY294002 (Yusta et al., 2000a). The anti-apoptotic actions of the rat GLP-2 receptor in transfected BHK fibroblasts are not strictly dependent on phosphatidylinositol 3-kinase or Akt, as GLP-2 directly promotes cell survival, enhances glycogen synthase kinase-3 and BAD phosphorylation, and reduces mitochondrial-associated BAD and Bax following LY294002-induced apoptosis in a PKA-dependent manner (Yusta et al., 2002).

E. Glucagon-Like Peptide-2 Receptor Expression and Function in Vivo

Despite the importance of GLP-2 for nutrient absorption, nutrients have only a modest impact on GLP-2 receptor expression in the gut (Yusta et al., 2000b). Whether GLP-2 is important for gut development or the period of intestinal adaptation during transition from the neonatal to the adult gut remains uncertain. GLP-2 immunoreactivity was detected in the fetal rat intestine, and fetal rat intestinal cell cultures secreted correctly processed GLP-2(1–33). High levels of bioactive GLP-2(1–33) were detected in the circulation of neonatal rats and GLP-2 receptor mRNA transcripts were detected in fetal rat intestine and in neonatal stomach, jejunum, ileum, and colon (Lovshin et al., 2000). The levels of GLP-2 receptor messenger RNA transcripts were comparatively higher in the fetal and neonatal intestine and declined to adult levels by postnatal day 21 (Lovshin et al., 2000). Similarly, little is known about the regulation of GLP-2 receptor expression in the brain. A 1.6-kilobase fragment of the mouse GLP-2 receptor promoter directs *LacZ* expression to multiple regions of the murine CNS that also coexpress the endogenous mouse GLP-2 receptor. In contrast, the promoter sequences specifying gut-specific GLP-2 receptor expression remain less well defined (Lovshin et al., 2001). Immunoneutralization of GLP-2 in the diabetic rat attenuates the adaptive response in the small bowel, implicating a role for GLP-2 as a component of the molecular signaling cascade controlling intestinal adaptation (Hartmann et al., 2002). The physiological importance of GLP-2 in the gut or brain remains uncertain, due to the lack of either potent GLP-2 receptor antagonists or murine models of disrupted GLP-2 receptor expression. Similarly, naturally occurring mutations of the GLP-2 receptor have not yet been reported. However, mice with disruption of the prohormone convertase-1 gene exhibited marked reductions in the intestinal levels of GLP-2, reduced somatic growth, and diarrhea, suggesting that GLP-2 action may contribute to regulation of murine intestinal function in vivo (Zhu et al., 2002).

F. Glucagon-Like Peptide-2 Receptor Activation and the Treatment of Intestinal Disease

The trophic and anti-apoptotic actions that ensue following activation of GLP-2 receptor signaling in the rodent intestine have fostered interest in the potential use of GLP-2 analogs for the treatment of intestinal disorders. Administration of GLP-2 receptor agonists in experimental rodent models of intestinal injury ameliorates short bowel syndrome secondary to intestinal resection, reverses mucosal hypoplasia associated with parenteral nutrition, attenuates inflammatory disease in the small and large intestine, and decreases mucosal damage secondary to vascular ischemia, and chemotherapy-induced enteritis (Chance et al., 1997, 2000; Scott et al., 1998; Boushey et al., 1999, 2001; Drucker et al., 1999b; Kato et al., 1999; Alavi et al., 2000; Prasad et al., 2000, 2001). As native GLP-2 exhibits a very short half-life in vivo due principally to enzymatic inactivation by dipeptidyl peptidase IV (Drucker et al., 1997b; Xiao et al., 1999, 2000a; Hartmann et al., 2000a,b), protease resistant GLP-2 analogs have been developed that exhibit longer durations of action and greater potency in vivo (Drucker et al., 1997b; DaCambra et al., 2000; Drucker, 2001b). A pilot study of GLP-2 administration in human subjects with short bowel syndrome demonstrated significant improvements in energy absorption, bone density, increased body weight, and lean body mass, which correlated with increased crypt plus villus height on intestinal biopsy sections (Jeppesen et al., 2001).

VI. The Glucose-Dependent Insulinotropic Peptide Receptor

GIP (gastric inhibitory polypeptide or glucose-dependent insulinotropic peptide) is a 42-amino acid peptide secreted by endocrine K cells of the duodenum. Secretion of GIP is triggered by nutrients, either glucose, amino acids, or fat. Initially, GIP was characterized as a factor inhibiting the secretion of hydrochloric acid from stomach parietal cells (gastric inhibitory polypeptide). A major physiological role as a potentiator of glucose-stimulated insulin secretion was later recognized hence its second more commonly used name (Dupre et al., 1973). This insulinotropic function is shared by GLP-1 and both peptides are thought to represent the major gluco-incretin hormones forming the enteroinsular axis, i.e., the gut hormonal response to ingested food that potentiates the effect of glucose on insulin secretion (Unger and Eisentraut, 1969). The regions of the receptor involved in peptide binding include the relatively long extracellular

amino-terminal domain (Gelling et al., 1997b); however, both the amino-terminal extracellular and the first transmembrane domains are necessary for binding and receptor activation. The carboxyl-terminal cytoplasmic part of the receptor, in particular serines 426 and 427 (Wheeler et al., 1999) and serine 406 and cysteine 411 (Tseng and Zhang, 1998), are important for receptor desensitization and internalization.

