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Structure-Function of the Glucagon Receptor Family of G Protein–Coupled Receptors: The Glucagon, GIP, GLP-1, and GLP-2 Receptors

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The glucagon-like peptides include glucagon, GLP-1, and GLP-2, and exert diverse actions on nutrient intake, gastrointestinal motility, islet hormone secretion, cell proliferation and apoptosis, nutrient absorption, and nutrient assimilation. GIP, a related member of the glucagon peptide superfamily, also regulates nutrient disposal via stimulation of insulin secretion. The actions of these peptides are mediated by distinct members of the glucagon receptor superfamily of G protein–coupled receptors. These receptors exhibit unique patterns of tissue-specific expression, exhibit considerable amino acid sequence identity, and share similar structural and functional properties with respect to ligand binding and signal transduction. This article provides an overview of the biology of these receptors with an emphasis on understanding the unique actions of glucagon-related peptides through studies of the biology of their cognate receptors.

Keywords Diabetes, GIP, GLP-1, GLP-2, Glucagon, Glucose, Intestine, Regulatory Peptides

GENERAL INTRODUCTION

The gastroenteropancreatic-brain axis expresses a diverse number of peptide hormones, including several that are structurally related to the pancreatic hormone, glucagon (Table 1). Within the mammalian glucagon superfamily of peptides, glucagon and the glucagon-like peptides, GLP-1 and GLP-2, are all encoded by a single proglucagon gene (Bell et al. 1983; Irwin 2001; White and Saunders 1986). Tissue-specific posttranslational processing of proglucagon by prohormone convertases results in the liberation of glucagon in the pancreatic A cell, and GLP-1 and GLP-2 in the intestinal L cell and brain (Mojsov et al. 1986; Orskov et al. 1987). As discussed below, all three proglucagon-derived peptides (PGDPs) play important roles in the physiologic regulation of nutrient homeostasis, through effects on energy intake and satiety, nutrient fluxes through and across the gastrointestinal tract, and energy assimilation. Several of these biological activities are shared by a fourth glucagon-related peptide hormone, glucose-dependent insulinotropic peptide (GIP) (Table 1). The diverse biological activities of these glucagon-related peptides are exerted through highly specific G protein–coupled receptors (GPCR), members of the glucagon receptor superfamily. For the purposes of this article, the biological activities and mechanisms of action of each of these peptides will be discussed individually.

Biological Actions of the Glucagon-Related Peptides

Glucagon

Glucagon is a 29 amino acid peptide hormone liberated from islet A cells in the endocrine pancreas. The principal actions of glucagon involve regulation of metabolic pathways involved in glucose homeostasis. Glucagon secretion from the A cell is coupled to the ambient levels of circulating glucose, with hypoglycemia and hyperglycemia leading to stimulation and inhibition of glucagon release, respectively (Unger 1985). Glucagon action in the liver impinges on numerous enzymes important for control of gluconeogenesis, glycogenolysis, and fatty acid metabolism, leading to restoration of circulating glucose concentrations in the setting of energy depletion. The actions of glucagon on hepatic glucose production are exerted at multiple levels, including regulation of gene transcription and modulation of enzyme activity (Pilkis and Granner 1992). Defective

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TABLE 1
The amino acid sequences of human glucagon, GLP-1, GLP-2, and GIP

Glucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG
GLP-2	HADGSFSDEMNTILDNLAARDFINWLIQTKITD
GIP	YAEGTFISDYSIAMDKIRQQDFVNWLLAQ

glucagon secretion contributes to the development of hypoglycemia in insulin-treated patients with diabetes (Gerich 1988; Taborsky, Jr. et al. 1998).

Glucagon receptors are widely expressed in multiple tissues including the liver, brain, pancreas, heart, kidney, and smooth muscle cell in the gastrointestinal tract and peripheral vasculature. In the heart, glucagon stimulates inotropic and chronotropic activity (Parmley et al. 1968), leading to the selective use of glucagon in medical emergencies characterized by refractory bradycardia due to beta blocker toxicity or cardiogenic shock (White 1999). Supraphysiological levels of glucagon also exert vasodilator effects by reducing vascular resistance in specific vascular beds (Farah 1983).

Glucagon infusion activates nephrogenic adenylyl cyclase (leading to increased urinary cAMP excretion), increases the glomerular filtration rate, and regulates ion transport and electrolyte excretion in the kidney (Ahloulay et al. 1992; Broadus et al. 1970; Schwartz Sorensen et al. 1993). Although hypoglycemia is associated with increased hepatic and renal glucose production, a role for glucagon in the regulation of renal gluconeogenesis is unclear (Cersosimo et al. 1999; Stumvoll et al. 1997, 1998). Whether glucagon actions on the kidney are essential for regulation of renal physiology remains to be determined.

Within the brain, PGDPs, including glucagon, are synthesized principally in the brainstem and, to a lesser extent, in the hypothalamus (Drucker and Asa 1988); however, glucagon-immunoreactive nerve fibers are widely distributed through the mammalian central nervous system (Dorn et al. 1983). Glucagon administration elicits a broad spectrum of actions, including stimulation of anterior pituitary hormone secretion (Spathis et al. 1974), hypothalamic somatostatin release (Shimatsu et al. 1983), and ketone utilization (Kirsch and D'Alecy 1984). Intracerebroventricular administration of glucagon potently suppresses food intake in the rat (Inokuchi et al. 1984) and enhances sympathetic nerve activity (Krzeski et al. 1989; Shimizu et al. 1993), whereas glucagon suppresses the activity of hypothalamic glucose-sensitive neurons but has no effect on cortical neurons in the rat (Inokuchi et al. 1986).

Glucagon stimulates lipolysis in isolated adipocyte preparations from young animals (Heckemeyer et al. 1983; Lefebvre and Luyckx 1969); however, these actions are significantly attenuated with aging or weight gain (Bertrand et al. 1980). The potential importance of glucagon for stimulation of human adipocyte lipolysis is less clear (Richter et al. 1989; Richter and Schwandt 1985). Glucagon withdrawal or physiological hyperglucagonemia did not produce significant changes in palmitate flux in normal or diabetic human subjects (Jensen et al. 1991). Similarly, subcutaneous infusion of glucagon into the gastrocnemius muscle or abdominal tissue had no effects on lipolysis rates or blood flow in normal human subjects (Bertin et al. 2001; Gravholt et al. 2001). Glucagon modulates electrical activity in gastrointestinal smooth muscle (Patel et al. 1979; Taylor et al. 1975), leading to its clinical use as spasmolytic agent for modulation of motility during endoscopic and radiologic examination of the gastrointestinal tract.

Glucagon-Like Peptide-1 and Glucose-Dependent Insulinotropic Peptide

The incretin concept was first developed in 1930 to describe the observation that administration of glucose by the oral route was associated with a greater increment in insulin secretion than was a euglycemic load given intravenously (Elrick et al. 1964; LaBarre and Still 1930). This concept was further refined by both Unger (Unger and Eisentraut 1969) and Creutzfeldt (Creutzfeldt 1979) to define an incretin as a gut hormone released in response to nutrient ingestion that stimulates glucose-dependent insulin secretion. The first incretin to be identified was the duodenal hormone, GIP. Although initially reported to possess gastric inhibitory actions (Brown 1982; Brown and Dryburgh 1971), GIP was subsequently discovered to be a potent stimulator of insulin secretion (Dupre et al. 1973). Subsequent studies using GIP receptor antagonists have clearly established that GIP fulfills all of the requirements for an incretin (Baggio et al. 2000; Lewis et al. 2000; Tseng, Kieffer et al. 1996). However, reports that immunoneutralization of GIP does not abolish the incretin effect of ingested nutrients (Ebert and Creutzfeldt 1982; Ebert et al. 1983), led to an ongoing search for additional insulinotropic gut hormones. It was not until 1987 that the second major incretin was discovered to be another member of the glucagon-family of peptides, GLP-1 (Kreymann et al. 1987; Mojsov et al. 1987; Orskov and Nielsen 1988). Numerous studies on the insulinotropic actions of GIP and GLP-1 have now established that, together, these peptides constitute the majority of the incretin effect following ingestion of a meal, and that the two hormones contribute equivalently to this effect (Kieffer and Habener 1999). The importance of GIP and GLP-1 in glucose homeostasis is suggested by the strong degree of sequence conservation that exists for each of these hormones (Irwin 2001). Consistent with this hypothesis, receptor antagonist studies and the analysis of mice with null mutations in the receptors for GIP (GIPR) or

GLP-1 (GLP-1R) have demonstrated that loss of GIP or GLP-1 action results in an impaired insulin response to oral glucose (Miyawaki et al. 1999; Scrocchi et al. 1996). Whether these peptides are the sole hormones mediating the incretin effect awaits the development of mice with combined defects in both GIP and GLP-1 receptor signaling. Finally, very recent studies have indicated an exciting role for GLP-1 as a β cell tropic factor. Administration of GLP-1, or exendin-4, a long-acting GLP-1R agonist, to mice or rats with diminished β cell reserve leads to enhancements in β cell mass and an increased capacity for insulin secretion (Stoffers et al. 2000; Xu et al. 1999). Consistent with these observations, mice with a null mutation in the GLP-1R exhibit alterations in β cell topography and reduced insulin secretion (Ling et al. 2001). Whether GIP also regulates β cell growth remains to be established.

