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The Glucagon-Like Peptides

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I. Introduction

IT HAS been 15 yr since the initial discovery of the glucagon-like peptides (GLPs) as potential bioactive peptides encoded in the preproglucagon gene. The GLPs and glucagon are formed by alternative tissue-specific cleavages in the L cells of the intestine, the α-cells of the endocrine pancreas, and neurons in the brain. Glucagon-like peptide-1 (GLP-1) is now known to be a potent glucose-dependent insulinotropic hormone, which has important actions on gastric motility, on the suppression of plasma glucagon levels, and possibly on the promotion of satiety and stimulation of glucose disposal in peripheral tissues independent of the actions of insulin. As a consequence of these properties, GLP-1 is under investigation as a potential treatment of diabetes mellitus. GLP-2 was recognized only recently to have potent growth-promoting activities on intestinal epithelium.

The interest in the GLPs has grown exponentially. By 1988 there were 170 publications describing the properties of the GLPs. Five years later this number grew to 426 and currently (1999) more than 1,000 publications appear in the database of the National Library of Medicine (PubMed).

Since the last comprehensive review of GLP-1 appeared in *Endocrine Reviews* in 1995 (1), many new developments have occurred and are described in this review. The purpose of this article is to emphasize the newer and what are perceived to be the more current and important aspects of the biology of the GLPs. For additional information and references, the reader is referred to several informative earlier reviews (1-12).

II. History of the Incretin Concept: Discovery of Gastric Inhibitory Polypeptide

As a result of their discovery of secretin in 1902, Bayliss and Starling (13) speculated that signals arising from the gut after ingestion of nutrients might elicit pancreatic endocrine

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responses and affect the disposal of carbohydrates. In 1906 Moore *et al.* (14) postulated that the duodenum produced a 'chemical excitant' for pancreatic secretion and attempted to treat diabetes by injecting gut extracts. Zunz and Labarre (15, 16) pursued this factor and prepared an intestinal extract free of secretin activity that was able to produce hypoglycemia in dogs. Labarre (16) introduced the term 'incretin' to describe the humoral activity of the gut that might enhance the endocrine secretion of the pancreas (16). Although other investigators also reported the presence of hypoglycemic factors in duodenal extracts (17-20), Loew and colleagues (21) were unable to lower blood glucose levels in dogs with extracts of dog or hog intestinal mucosa obtained by a number of methods. Although these later extracts were tested only in fasting animals, after this report, interest in isolating an intestinal hypoglycemic factor declined.

The development of a reliable RIA for insulin in the 1960s by Yalow and Berson (22), which allowed measurements of the circulating levels of this hormone, renewed interest in the search for incretins. It was demonstrated by both immunoassay (23, 24) and bioassay (25, 26) that the action of glucose on the pancreas could not account completely for the insulin response observed in the blood. These reports demonstrated that iv glucose administration resulted in a lower plasma insulin response than when given by intrajejunal infusion, even though lower blood glucose levels were achieved by the later (Fig. 1). Perley and Kipnis (27) estimated the alimentary component to be close to 50% by subtracting from the insulin secretory response seen after oral glucose that insulin response obtained with the infusion of iv glucose, which duplicated the oral blood glucose profile.

In 1969, Unger and Eisentraut (28) named the connection between the gut and the pancreatic islets the 'enteroinsular axis.' Creutzfeldt (29) suggested that this axis encompasses nutrient, neural, and hormonal signals from the gut to the islet cells secreting insulin, glucagon, somatostatin, or pancreatic polypeptide (Fig. 2). Furthermore, Creutzfeldt (29) defined the criteria for fulfillment of the hormonal or incretin part of the enteroinsular axis as: 1) it must be released by nutrients, particularly carbohydrates, and 2) at physiological levels, it must stimulate insulin secretion in the presence of elevated blood glucose levels.

One hormone that clearly fits the requirements to be an incretin is glucose-dependent insulinotropic polypeptide (GIP). GIP was originally isolated as an 'enterogastrone,' or hormone secreted in response to fat or its digestive products in the intestinal lumen that inhibits gastric acid secretion (30). Brown and colleagues (31-33) isolated GIP from impure preparations of cholecystokinin (CCK) that contained acid-inhibitory activity using the canine Heidenhain pouch as a bioassay. GIP was shown to be a potent inhibitor of gastric acid and pepsin secretion and was thus originally named 'gastric inhibitory polypeptide' (34, 35). Earlier, Dupré and Beck (26) had demonstrated that a crude preparation of CCK also possessed insulinotropic activity. In 1972, Rabinovitch and Dupré (36) found that this insulinotropic action could be removed by further purification of the CCK. This observation resembled the loss of the acid-inhibitory activity reported previously by Brown and Pederson (33) during the purification of GIP from CCK and led Dupré *et al.* (37) to the hypothesis that GIP may also possess insulin-releasing capabilities. In 1973, Dupré *et al.* (37) demonstrated that a purified preparation of porcine GIP infused intravenously in humans in concert with glucose stimulated the release of significantly greater quantities of immunoreactive insulin than when the same dose of glucose was administered alone. The insulin response was sustained for the duration of the GIP infusion and was not observed during the euglycemic state (37). The glucose-dependent nature for the insulinotropic activity of GIP was later demonstrated *in vivo* in dogs (38) and humans (39) and in the perfused rat pancreas (40). Furthermore, GIP released in response to the oral ingestion

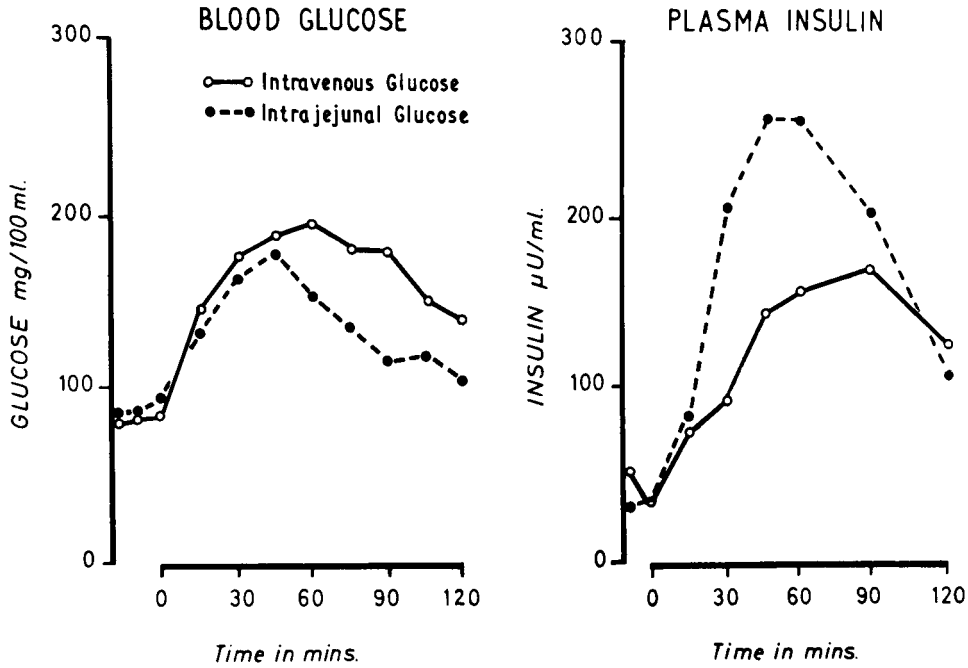

FIG. 1. Demonstration of the incretin concept. Blood glucose and insulin responses after either intravenous or intrajejunal glucose infusion in normal subjects. Although plasma glucose levels after intravenous glucose infusion were higher than those after intrajejunal glucose infusion, the latter generated a larger insulin response. Based on these results, McIntrye *et al.* (23) suggested that a humoral substance was released from the jejunum during glucose absorption, acting in concert with glucose to stimulate insulin release from pancreatic β-cells. [Reproduced with permission from N. McIntyre *et al.*: *Lancet* 2:20-21, 1964 (23) © The Lancet Ltd.].

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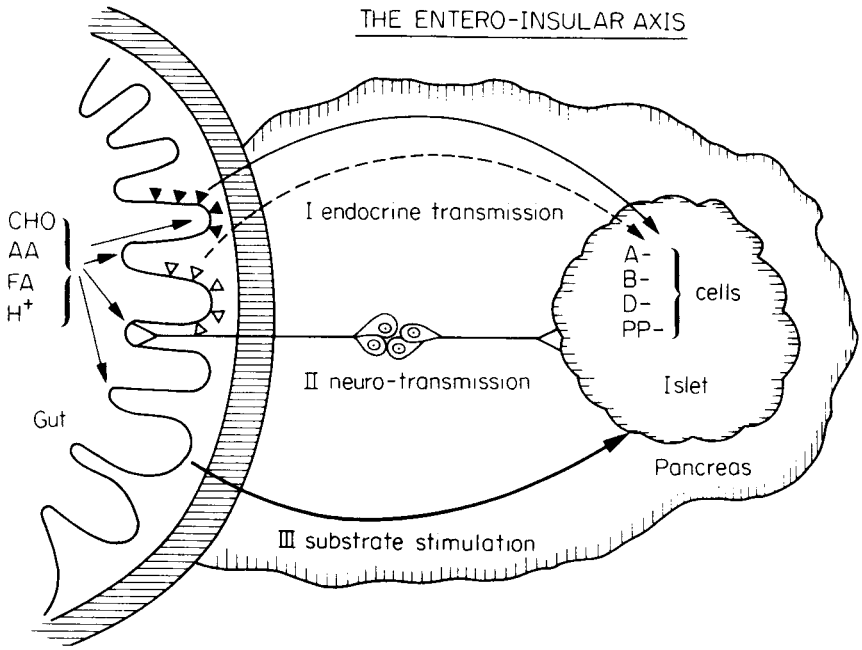

FIG. 2. The enteroinsular axis. After ingestion of nutrients, hormone secretion from different cell types of the pancreatic islets [A (α), B (β), D (δ), PP] may be modified by one or more modalities of: I, endocrine transmission; II, neurotransmission; and III, direct substrate stimulation. [Reproduced with permission from W. Creutzfeldt: *Diabetologia* 16:75-85, 1979 (29) © Springer-Verlag].

of fat yielded no increase in plasma insulin levels unless intravenous glucose was also administered (41-43). The glucose dependency of GIP-stimulated insulin secretion appeared to provide an important safeguard against inappropriate stimulation of insulin release during a high-fat, low-carbohydrate meal. The recognition of this additional important physiological function of GIP led to the alternate designation glucose-dependent insulinotropic polypeptide (GIP) (44).

In accordance with the roles of GIP as an enterogastrone and an incretin, immunoreactive GIP cells have been located in the upper small intestine of ruminants (45), humans, pigs, dogs (46), and rats (47). In the gastrointestinal tract of dog and man, immunoreactive GIP is present in cells predominantly in the midzone of the duodenal villi and, to a lesser extent, in the jejunum (48). Levels of GIP rise several fold shortly after ingestion of a meal containing fat (41-43) or glucose (38, 49, 50). It appears glucose may act directly at the level of the GIP-secreting K cells to stimulate GIP release (51, 52).

Studies employing GIP antisera to immunoneutralize endogenous GIP indicated that intestinal hormones other than GIP contribute substantially to the incretin effect (53, 54). These findings were supported by the observation that insulinotropic activity remained in intestinal extracts after removal of GIP by immunoadsorption (55). Finally, a major contribution to the incretin effect from the lower gastrointestinal tract was shown in studies of patients after varying degrees of resection of the small intestine (56). The incretin effect of oral glucose correlated positively to the total length of residual small bowel rather than to an integrated release of GIP. Patients with preserved ileal residues had much larger incretin effects than patients with no ileal residues, despite equal integrated increases in plasma GIP, findings indicative of the presence of incretins other than GIP in the ileum (56).

III. Discovery of GLP-1

In the interim between the discovery in the 1970s of GIP as an important intestinal incretin hormone to the actual discovery of GLP-1, it was suspected that there must be a second incretin hormone in addition to GIP (54-56). The ushering in of the era of recombinant DNA technology in the late 1970s provided the means necessary for the identification of the 'missing' incretin hormone. In the early 1980s, the cloning of cDNAs encoding the preproglucagons from pancreata of the anglerfish was accomplished (57, 58). The anglerfish was found to have two separate nonallelic preproglucagon genes, I and II, both encoding a glucagon and a glucagon-related peptide (GRP) (58). Notably, the glucagon-related peptide encoded in the anglerfish, preproglucagon-I, located carboxy proximal to the sequence of glucagon, bore a strong homology to the sequence of GIP, leading Lund *et al.* (57) to suggest that the anglerfish GRP-1 may be an intestinal incretin hormone. In support of this supposition Lund *et al.* (59) showed that similar preproglucagon mRNAs were expressed in the anglerfish pancreas and intestine, a finding that strongly supported the prediction that GRP could be an incretin hormone. Subsequently, preproglucagon mRNAs were cloned from human (60) and rat (61) gut and shown to be identical in sequences to the mRNAs in pancreas.