A. Structure

The GIP receptor cDNA was originally cloned from a rat brain cortex cDNA library (Usdin et al., 1993) and encodes a seven transmembrane domain protein consisting of 455 amino acids. Receptor cDNAs were subsequently isolated from human (466 amino acid) (Gremlich et al., 1995; Yamada et al., 1995) and hamster (462 amino acids) (Yasuda et al., 1994) cDNA libraries. The human receptor gene, located on chromosomal 19q13.3 (Gremlich et al., 1995), contains 14 exons spanning approximately 14 kb (Yamada et al., 1995). The rat gene contains 13 exons spanning approximately 10 kb of genomic DNA (Boylan et al., 1999).

B. Binding Affinity and Antagonists

The GIP receptor binds its ligand with a K_D of ~0.2 nM (Gremlich et al., 1995). Although the exact sites of interaction between the receptor and its ligand are not known, the NH_2-terminal part of GIP (residues 1–14 and 19–30) contains a bioactive domain that can activate cAMP formation (Hinke et al., 2001). GIP(6–30) has an affinity for the receptor similar to that of full-length GIP(1–42) but acts as an antagonist, inhibiting GIP-induced cAMP formation (Gelling et al., 1997a). GIP(7–30)-NH_2 is a potent antagonist that can markedly reduce glucose-stimulated insulin secretion in fasted rats or blunt the postprandial increase in plasma insulin levels (Tseng et al., 1996). The truncated lizard peptide exendin-(9–39) is also an inhibitor of GIP binding and cAMP formation by GIP-activated receptors (Gremlich et al., 1995).

GIP is rapidly degraded by the action of the ubiquitous enzyme dipeptidyl peptidase IV (Mentlein et al., 1993; Kieffer et al., 1995; Pauly et al., 1996). The substitution of the L-alanine in position 2 of GIP by a D-Ala residue renders the peptide resistant to degradation and confers greater insulinotropic activity to the peptide as revealed by improved glucose tolerance in control and obese diabetic rats (Hinke et al., 2002). The substitution of proline for glutamate in position 3 (Pro^3)GIP also generates a peptide resistant to degradation by dipeptidyl peptidase IV. This peptide however behaves as an antagonist that inhibits GIP-induced cAMP production and insulin secretion by β-cells (Gault et al., 2002).

C. Intracellular Signaling Pathways

GIP binding to its receptor primarily activates adenylyl cyclase and increases intracellular cAMP (Lu et al., 1993). Activation of the MAP kinase pathway (Kubota et al., 1997), phospholipase A2 (Ehses et al., 2001), as well as the phosphatidylinositol 3-kinase/protein kinase B pathway (Trumper et al., 2001) have also been reported in β-cell lines following GIP stimulation. These responses, as well as an increase in $[Ca^{2+}]_i$ (Wheeler et al., 1995), may at least in part be secondary to a rise in intracellular levels of cAMP. Studies using both transfected Chinese hamster ovary-K1 cells and a β-cell line INS-1 clone 832/13 demonstrated that GIP induced phosphorylation of Raf-1 (Ser259), Mek 1/2 (Ser217/Ser221), ERK 1/2 (Thr202, Tyr204), and p90 RSK (Ser380) in a concentration-dependent manner (Ehses et al., 2002). The GIP regulation of ERK 1/2 occurred via Rap1, but did not involve Gβγ subunits nor Src tyrosine kinase (Ehses et al., 2002). Site-directed mutagenesis was used to create a point mutation (T340P) in the sixth transmembrane-spanning domain of the GIP receptor, which confers constitutive, although submaximal, cAMP forming activity (Tseng and Lin, 1997).

The tissue distribution of the GIP receptor is broad, with high levels of expression in human, rat, and mouse pancreatic islet β-cells. The rat GIP receptor has also been detected by Northern blot analysis or by in situ hybridization in the gut, adipose tissue, heart, pituitary, adrenal cortex, osteoblasts (Bollag et al., 2000) endothelial cells (Zhong et al., 2000), cerebral cortex, hippocampus, and olfactory bulb (Usdin et al., 1993; Mazzocchi et al., 1999). Whether GIP is actually synthesized in the brain and acts locally within the central nervous system remains uncertain.

Activation of the receptor in pancreatic β-cells potentiates glucose-stimulated insulin secretion; stimulation of insulin promoter activity and β-cell proliferation may also be GIP-regulated responses (Fehmann and Goke, 1995; Trumper et al., 2001). These effects require initial elevations in intracellular cAMP. The stimulation of insulin secretion is mediated by both protein kinase A-dependent and independent pathways, the latter involving the cAMP binding protein cAMP-GEF, which is a regulator of the small G protein Rab3 (Ozaki et al., 2000; Kashima et al., 2001).

There is controversy regarding the action of GIP in adipose tissue. Some reports describe an effect of GIP on the stimulation of fatty acid synthesis and an increase in insulin-stimulated incorporation of fatty acids into triglycerides (Yip and Wolfe, 2000). Other reports describe GIP stimulation of lipolysis (McIntosh et al., 1999). In stomach, GIP inhibits gastric acid secretion (Brown, 1982) whereas GIP increases collagen and alkaline phosphatase expression in an osteoblast cell line (SaOS2), by a cAMP-dependent signaling pathway (Bollag et al., 2000). The effect of GIP on specific vascular beds may be either vasoconstrictor or vasodilatory (Zhong et al., 2000). In the rat adrenal cortex, GIP stimulates secretion of corticosterone (Mazzocchi et al., 1999), and in human adrenal glands, where expression of the GIP

receptor is low or absent, abnormal overexpression of the receptor underlies the development of food-dependent Cushing's syndrome (Reznik et al., 1992; de Herder et al., 1996).