Because of their insulinotropic and glucose-lowering actions, both GIP and GLP-1 are candidate peptides for the treatment of type 2 diabetes mellitus. However, only GLP-1 is currently in clinical trials for such therapeutic use, for two main reasons. First, although both GIP and GLP-1 inhibit gastric emptying (Nauck et al. 1996), an effect that delays postprandial rises in glycemia, only GLP-1 also induces satiety (Scrocchi et al. 1996; Turton et al. 1996). In addition, these peptides have opposing effects on glucagon release, with GLP-1 inhibiting, but GIP stimulating, glucagon secretion (Elahi et al. 1979; Komatsu et al. 1989). More importantly, although GLP-1 lowers glycemia in patients with type 2 diabetes (Gutniak et al. 1992; Nauck et al. 1996), the insulinotropic actions of GIP appear to be markedly abrogated in such individuals (Elahi et al. 1994; Nauck et al. 1993). These findings are consistent with previous observations that the incretin effect is diminished in patients with type 2 diabetes (Nauck et al. 1986), but, in addition, suggest that this defect occurs consequent to abnormalities at the level of the GIPR and/or in its postreceptor signaling cascade. Indeed, diabetic Zucker fatty rats, a rodent model of type 2 diabetes, exhibit decreased islet expression of the GIPR in association with diminished β cell responsiveness to GIP (Lynn et al. 2001).

Glucagon-Like Peptide-2

GLP-2, located carboxyterminal to GLP-1 in the proglucagon sequence, is cosecreted with GLP-1, oxyntomodulin, and glicentin from gut L cells, primarily in response to nutrient ingestion (Brubaker, Crivici et al. 1997; Xiao et al. 1999; Xiao, Boushey et al. 2000). Following clinical and experimental observations linking intestinal growth, injury, and gut adaptation to increased levels of circulating PGDPs (Drucker 1999, 2001), GLP-2 was subsequently identified as the PGDP exhibiting significant intestinotrophic properties in vivo (Drucker et al. 1996).

Administration of GLP-2 to normal mice and rats results in significant increases in mucosal thickness of the small and large intestine, with more prominent trophic effects consistently observed in the small bowel, especially in the jejunum (Drucker et al. 1996; Drucker, Deforest et al. 1997; Drucker, Shi et al. 1997; Tsai, Hill, Asa et al. 1997; Tsai, Hill, and Drucker 1997). The intestinotrophic effects of GLP-2 are mediated by stimulation of crypt cell proliferation and inhibition of apoptosis in both the crypt and villus compartments of the small bowel (Drucker et al. 1996; Tsai, Hill, Asa et al. 1997). GLP-2 administration also results in inhibition of gastric emptying, reduced gastric acid secretion, stimulation of intestinal hexose transport and nutrient absorption, and reduction in mucosal epithelial permeability in the small bowel (Benjamin et al. 2000; Brubaker, Izzo et al. 1997; Cheeseman and Tsang 1996; Wojdemann et al. 1998, 1999).

The reparative and antiapoptotic actions of GLP-2 have also been examined in the setting of experimental intestinal injury. GLP-2 reduces mucosal damage and decreases morbidity and mortality in a broad spectrum of rodent models of small and large bowel injury, including enteral nutrient deprivation, intestinal inflammation, ischemia, and chemotherapy-induced enteritis (Alavi et al. 2000; Boushey et al. 1999, 2001; Chance et al. 1997, 2001; Drucker et al. 1999). GLP-2 also enhances nutrient absorption and intestinal adaptation in rats with experimental small bowel resection (Scott et al. 1998) and in human subjects with short bowel syndrome (Jeppesen et al. 2001).

RECEPTOR CLONING: SPECIFICITY AND DISTRIBUTION

The GLP-1R was the first member of this group of receptors to be cloned (Thorens 1992). A 463 amino acid 7 transmembrane-spanning protein, the GLP-1R exhibits 27% to 40% sequence homology to the receptors for secretin, calcitonin, and parathyroid hormone. As these receptors shared higher identity with each other than with other members of the G protein–coupled receptor superfamily, they were classified together into a new family of receptors (Thorens 1992) now known as the type II receptor family. However, despite the strong sequence homology between GLP-1 and the other members of the glucagon-related family of peptides, the GLP-1R recognizes GLP-1 specifically, with no demonstrable binding by a number of related peptides, including secretin and vasoactive intestinal peptide (VIP) (Fehmann et al. 1994). Although binding of GIP and glucagon to the GLP-1R has been observed in some studies, this appears to occur only at micromolar concentrations (Thorens 1992). Furthermore, only GLP-1 appears to activate the GLP-1R, stimulating production of cAMP with an ED_{50} of 3 nM in COS cells transfected with the human receptor (Dillon et al. 1993). Consistent with the very high degree of sequence conservation found for GLP-1 in most mammalian species, the GLP-1R also exhibits conservation between species, with 90% homology between the rat and human receptors (Sivarajah et al. 2001). The distribution of the mammalian GLP-1 receptor also appears to be conserved, with RT-PCR and Northern blot analyses demonstrating expression in tissues known to be responsive to GLP-1, including the pancreatic β cells, the stomach, and the brain (Bullock et al. 1996; Campos et al. 1994; Thorens 1992).

The rat glucagon receptor (GR) cDNA was cloned in 1993 and encodes a 485 amino acid protein of ~54,962 daltons (Jelinek et al. 1993). Glucagon stimulates an increase in cAMP and intracellular calcium in transfected cells expressing the cloned receptor, with much weaker receptor activation noted only with pharmacological concentrations of the related peptides secretin, calcitonin, and parathyroid hormone (Jelinek et al. 1993). The human glucagon receptor cDNA encodes a protein of 477 amino acids that exhibits 82% identity to the rat receptor (Lok et al. 1994; MacNeil et al. 1994). Glucagon inhibits binding of [^{125}-I]-glucagon to the cloned human receptor with an IC_{50} of 5 nM, with tenfold lower tracer displacement exhibited by oxyntomodulin and GLP-1, whereas GIP, GLP-2, and secretin fail to displace binding of [^{125}-I]-glucagon binding at concentrations up to 3 uM (MacNeil et al. 1994). Mammalian glucagon receptor RNA transcripts are widely expressed in peripheral tissues, including liver, kidney, heart, adipose tissue, pancreatic islets, the gastrointestinal tract, spleen, thymus, adrenal gland, ovary, testes, and the central nervous system (Campos et al. 1994; Dunphy et al. 1998; Hansen et al. 1995; Svoboda et al. 1994).

Cloning of the GIPR in 1993 demonstrated that this 455 amino acid protein also belongs to the 7 transmembrane-spanning, glucagon receptor-related superfamily of receptors, sharing 44% sequence identity with the glucagon receptor (Usdin et al. 1993). The GIPR exhibits highly specific GIP binding, although some binding by exendin-4, a GLP-1R agonist, was noted at very high concentrations of 1–10 μM (Gremlich et al. 1995). However, there was no demonstrable response of the GIPR to other members of the glucagon-related family of hormones (Gremlich et al. 1995; Usdin et al. 1993). A high degree of sequence conservation (>80%) between the rat, hamster, and human GIPR's has been reported (Gremlich et al. 1995; Yamada et al. 1995). Consistent with its known sites of action, the GIP receptor is expressed in tissues such as the pancreas and upper gastrointestinal tract (Usdin et al. 1993). Interestingly, the GIPR is also expressed at low levels in the adrenal gland, where overexpression appears to contribute to the development of "Food-Dependent Cushing's Syndrome" (N'Diaye et al. 1998). The receptor is also found in adipose tissue, where it may play a role in lipolysis (Yip and Wolfe 2000), and in the brain, where GIP function remains to be ascertained. GIP also exerts anabolic actions in bone cells (Bollag et al. 2000), and GIP administration prevents ovariectomy-associated bone loss in vivo (Bollag et al. 2001).