Shortly after the discovery of anglerfish GRP, the preproglucagon cDNAs of mammals were cloned (62-64) as well as the human gene (65). It became clear that the anglerfish GRP-I is a homolog of the GLP-1s encoded in the mammalian preproglucagons, which were subsequently proven to be potent insulinotropic incretins. There was, however, some uncertainty regarding the identification of the bioactive isoform of GLP-1 that had true insulinotropic actions. Based on the amino acid sequence of the mammalian preproglucagons, the sites that would be predicted for posttranslational

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processing into peptide hormones were somewhat ambiguous. At the time it was generally believed that the yet-to-be-identified prohormone convertases (PCs) that enzymatically split prohormones into bioactive peptides required two adjacent basic amino acids, combinations of arginine, and lysine. The GLP-1 sequence begins with a histidine as the amino-terminal residue, as do most of the peptide hormones in the glucagon-related superfamily of hormones (Fig. 3). In the preproglucagon sequence, the first histidine is preceded by two basic amino acids, Lys-Arg, followed by four residues, another single basic residue, arginine, and a second histidine. The thinking at that time was that the putative bioactive peptide that would theoretically be cleaved from the preproglucagon during posttranslational processing would be at the Lys-Arg yielding a peptide of 37 or 36 amino acids, depending on whether the C-terminal glycine was present or absent and whether the penultimate C-terminal arginine was amidated in the absence of the C-terminal glycine. Thus, the 1-37 and 1-36 GLP-1 peptide isoforms were the first to be synthesized and tested for biological activity. The results of the experimental testing were disappointing. One report questioned whether GLP-1 had any relevant activity: 'How glucagon-like is glucagon-like peptide?' (66), as it had no effect on plasma glucose and insulin levels when administered to rabbits. Another report showed a weak stimulation of insulin secretion in cultured rat pancreatic islets at superpharmacological doses (25 nM) of GLP-1(1-36)amide and suggested that an N-terminally truncated peptide, GLP-1(7-36)amide, may be more active (67), as was suggested for GLP-1(7-37), an N-terminally truncated form of GLP-1(1-37) (67). These ideas were based upon alignment of the sequence of GLP-1 with the other members of the glucagon superfamily of peptide hormones (see Fig. 3), which revealed that the best alignment was with the histidine at position 7, and not position 1 of GLP-1 (12, 63, 67). In 1986 it was discovered that GLP-1 was indeed further N-terminally truncated by posttranslational processing in the intestinal L cells (68, 69). In contrast to GLP-1(1-37), GLP-1(7-37) and (7-36)amide were found to be potent insulinotropic hormones in the isolated perfused pancreas of rats (70) and pigs (71), and in humans (72). Further, it was suggested that the weak insulinotropic actions of GLP-1(1-37) at micromolar concentrations were probably artefactual due to a 0.1% level of nonspecific cleavage of GLP-1(1-37) to GLP-1(7-37) by nonspecific cathepsins in the serum-implemented tissue culture media (73). At present it is well established that the GLP-1 isoforms GLP-1(7-37) and GLP-1(7-36)amide are the bioactive insulinotropic peptides derived from preproglucagon in the intestine and the hind brain. The functions of the lesser GLP-1 isoforms GLP-1(1-37) and GLP-1(1-36)amide remain unknown.

IV. Structures of GLPs and Family of Glucagon-Related Peptides

The GLPs belong to a larger family referred to as the glucagon superfamily of peptide hormones. These hormones are classified within this family based on their considerable sequence homology, having anywhere from 21% to 48% amino acid identity with glucagon. Included in this family are: glucagon, GLP-1(7-37) and -(7-36)amide, GIP, exendin-3 and -4, secretin, peptide histidine-methionine amide (PHM), GLP-2, helospectin-1 and -2, helodermin, pituitary adenyl cyclase-activating polypeptides (PACAP)-38, and -27, PACAP-related peptide (PRP), GH-releasing factor (GRF), and vasoactive intestinal polypeptide (VIP) (Fig. 3). These peptide hormones are produced in the gut, pancreas, and the central and peripheral nervous systems and exhibit a wide variety of biological actions in which several act as neurotransmitters. Notably, even peptide hormones that are co-encoded within the same precursor, such as the peptide hormones derived from the cleavages of preproglucagon, differ significantly in the physiological processes that they regulate. For example, the major function of glucagon is to maintain blood glucose levels during fasting, whereas GLP-1 functions primarily during feeding to stimulate insulin release and to lower blood glucose levels. On the other hand, GLP-2 appears to regulate the growth of intestinal epithelial cells.

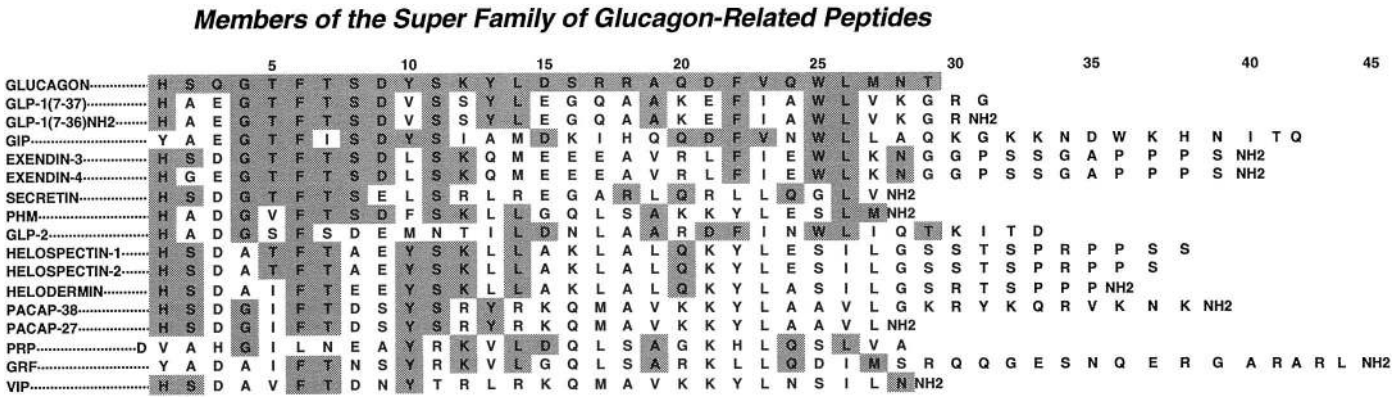

FIG. 3. Amino acid sequences of the members of the superfamily of glucagon-related peptides. Sequences include human glucagon, human GLPs, human GIP, exendins *(Heloderma horridum)*, human secretin, human peptide histidine methionine (PHM), helospectins *(Heloderma horridum)*, helodermin *(Heloderma suspectum)*, human PACAP, human PACAP-related peptide (PRP), human GRF, and human VIP. Residues identical to those of glucagon in the same position are *shaded*. *Standard single letter abbreviations* are used for amino acids (IUPAC-IUB Commission on Biochemical Nomenclature): A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Exendin-3, exendin-4, helospectin-1, helospectin-2, and helodermin were all isolated from lizard (*Heloderma*) venom. They are potent secretagogues of the exocrine pancreas (74). Helodermin shares 53% and 42% homology with human PACAP and VIP, respectively, and has high affinity for the VIP_2 receptor (75). Exendin-4 is 53% homologous to mammalian GLP-1 and acts as a high-affinity agonist on the GLP-1 receptor (76, 77). Exendin-4 and GLP-1 may interact with specific receptors yet to be identified in guinea pig pancreatic acini tissue (78). In contrast, the amino-terminally truncated form of exendin-3(9-39) is a potent antagonist of GLP-1 actions (76, 77). Lizard helodermin, exendin, VIP/PHI, PACAP, and glucagon/GLP-1 cDNAs have been cloned, revealing that separate genes exist for these peptides (79, 80). To date, no evidence has been uncovered for the existence of mammalian homologues of the lizard helodermin or exendin (79, 80). It appears that helodermin and exendin-4 are not the evolutionary precursors to mammalian PACAP/VIP or GLP-1 but represent a distinct family of peptides. It seems likely that the high-affinity and biological activities of helodermin and exendin-4 on the mammalian VIP_2 and GLP-1 receptors, respectively, are a result of convergent evolution (80).

It is proposed that the proglucagon gene arose by the duplication of an ancestral gene approximately 800-1,000 million years ago (81). The structural organization of the genes of the glucagon superfamily of peptide hormones suggests that the ancestral gene consisted of four exons, which encoded the 5′-untranslated region of the mRNA, the signal peptide, the hormone and the 3′-untranslated region of the mRNA, respectively (82). The glucagon superfamily of hormones may have arisen by duplication and amplification of this basic gene, followed by a further duplication and amplification of the exon encoding the glucagon hormone domain to generate the multiple GLPs observed in preproglucagon (82). Based on statistical analysis of DNA sequences of the preproglucagon genes from bovine, human, hamster, and anglerfish, Lopez *et al.* (83) postulated that the two anglerfish genes arose from gene duplication approximately 160 million years ago (83). Furthermore, this analysis suggested that the GLP-2 sequence originated by duplication of the glucagon or GLP-1 sequence before the earliest divergence of fish (83). However, until recently, it was believed that GLP-2 was not expressed in either fish or birds (11, 84). Irwin and Wong (85) discovered that, unlike pancreatic proglucagon of fish and birds, the intestinal proglucagon does contain the sequence of GLP-2. Therefore, fish and bird proglucagon mRNAs from pancreas and intestine have different 3′-ends that are due to alternative mRNA splicing (85). The recent cloning of the frog (*Xenopus*) proglucagon cDNAs revealed the presence of three distinct GLP-1 peptides in addition to glucagon and GLP-2 (86). It has been postulated that the first exon duplication event resulting in the appearance of glucagon and GLP occurred at least 405-800 million years ago (81, 83). A duplication of the GLP-containing exon, giving rise to GLP-1 and GLP-2, may have occurred between 365 (divergence of mammals and amphibians) and 405 (divergence of cartilaginous fish and tetrapods) million years ago (81). The amino acid sequences of the preproglucagon genes are highly conserved among mammals (Fig. 4), and the products derived from proglucagon, glucagon (Fig. 5), and GLP-1 (Fig. 6) are highly conserved throughout the evolution of animal species. The amino acid sequence of glucagon is highly conserved during the evolution of tetrapods (3 substitutions between salamander and human), even more than the sequences of either GLP-1 (7 substitutions) or GLP-2 (15 substitutions). The high degree of conservation of the glucagon and GLP sequences during evolution indicates the importance of the physiological processes regulated by these hormones.

The conservation of GLP-1 also reflects the fact that essentially the entire amino acid sequence of GLP-1 is required for full biological activity. Removal of the N-terminal histidine (= GLP-1 8-37) results in drastic loss of receptor binding and insulinotropic activity (87-90). The positive charge of the imidazole side chain of the histidine residue appears to be crucial for GLP-1 actions (91). Likewise, N-terminal truncation of this histidine from the related insulinotropic peptide exendin-4(1-39) reduces agonist activity by approximately 10-fold (92). Notably, N-terminal truncation of exendin-4 by two residues yields a peptide that binds with the same affinity as full-length exendin but antagonizes GLP-1 action (92). In contrast, an N-terminal truncation of GLP-1 by two residues reduces binding affinity to approximately 1% that of the intact molecule (92, 93). Also, addition of an amino acid to the N terminus of GLP-1(6-37) also reduces biological activity (87, 89). Truncation at the C terminus also reduces the biological activity of GLP-1 considerably (87, 88, 90, 93). Substitution in the N-terminal part of the GLP-1 molecule with the corresponding glucagon residues impaired the affinity for the GLP-1 receptor only moderately whereas exchanges in the C-terminal portion of GLP-1 decreased the affinity for the GLP-1 receptor more than 100-fold (94). In contrast, the binding affinity of GLP-1 to its receptor is more sensitive to GIP-like changes in the N-terminal region than to changes in the C-terminal region (95).

Another approach to understanding the structure-activity relationships of GLP-1 has been obtained from studies of peptide analogs in which individual amino acids are substituted. These studies revealed that the residues in positions 1 (His), 4 (Gly), 6 (Phe), 7 (Thr), 9 (Asp) 22 (Phe), and 23 (Ile) are important for the binding affinity and biological activity of GLP-1 (96-98). Two-dimensional nuclear magnetic resonance of GLP-1 in a membrane-like environment (a dodecylphosphocholine micelle) revealed that GLP-1 consists of an N-terminal random coil segment (residues 1-7), two helical segments (7-14 and 18-29), and a linker region (15-17) – a structure similar to that observed for glucagon (99). Thus far, attempts to generate smaller active fragments of GLP-1 that retain potent insulinotropic activity have failed (89, 98, 100).