D. Gene Knockout

The physiological importance of the GIP receptor in glucose homeostasis was assessed in mice with a targeted disruption of the receptor gene (Miyawaki et al., 1999). GIPR$^{-/-}$ mice show impaired oral but normal intraperitoneal glucose tolerance, and glucose tolerance deteriorated following 3 weeks of high fat feeding. Remarkably, following several months of high fat feeding, GIPR$^{-/-}$ mice gained less weight, and exhibited a reduction in adipose tissue mass and levels of circulating leptin compared with wild-type controls fed an identical high fat diet (Miyawaki et al., 2002). Furthermore, transgenic mice harboring mutations in both the leptin and GIP receptor genes (ob/ob:GIPR$^{-/-}$) weighed less and had decreased levels of blood glucose compared with ob/ob mice alone, suggesting that elimination of GIP receptor signaling may protect against the development of murine obesity (Miyawaki et al., 2002). A combination of food intake and metabolic expenditure studies suggested that reduced weight gain is attributable primarily to increased substrate oxidation, with no differences in food intake observed in mice with the absence of GIP receptor function (Miyawaki et al., 2002). Double gene inactivation for both the GIP and GLP-1 receptors leads to comparatively greater glucose intolerance compared with mice with single incretin receptor knockouts, indicating that GIP and GLP-1 play additive roles in the control of glucose homeostasis through the entero-insular axis.

E. Glucose-Dependent Insulinotropic Peptide Receptor and Type 2 Diabetes

Type 2 diabetes mellitus is characterized by a decrease in glucose-stimulated insulin secretion in association with a reduced incretin effect (Ebert and Creutzfeldt, 1987). However, the levels of intact circulating GIP are only minimally reduced or normal in subjects with type 2 diabetes (Rask et al., 2001; Vilsboll et al., 2001). This, therefore, suggests that GIP action on pancreatic β-cells may be reduced in the setting of type 2 diabetes. The role of β-cell resistance to GIP has been evaluated, and it was demonstrated that pharmacological doses of GIP infused intravenously failed to stimulate insulin secretion in diabetic human subjects, whereas GLP-1, at the same infusion rates, produced very robust secretory responses (Nauck et al., 1993). Hence a defect in GIP receptor expression and/or signaling may be associated with β-cell dysfunction in type 2 diabetes (Holst et al., 1997). In the diabetic Zucker rat, decreased responsiveness to GIP is associated with a decrease in islet GIP receptor expression (Lynn et al., 2001).

The data demonstrating a link between reduced sensitivity of β-cells to the insulinotropic effect of GIP and type 2 diabetes suggest that mutations in this receptor may be diabetogenic. Intriguingly, first degree relatives of subjects with type 2 diabetes also demonstrate reduced insulinotropic responses to exogenous GIP (Meier et al., 2001). Analysis of the GIP receptor gene in Danish and Japanese populations revealed the existence of three allelic variants: Gly198Cys (second extracellular domain), Ala207Val (second extracellular domain), and Glu354Gln (sixth transmembrane domain) (Kubota et al., 1996; Almind et al., 1998). The Gly198Cys variant leads to decreased cAMP coupling when tested in stably transfected Chinese hamster ovary cells (Kubota et al., 1996). No significant association of these variants was found, however, with type 2 diabetes, except for a small decrease in fasting levels of C-peptide in individuals homozygous for Glu354Gln (Almind et al., 1998).

VII. The Growth Hormone-Releasing Hormone Receptor

The regulation of somatic growth in vertebrate species is under complex hormonal control. Growth hormone, secreted from the pituitary gland, acts on many peripheral target tissues to alter cellular metabolism, proliferation, and differentiation. Many of the growth-promoting actions of growth hormone are ascribed to its stimulation of the synthesis of insulin-like growth factor-1 (IGF-1), a potent mitogen for many cells. Pituitary growth hormone synthesis and secretion is regulated by direct neuroendocrine signals from the brain, as well as numerous peripheral feedback cues. The predominant neuroendocrine peptides regulating growth hormone secretion are GHRH, which stimulates growth hormone synthesis and secretion, and somatostatin, which suppresses growth hormone secretion. The first section below gives a brief introduction to the ligand GHRH, its biosynthesis and activities, and the status of GHRH agonists and antagonists. This is followed by a discussion of the GHRH receptor, including its hormone binding and signaling properties, expression and regulation, and involvement in disease of the growth hormone axis.

A. Growth Hormone-Releasing Hormone

GHRH (sometimes referred to as GRF or GHRF) was initially isolated from pancreatic tumors that caused acromegaly (Guillemin et al., 1982; Rivier et al., 1982) and later characterized from the hypothalamus (Spiess et al., 1983; Ling et al., 1984b) based on its ability to stimulate growth hormone secretion from primary cultures of rat pituitary cells. GHRH also stimulates growth hormone gene transcription (Barinaga et al., 1983; Gick et al., 1984) and pituitary somatotroph cell proliferation (Billestrup et al., 1986; Mayo et al., 1988). GHRH is released from neurosecretory cells in the arcuate nuclei of the hypothalamus (Merchenthaler et al.,

1984; Sawchenko et al., 1985) and along with the inhibitory peptide somatostatin mediates the neuroendocrine regulation of pituitary growth hormone synthesis and secretion.