The human and rat GLP-2 receptor cDNAs were isolated from intestinal and hypothalamic cDNA libraries in 1999 (Munroe et al. 1999). The rat GLP-2R cDNA comprises 2357 nucleotides and encodes a 550 amino acid receptor precursor protein. Two putative functionally identical translation start sites are present at the 5′end of the rat receptor, whereas only a single upstream ATG is present in the human sequence; the mouse GLP-2R sequence contains only the downstream ATG codon (Lovshin et al. 2001). The GLP-2R specifically recognizes GLP-2 with an EC_{50} for cAMP stimulation of 0.58 nM, with no significant stimulation of cAMP generation detected with related glucagon-like peptides and peptide members of the glucagon-secretin superfamily at concentrations of 10 nM (DaCambra et al. 2000; Munroe et al. 1999). Unlike the more widely expressed GLP-1 and glucagon receptors, GLP-2R expression is more restricted, predominantly to endocrine cells and enteric neurons in the gastrointestinal tract and selected brain neurons (Bjerknes and Cheng 2001; Lovshin et al. 2001; Munroe et al. 1999; Yusta, Huang et al. 2000). GLP-2R expression has been localized to distinct subsets of enteroendocrine cells in the human stomach and both small and large intestine, implying that GLP-2R receptor signaling in gut endocrine cells stimulates the activation and/or repression of endocrine-derived downstream mediators of GLP-2 action (Yusta, Huang et al. 2000).

GENOMIC ORGANIZATION

The human, mouse, and rat glucagon receptor genes contain 13 exons, and the human gene has been mapped to chromosome 17q24 (Burcelin et al. 1995; Lok et al. 1994; Maget et al. 1994), with the relative location of introns conserved among members of the receptor superfamily. Although RT-PCR studies have identified incompletely processed glucagon receptor transcripts, with the potential to give rise to variant glucagon receptor proteins (Maget et al. 1994), variant receptor transcripts have not been detected in subsequent studies (Dunphy et al. 1998; Hansen et al. 1995), hence the significance of potential glucagon receptor splice variants requires further clarification.

The human GIPR gene contains 14 exons (Yamada et al. 1995), whereas the rat gene contains 16 exons (Boylan et al. 1999). The human GIPR gene is located on chromosome 19q13.3 (Gremlich et al. 1995). As is the case with the glucagon, GLP-1, and GLP-2 receptor genes (Geiger et al. 2000), the GIPR 5′-flanking region contains several Sp1 binding sites, but no functional TATA or CAAT boxes (Boylan et al. 1999; Geiger et al. 2000; Lovshin et al. 2001; Wildhage et al. 1999). The human GLP-1R gene is located on band 21 of chromosome 6 (Stoffel et al. 1993), whereas the GLP-2R gene has been localized to human chromosome 17p13.3 (Munroe et al. 1999). The structural organization of the GLP-1 and GLP-2 receptor genes remains incompletely characterized.

ALTERNATIVE RNA SPLICING

RNA splicing resulting in functionally distinct receptor isoforms has not been conclusively proven for any of the glucagon-receptor related receptors with the exception of the GIPR. Genomic analysis of the GIPR identified one exon, designated #8a, that appears to be alternatively spliced, resulting in a variant mRNA transcript that encodes a truncated receptor (Boylan et al. 1999). Although the functional properties of the variant receptor are unknown, an internally deleted GIPR, missing nucleotides 793-924, that does not bind GIP in transient expression assays, has been isolated (Gremlich et al. 1995). A second mutant

receptor cDNA, isolated from human insulinoma cells, lacks an internal 62 bp exon, resulting in a premature stop codon that encodes a truncated receptor RNA (Volz et al. 1995). Similar variant GIP receptor transcripts were detected in RNA from endothelial cell lines (Zhong et al. 2000). In contrast, a second human GIPR with a 27 amino acid insertion in the C-terminal tail appears to be fully functional (Gremlich et al. 1995); however, the tissue distribution and functional significance of this receptor has not been examined in human tissues in vivo.

SIGNALING/G PROTEIN INTERACTIONS

Glucagon activates multiple G protein–mediated signal transduction pathways in liver cells, leading to stimulation of adenylyl cyclase (Pohl et al. 1969) and phosphoinositol turnover (Wakelam et al. 1986). The importance of dual signaling systems is exemplified by studies with glucagon analogues that fail to stimulate cAMP formation, yet activate glycogenolysis and gluconeogenesis, via stimulation of glucagon receptor signaling, coupled to inositol phosphate production (Wakelam et al. 1986). Although both glucagon and β-adrenergic agonists stimulate adenylyl cyclase in liver cells, the glucagon receptor is coupled to both $G_{s\alpha\text{-s}}$ and $G_{s\alpha\text{-L}}$ whereas hepatic β-adrenergic receptors are primarily coupled to $G_{s\alpha\text{-L}}$ (Yagami 1995). Generation of the (H178R) mutation in the glucagon receptor results in increased levels of basal cAMP and increased glucagon binding affinity (Hjorth et al. 1998). However, glucagon-mediated stimulation of cAMP or intracellular calcium appears to be dependent on amino acid sequences residing within the second and third intracellular loops (Cypess et al. 1999). Furthermore, deletional studies have demonstrated that the majority of carboxyterminal amino acid sequences distal to TM7 are not required for glucagon binding or adenylyl cyclase activation (Unson et al. 1995).

Differential interaction of the glucagon receptor with specific Gs isoforms has been detected in studies using GR-Gα_s fusion proteins. The affinity of the wild-type GR for glucagon ($\sim$IC$_{50}$ of $\sim$46 nM) was comparable to the affinity observed with the GR-Gα_s-S hybrid receptor, whereas the GR-Gα_s-L receptor exhibited a lower IC$_{50}$ for glucagon binding of $\sim$3.2 nM and a fourfold higher IC$_{50}$ for adenylyl cyclase activation compared to GR-Gα_s-L (Unson et al. 2000). The higher affinity reversed in the presence of GTPγS, suggesting that specific coupling of the GR to distinct Gs isoforms may account for the dual class of high and low affinity glucagon receptors described in previous studies (Horwitz et al. 1985, 1986).

Although coupling of the GLP-1R to the adenylyl cyclase pathway has been well established (Drucker et al. 1987), GLP-1 also activates phospholipase C and MAPK in some cell types (Montrose-Rafizadeh et al. 1999; Wheeler et al. 1993), and stimulates calcium influx leading to increases in cytosolic free calcium $[Ca^{2+}]_i$. Consistent with this observation, the GLP-1R couples to multiple G proteins, including Gα_s, G$\alpha_{q/11}$ and G$\alpha_{i1,2}$ (Montrose-Rafizadeh et al. 1999) and a GLP-1R-G protein interaction domain includes amino acid sequences within the third intracellular loop (Hallbrink et al. 2001). Consistent with these findings, structural comparison of the GLP-1R, with related members of the GPCR superfamily, implicated the sequence K334-L335-K336 in the 3rd intracellular loop as a potential site for G protein coupling, and deletion of these amino acids abolishes both coupling to adenylyl cyclase and insulin secretion (Salapatek et al. 1999; Takhar et al. 1996). Alanine scanning mutagenesis further identified amino acids V327, I328, V331, and K334 as essential for the stimulation of adenylyl cyclase by GLP-1 (Mathi et al. 1997). Interestingly, 3-dimensional modeling of the 3rd intracellular loop demonstrates that these amino acids form one face of an α-helix, similar to that reported for the G protein coupling region of the m5 muscarinic receptor (Hill-Eubanks et al. 1996). The importance of amino acids within the third intracellular loop for G protein coupling is further emphasized by experiments using synthetic peptide sequences that demonstrate activation of both pertussis- and cholera toxin-sensitive G proteins as assessed by measurement of GTPase activity (Hallbrink et al. 2001).

GIP also stimulates adenylyl cyclase activation in islet and intestinal cells and in heterologous cells expressing a transfected receptor (Amiranoff et al. 1984; Emami et al. 1986). GIP increases intracellular calcium in endothelial and some (Wheeler et al. 1995) but not all (Gremlich et al. 1995; Volz et al. 1995) cell lines transfected with the GIPR cDNA. A T340P mutation in the rat GIP receptor expressed in human embryonal kidney cells results in constitutive activation of adenylyl cyclase yet retention of GIP responsivity (Tseng and Lin 1997). Intriguingly, GIP also stimulates arachidonic acid production and release in Chinese hamster ovary cells expressing the rat GIPR, and in islet βTC-3 cells (Ehses et al. 2001).

GLP-2 receptor activation is also coupled to activation of cAMP-dependent pathways in heterologous cell lines transfected with the rat or human GLP-2R (Munroe et al. 1999; Yusta et al. 1999). GLP-2 activates AP-1-dependent signaling pathways, immediate early gene expression, and p70 S6 kinase activity, but does not stimulate intracellular calcium accumulation in BHK cells expressing the rat GLP-2R (Yusta et al. 1999). The GLP-2 effects on AP-1-dependent pathways are likely indirect, as GLP-2-stimulated induction of AP-1 luciferase activity was markedly attenuated in the presence of PKA inhibition (Yusta et al. 1999). GLP-2R signaling in BHK-GLP-2R cells is also coupled to activation of an antiapoptotic signaling program in a PKA-, phosphatidyl inositol 3-kinase-, and mitogen-activated protein kinase-independent manner (Boushey et al. 2001; Yusta, Boushey et al. 2000).