V. Tissue Distribution of the Expression of GLPs

A. Pancreatic α-cells

Pancreatic α-cells were discovered in 1907 as histologically distinct cells from the β-cells of the islets of Langerhans (101). It was not until 1962 that α-cells were shown by immunofluorescence staining studies to be the source

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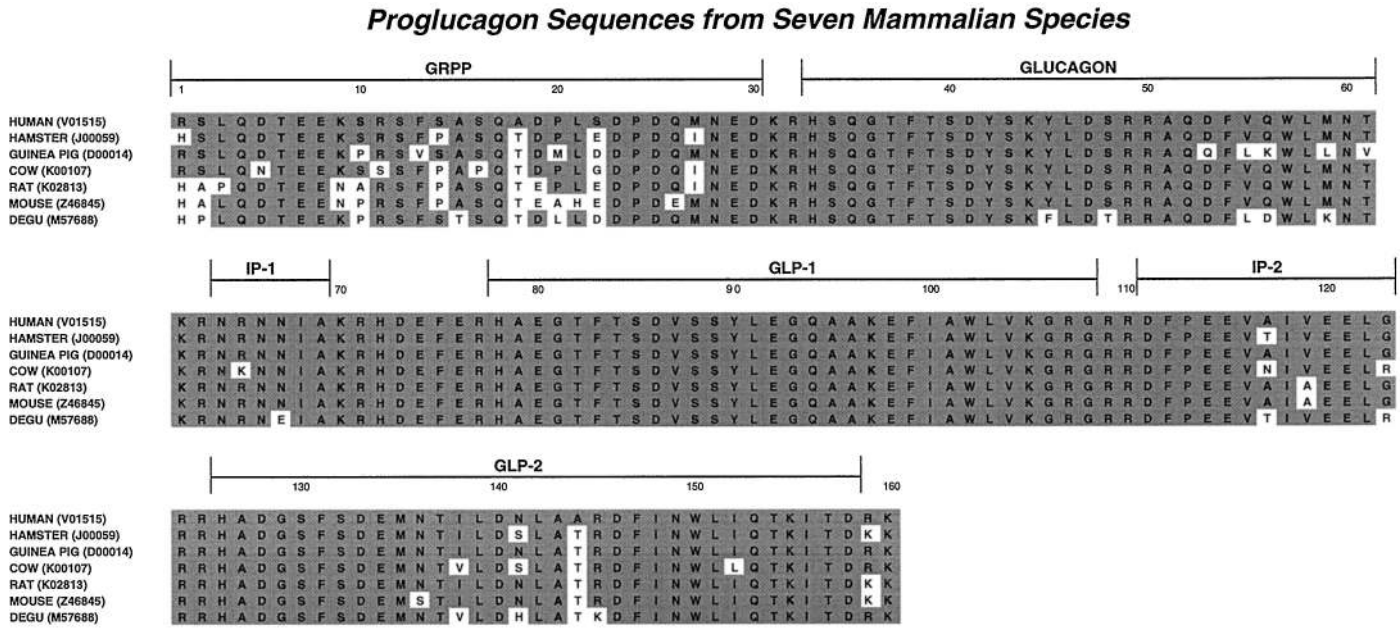

FIG. 4. Amino acid sequences of proglucagon from seven mammalian species. GenBank accession numbers are given in *parentheses*. Major proglucagon products are indicated by *bars*; GRPP, glicentin-related pancreatic peptide; IP-1 and IP-2, intervening peptides; GLP-1 and GLP-2, GLPs. *Shaded residues* are completely conserved between the seven species. Standard *single letter abbreviations* are used for amino acids (IUPAC-IUB Commission on Biochemical Nomenclature): A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

of glucagon (102). The α-cells are one of four distinct polypeptide-secreting islet cell types: glucagon-secreting α-cells, insulin- and amylin-secreting β-cells, somatostatin-secreting δ-cells, and pancreatic-polypeptide-secreting F cells. These cells are arranged in highly organized patterns within the islets. In rodents, α-cells and δ-cells exist on the surface or mantle of the islet surrounding the core of β-cells, although the patterns of distribution of the α-, δ-, and β-cells differ among animal species (103-105). Uncertainties in the islet vascular architecture and the direction of blood flow within the islets (106) still cloud the functional significance of the anatomic arrangement of hormone-producing islet cells.

mRNA encoding proglucagon can be detected by PCR early in the wall of the embryonic foregut at the 20-somite stage and is restricted to the area of the duodenum from which the pancreas will develop 10-12 h later (107). The pancreas arises as two outbuddings of the gut tube shortly after it is formed early in development at embryonic day 9 (ED 9) in the mouse (for review see Ref. 108). By ED 10, the budding anlaga fuse to become the dorsal and ventral pancreas with their respective ducts. By immunofluorescence, glucagon-positive cells are identifiable at ED 10.5 in the dorsal bud and at ED 11.5 in the ventral bud (109). Individual hormone-containing cells are located within the epithelium of pancreatic ducts, and clusters of endocrine cells are found in the pancreatic interstitium. Starting on ED 16.5, islets begin to form, and by day 18.5 the islets consist of centrally located β-cells with the adult 'one cell-one hormone' phenotype (109). The expression of specific transcription factors is involved in the determination of cell lineages that determine the development of islet-specific cells of the endocrine pancreas. For example, recent findings indicate that disruption of the gene for the transcription factor Pax6 in mice results in a near-complete failure in the development of α-cells (110), whereas disruption of Pax4 results in the absence of mature β- and δ-cells (111) (Fig. 7). Mice lacking the transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic β-cells (112).

As illustrated in Fig. 8, cell-specific processing of proglucagon in pancreatic α-cells leads primarily to the production of glucagon. However, immunoreactive GLP-1 is detectable in rat pancreatic α-cells by immunocytochemistry (113). Fully processed GLP-1 (7-36 amide and 7-37) is also visualized in pancreatic rat extracts by using chromatographic techniques and RIAs (114, 115). A recent investigation detected predominantly GLP-1 (1-36) amide in extracts of rat pancreas (116). Using similar techniques, small amounts of N-terminally extended GLP-1 (1-36 amide and 1-37) are also found in extracts from porcine and human pancreas (117, 118). In addition, immunoreactive GLP-1 is secreted from the arginine-perfused rat pancreas and glucose-stimulated isolated rat islets, as detected by RIA (113, 114). The relatively small quantity of GLP-1 produced by the pancreas might have important local actions within the islets.

B. Intestinal L cells

Intestinal cells are reported to react with glucagon-specific C-terminal antisera, although the intestinal immunodeterminant responsible for the immune reaction appears to differ chemically from pancreatic glucagon (152, 153). Antibodies directed against the midpart of glucagon and antibodies against the nonglucagon part of the glicentin molecule reveal a large population of endocrine cells in the small and large bowel that express proglucagon and its fragments (154-156).

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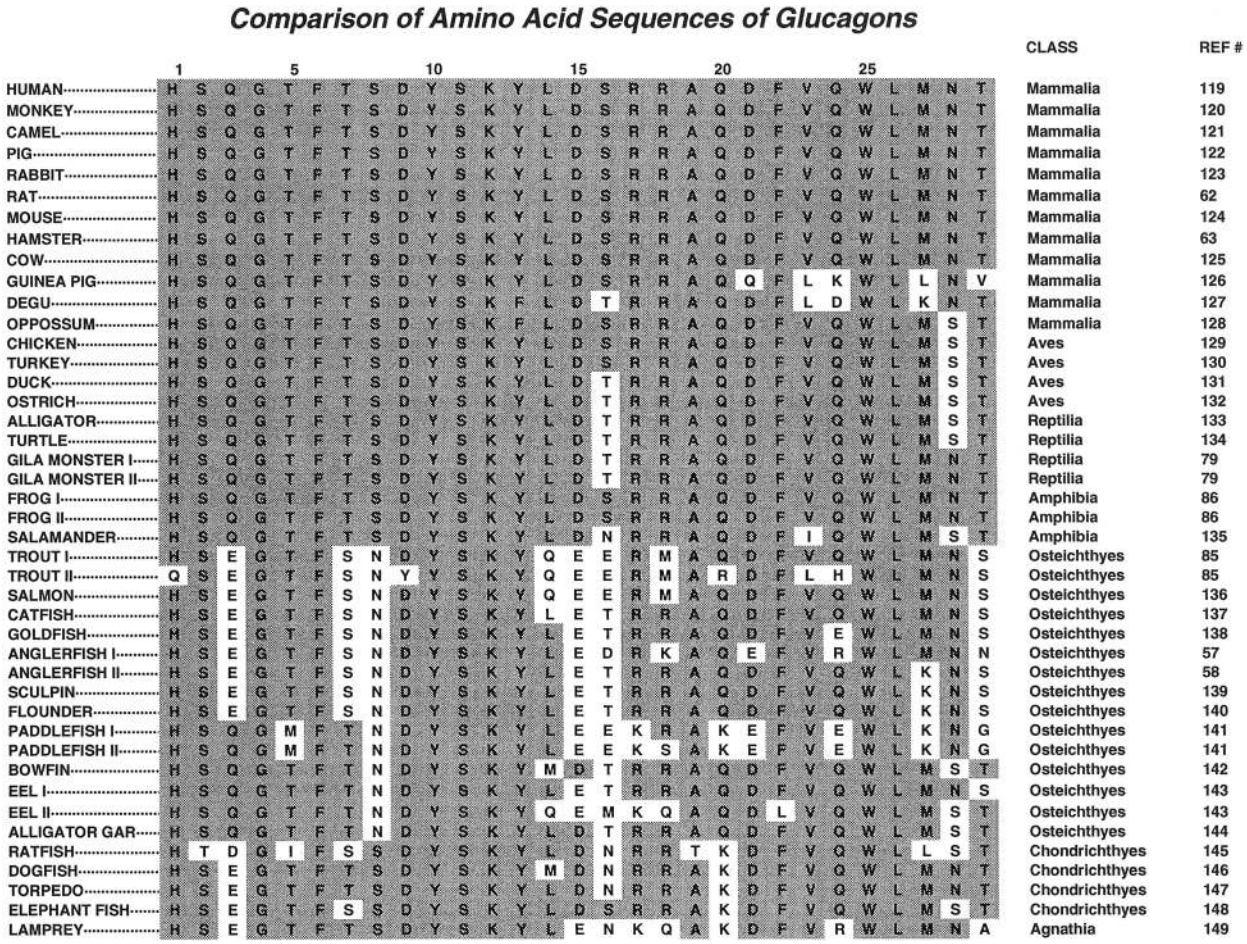

FIG. 5. Amino acid sequences of vertebrate glucagons. Classes are as indicated and residues identical to those of human glucagon in the same position are *shaded*. Standard *single letter abbreviations* are used for amino acids (IUPAC-IUB Commission on Biochemical Nomenclature): A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; Q, Gln, R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Reference numbers indicate the source of the corresponding sequence.

In contrast to the pancreas where GLPs represent minor products, GLPs are fully processed in abundance in the intestine, representing the major source of circulating GLPs (115-117). By virtue of their ultrastructure as assessed by electron microscopy, these cells are designated as L cells that clearly differ from pancreatic α-cells in the morphology of the granules (154, 156-158). The intestinal L cells are flask shaped and open-type, the microvilli reach the intestinal lumen, and a domain rich in endocrine granules exists near the basal lamina (159, 160) (Fig. 9). The shape of the L cells suggests that the cells can respond to changes in the environment within the intestinal lumen, resulting in a basal discharge of their granular contents.

The L cells are the second most abundant population of endocrine cells in the human intestine, exceeded only by the population of enterochromaffin cells. A high abundance of L cells is present in the distal jejunum and ileum, and an increasing abundance of L cells is demonstrable along the colon, with the highest concentration in the rectum (160-163). L cells first appear in human fetuses at the 8th week of gestation in the ileum, the 10th week in the oxyntic mucosa and proximal small intestine, and at the 12th week in the colon (164). This distribution of L cells differs greatly from that of the cells that secrete GIP, which are located in the more proximal regions of the jejunum (8, 45-48, 165, 166). The L cells of the small and large bowel are thought to arise from pluripotent stem cells in the crypts that also give rise to enterocytes, goblet cells, and Paneth cells (167). The L cells have a lower rate of turnover than other cell types in the crypts (168). Most of the L cells reside in the crypts of Lieberkühn, but a few cells can also be observed in the intestinal villi.