GHRH is also expressed in the placenta, where it may have paracrine functions or contribute to fetal growth (Suhr et al., 1989; Margioris et al., 1990); in the gonads, where it may be an autocrine or paracrine regulator of steroidogenesis and granulosa or Sertoli cell function (Berry and Pescovitz, 1988; Bagnato et al., 1992; Ciampani et al., 1992); and in lymphocytes, where it may modulate lymphocyte activation and immune function (Stephanou et al., 1991). An important role for GHRH in post-embryonic growth is suggested by clinical studies of tumors that secrete GHRH (Frohman and Szabo, 1981; Thorner et al., 1982; Frohman and Jansson, 1986), and by animal studies using transgenic mice that overexpress GHRH (Hammer et al., 1985; Stefaneanu et al., 1989). In both settings, growth hormone hypersecretion, pituitary somatotroph cell hyperplasia, and inappropriate patterns of growth (acromegaly or gigantism) are observed.

GHRH is a peptide hormone of 42–44 amino acids, depending on the species, which is proteolytically processed from a larger precursor protein of 103–108 amino acids (Fig. 3C) (Mayo et al., 1983, 1985; Frohman et al., 1989b; Suhr et al., 1989). The GHRH precursor also encodes an additional carboxyl-terminal peptide that is reported to modulate Sertoli cell activity in the testis (Breyer et al., 1996). In submammalian vertebrate species, a single gene and precursor protein encodes both GHRH and PACAP, and PACAP plays a role in growth regulation that is probably equal to that of GHRH in most submammalian vertebrate species, except birds (Montero et al., 2000).

The mature GHRH peptide is amidated at the carboxyl terminus in many species, but not in rodents. Shorter processed forms of the full-length human peptide GHRH(1–44)NH$_2$ have been characterized, with the predominant forms being GHRH(1–40)OH in hypothalamus (Ling et al., 1984b) and GHRH(1–37)NH$_2$ in a pancreatic tumor (Guillemin et al., 1982; Rivier et al., 1982). Carboxyl-terminally truncated peptides as short as GHRH(1–29)NH$_2$ display growth hormone-releasing activity comparable to that of the full-length peptide (Ling et al., 1984a; Campbell et al., 1991), and GHRH(1–29)NH$_2$ has therefore served as the template for the design of most GHRH agonists and antagonists.

Several modifications to GHRH, including substitution of the conserved alanine at position 2 with other residues such as D-alanine, improve in vivo potency (Lance et al., 1984) largely by inhibition of proteolytic degradation by dipeptidyl peptidase IV, which rapidly hydrolyzes the Ala^2-Asp^3 bond and inactivates GHRH in serum (Frohman et al., 1989a). Enhancement of the amphipathic α-helical properties of GHRH by alanine replacement results in enhanced receptor affinity and increased potency in in vitro assays (Coy et al., 1996; Cervini et al., 1998), and an analog with 48% alanine content, [D-Ala^2, Ala$^{8,9,15,16,18,22,24–28}$]GHRH(1–29)NH$_2$ (NC-9–45), is 1.9 times more potent than the parent compound (Coy et al., 1996). Analogs combining the degradation stabilizing replacements at position 2 with α-helix-enhancing modifications such as the Ala^{15} substitution have been particularly effective for increasing activity in vitro and in vivo (Kubiak et al., 1996). Table 3 summarizes the structures and activities of several of these GHRH agonists.

Replacement of the conserved alanine at position 2 of GHRH with D-arginine converts the hormone into a competitive antagonist (Robberecht et al., 1985). Working with this compound, a subsequent generation of potent GHRH antagonists were developed (the MZ series) containing the helix-stabilizing substitutions Phe(4-Cl) at position 6, α-aminobutyric acid at position 15, and norleucine at position 27, together with a hydrophobic NH$_2$-terminal acyl moiety and a carboxyl-terminal agmatine (Zarandi et al., 1994). Representative examples include MZ-4-71 and MZ-5-156. A more recent series of antagonists, the JV series, incorporate arginine or homoarginine at position 9 and an enzymatically resistant carboxyl-terminal D-Arg^{28}-Har^{29}-NH$_2$ group (Varga et al., 1999). Representative examples include JV-1-36 and JV-1-38. These antagonists are being developed largely as potential antitumor agents, in that they inhibit the growth of many tumor cells, probably by suppression of IGF-1 or IGF-2 production (Schally and Varga, 1999; Kineman, 2000). Table 3 summarizes the structures and activities of several of these GHRH antagonists.

B. Structure of the Growth Hormone-Releasing Hormone Receptor

The GHRH receptor was initially cloned from human, rat, and mouse pituitary, and in these species the isolated cDNAs encode a 423-amino acid protein (Fig. 5) (Lin et al., 1992; Mayo, 1992; Gaylinn et al., 1993). The porcine receptor was later identified as a 451-amino acid protein, but it appears that there are several isoforms with differing carboxyl-terminal ends, presumably generated by alternative RNA processing, one of which corresponds to the 423-amino acid form (Hsiung et al., 1993; Hassan, 2001). Bovine and ovine receptors have also been cloned, and although the bovine GHRH receptor is similar to other mammalian species, the ovine receptor has a 16-amino acid truncation at its carboxyl terminus, a characteristic shared with the caprine receptor (Horikawa et al., 2001). The predicted GHRH receptor protein has the molecular size expected from GHRH photoaffinity cross-linking studies, and it is expressed predominantly in the anterior pituitary gland, the site of GHRH action (Mayo et al., 1995; Gaylinn, 1999). Figure 5 shows a schematic structure of the GHRH receptor and shows key features of the receptor