RECEPTOR DESENSITIZATION

The glucagon receptor undergoes both homologous and heterologous desensitization in hepatocytes in vitro, and protein kinase C–selective inhibitors abrogate the heterologous desensitization process (Savage et al. 1995). Agonist occupancy of related hormone receptors, coupled to adenylyl cyclase in hepatocytes, results in heterologous desensitization with kinetics

somewhat slower than those observed for homologous desensitization (Premont and Iyengar 1988). Both homologous and heterologous glucagon receptor desensitization are independent of increases in intracellular calcium (Savage et al. 1995); receptor desensitization is also independent of cAMP activation and is induced by activators of inositol phospholipid metabolism such as protein kinase C, independent of the inhibitory guanine nucleotide regulatory protein Gi. (Murphy et al. 1987, 1989).

Prolonged activation of the GLP-1R by GLP-1 agonists results in a diminished response to subsequence ligand binding in vitro (Fehmann and Habener 1991). Mutagenesis studies have identified three pairs of serine residues in the C-terminal tail of the receptor (S441/S442, S444/S445, and S451/S452) important for ligand-induced desensitization of the GLP-1R (Widmann et al. 1996a, 1996b, 1997). These amino acids are also necessary for internalization of the receptor, while phosphorylation of an additional serine pair (S431/S432) by protein kinase C appears to be involved in heterologous desensitization of the GLP-1R (Widmann et al. 1996a, 1996b, 1997). It has been suggested that this heterologous desensitization may occur in the β cell in response to ligand-mediated activation of phospholipase C, such as occurs in response to physiological regulators, including acetylcholine (Widmann et al. 1996b). Nevertheless, diminution of GLP-1 responsivity has not yet been clearly described in vivo, suggesting that other compensatory mechanisms may serve to protect the receptor from downregulation.

Homologous and/or heterologous desensitization of the GIPR may account for the reduced response to GIP that has been observed in patients with type 2 diabetes (Elahi et al. 1994; Nauck et al. 1993). The GIPR undergoes homologous desensitization (Fehmann and Habener 1991; Tseng, Boylan et al. 1996; Tseng and Zhang 2000), while incubation of islets with high glucose also reduces the response to GIP in a manner that is independent of protein kinases A and C (Hinke et al. 2000). The rat GIPR possesses only a single pair of serine residues in its C-terminal tail (S426/S427), and a combination of site directed mutagenesis and carboxyterminal deletions have implicated a role for these residues in receptor internalization, whereas S406 and C411 contribute to receptor desensitization (Tseng and Zhang 1998; Wheeler et al. 1999). Cotransfection of a cDNA encoding β-arrestin-1, or the G protein–coupled receptor kinase 2 (GRK2), but not GRK5 or GRK6, attenuated GIP-stimulated cAMP accumulation in L293-GIPR and βTC3 cells (Tseng and Zhang 2000). However, none of these proteins appear to be involved in GIPR internalization.

In contrast to the information available for the glucagon, GLP-1, and GIP receptors, GLP-2 receptor desensitization has not yet been examined in cells expressing a GLP-2R in vitro. Although a diminished response to supraphysiological levels of GLP-2 is apparent in heterologous cells expressing the GLP-2R (Lovshin et al. 2001), mice treated with daily injections of GLP-2 for 12 weeks continued to exhibit enhanced intestinal mass, consistent with a lack of GLP-2R desensitization in vivo (Tsai, Hill, Asa et al. 1997).

MOLECULAR BASIS OF LIGAND RECEPTOR-INTERACTION

A series of N-terminal truncation and internal deletion mutants has been used to demonstrate that glycosylation is unlikely to be important for glucagon receptor intracellular trafficking and plasma membrane insertion (Unson et al. 1995). Remarkably, deletion of the intracellular carboxyterminal tail of the rat receptor does not abrogate glucagon binding or adenylyl cyclase generation in transiently transfected COS-1 or CHO cells (Buggy et al. 1997; Unson et al. 1995). Similarly, the carboxyterminal glucagon receptor tail is not required for calcium mobilization in CHO cells expressing the human glucagon receptor (Buggy et al. 1997). By contrast, mutation of a highly conserved D64 residue in the N-terminal portion of the rat glucagon receptor completely eliminates glucagon binding (Carruthers et al. 1994). This residue corresponds to a mutated Asp residue at amino acid 60 in the growth hormone releasing hormone receptor that gives rise to the *little* mouse phenotype (Lin et al. 1993). Antisera directed against amino acid sequences 126–137 of the N-terminal region, and agai residues 206–219 of the first extracellular loop block [^{125}I]-glucagon binding and interfere with glucagon-induced adenylyl cyclase generation in rat liver membranes (Unson et al. 1996). Similarly, domain swap experiments that exchanged comparable regions of the human GLP-1 and glucagon receptors identified the more proximal portion of the N-terminal extracellular extension, and sequences within the first extracellular loop and transmembrane domains III and IV, as critical for glucagon receptor binding (Buggy et al. 1995). In contrast, domain swap experiments involving the N-terminal regions of the glucagon and calcitonin receptors demonstrated that the GR N-terminus alone is not always sufficient for conferring 125-I-glucagon binding to a chimeric receptor (Stroop et al. 1995).

Although glycosylation of the GLP-1 receptor affects the number of GLP-1 binding sites detected in islet Rin5Fm cells, inhibition of N-linked glycosylation with tunicamycin did not change the affinity of the receptor for GLP-1 binding (Goke et al. 1994). Remarkably, the isolated N-terminal portion of the GLP-1 receptor competes for GLP-1 binding with the intact receptor expressed in CHL cells, and binds radiolabeled GLP-1 in crosslinking experiments in vitro (Wilmen et al. 1996). Within the N-terminal domain, mutagenesis studies have identified residues W39, W72, W91, W110, and W120 as important determinants of ligand binding (Van Eyll et al. 1996; Wilmen et al. 1997). Although the N-terminal domain of the GLP-1R appears sufficient for GLP-1 binding, GLP-1-stimulated receptor activation and cAMP formation is detectable despite complete absence of the extracellular N-terminal domain (Gelling et al. 1997). Furthermore, mutation of amino acid residues K197, D198, K202, D215, and R227 in the 1st extracellular loop results

in significantly reduced ligand binding affinity, suggesting that these residues likely interact with sequences in the N-terminal domain for optimal binding of GLP-1R ligands and/or are important for the conformational structure of the ligand binding domain (Xiao, Jeng et al. 2000).

Experiments employing a domain swap paradigm with the GIP receptor have identified the N-terminal domain extending to amino acid 132 as sufficient for binding of GIP agonists (Gelling et al. 1997). Nevertheless, sequences extending to amino acid 222 within transmembrane domain 3 are required for both ligand binding and G protein activation (Gelling et al. 1997). In contrast to data obtained with the GLP-1 and glucagon receptors, truncation of the GIP receptor sequences immediately carboxyterminal to the 7th transmembrane domain to amino acid 395 results in complete loss of ligand binding (Tseng and Zhang 1998; Wheeler et al. 1999), likely due to the requirement for a minimum number of carboxyterminal residues for efficient receptor synthesis and insertion into the cell membrane (Wheeler et al. 1999).

RECEPTOR REGULATION

Glucagon receptor mRNA transcripts are expressed at comparable levels in the liver of normal versus diabetic mice, although ligand binding studies provide evidence for increased receptor number in the livers of db/db mice (Yoo-Warren et al. 1994). The levels of glucagon receptor mRNA transcripts are increased in the presence of high glucose concentrations following 24 h culture of rat hepatocytes and islets, whereas glucagon itself and induction of adenylyl cyclase with heterologous agents significantly reduced expression of the hepatic glucagon receptor (Abrahamsen et al. 1995; Abrahamsen and Nishimura 1995). The glucose-induction of GR expression is partially antagonized by insulin and is dependent on local oxygen tension (Krones et al. 1998). GR expression is regulated by thyroid hormone in a tissue-specific manner (Morales et al. 1998) and is downregulated in brown adipose tissue by cold exposure in a sympathetic nervous system–dependent manner (Morales et al. 2000). These findings, taken together with the reduction of glucagon receptor RNA transcripts in tumor-bearing rats with hyperglucagonemia (Nishimura et al. 1996), suggest that glucagon regulates the number of glucagon receptors in part through autoregulation of glucagon receptor gene expression.