C. Central nervous system

Before the identification of GLP-1 as a separate product of the posttranslational processing of proglucagon, it was recognized that gut-type glucagon immunoreactivity existed within the central nervous system of several mammalian species (169-173). The subsequent development of specific antisera allowed for the analysis of the precise anatomical distribution of GLP-1. GLP-1-immunoreactive nerve fibers and terminals are widely distributed throughout the brain; the highest density occurs in the hypothalamus, thalamus, and septal regions, and the lowest occurs in the cortex and hindbrain (174-179). Chromatographic analyses of extracts of

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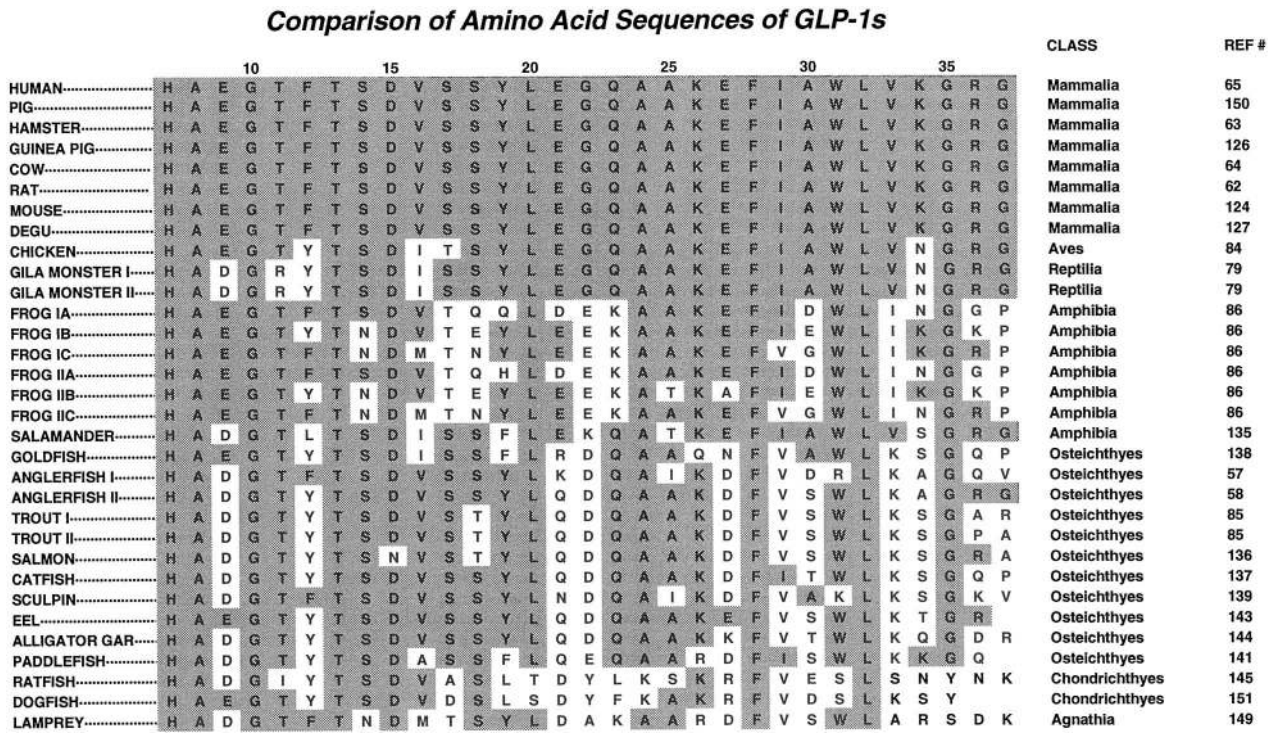

FIG. 6. Amino acid sequences of vertebrate GLP-1s. Classes are as indicated and residues identical to those of human GLP-1s in the same position are *shaded*. Standard *single letter abbreviations* are used for amino acids (IUPAC-IUB Commission on Biochemical Nomenclature): A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Reference numbers indicate the source of the corresponding sequence.

rat brainstem and hypothalamus revealed that proglucagon is processed in a manner similar to that in the intestine, preferentially giving rise to oxyntomodulin, glicentin, GLP-1, and GLP-2 (116, 177, 179-181). Posttranslational processing of proglucagon may undergo changes during development, as the predominance of glicentin and oxyntomodulin over glucagon in the rat hypothalamus increases dramatically from fetus to adult (182). In rats, monkeys, and man, GLP-1 has been detected in neuronal cell bodies within the medulla oblongata (174-176, 179, 183). Within the caudal medulla, immunostained cell bodies were located within the nucleus of the solitary tract and the dorsal and ventral parts of the medullary reticular nucleus (175). GLP-1 neurons of the solitary tract constitute a distinct noncatecholaminergic cell group that projects to many sites within the brain, one of which is the hypothalamic paraventricular nucleus (179). *De novo* synthesis of proglucagon occurs in these cell bodies as proglucagon mRNA is detected by *in situ* hybridization experiments using oligonucleotide probes (176, 179, 184).

VI. Proglucagon Biosynthesis

A. Organization/structure of the proglucagon gene

The gene encoding glucagon and the GLPs is expressed as a 2-kb transcript that consists of a 5′-untranslated region, the protein-coding region comprised of the N-terminal signal sequence, the proglucagon consisting of the glicentin-specific peptide and followed in order by the sequences that encode glucagon, GLP-1, and GLP-2 (Fig. 10). The glucagon, GLP-1, and GLP-2 sequences are interrupted by short spacer sequences that encode intervening peptides (Fig. 10). The N-terminal signal sequence is typical of preprohormones that are destined to cross-membranes during the biosynthesis of the protein, and the details of their function are well documented [for review see Ref. 185). It is somewhat remarkable that the six exons that comprise the transcribed region of the gene consist of distinct functional domains of the mRNA and encoded preproglucagon (65, 69). Namely, these regions are the 5′-untranslated sequence, the signal and N-terminal glicentin sequence, glucagon, GLP-1, GLP-2, and the 3′-untranslated region (Fig. 10). This exonic arrangement of the preproglucagon gene is a representative example of the modular arrays of exons that often encode specific functional domains of proteins (186).

B. Regulation of glucagon gene expression

There are three known sites of expression of the proglucagon gene: the α-cells of the pancreatic islets, the L cells predominantly located in the distal ileum, colon, and rectum, and the nucleus tractus solitarius in the hindbrain, which is the nucleus of the vagus (X) nerve. There is also expression of the proglucagon gene in magnacellular neurons of the hypothalamus. In many instances, nutrients and effectors that either stimulate or suppress secretion of glucagon or GLPs (see below) also likewise similarly control expression of the proglucagon gene at one or more levels of gene transcription, mRNA stability, or translation. Several factors that stimulate the secretion of rat intestinal glucagon-like immu-

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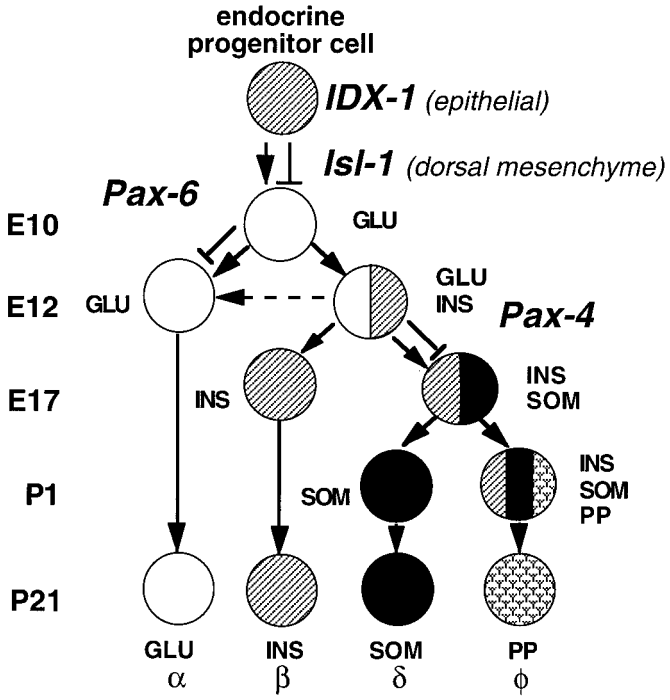

FIG. 7. Proposed developmental pathway of the endocrine pancreas in the mouse, showing interruptions of development in response to disruptions of the transcription factor genes, IDX-1, Isl-1, Pax-4, and Pax-6. Knockouts of IDX-1 and Isl-1 result in early failure of the development of epithelial cells derived from the endodermal stem cell. IDX-1 is a key factor in the very early development of all pancreatic epithelial cells, whereas Isl-1 is required for the development of the dorsal mesenchyme, and its failure leads to a specific arrest of development of the epithelial cells of the dorsal pancreas; the mice die at ED 9.5. Inactivation of Pax-4 by homologous recombination prevents development of the β- and δ-cells and shunts development to the α-cell lineage. The Pax-6 knockout does the opposite: α-cells do not develop, but some development occurs in β- and δ-cells. Recently, the knockout in mice of transcription factor Nkx2.2 showed a phenotype of arrested differentiation of β-cells (112). GLU, Glucagon; INS, insulin; SOM, somatostatin; PP, pancreatic polypeptide. Days of embryonic development are indicated on the *left of the figure* (E10-E17) and postnatal days are indicated by P1-P21. [Reproduced with permission from J. F. Habener and D. A. Stoffers: *Proc Assoc Am Phys* 110:12-21, 1998 (585)].

noreactive peptides, such as $(Bu)_2$cAMP, forskolin, and cholera toxin, also elevate intestinal proglucagon mRNA levels, whereas other factors, phorbol esters and bombesin, stimulate secretion but not mRNA levels in intestine (61, 187-189). It is also theoretically possible that regulation may be exerted at the level of posttranslational processing of proglucagon to glucagon and GLPs, but that has not been demonstrated yet. Actually, there is not a great deal known about the mechanisms involved in the control of proglucagon gene expression.

The promoter of the proglucagon gene has been analyzed in some detail by several groups of investigators over the past 10-12 yr. Five important transcriptional DNA control elements have been identified in the 2.5-kb of DNA sequence that 5′ flanks the initiation of transcription of the rat preproglucagon gene (Fig. 11). The five DNA control sequences of approximately 20-40 bp have been designated G1, G2, G3, CRE (cAMP response element), and ISE (intestinal specific element) or GUE (glucagon upstream enhancer) (190). A sixth subelement within G1 has been designated G4 (191). The G1 element confers α-cell-specific expression of the glucagon gene in the pancreas, the G2 and G3 elements are enhancers specific to islet cells (192), the CRE lends cAMP responsivity to transcription of the preproglucagon gene (193), and ISE is a determinant for the transcriptional expression of the gene in intestinal L cells (194). One caveat is that essentially all of the information on the *cis*-acting control elements and the transactivating DNA-binding proteins has been derived from studies in cultured insulinoma cells that express the glucagon gene. These are transformed immortalized cells that may or may not be entirely representative of normal α- or L cells in the context of the living animal.

The G1 element of the preproglucagon gene promoter has been the most extensively studied of the five control elements identified so far. It consists of 30-40 nucleotides, is located close to the TATA-box to which the basal RNA polymerase complex of basal transcription factors are assembled, and seems clearly to be responsible for allowing the expression of the preproglucagon gene in α-cells (192, 195). The exclusion of, or mutations within, the G1 element precludes expression of the gene in α-cells. Deoxyribonuclease I (DNase I) footprint analysis of the G1 element indicates that a large, complex array of DNA-binding proteins interacts in this region of the promoter (192). Some progress has been made in the identification of the specific DNA-binding proteins involved in interactions with the G1 element. As might have been anticipated, three of the five DNA-binding proteins thus far identified to act on G1, Brn4 (196), Cdx2 (196-199), and Pax6 (200), are homeotic selector proteins, so called homeoproteins, critically involved in the determination of the body plan and organogenesis during development. The other two proteins, E47 and Beta2/NeuroD, are basic helix-loop-helix proteins also known to be important in development (201). After completing their roles in embryonic development, homeodomain proteins typically exert a second role in the regulation of the expression of key genes in the fully differentiated cells, namely the α-cells with respect to Brn4, Cdx2, and Pax6. In this regard, it is noteworthy that targeted disruptions of either the Brn4 (M. G. Rosenfeld, University of California, San Diego, personal communication) or Pax6 (110, 202) genes in mice results in the failure of α-cells to develop in the pancreas.

The G2 and G3 DNA-control elements in the promoter are enhancers of proglucagon gene transcription function in islet cells, but are not restricted to islet cells. Transcription factors in the hepatocyte nuclear factor family (HNF), HNF3β and HNFα, have been shown to interact with G2 and G3 (203, 204). Isoforms of HNF-3 thereby either enhance or repress transcription of the proglucagon gene promoted by the G1 element and its cognate DNA-binding proteins described above. The involvement of HNF transcription factors in the regulation of the expression of the glucagon gene is interesting because the liver and pancreas (and spleen) are derived from adjacent regions of the gut endoderm during development [for review see Ref. 108). In conditions of chronic injury to the pancreas, such as invoked by dietary deficiencies of methionine or copper, pancreatic acinar tissue undergoes metaplasia to liver tissue. Evidence has been re-

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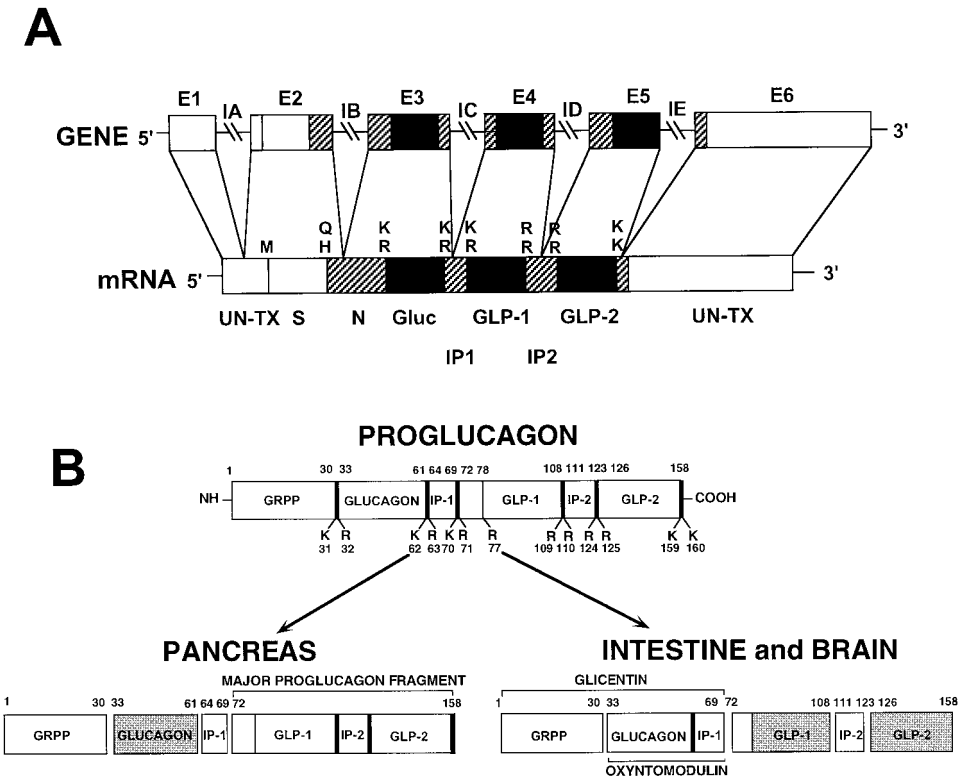