TABLE 3
GHRH agonists and antagonists

Agonists	Endogenous ligands (human):	
	GHRH(1–44)NH$_2$, GHRH(1–40)OH, GHRH(1–37)NH$_2$	
	Selective agonists (only a few representative compounds are listed):	
	Standard GHRH(1–29)NH$_2$	
	BIM 28011	[D-Ala^2, Ala^{8,9,15,27}, D-Arg^{29}]hGHRH(1–29)NH$_2$ (Coy et al., 1996)
	NC-9-96	[D-Ala^2, Aib^{8,18,24}, Ala$^{9,15,16,18,22,24–28}$]hGHRH(1–29)NH$_2$ (Coy et al., 1996)
	Standard hGHRH(1–40)OH	
	No. 15	[Ala^{15}, Nle^{27}]hGHRH(1–29)NH$_2$ (Cervini et al., 1998)
	No. 29	[MeTyr^1, Ala^{15,22}, Nle^{27}]hGHRH(1–29)NH$_2$ (Cervini et al., 1998)
	No. 46	Cyclo(25–29)[MeTyr^1, Ala^{15}, D-Asp^{25}, Nle^{27}, Orn^{29}] hGHRH(1–29)NH$_2$ (Cervini et al., 1998)
Agonist potencies	GHRH(1–29)NH$_2$	1.0
	BIM 28011	4.9
	NC-9-96	2.6
	hGHRH(1–40)OH	1.0
	No. 15	5.6
	No. 29	25.6
	No. 26	16.7
	Relative growth hormone-releasing potency in vitro on rat pituitary cell cultures compared to either the GHRH(1–29)NH$_2$ (Coy et al., 1996) or hGHRH(1–40)OH (Cervini et al., 1998) standards	
Antagonists	Only a few representative compounds are listed:	
	Standard	[Ac-Tyr^1, D-Arg^2]hGHRH(1–29)NH$_2$ (Robberecht et al., 1985)
	MZ-4-71	[Ibu-Tyr^1, D-Arg^2, Phe(4-Cl)^6, Abu^{15}, Nle^{27}]hGHRH(1–28)Agm (Zarandi et al., 1994)
	MZ-5-156	[PhAc-Tyr^1, D-Arg^2, Phe(4-Cl)^6, Abu^{15}, Nle^{27}]hGHRH(1–28)Agm (Zarandi et al., 1994)
	JV-1-36	[PhAc-Tyr^1, D-Arg^2, Phe(4-Cl)^6, Arg^9, Abu^{15}, Nle^{27}, D-Arg^{28}, Har^{29}]hGHRH(1–29)NH$_2$ (Schally and Varga, 1999)
	JV-1-38	[PhAc-Tyr^1, D-Arg^2, Phe(4-Cl)^6, Har^9, Tyr(Me)^{10}, Abu^{15}, Nle^{27}, D-Arg^{28}, Har^{29}]hGHRH(1–29)NH$_2$ (Schally and Varga, 1999)
Antagonist potencies	[Ac-Tyr^1, D-Arg^2]hGHRH(1–29)NH$_2$	52% 3.22 nM
	MZ-4-71	83% 0.12 nM
	MZ-5-156	95% 0.069 nM
	JV-1-36	100% 0.042 nM
	JV-1-38	98% 0.079 nM
	Inhibition of *in vitro* growth hormone release induced by 1 nM hGHRH(1-29)NH$_2$ by 30 nM antagonist, and dissociation constant of the inhibitor-receptor complex (Schally and Varga, 1999)	

protein that are referred to in the discussion that follows.

C. Hormone Binding by the Growth Hormone-Releasing Hormone Receptor

A variety of radioligands have been used to assess GHRH binding to its receptor, including [His^1, ^{125}I-Tyr^{10}, Nle^{27}]hGHRH(1–32)NH$_2$ (Seifert et al., 1985), ^{125}I-hGHRH(1–40)OH (Velicelebi et al., 1985), and [^{125}I-Tyr^{10}]hGHRH(1–44)NH$_2$ (Abribat et al., 1990). The GHRH receptor binds GHRH with subnanomolar affinity (Mayo, 1992; Gaylinn et al., 1993). Related peptides such as PACAP and VIP can displace GHRH binding but only at micromolar concentrations. Ligand-binding determinants within the GHRH receptor remain largely unidentified, but several lines of evidence indicate that the NH$_2$-terminal extracellular domain plays an important role in GHRH binding. GHRH interaction with this domain has been demonstrated using yeast two-hybrid assays (Kajkowski et al.,

1997) and using photoaffinity cross-linking (Gaylinn et al., 1994a). It is in this domain that a mutation of the receptor that disrupts GHRH binding occurs in the little mouse (Gaylinn et al., 1999). Deletion of the NH$_2$-terminal extracellular domain, or insertion of a short epitope tag into this region, also abolishes GHRH binding (DeAlmeida and Mayo, 1998). Whereas these studies indicate an important role for the NH$_2$-terminal extracellular domain in ligand binding, analysis of chimeric GHRH-VIP and GHRH-secretin receptors suggest that critical determinants for GHRH interaction are also found in the membrane-spanning domains and associated extracellular loops of the receptor (DeAlmeida and Mayo, 1998).