The levels of islet GLP-1 receptor mRNA transcripts are modestly decreased following exposure to 10 mM dexamethasone and high (20 mM) glucose, but islet GLP-1R mRNA transcripts were not affected by agents that altered intracellular cAMP (Abrahamsen and Nishimura 1995). In contrast, neonatal rats fed a high carbohydrate diet for 12 days develop hyperinsulinemia in association with increased levels of plasma GLP-1 and enhanced expression of islet GLP-1R mRNA transcripts (Srinivasan et al. 2000). Induction of GLP-1R expression has also been observed in glial cells following experimental brain injury in the rat (Chowen et al. 1999).

The regulation of GIP and GLP-2 receptor expression has not been extensively examined. Consistent with the defect in GIP action observed in experimental models and human subjects with diabetes, GIP receptor mRNA transcripts and receptor protein are decreased in islets from Zucker diabetic rats (Lynn et al. 2001); however, the mechanisms regulating GIP receptor expression remain unknown.

RECEPTOR POLYMORPHISMS AND DISEASE

Glucagon receptor polymorphisms, principally a Gly to Ser missense mutation in exon 2 at amino acid 40, have been observed with higher frequency in some but not all diabetic populations (Ambrosch et al. 1999; Babadjanova et al. 1997; Fujisawa et al. 1995; Gough et al. 1995; Hager et al. 1995; Huang et al. 1999). Paradoxically, the G40S mutation results in a receptor with reduced sensitivity to glucagon in vitro (Hansen et al. 1996), and carriers of this mutation exhibit a reduced glycemic response to glucagon infusion in vivo (Tonolo et al. 1997). Similarly, the G40S mutation has also been detected with increased frequency in some subjects with hypertension (Chambers and Morris 1996), including patients in the Olivetti heart study from Italy, where the Gly to Ser mutation was associated with a twofold increased risk of hypertension, and those carrying the Gly40Ser allele had higher serum uric acid and lower fractional excretion of uric acid and exogenous lithium, independently of age, body mass, and current pharmacological treatment (Strazzullo et al. 2001). Nevertheless, this association has not been uniformly confirmed in different hypertensive populations (Brand et al. 1999; Huang et al. 1999). Several polymorphisms have also been identified in the human GLP-1 receptor gene (Stoffel et al. 1993; Tanizawa et al. 1994); however, no significant linkage associations to diabetes or obesity have yet been detected (Norman et al. 1999; Tanizawa et al. 1994; Yagi et al. 1996; Zhang et al. 1994). Similarly, although several polymorphisms have been identified in the human GIP receptor gene, the variant receptors are not associated with significantly altered GIP binding or signal transduction and have not yet been consistently linked to an increased frequency of diabetes in human subjects (Almind et al. 1998; Kubota et al. 1996).

SUMMARY

Glucagon, GLP-1, GLP-2, and GIP represent physiologically important regulators of nutrient intake and assimilation and are currently being used in the clinic (glucagon) or are under active investigation in Phase 2 human trials for the treatment of diabetes and intestinal disease (GLP-1 and GLP-2, respectively). Hence, their receptors represent not only important molecules essential for peptide hormone action, but also potential pharmaceutical targets for development of peptidomimetic agonists and antagonists. Given the increasing interest in the physiology and biochemistry of these regulatory peptides, it seems likely that ongoing studies will significantly enhance our understanding of

the regulation and function of members of the glucagon receptor superfamily.

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REFERENCES

Abrahamsen, N., Lundgren, K., and Nishimura, E. 1995. *J. Biol. Chem.* 270:15853–15857.

Abrahamsen, N., and Nishimura, E. 1995. *Endocrinology* 136:1572–1578.

Ahloulay, M., Bouby, N., Machet, F., Kubrusly, M., Coutaud, C., and Bankir, L. 1992. *Am. J. Physiol.* 263:F24–F36.

Alavi, K., Schwartz, M. Z., Palazzo, J. P., and Prasad, R. 2000. *J. Pediatr. Surg.* 35:847–851.

Almind, K., Ambye, L., Urhammer, S. A., Hansen, T., Echwald, S. M., Holst, J. J., Gromada, J., Thorens, B., and Pedersen, O. 1998. *Diabetologia* 41:1194–1198.

Ambrosch, A., Lobmann, R., Dierkes, J., Konig, W., Luley, C., and Lehnert, H. 1999. *Clin. Chem. Lab. Med.* 37:719–721.

Amiranoff, B., Vauclin-Jacques, N., and Laburthe, M. 1984. *Biochem. Biophys. Res. Commun.* 123:671–676.

Babadjanova, G., Reincke, M., Mora, P., Chuchalin, A., and Allolio, B. 1997. *Exp. Clin. Endocrinol. Diabetes* 105:225–226.

Baggio, L., Kieffer, T. J., and Drucker, D. J. 2000. *Endocrinology* 141:3703–3709.

Bell, G. I., Sanchez-Pescador, R., Laybourn, P. J., and Najarian, R. C. 1983. *Nature* 304:368–371.

Benjamin, M. A., McKay, D. M., Yang, P. C., Cameron, H., and Perdue, M. H. 2000. *Gut* 47:112–119.

Bertin, E., Arner, P., Bolinder, J., and Hagstrom-Toft, E. 2001. *J. Clin. Endocrinol. Metab.* 86:1229–1234.

Bertrand, H. A., Masoro, E. J., and Yu, B. P. 1980. *Endocrinology* 107:591–595.

Bjerknes, M., and Cheng, H. 2001. *Proc. Natl. Acad. Sci. USA*.

Bollag, R. J., Zhong, Q., Ding, K. H., Phillips, P., Zhong, L., Qin, F., Cranford, J., Mulloy, A. L., Cameron, R., and Isales, C. M. 2001. *Mol. Cell Endocrinol.* 177:35–41.

Bollag, R. J., Zhong, Q., Phillips, P., Min, L., Zhong, L., Cameron, R., Mulloy, A. L., Rasmussen, H., Qin, F., Ding, K. H., and Isales, C. M. 2000. *Endocrinology* 141:1228–1235.

Boushey, R. P., Yusta, B., and Drucker, D. J. 1999. *Am. J. Physiol.* 277:E937–E947.

Boushey, R. P., Yusta, B., and Drucker, D. J. 2001. *Cancer Res.* 61:687–693.

Boylan, M. O., Jepeal, L. I., and Wolfe, M. M. 1999. *Peptides* 20:219–228.

Brand, E., Bankir, L., Plouin, P. F., and Soubrier, F. 1999. *Hypertension* 34:15–17.

Broadus, A. E., Kaminsky, N. I., Northcutt, R. C., Hardman, J. G., Sutherland, E. W., and Liddle, G. W. 1970. *J. Clin. Invest.* 49:2237–2245.

Brown, J. C. 1982. *Gastric Inhibitory Polypeptide. Monographs in Endocrinology.* Heidelberg: Springer-Verlag.

Brown, J. C., and Dryburgh, J. R. 1971. *Can. J. Biochem.* 49:867–872.

Brubaker, P. L., Crivici, A., Izzo, A., Ehrlich, P., Tsai, C.-H., and Drucker, D. J. 1997. *Endocrinology* 138:4837–4843.

Brubaker, P. L., Izzo, A., Hill, M., and Drucker, D. J. 1997. *Am. J. Physiol.* 272:E1050–E1058.

Buggy, J. J., Heurich, R. O., MacDougall, M., Kelley, K. A., Livingston, J. N., Yoo-Warren, H., and Rossomando, A. J. 1997. *Diabetes* 46:1400–1405.

Buggy, J. J., Livingston, J. N., Rabin, D. U., and Yoo-Warren, H. 1995. *J. Biol. Chem.* 270:7474–7478.

Bullock, B. P., Heller, R. S., and Habener, J. F. 1996. *Endocrinology* 137:2968–2978.

Burcelin, R., Li, J., and Charron, M. J. 1995. *Gene* 164:305–310.

Campos, R. V., Lee, Y. C., and Drucker, D. J. 1994. *Endocrinology* 134:2156–2164.

Carruthers, C. J. L., Unson, C. G., Kim, H. N., and Sakmar, T. P. 1994. *J. Biol. Chem.* 269:29321–29328.

Cersosimo, E., Garlick, P., and Ferretti, J. 1999. *Diabetes* 48:261–266.

Chambers, S. M., and Morris, B. J. 1996. *Nat. Genet.* 12:122.

Chance, W. T., Foley-Nelson, T., Thomas, I., and Balasubramaniam, A. 1997. *Am. J. Physiol.* 273:G559–G563.

Chance, W. T., Sheriff, S., McCarter, F., and Ogle, C. 2001. *J. Burn. Care Rehabil.* 22:136–143.

Cheeseman, C. I., and Tsang, R. 1996. *Am. J. Physiol. Gastrointest. Liver Physiol.* 271:G477–G482.

Chowen, J. A., de Fonseca, F. R., Alvarez, E., Navarro, M., Garcia-Segura, L. M., and Blazquez, E. 1999. *Neuropeptides* 33:212–215.

Creutzfeldt, W. 1979. *Diabetologia* 16:75–85.