FIG. 8. Expression of the preproglucagon gene. A, Diagram of the proglucagon gene and encoded mRNA. The gene consists of six exons (E1-E6) and five introns (IA-IE). Alternative splicing of exons E4 and E5 occurs in salmonid fishes but not in mammals. The exons encode functional domains of the preproglucagon: S, signal peptide; N, amino-terminal sequence of proglucagon; Gluc, glucagon; IP, intervening peptides. The pairs of basic residues that serve as posttranslational sites of processing of the preproglucagon encoded by the mRNA are shown. M, Methionine encoded by AUG codon that initiates translation; Q, glutamine; H, histidine; K, lysine; R, arginine; UN-TX, untranslated regions of mRNA [Adapted from S. Mojsov *et al.*: *J Biol Chem* 261:11880-11889, 1986 (69)]. B, Alternative posttranslational processing of proglucagon in pancreas, intestine, and brain. Enzymatic cleavages at specific pairs of basic residues in proglucagon produces numerous multifunctional peptide hormones involved in nutrient metabolism. K, Lysine; R, arginine. The major bioactive hormones derived from proglucagon are glucagon in the pancreatic α-cells and GLP-1 (two isoforms, 7-37 and 7-36 NH_2) and GLP-2 in the intestinal L cells and brain. Numbers on proglucagon denote amino acid positions. GRPP, Glicentin-related pancreatic peptide; Gluc, glucagon; IP-1 and IP-2, intervening peptides; MPF, major proglucagon fragment. GLP-1(7-36) is α-amidated on the carboxyl-terminal arginine residue.

ported that the G3 element may serve as a negative insulin response element, and thereby may account for the paracrine actions of insulin to suppress glucagon gene expression (194). Transcription of the proglucagon gene in islet α-cell lines is enhanced by phorbol ester-mediated activation of protein kinase C (205). However, in rat intestine, phorbol esters have no effect on proglucagon mRNA levels (61). Recent studies identify the G2 element as the target of the stimulatory actions of phorbol esters and the interactions of the transcription factors HNF-3β and members of the Ets-related transcription factors (206).

The cAMP response element (CRE) is located in the promoter of the proglucagon gene adjacent to the G3 element. The CRE confers cAMP responsivity to the transcription of the proglucagon gene (193, 207). In studies *in vitro* in glucagon-producing insulinoma cells, it is clear that the CRE in the promoter of the proglucagon gene is a target for interactions with CRE-binding protein (CREB), the CRE-binding protein involved in mediating cAMP responses of multiple genes (193, 207-210). Proteins that bind to sites adjacent to the CRE and inhibit the CREB-mediated cAMP stimulation of glucagon expression, designated CAPs (CREB-associated proteins), have been described (210). Notably, NF-Y is reported to bind to DNA sites immediately adjacent to the CRE of the rat insulin-1 gene promoter and to inhibit cAMP-responsive gene transcription (211). The identification of a functional CRE in the promoter of the proglucagon gene is consistent with the reported findings of cAMP-coupled GLP-1 and GIP receptors on pancreatic α-cells and that GLP-1 and GIP stimulate the secretion of glucagon from α-cells by cAMP-dependent mechanisms (212-214).

The intestinal L cell permissive enhancer in the promoter of the proglucagon gene is less well defined compared with the pancreatic α-cell-specific promoter element G1. The identification of an intestinal-specific promoter element (ISE) was accomplished by transient transfection-expression studies in SV40 transformed cell lines obtained from intestinal L cell tumors that arose in mice made transgenic with a SV40 large T antigen driven by the 2.5-kb 5′-flanking region of the proglucagon gene (190, 208). The expression of the proglucagon gene in L cells requires DNA control elements located between 1,300 and 2,300 bp 5′ upstream of the G1, G2, G3, and CRE elements of the promoter.

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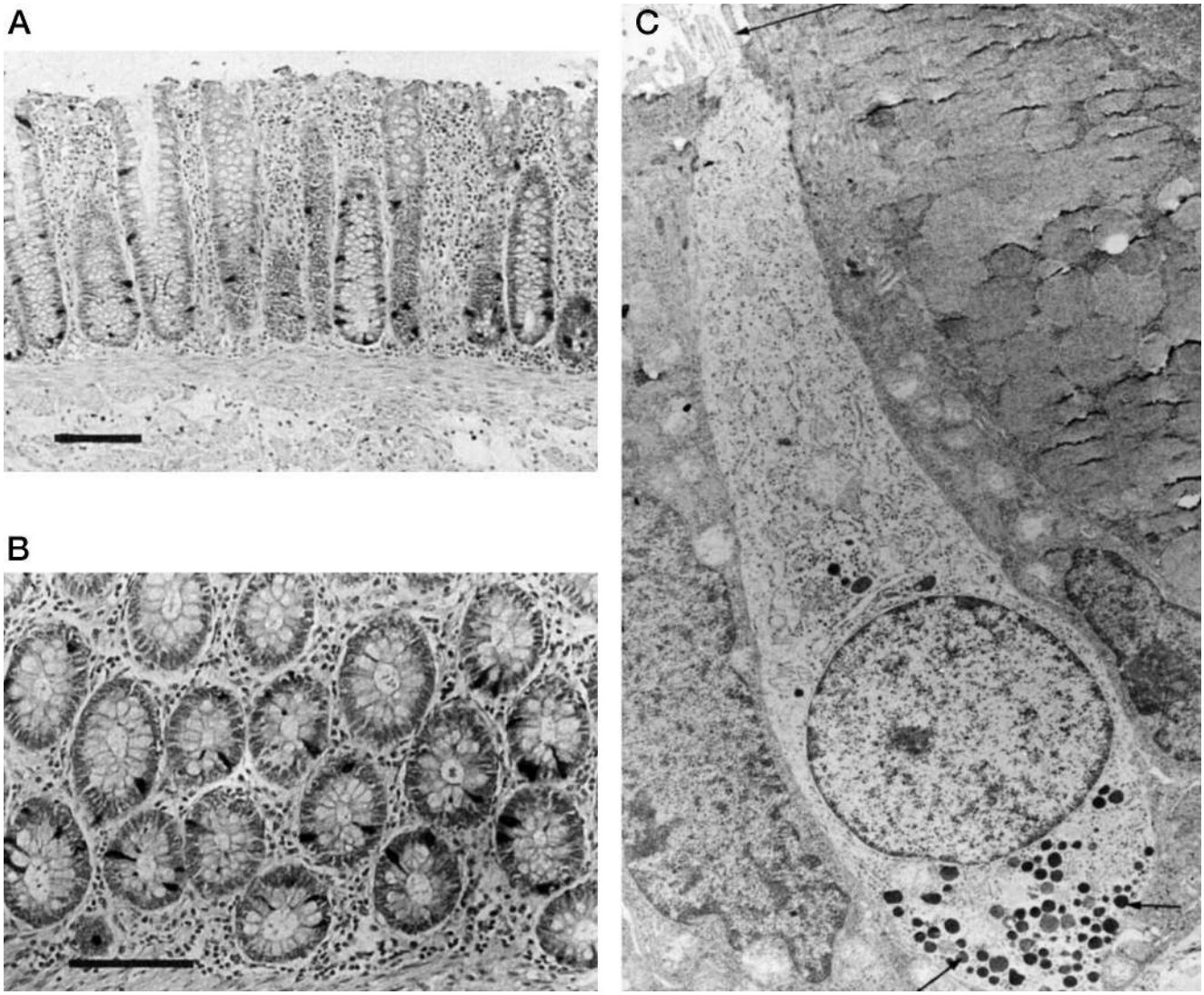

FIG. 9. GLP-1-immunoreactive cells in the human rectal mucosa. The cells occur in all regions of the crypts with a predominance in the basal region (A). They reach the lumen via slender apical processes (B and C). *Bars* = 25 μm. *Short arrows* indicate basolateral secretory vesicles; *long arrow* indicates luminal villi. [Reproduced with permission from R. Eissele *et al*: *Eur J Clin Invest* 22:283-291, 1992 (160).]

C. Posttranslational processing of proglucagon

When the modular exonic arrangement of the proglucagon gene was first noted (65), it seemed likely that alternative exon splicing would generate distinct mRNAs each encoding either glucagon or the GLPs. Indeed, in the frog, lizard, chicken, and fish, alternative RNA splicing of two proglucagon genes generates proglucagon mRNA transcripts that encode glucagon and GLP-1, but not GLP-2 in the pancreas, whereas mRNAs for all three are generated in the intestine (85). However, in mammals, the diversification of the expression of the preproglucagon gene occurs at the level of alternative posttranslational processing of proglucagon (60, 68, 69, 215) (Fig. 8). There is a remarkably specific alternative processing of proglucagon: the predominant bioactive peptide produced in the pancreatic α-cells is glucagon, whereas in the intestines and the brain the bioactive products produced are predominantly GLPs. Thus, the alternative processing reflects a dichotomy between the expression of hormones essential for the regulation of glucose metabolism in the fasting *vs.* the fed state. Glucagon is operative during fasting in mobilizing glucose from peripheral tissues to maintain blood glucose levels, whereas GLP-1 comes into play during feeding to augment glucose-dependent insulin release, and possibly to promote satiety.

Several of the enzymes that posttranslationally cleave proproteins into peptides or hormones have been identified. These enzymes comprise a family known as subtilisins or subtilisin-like proprotein convertases, otherwise known as prohormone convertases (PCs) (216). Two of the five or six identified convertases, PC2 and PC1/3, are expressed at high levels in pancreatic islets. Several studies using cell culture models transfected with expression vectors for recombinant PC2 and PC1/3 have uniformly established that processing by PC1/3 results in the formation of GLPs similar to those found in the intestine and PC2 (and possibly another yet-to-be-identified convertase) contributes to processing pro-

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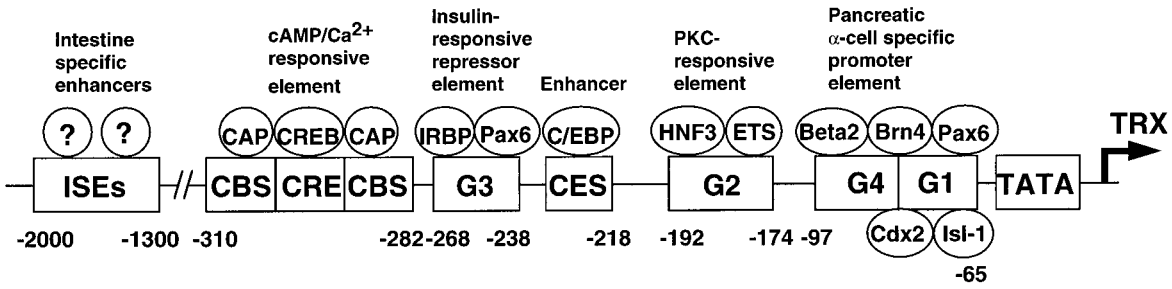

FIG. 10. DNA control elements and interactive transacting protein factors in the 2,300-bp promoter of the rat glucagon gene. ISEs, Intestine-specific enhancers [includes the glucagon upstream enhancer (190)]; CAP, CREB-associated protein; CBS, CAP-binding site; CREB, cAMP response element-binding protein; CRE, cAMP response element; IRBP, insulin responsive binding protein; CES, C/EBP enhancer site; HNF3, hepatic nuclear factor-3; ETS, ubiquitous developmental transcription factors; Beta2, Beta2/NeuroD basic helix-loop-helix factor; Isl-1, islet lim-homeodomain protein; Brn4, brain-4; Cdx2, caudal-related homeobox-2; Pax6, paired homeobox-6; G1, G2, G3, G4, major α-cell/islet enhancers; TATA, TATA box; TRX, transcription. [Adapted from J. F. Habener, In: H. C. Fehmann, B. Göke (eds) *The Insulinotropic Gut Hormone Glucagon-Like Peptide 1*, vol 13:15-23, 1997 (586)].

glucagon in the pancreatic pattern to produce glucagon (124, 217-222). Further, PC2 null mice defective in the expression of PC2 manifest severe fasting hypoglycemia and a reduced rise in blood glucose levels during an intraperitoneal glucose tolerance test, consistent with a deficiency of circulating glucagon (223).