D. Signaling by the Growth Hormone-Releasing Hormone Receptor

When the GHRH receptor protein is expressed in transfected cells, these cells acquire the ability to bind GHRH with high affinity and selectivity and to respond

Multiple GLP-2 Actions on the Gastrointestinal Mucosal Epithelium
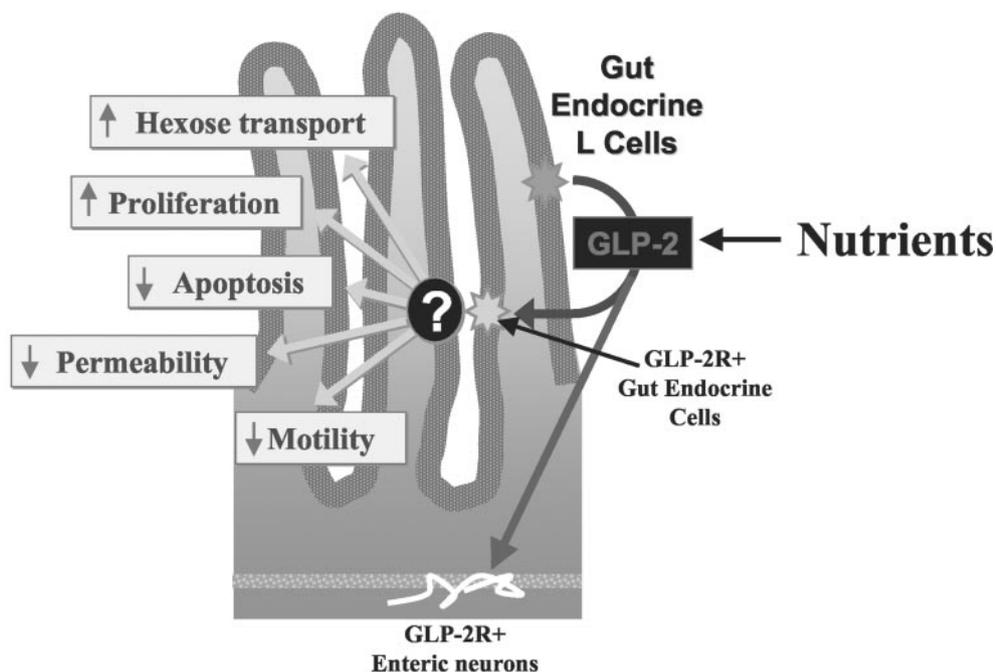
FIG. 4. Schematic representation of GLP-2 action in the gastrointestinal epithelium via GLP-2 receptors expressed in the enteric nervous system (mouse) or enteroendocrine cells (human).

to GHRH to activate adenylyl cyclase and increase intracellular levels of the second messenger cAMP (Lin et al., 1992; Mayo, 1992; Gaylinn et al., 1993; Hassan et al., 1995; DeAlmeida and Mayo, 1998). In somatotroph cells, GHRH also stimulates an influx of calcium (Holl et al., 1988; Lussier et al., 1991) most likely through voltage-sensitive Ca^{2+} channels (Chen et al., 1994). Although GHRH is reported to stimulate the phospholipase C-inositol phosphate-calcium mobilization pathway in pituitary cells in some studies (Canonico et al., 1983; Ohlsson and Lindstrom, 1990), other studies report no activation of this pathway (Escobar et al., 1986; French et al., 1990), and no coupling of the cloned GHRH receptor to this signaling pathway has yet been detected in transfected cells (Miller et al., 1999). One study suggests that distinct somatotroph cell subpopulations may respond differently to GHRH with respect to activation of the phospholipid turnover signaling pathway (Ramirez et al., 1999). Recent studies demonstrate that GHRH receptor signaling leads to activation of the MAP kinase pathway and ERK phosphorylation, at least in some cell types (Pombo et al., 2000; Zeitler and Siriwardana, 2000), an activity that likely relates to the ability of GHRH to stimulate somatotroph cell proliferation.

There is little available information on the regulation of GHRH receptor signaling, but one report indicates that the receptor undergoes desensitization following short exposures to ligand (Hansen et al., 2001). In transfected cells, this is a homologous desensitization, but in rat pituitary cells it appears to be a mixed homologous and heterologous desensitization. Desensitization of the GHRH receptor is associated with a rapid internalization from the cell surface.