Cypess, A. M., Unson, C. G., Wu, C. R., and Sakmar, T. P. 1999. *J. Biol. Chem.* 274:19455–19464.

DaCambra, M. P., Yusta, B., Sumner-Smith, M., Crivici, A., Drucker, D. J., and Brubaker, P. L. 2000. *Biochemistry* 39:8888–8894.

Dillon, J. S., Tanizawa, Y., Wheeler, M. B., Leng, X.-H., Ligon, B. B., Rabin, D. U., Yoo-Warren, H., Permutt, M. A., and Boyd, A. E., III. 1993. *Endocrinology* 133:1907–1910.

Dorn, A., Rinne, A., Bernstein, H. G., Ziegler, M., Hahn. H. J., and Rasanen, O. 1983. *Exp. Clin. Endocrinol.* 81:24–32.

Drucker, D. J. 1999. *Trends in Endocrinology and Metabolism* 10:153–156.

Drucker, D. J. 2001. *J. Clin. Endocrinol. Metab.* 86:1759–1764.

Drucker, D. J., and Asa, S. 1988. *J. Biol. Chem.* 263:13475–13478.

Drucker, D. J., Deforest, L., and Brubaker, P. L. 1997. *Am. J. Physiol.* 273:G1252–G1262.

Drucker, D. J., Ehrlich, P., Asa, S. L., and Brubaker, P. L. 1996. *Proc. Natl. Acad. Sci. USA* 93:7911–7916.

Drucker, D. J., Philippe, J., Mojsov, S., Chick, W. L., and Habener, J. F. 1987. *Proc. Natl. Acad. Sci. USA* 84:3434–3438.

Drucker, D. J., Shi, Q., Crivici, A., Sumner–Smith, M., Tavares, W., Hill, M., Deforest, L., Cooper, S., and Brubaker, P. L. 1997. *Nature Biotechnology* 15:673–677.

Drucker, D.J., Yusta, B., Boushey, R. P., Deforest, L., and Brubaker, P. L. 1999. *Am. J. Physiol*. 276:G79– G91.

Dunphy, J. L., Taylor, R. G., and Fuller, P. J. 1998. *Mol. Cell Endocrinol.* 141:179–186.

Dupre, J., Ross, S. A., Watson, D., and Brown, J. C. 1973. *J. Clin. Endocrinol. Metab.* 37:826–828.

Ebert, R., and Creutzfeldt, W. 1982. *Endocrinology* 111:1601–1606.

Ebert, R., Unger, H., and Creutzfeld, W. 1983. *Diabetologia* 24:449–454.

Ehses, J. A., Lee, S. S., Pederson, R. A., and McIntosh, C. H. 2001. *J. Biol. Chem.* 276:23667–23673.

Elahi, D., Andersen, D. K., Brown, J. C., Debas, H. T., Hershcopf, R. J., Raizes, G. S., Tobin, J. D., and Andres, R. 1979. *Am. J. Physiol.* 237:E185–E191.

Elahi, D., McAloon-Dyke, M., Fukagawa, N. K., Meneilly, G. S., Sclater, A. L., Minaker, K. L., Habener, J. F., and Andersen, D. K. 1994. *Regul. Pept.* 51:63–74.

Elrick, H., Stimmler, L., Hlad, C. J., and Arai, Y. 1964. *J. Clin. Endocrinol. Metab.* 24:1076–1082.

Emami, S., Chastre, E., Bodere, H., Gespach, C., Bataille, D., and Rosselin, G. 1986. *Peptides* 7 (Suppl 1):121–127.

Farah, A. E. 1983. *Pharmacological Reviews* 35:181–217.

Fehmann, H. C., and Habener, J. F. 1991. *Endocrinology* 128:2880–2888.

Fehmann, H. C., Jiang, J., Schweinfurth, J., Dorsch, K., Wheeler, M. B., Boyd, A. E., 3rd, and Goke, B. 1994. *Z. Gastroenterol.* 32:203–207.

Fujisawa, T., Ikegami, H., Yamato, E., Takekawa, K., Nakagawa, Y., Hamada, Y., Ueda, H., Fukuda, M., and Ogihara, T. 1995. *Diabetologia* 38: 983–985.

Geiger, A., Decaux, J. F., Burcelin, R., Le Cam, A., Salazar, G., Charron, M. J., Girard, J., and Kervran, A. 2000. *Biochem. Biophys. Res. Commun.* 272:912–921.

Gelling, R. W., Wheeler, M. B., Xue, J., Gyomorey, S., Nian, C., Pederson, R. A., and McIntosh, C. H. 1997. *Endocrinology* 138:2640–2643.

Gerich, J. E. 1988. *Diabetes* 37:1608–1617.

Goke, R., Just, R., Lankat-Buttgereit, B., and Goke, B. 1994. *Peptides* 15:675–681.

Gough, S. C. L., Saker, P. J., Pritchard, L. E., Merriman, T. R., Merriman, M. E., Rowe, B. R., Kumar, S., Aitman, T., Barnett, A. H., Turner, R. C., Bain, S. C., and Todd, J. A. 1995. *Hum. Mol. Genet.* 4:1609–1612.

Gravholt, C. H., Moller, N., Jensen, M. D., Christiansen, J. S., and Schmitz, O. 2001. *J. Clin. Endocrinol. Metab.* 86:2085–2089.

Gremlich, S., Porret, A., Hani, E. H., Cherif, D., Vionnet, D., Froguel, P., and Thorens, B. 1995. *Diabetes* 44:1202–1208.

Gutniak, M., Orskov, C., Holst, J. J., Ahren, B., and Efendic, S. 1992. *N. Engl. J. Med.* 326:1316–1322.

Hager, J., Hansen, L., Vaisse, C., Vionnet, N., Philippi, A., Poller, W., Velho, G., Carcassi, C., Contu, L., Julier, C., et al. 1995. *Nat. Genet.* 9: 299–304.

Hallbrink, M., Holmqvist, T., Olsson, M., Ostenson, C. G., Efendic, S., and Langel, U. 2001. *Biochim. Biophys. Acta* 1546:79–86.

Hansen, L. H., Abrahamsen, N., Hager, J., Jelinek, L., Kindsvogel, W., Froguel, P., and Nishimura, E. 1996. *Diabetes* 45:725–730.

Hansen, L. H., Abrahamsen, N., and Nishimura, E. 1995. *Peptides* 16:1163–1166.

Heckemeyer, C. M., Barker, J., Duckworth, W. C., and Solomon, S. S. 1983. *Endocrinology* 113:270–276.

Hill-Eubanks, D., Burstein, E. S., Spalding, T. A., Brauner-Osborne, H., and Brann, M. R. 1996. *J. Biol. Chem.* 271:3058–3065.

Hinke, S. A., Pauly, R. P., Ehses, J., Kerridge, P., Demuth, H. U., McIntosh, C. H., and Pederson, R. A. 2000. *J. Endocrinol.* 165:281–291.

Hjorth, S. A., Orskov, C., and Schwartz, T. W. 1998. *Mol. Endocrinol.* 12:78–86.

Horwitz, E. M., Jenkins, W. T., Hoosein, N. M., and Gurd, R. S. 1985. *J. Biol. Chem.* 260:9307–9315.

Horwitz, E. M., Wyborski, R. J., and Gurd, R. S. 1986. *J. Biol. Chem.* 261:13670–13676.

Huang, C. N., Lee, K. C., Wu, H. P., Tai, T. Y., Lin, B. J., and Chuang, L. M. 1999. *Pancreas* 18:151–155.

Inokuchi, A., Oomura, Y., and Nishimura, H. 1984. *Physiol. Behav.* 33:397–400.

Inokuchi, A., Oomura, Y., Shimizu, N., and Yamamoto, T. 1986. *Am. J. Physiol.* 250:R120–R126.

Irwin, D. M. 2001. *Regul. Pept.* 98:1–12.

Jelinek, L. J., Lok, S., Rosenberg, G. B., Smith, R. A., Grant, F. J., Biggs, S., Bensch, P. A., Kuijper, J. L., Sheppard, P. O., Sprecher, C. A., et al. 1993. *Science* 259:1614–1616.

Jensen, M. D., Heiling, V. J., and Miles, J. M. 1991. *J. Clin. Endocrinol. Metab.* 72:308–315.

Jeppesen, P. B., Hartmann, B., Thulesen, J., Graff, J., Lohmann, J., Hansen, B. S., Tofteng, F., Poulsen, S. S., Madsen, J. L., Holst, J. J., and Mortensen, P. B. 2001. *Gastroenterology* 120:806–815.

Kieffer, T. J., and Habener, J. F. 1999. *Endocr. Rev.* 20:876–913.

Kirsch, J. R., and D'Alecy, L. G. 1984. *Stroke* 15:324–328.