VII. Regulation of GLP Secretion

A. Overview

Determinations of circulating profiles of immunoreactive GLP-1 levels have provided information regarding the physiological processes that regulate GLP-1 secretion. Before the development of specific GLP-1 RIAs in the late 1980s, L cell secretion was usually quantified as gut glucagon-like immunoreactivity (gGLI), which includes glicentin plus oxyntomodulin. Because gGLI is produced in quantitatively identical amounts to GLP-1 after posttranslational processing of proglucagon (69, 115, 117, 224-227), studies reporting secretion of gGLI reflect that of GLP-1. More recently, assays have been developed utilizing antisera that specifically detect GLP-1. These procedures may detect both pancreatic and intestinal derived GLP-1. Although small quantities of GLP-1 (7-37 and 7-36 amide) may be produced by pancreatic α-cells and cosecreted with glucagon (113-115), the major source of circulating GLP-1 is the intestinal L cell (116-118). As discussed below, numerous studies have revealed that the release of GLP-1 is under the control of nutrients, hormones, and neural inputs. The result is a biphasic mechanism of release, with both hormonal and neural mediation of early GLP-1 release (15-30 min), and direct nutrient contact with L cells mediating later GLP-1 secretion (30-60 min).

To allow for a direct assessment of the interactions among paracrine, endocrine, neural, and luminal influences at the level of the L cell, new *in vitro* techniques were required. A major factor impeding studies at the cellular level is the diffuse nature of the distribution of intestinal L cells. However, models consisting of primary cultures of rat intestinal cells or canine ileal mucosal cells have been successfully developed as *in vitro* strategies to study the production of GLP-1 (188, 189, 228, 229). The limited numbers and viability of cells obtained by these techniques in addition to the heterogeneity of the isolated cells prevent extensive analysis of proglucagon gene regulation. The development of tumor-derived cell lines that express proglucagon-derived peptides has aided in this regard. The GLUTag cell line was developed from intestinal tumors in proglucagon-SV40 large T antigen transgenic mice (230, 231) whereas the STC-1 cell line was derived from an intestinal endocrine tumor that developed in mice carrying the transgenes for the rat insulin promoter linked to SV40 large T antigen and the polyoma virus small T antigen (232).

B. Intracellular signals

The development of *in vitro* methods to study GLP-1 release at the cellular level has enabled the analysis of intracellular signal pathways that regulate the secretion and expression of GLP-1. Studies with intestinal cell cultures and the L cell line, GLUTag, indicate that the activation of protein kinase A stimulates both GLP-1 release and synthesis (61, 188, 189, 208, 229, 233-235). In contrast, activation of protein kinase C results in an increased secretion of GLP-1 in intestinal cell cultures (188, 189, 233, 236) and the GLUTag and STC-1 cell lines (208, 235, 237), but does not appear to increase transcription of the proglucagon gene (61, 235). Treatment with the phospholipase C activator α-ketoisocaproic acid does not enhance GLP-1 secretion by either fetal rat intestinal cultures or GLUTag cells (235). Inhibition of GLP-1 secretion by a calcium channel blocker ($CoCl_2$) and stimulation of GLP-1 release by increasing intracellular calcium concentrations indicate a primary role of calcium in basal secretion by the L cell (233). Thus there may be multiple signals involved in the L cell response that are perhaps important in allowing for an integrated response to a variety of different L cell effectors.

C. Carbohydrates

GLP-1 is released into the circulation after a meal (72, 117, 225, 238-243). Significantly more GLP-1 is released after a liquid meal than a solid meal of identical composition (244). The majority of GLP-1 released appears to be in the form of GLP-1 (7-36 amide) with levels reaching approximately 50

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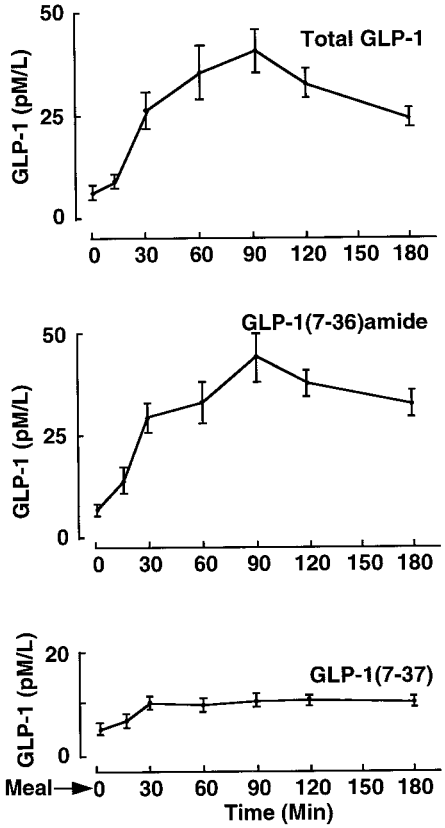

FIG. 11. Secretory responses of GLP-1 isopeptides GLP-1(7-37) and GLP-1(7-36)amide to a meal in six nondiabetic subjects. RIAs are relatively specific for detection of the differences in the COOH-termini of the two isopeptides. Approximately 80% of the total GLP-1 consists of the GLP-1(7-36)amide. [Adapted with permission from C. Orskov *et al.*: *Diabetes* 43:535-539,1994 (117)].

pM, whereas GLP-1 (7-37) rises to 10 pM (Fig. 12). In keeping with the role of GLP-1 as an incretin hormone, the oral intake of glucose alone stimulates GLP-1 release in humans (72, 241, 245-250), pigs (251, 252), dogs (253-255), and rats (116, 256). In contrast to oral glucose administration, elevation of plasma glucose by the administration of glucose systemically does not stimulate GLP-1 secretion, indicating the glucose sensing machinery is distributed on the luminal side of the intestine (236, 241, 257). Infusion of glucose into the intestinal lumen stimulates GLP-1 release in humans (249), rats (241, 257-261), dogs (255, 262, 263), and pigs (224). These observations are consistent with the role of GLP-1 as an important incretin hormone acting on the pancreatic β-cells to stimulate appropriate insulin release after glucose absorption.

The release of GLP-1 from the isolated perfused ileum requires sodium (241, 264), implicating the brush-border sodium/glucose cotransporter in the glucose effect. Consistent with these findings, other sugars that utilize this cotransporter for absorption across the intestinal epithelium, *e.g.*, galactose, also stimulate GLP-1 release (241, 261, 262). Nontransportable sugars, *e g.*, 2-deoxyglucose, or sugars using a different mechanism of transport, *e.g.*, fructose and lactose, do not stimulate the release of GLP-1 (255, 262). Furthermore, GLP-1 release from canine or rat ileum perfused in response to the carbohydrates methyl-α D-glucoside and 3-O-methyl-D-glucose indicate that intracellular metabolism and intracellular removal, respectively, are not essential to induce GLP-1 secretion in rats (261, 262).

Although high concentrations of glucose (28 mM) have been demonstrated to stimulate GLP-1 secretion from isolated rat intestinal cell cultures (228), it is unlikely that glucose normally acts directly on L cells. Indeed, a recent study did not observe any effect of glucose (5-25 mM) on GLP-1 secretion from isolated canine intestinal cells (189). Under normal feeding conditions, the majority of glucose is absorbed before reaching the ileum (265). In addition, the rapid GLP-1 secretory response to oral glucose (72, 240, 241, 245, 247) suggests that glucose must activate the release of GLP-1 by means other then a direct effect on L cells.

D. Fats

In addition to glucose, fats appear to stimulate the release of proglucagon-derived peptides, perhaps related to the roles of both oxyntomodulin and GLP-1 as enterogastrones, or inhibitors of gastric function (266-271). The secretion of GLP-1 is increased by ingestion of mixed fats or triglycerides in humans (241, 247, 249, 272-275), and dogs (274) and by placement of mixed fats directly into the intestinal lumen of rats (257, 276) and pigs (252). Interestingly, Roberge and Brubaker (276) discovered that placement of fat in the duodenum of rats stimulates GLP-1 secretion independently of the contact of nutrients with the distal L cells. Furthermore, duodenal fat increased the secretion of GLP-1 into the circulation to the same extent as was observed after the direct administration of fat into the ileum (257, 276). These observations suggest the existence of a proximal-distal loop regulating the L cell response to ingested nutrients (276). Such a mechanism could contribute to the significant increase in circulating GLP-1 levels observed within 5-10 min of ingesting a meal, before contact of nutrients with the L cells (72, 240, 241, 247, 248, 250, 277). As discussed below, among the potential mediators of such a loop are various endocrine and neuroendocrine peptides, as well as neurotransmitters.

The observation of fatty acid-induced GLP-1 release from isolated intestinal cell cultures suggests that fatty acids can act directly on the L cell (187, 278, 279). Interestingly, bile acids appear to increase the secretion of proglucagon-derived peptides in humans (280), dogs (281), and rats (260), suggesting that the arrival of bile into the ileum may play an important feedback message for the release of GLP-1. Results obtained with fatty acids indicate that both the chain length and degree of saturation of the fatty acids affect the ability of fats to stimulate GLP-1 secretion. Monounsaturated long-chain fatty acids (=C16) are preferred over short-chain or medium-chain, polyunsaturated or saturated fatty acids (187, 233, 235, 251, 273, 278, 279). However, long-term exposure of rats to short-chain fatty acids derived from a diet containing readily fermentable fibers increases proglucagon mRNA levels and secretion of GLP-1 in response to a glucose challenge (282).

E. Proteins

Mixed meals that contain proteins increase GLP-1 secretion in humans (72, 117, 225, 238-243, 247) and rats (227, 283).

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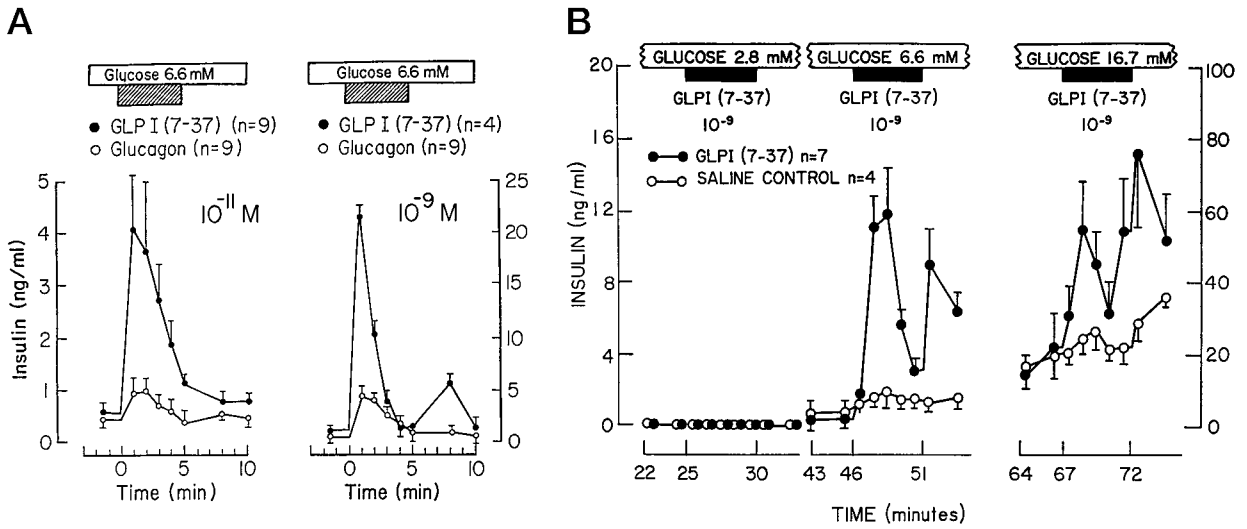

FIG. 12. A, Effects of different concentrations of synthetic GLP-1(7-37) and glucagon on insulin secretion from the perfused rat pancreas. Background perfusate contains 6.6 mM glucose. B, Glucose dependency of effect of 10^{-9} M GLP-1(7-37) on insulin secretion from isolated perfused rat pancreas. Insulin responses at 2.8 and 6.6 mM glucose determined by scale at *left*; those at 16.7 mM determined by scale at *right*. [Adapted with permission from G. C. Weir *et al.*: *Diabetes* 38:338-342, 1989 (587)].

However, either amino acids or protein alone did not consistently increase GLP-1 release in *in vivo* studies in humans (241, 247, 249), dogs (253, 284), or rats (260, 285). Recently, it was discovered that unlike protein or an amino acid mixture, protein hydrolysates (peptones) stimulate GLP-1 secretion from isolated vascularly perfused rat intestine and the murine enteroendocrine cell line STC-1 (285). It was argued that the peptones (mixtures of oligopeptides of various molecular weights) are more likely to closely mimic the protein-derived components of the intestinal chyme than would undigested proteins or amino acids. Furthermore, peptone treatment of STC-1 and GLUTag cells with peptones resulted in a significant increase in proglucagon RNA levels as a result of increased transcription of the glucagon gene (285). There was no effect of peptones on proglucagon RNA levels in pancreatic glucagon-producing cell lines (285). Therefore, the protein content of a mixed meal may contribute to GLP-1 secretion and synthesis via the production of peptones that contact L cells in the jejunum.