E. Expression and Regulation of the Growth Hormone-Releasing Hormone Receptor

The predominant site of GHRH receptor expression is the pituitary gland, where the mRNA has been localized by RNA blotting and in situ hybridization assays (Lin et al., 1992; Mayo, 1992; Gaylinn et al., 1993; Godfrey et al., 1993; Hsiung et al., 1993). Within the pituitary gland, expression is confined to the anterior lobe (Lin et al., 1992; Mayo, 1992). It remains uncertain whether pituitary cells other than growth hormone-secreting somatotrophs express the GHRH receptor mRNA. The receptor mRNA has also been found in the placenta, a site of GHRH production (Mayo et al., 1996), the kidney (Matsubara et al., 1995; Mayo et al., 1996) and the hypothalamus (Takahashi et al., 1995). Using sensitive RT-PCR/Southern blotting assays, the GHRH receptor transcript has been found in an extremely wide range of rat tissues at low levels (Matsubara et al., 1995) al-
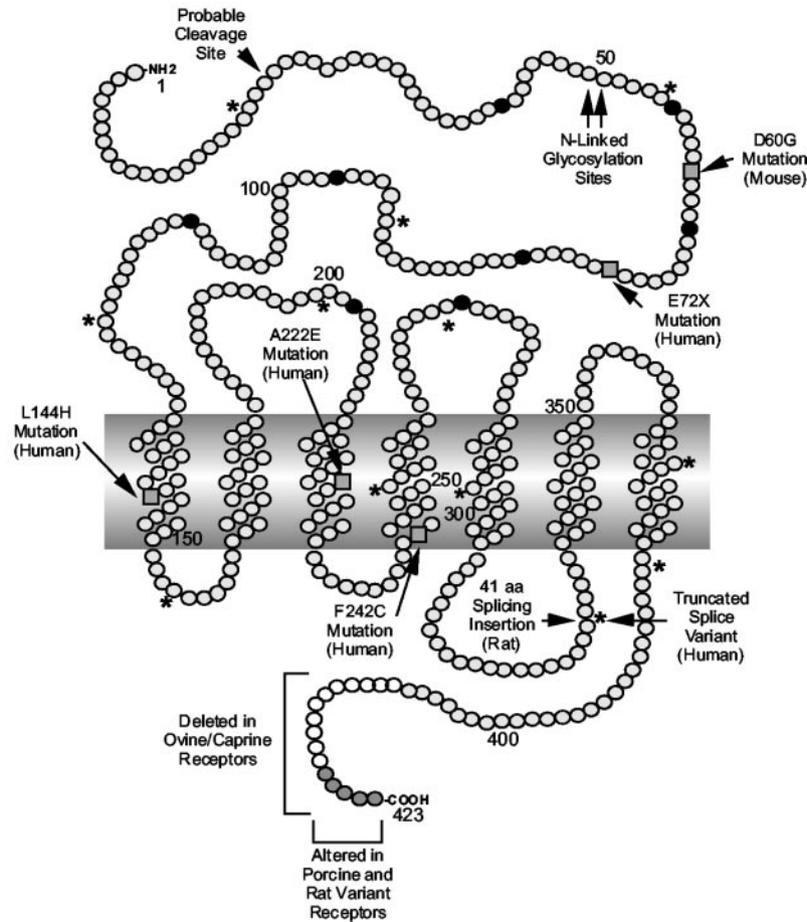
FIG. 5. Schematic structure of the GHRH receptor. Key features referred to in the text are overlaid onto this linear representation of a generic GHRH receptor. The black filled amino acids represent the six cysteine residues in the NH₂-terminal extracellular domain conserved in this receptor family, as well as the cysteines in extracellular loops one and two conserved in most GPCRs. Asterisks indicate positions at which introns interrupt the GHRH coding sequences within the gene. Characterized inactivating mutations in the receptor are indicated by the squares. Also indicated are several of the best characterized receptor variants, although others have been reported.

though the physiological significance of this broad expression remains unclear. GHRH receptor immunoreactive protein has been demonstrated only in the pituitary and the kidney (Morel et al., 1999; Boisvert et al., 2002).

GHRH receptor expression in the pituitary is developmentally regulated (Korytko et al., 1996; Sato and Takahara, 1997) and sexually dimorphic (Ono et al., 1995; Mayo et al., 2000). Receptor gene expression is positively regulated by glucocorticoids (Lam et al., 1996; Korytko and Cuttler, 1997; Miller and Mayo, 1997) and thyroid hormone (Miki et al., 1995; Korytko and Cuttler, 1997) and negatively regulated by estrogen (Lam et al., 1996). GHRH itself may modulate the expression of its receptor, although investigators have reached somewhat different conclusions regarding whether this is a positive or negative regulation, and the effects are likely dependent on the age of the animal and the duration of GHRH exposure (Horikawa et al., 1996; Miki et al., 1996; Aleppo et al., 1997; Girard et al., 1999; Lasko et al., 2001). In transgenic models with varying levels of growth hormone expression, the level of circulating growth hormone may contribute to regulation of GHRH receptor expression in some tissues (Peng et al., 2001).

F. The Growth Hormone-Releasing Hormone Receptor Gene and Receptor Splice Variants

The GHRH receptor gene maps to the centromeric region of mouse chromosome 6 (Godfrey et al., 1993; Lin et al., 1993) and to human chromosome 7p14/15 (Gaylinn et al., 1994b; Wajnrajch et al., 1994). The genes for the GHRH and PAC1 receptors are adjacent genes in the human genome and probably evolved by gene duplication (Vaudry et al., 2000). The gene has been characterized in detail in the human (Petersenn et al., 1998), mouse (Lin et al., 1993), and rat (Miller et al., 1999) and consists of 13 major exons spanning approximately 15 kb of DNA. There is no clear correspondence between exons of the gene and functional domains of the receptor protein. Functional analysis of the gene promoter in cell transfection assays reveals a selective preference for expression in pituitary cells (Iguchi et al., 1999; Miller et al., 1999) likely due to the presence of the transcription factor Pit-1, which positively regulates GHRH receptor gene expression (Lin et al., 1992; Iguchi et al., 1999; Miller et al., 1999; Salvatori et al., 2002). Glucocorticoid regulation has also been mapped to a composite regula-

tory element in the receptor gene promoter (Nogami et al., 2002).