Komatsu, R., Matsuyama, T., Namba, M., Watanabe, N., Itoh, H., Kono, N., and Tarui, S. 1989. *Diabetes* 38:902–905.

Kreymann, B., Ghatei, M. A., Williams, G., and Bloom, S. R. 1987. *Lancet* ii:1300–1304.

Krones, A., Kietzmann, T., and Jungermann, K. 1998. *FEBS Lett.* 421:136–140.

Krzeski, R., Czyzyk-Krzeska, M. F., Trzebski, A., and Millhorn, D. E. 1989. *Brain Res.* 504:297–300.

Kubota, A., Yamada, Y., Hayami, T., Yasuda, K., Someya, Y., Ihara, Y., Kagimoto, S., Watanabe, R., Taminato, T., Tsuda, K., and Seino, Y. 1996. *Diabetes* 45:1701–1705.

LaBarre, J., and Still, E. U. 1930. *Am. J. Physiol.* 91.

Lefebvre, P. J., and Luyckx, A. S. 1969. *Diabetologia* 5:195–197.

Lewis, J. T., Dayanandan, B., Habener, J. F., and Kieffer, T. J. 2000. *Endocrinology* 141:3710–3716.

Lin, S. C., Lin, C. R., Gukovsky, I., Lusis, A. J., Sawchenko, P. E., and Rosenfeld, M. G. 1993. *Nature* 364:208–213.

Ling, Z., Wu, D., Zambre, Y., Flamez, D., Drucker, D. J., Pipeleers, D. G., and Schuit, F. C. 2001. *Virchows Arch.* 438:382–387.

Lok, S., Kuijper, J. L., Jelinek, L. J., Kramer, J. M., Whitmore, T. E., Sprecher, C. A., Mathewes, S., Grant, F. J., Biggs, S. H., Rosenberg, G. B., et al. 1994. *Gene* 140:203–209.

Lovshin, J., Estall, J., Yusta, B., Brown, T. J., and Drucker, D. J. 2001. *J. Biol. Chem.* 276:21489–21499.

Lynn, F. C., Pamir, N., Ng, E. H., McIntosh, C. H., Kieffer, T. J., and Pederson, R. A. 2001. *Diabetes* 50:1004–1011.

MacNeil, D. J., Occi, J. L., Hey, P. J., Strader, C. D., and Graziano, M. P. 1994. *Biochem. Biophys. Res. Commun.* 198:328–334.

Maget, B., Tastenoy, M., and Svoboda, M. 1994. *FEBS Lett.* 351:271–275.

Mathi, S. K., Chan, Y., Li, X., and Wheeler, M. B. 1997. *Mol. Endocrinol.* 11:424–432.

Miyawaki, K., Yamada, Y., Yano, H., Niwa, H., Ban, N., Ihara, Y., Kubota, A., Fujimoto, S., Kajikawa, M., Kuroe, A., Tsuda, K., Hashimoto, H., Yamashita, T., Jomori, T., Tashiro, F., Miyazaki, J., and Seino, Y. 1999. *Proc. Natl. Acad. Sci. USA* 96:14843–14847.

Mojsov, S., Heinrich, G., Wilson, I. B., Ravazzola, M., Orci, L., and Habener, J. F. 1986. *J. Biol. Chem.* 261:11880–11889.

Mojsov, S., Weir, G. C., and Habener, J. F. 1987. *J. Clin. Invest.* 79:616–619.

Montrose-Rafizadeh, C., Avdonin, P., Garant, M. J., Rodgers, B. D., Kole, S., Yang, H., Levine, M. A., Schwindinger, W., and Bernier, M. 1999. *Endocrinology* 140:1132–1140.

Morales, A., Lachuer, J., Duchamp, C., Vera, N., Georges, B., Cohen-Adad, F., Moulin, C., and Barre, H. 1998. *Mol. Cell Endocrinol.* 144:71–81.

Morales, A., Lachuer, J., Geloen, A., Georges, B., Duchamp, C., and Barre, H. 2000. *Mol. Cell Biochem.* 208:139–142.

Munroe, D. G., Gupta, A. K., Kooshesh, P., Rizkalla, G., Wang, H., Demchyshyn, L., Yang, Z.-J., Kamboj, R. K., Chen, H., McCallum, K., Sumner-Smith, M., Drucker, D. J., and Crivici, A. 1999. *Proc. Natl. Acad. Sci. USA* 96:1569–1573.

Murphy, G. J., Gawler, D. J., Milligan, G., Wakelam, M. J. O., Pyne, N. J., and Houslay, M. D. 1989. *Biochem. J.* 259:191–197.

Murphy, G. J., Hruby, V. J., Trivedi, D., Wakelam, M. J., and Houslay, M. D. 1987. *Biochem. J.* 243:39–46.

Nauck, M., Stockmann, F., Ebert, R., and Creutzfeldt, W. 1986. *Diabetologia* 29:46–52.

Nauck, M. A., Heimesaat, M. M., Orskov, C., Holst, J. J., Ebert, R., and Creutzfeldt, W. 1993. *J. Clin. Invest.* 91:301–307.

Nauck, M. A., Wollschlager, D., Werner, J., Holst, J. J., Orskov, C., Creutzfeldt, W, and Willms, B. 1996. *Diabetologia* 39:1546–1553.

N'Diaye, N., Tremblay, J., Hamet, P., De Herder, W. W., and Lacroix, A. 1998. *J. Clin. Endocrinol. Metab.* 83:2781–2785.

Nishimura, E., Abrahamsen, N., Hansen, L. H., Lundgren, K., and Madsen, O. 1996. *Acta Physiol. Scand.* 157:329–332.

Norman, R. A., Permana, P., Tanizawa, Y., and Ravussin, E. 1999. *Int. J. Obes. Relat. Metab. Disord.* 23:163–165.

Orskov, C., Holst, J. J., Poulsen, S. S., and Kirkegaard, P. 1987. *Diabetologia* 30:874–881.

Orskov, C., and Nielsen, J. H. 1988. *FEBS Lett.* 229:175–178.

Parmley, W. W., Glick, G., and Sonnenblick, E. H. 1968. *N. Engl. J. Med.* 279:12–17.

Patel, G. K., Whalen, G. E., Soergel, K. H., Wu, W. C., and Meade, R. C. 1979. *Dig. Dis. Sci.* 24:501–508.

Pilkis, S. J., and Granner, D. K. 1992. *Annu. Rev. Physiol.* 54:885–909.