F. Endocrine

In addition to nutrients, hormones regulate GLP-1 secretion. Insulin has been reported to inhibit GLP-1 release both *in vitro* (228) and *in vivo* (286), perhaps acting as part of a feedback loop. Somatostatin-28 is an intestinal peptide that inhibits release from many endocrine cells through an inhibitory G protein (287, 288). Indeed, somatostatin-28 has been shown to inhibit GLP-1 release *in vivo* in the rat (289) and dog (253, 254) and *in vitro* with rat and canine intestinal cell cultures (187, 189, 279, 290). Of the endocrine peptides tested for effects on the L cell thus far, only GIP has been found to stimulate GLP-1 release (187, 234, 235, 257, 291-294). As discussed above earlier in this article, GIP is an intestinal hormone that acts both as an enterogastrone to inhibit gastric acid production and an incretin hormone that stimulates insulin release (see Ref. 295 for a recent GIP review). However, in contrast to the GLP-1-producing ileal L cells, GIP is secreted from K cells that are primarily located in the duodenum, and thereby are in an ideal location for regulation by nutrients. GIP is released rapidly in response to ingestion of nutrients, which are thought to act directly on the K cell. In rats, GIP was found to stimulate intestinal GLP-1 secretion when infused *in vivo* to mimic postprandial GIP concentrations (257). GIP also increases GLP-1 release from the isolated vascularly perfused rat ileum (292-294). Furthermore, GIP is a potent stimulator of both GLP-1 synthesis and secretion from rat intestinal cells *in vitro* (187, 188, 234) and isolated canine L cells (189). The mechanism of GIP-induced GLP-1 release appears to occur, at least in part, by activation of protein kinase A (189). These observations support the concept of a proximal-distal loop whereby nutrients entering the duodenum stimulate the release of GIP, which then circulates to the L cells of the ileum promoting the secretion of GLP-1. Currently, however, studies do not support the existence of a similar proximal-distal loop pathway in humans (72, 248, 296). Furthermore, infusion of an antagonist to the neuropeptide gastrin-releasing peptide (bombesin) concomitant with the placement of fats in the duodenum abrogated the stimulatory effects of the proximal nutrient on the distal L cell (297). These findings suggest that physiological doses of GIP act through the nervous system (either vagal or myenteric) to indirectly stimulate GLP-1 secretion, rather than acting directly at the level of the L cell.

G. Neural

In support of neural regulation of GLP-1 release, Rocca and Brubaker (298) have recently demonstrated that bilateral subdiaphragmatic vagotomy in conjunction with gut transection completely abolishes fat-induced GLP-1 release in rats (298). Consistent with a role for the vagus in the regulation of the L cell, stimulation of the distal end of the celiac branch of the subdiaphragmatic vagus nerve significantly

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stimulates the release of GLP-1 (298). Furthermore, GLP-1 secretion induced by exogenous GIP administration is abolished by selective hepatic branch vagotomy (298). Collectively, these findings indicate that GIP acts through vagal afferent pathways to stimulate the L cells indirectly. This stimulation is carried to the L cells by efferent pathways located in the celiac branch of the vagus nerve. Gastrin-releasing peptide is a major component of the nonadrenergic/noncholinergic branch of the vagus nerve, as well as of the enteric nervous system (299, 300), and is a candidate transmitter in these pathways. Gastrin-releasing peptide stimulates GLP-1 release *in vivo* in humans (301), rats (297, 302), and dogs (303, 304); in the perfused intestinal rat loop (291, 292, 305) and pig loop (224); and in rat and isolated canine intestinal cells (61, 187-189). Interestingly, the neuropeptide galanin inhibits both basal and gastrin-releasing peptide-induced GLP-1 secretion from isolated rat ileal cells through pertussis toxin-sensitive G protein and ATP-dependent potassium channels (188). Additional neurotransmitters and neuropeptides also likely mediate early secretion of GLP-1. Indeed, acetylcholine and muscarinic cholinergic agonists appear to stimulate GLP-1 secretion in the rat (187, 291-293, 305). In addition, the cholinergic agonist carbachol stimulates GLP-1 release from the murine cell lines STC-1 and GLUTag, evidently by activation of the muscarinic M3-subtype receptors (235, 237). In humans, the infusion of atropine reduces the secretion of GLP-1 in response to oral glucose, findings consistent with a direct cholinergic (muscarinic) control of L cells (250). Epinephrine and the β-adrenergic agonist, isoproterenol, stimulate GLP-1 secretion when infused into the isolated rat ileum or colon (291, 292) but not when tested for direct effects with GLUTag or rat intestinal cells *in vitro* (187, 235). Epinephrine also stimulates GLP-1 release in the dog *in vivo* (306, 307) and is stimulatory when added directly to isolated canine L cells *in vitro* (229). Collectively, these findings underscore the complexity of mechanisms regulating GLP-1 release from the distal L cells in response to the presence of nutrients in the proximal duodenum, involving an interaction of neural and endocrine pathways.

H. GLP-2

During the mid to late 1980s it was recognized that GLP-2 was specifically processed from preproglucagon in the intestine and was not liberated in appreciable quantities in pancreatic α-cells (69, 224, 238, 308-310). Although it would be predicted that GLP-2 should be secreted in parallel with GLP-1 in equal molar quantities, few studies have attempted to measure GLP-2 levels in the circulation. Furthermore, it is possible that GLP-2 is cleared and/or metabolized differently in the circulation, raising the possibility that circulating profiles differ from that of GLP-1. Ørskov and Holst developed specific RIAs for GLP-1 and GLP-2 and reported basal plasma levels of 107 ± 7 pM and 151 ± 14 pM, respectively, with levels reaching 145 ± 13 and 225 ± 15 pM 2 h after a mixed meal (225). More recently, Brubaker *et al.* (311) used RIA and HPLC techniques to more closely examine the plasma GLP-2; these authors reported a 1.5- to 3.6-fold increase in immunoreactive GLP-2 levels in fed compared with fasted rats and humans. Further, as discussed below, the inactive truncated GLP-2(3-33) peptide may account for approximately 50% of the total circulating GLP-2 (311).

VIII. Metabolism of GLPs

A. GLP-1

After its secretion, the metabolism of GLP-1 represents an important process in determining the levels of bioactive hormone in the circulation and may possibly be a means for further proteolytic processing. Elimination of bioactive GLP-1 from the circulation may occur via at least three different mechanisms: renal clearance, hepatic clearance, and degradation in the circulation. In support of an important role for the kidneys in the clearance of GLP-1, the levels of immunoreactive GLP-1 are significantly elevated in uremic patients (312). Renal extraction of endogenous and exogenous GLP-1 was also detected in anesthetized pigs (313). Nephrectomy or uretal ligation in rats increases the circulating half-life of GLP-1, and GLP-1 is extracted from perfusate of isolated rat kidneys (314). Collectively, the findings suggest that kidneys remove GLP-1 from the peripheral circulation by a mechanism that involves glomerular filtration and tubular catabolism (313, 314). Although no net extraction of endogenous GLP-1 across the liver has been detected, significant hepatic extraction of GLP-1 during a systemic infusion was identified in anesthetized pigs (313). The MCR, or least amount of plasma totally cleared of GLP-1 per unit of time, in humans is approximately 10 $ml \cdot kg^{-1} \cdot min^{-1}$ (72, 267, 315, 316). In accordance with this MCR, GLP-1 is eliminated relatively rapidly from plasma, with a half-life of approximately 5 min in humans (72, 267, 315, 316), pigs (313), dogs (317), and rats (314, 318). It is noteworthy that, because postsecretory degradation of the GLP hormones in the circulation may generate products that are immunoreactive in assays but are no longer biologically active, these assay values of circulating levels of GLP-1 and GLP-2 may overestimate the true biological half-life of these hormones. Indeed, as described below, the biological half-life of GLPs appears to be in the range of 1-2 min.

Degradation of GLP-1 in the circulation appears to occur initially by dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) cleavage at the amino terminus (histidine-alanine), resulting in GLP-1 (9-36)amide and GLP-1(9-37). These truncated forms of GLP-1 have been demonstrated to be the major metabolites of GLP-1 formed in human (319-321), canine (93), porcine (313), and rat (322) serum. In *in vivo* studies with rats, it was estimated that DPP IV cleaved 50% of a bolus GLP-1 infusion within 2 min (322). In contrast, GLP-1 remained intact for at least 10 min in rats that were DPP IV-deficient (322). Thirty minutes after subcutaneous GLP-1 administration to healthy humans, GLP-1(9-36) amide accounted for approximately 78% of immunoreactive GLP-1 (323). It is likely that there is subsequent enzymatic degradation of GLP-1 after cleavage by DPP IV by other enzymes (93, 321, 322). Multiple degradation products were observed by incubation of GLP-1 with purified human neutral endopeptidase (NEP-24.11; EC 3.4.24.11) and with RINm5F plasma

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membranes containing NEP-24.11 activity, suggesting this enzyme may also be involved in the metabolism of GLP-1 (324, 325).

In pigs, inhibition of DPP IV activity potentiates the insulin response to GLP-1, indicating that the intact N terminus of GLP-1 is important for its insulinotropic activity (326). Furthermore, the oral administration of a DPP IV inhibitor to Zucker fatty rats improves glucose tolerance by increasing the circulating half-lives of the endogenously released incretins GIP and, particularly, GLP-1 (327). Thus, analogs of GLP-1 that are DPP IV resistant have extended metabolic stability and may have extended insulinotropic activity *in vivo* (328). It remains possible, however, that the metabolic products of GLP-1 have important biological actions different from those of the parent peptides. Receptor-binding studies suggest that the DPP IV metabolite GLP-1(9-36)amide can bind to the pancreatic GLP-1 receptor, albeit with only 1% the affinity of native GLP-1 (92, 93). Further, GLP-1 (9-36)amide can antagonize the ability of native GLP-1 to generate adenyl cyclase activity by the pancreatic GLP-1 receptor (93). Recently, it was shown that GLP-1(9-36)amide could antagonize the inhibitory effect of GLP-1(7-36)amide on antral motility in anesthetized pigs (329). Whether sufficient quantities of this metabolite GLP-1(9-36)amide exist *in vivo* to act as an antagonist of GLP-1, or possibly to mediate other biological activities, remains to be determined.

B. GLP-2

GLP-2(1-33) is liberated from proglucagon in the intestinal L cells (69, 224, 238, 308). The MCR for GLP-2 has presently not yet been estimated, and the sites of clearance have not been investigated. However, GLP-2 levels are elevated in patients with chronic renal failure, indicating a role for the kidney in the clearance of circulating immunoreactive GLP-2 (311). Recently, GLP-2 (3-33) was identified in rat ileum and in plasma, where it accounts for as much as 50% of the total circulating GLP-2 (311). Similar to GLP-1 (9-36)amide, this truncated form of GLP-2 is a result of cleavage by DPP IV (311, 330). The expression of DPP IV within the intestinal epithelium (331, 332) could account for the detection of GLP-2 (3-33) in extracts of ileum (311). Likewise, the truncated GIP (3-42) has been detected in extracts of duodenal mucosa (333). DPP IV-mediated cleavage of GLP-2 appears to limit the intestinotrophic activity of the GLP-2 hormone (330). A GLP-2 analog containing glycine at position 2, thereby resistant to DPP IV, had greater intestinotrophic activity in rats compared with the native rat peptide (330).

IX. Physiological Actions of GLPs

A. Overview

The physiological actions of GLP-1 reflect the functions of organs in which specific GLP-1 receptors are expressed. These organs include the pancreatic islets, stomach, lung, brain, kidney, pituitary gland, cardiovascular system (heart), kidney, and small intestine (334, 335). However, there are reports of actions of GLP-1 on organs such as liver, adipose tissue, and skeletal muscle in which attempts to definitively identify GLP-1 receptors have not succeeded. This circumstance suggests the existence of as-yet-unidentified GLP-1 receptors that are distinct from the known, well characterized receptor.

B. Pancreatic islets

The earliest discovered biological actions of GLP-1 were on the pancreatic β-cells in which GLP-1(7-37) and GLP-1(7-36)amide were shown to be highly equipotent secretagogues for glucose-dependent insulin secretion (70-72) (Fig. 13). Studies employing exendin (9-39) as an antagonist *in vivo* have confirmed that the insulinotropic nature of GLP-1 makes an important contribution to the enteroinsular axis in rats (256, 336), baboons (337), and humans (338). Furthermore, mice with a null mutation in the GLP-1 receptor are glucose intolerant (339). Heterozygous GLP-1 receptor +/− mice also exhibit an abnormal glycemic response to an oral glucose challenge in association with reduced circulating levels of glucose-stimulated insulin secretion (340). Importantly, this insulinotropic action of GLP-1 is attenuated as ambient glucose levels fall (Fig. 13). The glucose-dependent nature of the incretin hormones GLP-1 and GIP is an efficient protective measure against hypoglycemia. The interdependence between glucose and incretin actions involves a cross-talk between glycolysis (glucose metabolism) and cAMP signaling pathways of the activated GLP-1 or GIP receptor. The glucose competence concept has been used to describe the mutual interdependence between glucose metabolism and GLP-1 actions on β-cells (*i.e*, glucose is required for GLP-1 action, and GLP-1 is required to render β-cells competent to respond to glucose) (341) (Fig. 14). This property of GLP-1 may improve the ability of β-cells to sense and respond to glucose in subjects with impaired glucose tolerance (342). It has been demonstrated recently that the glucose responsiveness of β-cells is well preserved in islets isolated from GLP-1 receptor −/− mice (343). However, in the absence of GLP-1 signaling, *i.e*., GLP-1 receptor −/− mice, it is interesting to note that there are compensatory changes in the enteroinsular axis via increased secretion and action of GIP (344).