Alternative RNA processing generates a variety of species-specific GHRH receptor transcripts. In the rat, an additional exon 11 is included in an alternatively spliced variant mRNA, resulting in the insertion of 41 amino acids into the third intracellular domain of the receptor. This variant receptor binds GHRH, but does not mediate signaling through the cAMP pathway, and neither receptor isoform is able to stimulate calcium mobilization from internal stores after GHRH treatment (Miller et al., 1999). An alternatively spliced form of the human GHRH receptor that is truncated following the fifth transmembrane domain has been identified both in normal pituitary and in pituitary adenomas (Hashimoto et al., 1995; Tang et al., 1995) and is reported to exert a dominant inhibitory effect on signaling by the normal receptor in cotransfection experiments (Motomura et al., 1998). Additional human GHRH receptor splice variants have been reported in several different cancers (Rekasi et al., 2000), and the detection of both GHRH and GHRH splice variants in human cancers raises the possibility that GHRH may exert an autocrine effect on regulation of neoplastic cell growth (Busto et al., 2002; Halmos et al., 2002). Alternative RNA processing also likely contributes to the carboxyl-terminal heterogeneity observed for the porcine GHRH receptor (Hsiung et al., 1993) and for the dwarf rat GHRH receptor (Zeitler et al., 1998). The two porcine receptor variants differ in their ability to bind GHRH and activate cAMP production in transfected cells (Hassan, 2001).

G. Mutation of the Growth Hormone-Releasing Hormone Receptor in Diseases of Growth

An inactivating mutation of the GHRH receptor was first reported in the *little* mouse (Godfrey et al., 1993; Lin et al., 1993). This is an autosomal recessive mutation mapping to chromosome 6 that results in somatotroph hyperplasia, growth hormone deficiency, and a dwarf phenotype in the homozygous mutant animals (Eicher and Beamer, 1976; Godfrey et al., 1993; Lin et al., 1993). There is a missense mutation in the GHRH receptor gene of the *little* mouse, resulting in replacement of the aspartic acid at position 60 in the NH_2-terminal extracellular domain of the receptor with glycine (Godfrey et al., 1993; Lin et al., 1993). This mutation does not affect the expression or cellular localization of the mutant receptor protein, but it abolishes binding of GHRH by the mutant receptor, resulting in a loss of GHRH signaling and subsequent defects in somatotroph proliferation and function (Gaylinn et al., 1999).

Several mutations leading to inactivation of the GHRH receptor have been reported in humans. Three distinct kindreds from India (Wajnrajch et al., 1996), Pakistan (Baumann and Maheshwari, 1997), and Sri Lanka (Netchine et al., 1998) have been reported that have a nonsense mutation truncating the GHRH recep-

tor at position 72 in the NH_2-terminal extracellular domain. A Brazilian kindred has a mutation in a splice donor site that leads to retention of the first intron, a shift in the translational reading frame, and truncation of the receptor protein at position 20, near the probable signal sequence cleavage site (Salvatori et al., 1999). A Japanese boy with a four base deletion in exon 12, resulting in a premature translational stop codon, has been described (Horikawa, 2002). Three new point mutations in the GHRH receptor gene in familial isolated growth hormone deficiency 1B have recently been reported (Salvatori et al., 2001a) and all three mutant receptors are inactive in cell transfection assays. There is one report of a regulatory mutation in which a Pit-1 binding site in the GHRH receptor promoter is disrupted (Salvatori et al., 2002). Activating mutations of the GHRH receptor in human disease have been looked for, focusing on growth hormone-secreting pituitary adenoma and changes within the third cytoplasmic loop and sixth transmembrane domain of the receptor, but to date no clear mutations have been identified (Lee et al., 2001; Salvatori et al., 2001b).

VIII. Summary

Peptide hormone receptors within the class 2 secretin receptor family exert multiple biologically important actions that regulate somatic growth, energy intake, nutrient absorption and disposal, and cell proliferation and apoptosis. Remarkably, multiple receptor agonists within this family are currently approved drugs utilized for diagnostic or therapeutic purposes, or under active clinical investigation for the treatment of specific human disorders. Hence, secretin may be used clinically for assessment of pancreatic function or for analysis of gastrin secretion in subjects with gastrinomas, and secretin-based therapies remain under investigation for the treatment of human diseases such as autism (Coniglio et al., 2001). GHRH is administered as a diagnostic challenge for the assessment of pituitary growth hormone reserve, and GHRH analogs, as well as GHRH receptor ligands, are under active investigation for conditions characterized by relative or absolute growth hormone deficiency (Gaylinn, 1999). Glucagon is utilized in the radiology suite for manipulation of gastrointestinal motility and as an agent to restore blood glucose in the setting of insulin-induced hypoglycemia (Muhlhauser et al., 1985). Conversely, glucagon receptor antagonists may potentially be useful for the treatment of diabetes mellitus (Zhang and Moller, 2000; Petersen and Sullivan, 2001; Ling et al., 2002). Similarly, GIP analogs continue to be assessed for potential utility in the treatment of type 2 diabetes (O'Harte et al., 1999, 2000), and disruption of the murine GIP receptor gene promotes resistance to weight gain and enhanced energy expenditure, suggesting that GIP receptor antagonism merits consideration for the treatment of obesity (Miyawaki et

al., 2002). Similarly, dipeptidyl peptidase-IV-resistant analogs of GLP-1 and GLP-2 are in clinical trials for the treatment of diabetes and intestinal disorders, respectively (Drucker, 1999a, 2001a,b, 2002). Hence, additional insights into the structural and functional properties of class 2 secretin receptor family members may provide information important not only for basic understanding of peptide hormone action but should form a rational scientific basis for design of novel agents targeted toward treatment of a diverse set of human diseases.

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