Pohl, S. L., Birnbaumer, L., and Rodbell, M. 1969. *Science* 164:566–567.
Premont, R. T., and Iyengar, R. 1988. *J. Biol. Chem.* 263:16087–16095.
Richter, W. O., Robl, H., and Schwandt, P. 1989. *Peptides* 10:333–335.
Richter, W. O., and Schwandt, P. 1985. *Int. J. Obes.* 9:25–27.
Salapatek, A. M., MacDonald, P. E., Gaisano, H. Y., and Wheeler, M. B. 1999. *Mol. Endocrinol.* 13:1305–1317.
Savage, A., Zeng, L., and Houslay, M. D. 1995. *Biochem. J.* 307:281–285.
Schwartz Sorensen, S., Eiskjaer, H., Orskov, H., and Bjerregaard, Pedersen, E. 1993. *Scand. J. Clin. Lab. Invest.* 53:25–34.
Scott, R. B., Kirk, D., MacNaughton, W. K., and Meddings, J. B. 1998. *Am. J. Physiol.* 275:G911–G921.
Scrocchi, L. A., Brown, T. J., MacLusky, N., Brubaker, P. L., Auerbach, A. B., Joyner, A. L., and Drucker, D. J. 1996. *Nature Med.* 2:1254–1258.
Shimatsu, A., Kato, Y., Matsushita, N., Ohta, H., Kabayama, Y., and Imura, H. 1983. *Neurosci. Lett.* 37:285–289.
Shimizu, H., Egawa, M., Yoshimatsu, H., and Bray, G. A. 1993. *Brain. Res.* 630:95–100.
Sivarajah, P., Wheeler, M. B., and Irwin, D. M. 2001. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 128:517–527.
Spathis, G. S., Bloom, S. R., Jeffcoate, W. J., Millar, J. G., Kurtz, A., Pyasena, M. R., Smith, J. A., and Nabarro, J. D. 1974. *Clin. Endocrinol. (Oxf.)* 3:175–186.
Srinivasan, M., Aalinkeel, R., Song, F., Lee, B., Laychock, S. G., and Patel, M. S. 2000. *Am. J. Physiol. Endocrinol. Metab.* 279:E1347–E1357.
Stoffel, M., Espinosa, R., III, Le Beau, M. M., and Bell, G. I. 1993. *Diabetes* 42:1215–1218.
Stoffers, D. A., Kieffer, T. J., Hussain, M. A., Drucker, D. J., Egan, J. M., Bonner-Weir, S., and Habener, J. F. 2000. *Diabetes* 49:741–748.
Strazzullo, P., Iacone, R., Siani, A., Barba, G., Russo, O., Russo, P., Barbato, A., D'Elia, L., Farinaro, E., and Cappuccio, F. P. 2001. *J. Mol. Med.* 79:574–580.
Stroop, S. D., Kuestner, R. E., Serwold, T. F., Chen, L., and Moore, E. E. 1995. *Biochemistry* 34:1050–1057.
Stumvoll, M., Meyer, C., Kreider, M., Perriello, G., and Gerich, J. 1998. *Metabolism* 47:1227–1232.
Stumvoll, M., Meyer, C., Mitrakou, A., Nadkarni, V., and Gerich, J. E. 1997. *Diabetologia* 40:749–757.
Svoboda, M., Tastenoy, M., Vertongen, P., and Robberecht, P. 1994. *Mol. Cell Endocrinol.* 105:131–137.
Taborsky, Jr., G. J., Ahren, B., and Havel, P. J. 1998. *Diabetes* 47:995–1005.
Takhar, S., Gyomorey, S., Su, R. C., Mathi, S. K., Li, X., and Wheeler, M. B. 1996. *Endocrinology* 137:2175–2178.
Tanizawa, Y., Riggs, A. C., Elbein, S. C., Whelan, A., Donis-Keller, H., and Permutt, M. A. 1994. *Diabetes* 43:752–757.
Taylor, I., Duthie, H. L., Cumberland, D. C., and Smallwood, R. 1975. *Gut* 16:973–978.
Thorens, B. 1992. *Proc. Natl. Acad. Sci. USA* 89:8641–8645.
Tonolo, G., Melis, M. G., Ciccarese, M., Secchi, G., Atzeni, M. M., Maioli, M., Pala, G., Massidda, A., Manai, M., Pilosu, R. M., Li, L. S., and Luthman, H. 1997. *Diabetologia* 40:89–94.
Tsai, C.-H., Hill, M., Asa, S. L., Brubaker, P. L., and Drucker, D. J. 1997. *Am. J. Physiol.* 273:E77–E84.
Tsai, C.-H., Hill, M., and Drucker, D. J. 1997. *Am. J. Physiol.* 272:G662–G668.
Tseng, C. C., Boylan, M. O., Jarboe, L. A., Usdin, T. B., and Wolfe, M. M. 1996. *Am. J. Physiol.* 270:E661–E666.
Tseng, C. C., Kieffer, T. J., Jarboe, L. A., Usdin, T. B., and Wolfe, M. M. 1996. *J. Clin. Invest.* 98:2440–2445.
Tseng, C. C., and Lin, L. 1997. *Biochem. Biophys. Res. Commun.* 232:96–100.
Tseng, C. C., and Zhang, X. Y. 1998. *Mol. Cell Endocrinol.* 139:179–186.
Tseng, C. C., and Zhang, X. Y. 2000. *Endocrinology* 141:947–952.
Turton, M. D., O'Shea, D., Gunn, I., Beak, S. A., Edwards, C. M. B., Meeran, K., Choi, S. J., Taylor, G. M., Heath, M. M., Lambert, P. D., Wilding, J. P. H., Smith, D. M., Ghatei, M. A., Herbert, J., and Bloom, S. R. 1996. *Nature* 379:69–72.
Unger, R. H. 1985. *Diabetologia* 28:574–578.
Unger, R. H., and Eisentraut, A. M. 1969. *Arch. Int. Med.* 123:261–266.
Unson, C. G., Cypess, A. M., Kim, H. N., Goldsmith, P. K., Carruthers, C. J. L., Merrifield, R. B., and Sakmar, T. P. 1995. *J. Biol. Chem.* 270:27720–27727.
Unson, C. G., Cypess, A. M., Wu, C. R., Goldsmith, P. K., Merrifield, R. B., and Sakmar, T. P. 1996. *Proc. Natl. Acad. Sci. USA* 93:310–315.
Unson, C. G., Wu, C. R., Sakmar, T. P., and Merrifield, R. B. 2000. *J. Biol. Chem.* 275:21631–21638.
Usdin, T. B., Mezey, E., Button, D. C., Brownstein, M. J., and Bonner, T. I. 1993. *Endocrinology* 133:2861–2870.
Van Eyll, B., Goke, B., Wilmen, A., and Goke, R. 1996. *Peptides* 17:565–570.
Volz, A., Goke, R., Lankat-Buttgereit, B., Fehmann, H. C., Bode, H. P., and Goke, B. 1995. *FEBS Lett.* 373:23–29.
Wakelam, M. J., Murphy, G. J., Hruby, V. J., and Houslay, M. D. 1986. *Nature* 323:68–71.
Wheeler, M. B., Gelling, R. W., Hinke, S. A., Tu, B., Pederson, R. A., Lynn, F., Ehses, J., and McIntosh, C. H. 1999. *J. Biol. Chem.* 274:24593–24601.
Wheeler, M. B., Gelling, R. W., McIntosh, C. H., Georgiou, J., Brown, J. C., and Pederson, R. A. 1995. *Endocrinology* 136:4629–4639.
Wheeler, M. B., Lu, M., Dillon, J. S., Leng, X.-H., Chen, C., and Boyd, A. E., III. 1993. *Endocrinology* 133:57–62.
White, C. M. 1999. *J. Clin. Pharmacol.* 39:442–447.
White, J. W., and Saunders, G. F. 1986. *Nucl. Acids Res.* 14:4719–4730.
Widmann, C., Dolci, W., and Thorens, B. 1996a. *Mol. Endocrinol.* 10:62–75.
Widmann, C., Dolci, W., and Thorens, B. 1996b. *J. Biol. Chem.* 271:19957–19963.
Widmann, C., Dolci, W., and Thorens, B. 1997. *Mol. Endocrinol.* 11:1094–1102.
Wildhage, I., Trusheim, H., Goke, B., and Lankat-Buttgereit, B. 1999. *Endocrinology* 140:624–631.
Wilmen, A., Goke, B., and Goke, R. 1996. *FEBS Lett.* 398:43–47.
Wilmen, A., Van Eyll, B., Goke, B., and Goke, R. 1997. *Peptides* 18:301–305.
Wojdemann, M., Wettergren, A., Hartmann, B., Hilsted, L., and Holst, J. J. 1999. *J. Clin. Endocrinol. Metab.* 84:2513–2517.
Wojdemann, M., Wettergren, A., Hartmann, B., and Holst, J. J. 1998. *Scand. J. Gastroenterol.* 33:828–832.
Xiao, Q., Boushey, R. P., Cino, M., Drucker, D. J., and Brubaker, P. L. 2000. *Am. J. Physiol.* 278:R1057–R1063.
Xiao, Q., Boushey, R. P., Drucker, D. J., and Brubaker, P. L. 1999. *Gastroenterology* 117:99–105.
Xiao, Q., Jeng, W., and Wheeler, M. B. 2000. *J. Mol. Endocrinol.* 25:321–335.
Xu, G., Stoffers, D. A., Habener, J. F., and Bonner-Weir, S. 1999. *Diabetes* 48:2270–2276.
Yagami, T. 1995. *Mol. Pharmacol.* 48:849–854.
Yagi, T., Nishi, S., Hinata, S., Murakami, M., and Yoshimi, T. 1996. *Diabet. Med.* 13:902–907.
Yamada, Y., Hayami, T., Nakamura, K., Kaisaki, P. J., Someya, Y., Wang, C. Z., Seino, S., and Seino, Y. 1995. *Genomics* 29:773–776.
Yip, R. G., and Wolfe, M. M. 2000. *Life Sci.* 66:91–103.
Yoo-Warren, H., Willse, A. G., Hancock, N., Hull, J., McCaleb, M., and Livingston, J. N. 1994. *Biochem. Biophys. Res. Commun.* 205:347–353.
Yusta, B., Boushey, R. P., and Drucker, D. J. 2000. *J. Biol. Chem.* 275:35345–35352.
Yusta, B., Huang, L., Munroe, D., Wolff, G., Fantaske, R., Sharma, S., Demchyshyn, L., Asa, S. L., and Drucker, D. J. 2000. *Gastroenterology* 119:744–755.
Yusta, B., Somwar, R., Wang, F., Munroe, D., Grinstein, S., Klip, A., and Drucker, D. J. 1999. *J. Biol. Chem.* 274:30459–30467.
Zhang, Y., Cook, J. T., Hattersley, A. T., Firth, R., Saker, P. J., Warren-Perry, M., Stoffel, M., and Turner, R. C. 1994. *Diabetologia* 37:721–724.
Zhong, Q., Bollag, R. J., Dransfield, D. T., Gasalla-Herraiz, J., Ding, K. H., Min, L., and Isales, C. M. 2000. *Peptides* 21:1427–1432.