Not only does GLP-1 stimulate insulin secretion, but it also stimulates transcription of the proinsulin gene and the biosynthesis of insulin (73, 345) (Fig. 15). However, this property of GLP-1 is not an absolute requirement for the maintenance of normal proinsulin gene transcription because amounts of pancreatic insulin mRNA transcripts were similar in wild-type and GLP-1 receptor −/− mice (340). Nevertheless, these properties clearly distinguish GLP-1 from those of the sulfonylurea class of hypoglycemic drugs that effectively stimulate insulin secretion but do not stimulate biosynthesis of proinsulin (346). GIP stimulates both insulin secretion and production (347, 348) in conditions of normoglycemia, but unlike GLP-1, GIP is ineffective in the stimulation of insulin secretion in individuals with type 2 diabetes (277, 349). Recent evidence indicates that GLP-1 may stimulate the proliferation and neogenesis of β-cells from ductal epithelium of mice and rats (350, 351). In the β-cell line INS-1, GLP-1 synergizes with glucose to activate expression of immediate-early response genes coding for transcription factors impli-

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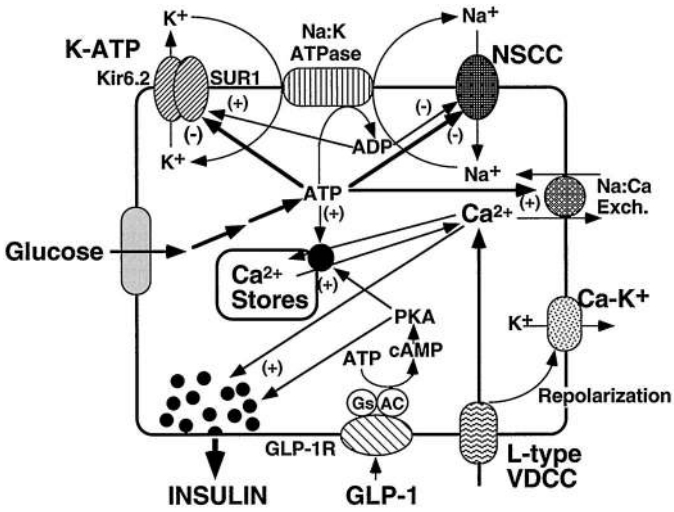

FIG. 13. Model of the proposed ion channels and signal transduction pathways in a pancreatic β-cell involved in the mechanisms of insulin secretion in response to glucose and GLP-1. The key elements of the model are the requirement of dual inputs of the glucose-glycolysis signaling pathway resulting in the generation of ATP and an increase in the ATP:ADP ratio, and the GLP-1 receptor (GLP-1R)-mediated cAMP PKA pathways to effect closure of ATP-sensitive potassium channels (K-ATP) consisting of the inward rectifier Kir6.2 and the sulfonylurea receptor SUR1. The closure of these channels results in a rise in the resting potential (depolarization) of the β-cell, leading to opening of voltage-sensitive calcium channels (L-type VDCC). A major component of the depolarizing current is carried by NSCCs that import Na^+ (and Ca^{2+}). In response to activation of NSCC and influx of Na^+ there is import of Ca^{2+} by the Na^+/Ca^{2+} exchanger (Na:Ca Exch). Release of intracellular membrane stores of calcium (Ca^{2+} stores) is induced by intracellular free Ca^{2+}, so called calcium-induced calcium release. The influx of Ca^{2+} through the open-end L-type VDCC triggers vesicular insulin secretion by the process of exocytosis. Phosphorylation of vesicular (granule) proteins by PKA may also trigger insulin secretion. Repolarization of the β-cell is achieved by opening of calcium-sensitive potassium channels (Ca-K). It is believed that the GLP-1 receptor is coupled to a stimulatory G-protein (Gs) and a calcium-calmodulin-sensitive adenylate cyclase.

cated in cell proliferation and differentiation (c-*fos*, c-*jun*, junB, zif-268, nur-77) (352). Moreover, administration of GLP-1 to aged rats that characteristically develop glucose intolerance between 18 and 20 months of age reverses the glucose intolerance (353). Thus GLP-1 may have potent pleiotrophic actions on both mature β-cells and duct cells that are progenitors of β-cells.

Receptors for GLP-1 have been detected also on α-cells and δ-cells (113, 213, 354, 355). The secretion of somatostatin increases in response to GLP-1 in rat islets (113) and in isolated perfused rat and canine pancreases (356, 357). Although GLP-1 appears to inhibit glucagon secretion *in vivo* (87, 356, 358-362), it stimulates glucagon release *in vitro* (213, 214). We speculated that the small amounts of biologically active GLP-1 produced in islets during the fasting state might exert autocrine/paracrine effects on a subset of α-cells containing GLP-1 receptors to increase glucagon biosynthesis via the cAMP pathway (213). During feeding, such an effect would be overcome by the combination of elevated insulin, somatostatin, and glucose, which collectively inhibit glucagon secretion. Thus the suppression of glucagon release observed *in vivo* may be indirectly attributable to the paracrine actions of the intraislet release of insulin and somatostatin. However, maintenance of glucagon secretion does not appear to be dependent upon functional GLP-1 signaling, as levels of pancreatic proglucagon mRNA and fasting and postabsorptive glucagon levels are normal in GLP-1 receptor −/− mice (340).

C. Counterregulatory actions of GLP-1 and leptin on β-cells

Leptin, the obesity hormone produced by adipose tissue, has opposing actions to GLP-1 on pancreatic β-cells. Leptin suppresses insulin secretion and gene expression (363-366), both of which are stimulated by GLP-1. However, it is worth noting that the inhibition of insulin secretion by leptin may be overridden by GLP-1, thereby assuring adequate insulin secretion in response to meals (363, 364). The feedback loop between leptin (fat) and insulin (pancreatic β-cells) constitutes an adipoinsular axis (367) that operates physiologically in parallel with the enteroinsular axis feedback loop involving GLP-1 (intestine) and insulin. Disruption of either axis appears to result in glucose intolerance and reveals the opposing actions of leptin and GLP-1. For example, mice with a null mutation in the GLP-1 receptor are more sensitive than wild-type mice to the insulin lowering effect of leptin, reflecting the interaction of GLP-1 and leptin in the regulation of insulin secretion (368). Because of the role of GLP-1 as a stimulator of insulin secretion, disruption of GLP-1 action in the GLP-1 receptor −/− mouse, may lead to unopposed inhibitory actions of leptin on the β-cell in the absence of functional GLP-1 receptors (368). Similarly, rats that express mutated leptin receptors (*fa/fa*) secrete approximately 5 times as much insulin as controls in response to GLP-1 (369). Therefore, because of the role of leptin as an inhibitor of insulin secretion, disruption of leptin action in the *fa/fa* rat may lead to unopposed stimulatory actions of GLP-1 on the β-cell in the absence of completely functional leptin receptors. Such a mechanism could contribute to the profound hyperinsulinemia in these animals and possibly in subjects with type 2 diabetes (370).

D. Stomach

It is well recognized that gastric function can be regulated by the distal portion of the small intestine. In humans, diversion of chyme from the ileum reduces the gastric secretory response compared with exposure of chyme to the entire small intestine (371). The presence of chyme or partially digested fat in the ileum of humans inhibits gastric emptying and jejunal motility – the so-called 'ileal brake' (249, 273, 372-376). As reviewed earlier, chyme and fats are potent stimulators of GLP-1, indicating GLP-1 may be a candidate hormone for regulating gastric function. Indeed, GLP-1 inhibits gastric acid secretion (pentagastrin- as well as meal-induced) and gastric emptying when infused in quantities that result in plasma concentrations similar to those observed after meals (267, 271, 315, 377-380). In rats, this effect of GLP-1 may be mediated by inhibition of gastrin secretion and stimulation of the release of gastric somatostatin (381, 382). However, in pigs and humans, GLP-1 does not seem to regulate the release of either gastrin or somatostatin (72, 267, 315, 316,

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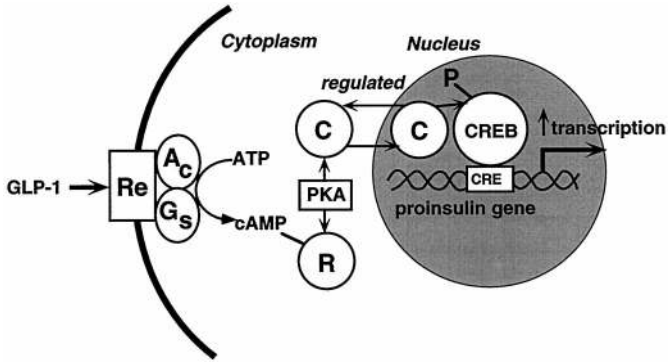

FIG. 14. Insulinotropic actions of GLP-1 on β-cells mediated by activation of the cAMP-signaling pathway. The binding of GLP-1 to its receptor (Re) activates adenylyl cyclase (A_c), resulting in the formation of cAMP. Binding of cAMP to the regulatory (R) subunit of PKA results in the release of the active catalytic (C) subunit. The active kinase then translocates to the nucleus and phosphorylates, and therefore activates, the nuclear transcriptional activator CREB bound to the CRE located in the promoter of the proinsulin gene. This cascade of signaling results in a stimulation of transcription of the proinsulin gene and increased insulin biosynthesis to replete stores of insulin secreted in response to nutrients (glucose) and incretins (GLP-1, GIP). [Adapted with permission from J. F. Habener: In *Diabetes Mellitus*, pp 68-78, 1996 (588)].

383). In these species, the inhibitory effect of GLP-1 on upper gastric functions could involve receptors located either in the central nervous system or associated with afferent pathways to the brain stem (380). These possibilities are supported by the observations that the inhibitory effect of GLP-1 on gastric emptying requires intact vagal enervation (269, 384, 385). Therefore, despite the known insulinotropic actions of GLP-1, the net effect of administering GLP-1 with a meal in healthy humans is a reduction in meal-related integrated incremental glucose and insulin responses (379). This observation supports the concept that the primary physiological role of GLP-1 may be as a mediator of ileal brake mechanisms, rather than as a incretin hormone (386). The actions of GLP-1 to delay gastric emptying are under investigation as an aspect of therapy for diabetes to attenuate the postprandial glucose excursion.

E. Lung

GLP-1 receptors are expressed at high density in rat lung membranes (335, 387, 388) and on vascular smooth muscle (389). The treatment of rat trachea and pulmonary artery with GLP-1 results in inhibition of mucous secretion and relaxation of smooth muscle (389). The sequence of the cDNA for the GLP-1 receptor expressed in rat lung is identical to the β-cell receptor except for one codon (390). When expressed in Chinese hamster ovary (CHO) cells, this receptor displays a pharmacological profile similar to that seen with cells expressing the β-cell-derived cDNA (390). Notably, GLP-1 receptor mRNA is detected in type II pneumocytes (334) and stimulates the secretion of surfactant from these cells (391). The overall physiological role of GLP-1 actions on the lung remains uncertain. The unusually high abundance of receptors in the lung suggests important actions of GLP-1 in pulmonary physiology. It is difficult to envision how GLP-1 actions on the lung would relate to the release of GLP-1 from the intestine in response to meals. One possibility is the local production of proglucagon and GLP-1 within the lung to establish a paracrine loop, but proglucagon expression has not yet been detected in the lung.

F. Brain

Perhaps the most surprising and unexpected actions of GLP-1, discovered only recently, are on the hypothalamus to inhibit food and water intake. GLP-1 appears now to be an anorexigenic hormone similar in action to the obesity hormone leptin and to antagonize orexigenic hormones such as CRF and neuropeptide Y. The discovery of these actions of GLP-1 on the promotion of satiety and the suppression of energy intake are recent and are somewhat controversial.

It had been known from earlier studies that binding sites for GLP-1 exist in plasma membranes prepared from rat brain (174, 387, 392), and by *in situ* binding studies that receptors exist in and around the hypothalamus and arcuate nucleus (393, 394). The density of GLP-1 receptors is particularly high in the arcuate nucleus, the paraventricular and supraoptic nuclei, and in the sensory circumventricular organs such as the subfornical organ, organum vascularum, laminae terminus, and the area postrema. The expression of GLP-1 receptors in the brain was confirmed by RT-PCR cloning of the GLP-1 receptor from mRNA prepared from rat brain (395). It was also shown in earlier studies that proglucagon and proglucagon-derived peptides are produced locally in the brain (see *Section V*). High densities of GLP-1-immunoreactive nerve fibers are present in paraventricular nucleus, dorsomedial hypothalamic nucleus, and the subfornical organ.

Several studies have now shown that the administration of GLP-1 into the third intracerebral ventricles of rats results in a profound decrease in food consumption (396-401). These effects of GLP-1 appear to be mediated by interactions on specific GLP-1 receptors because the reduction in food intake is greatly attenuated by prior or coadministration of the GLP-1 receptor antagonist, exendin 9-39 (396). The intracerebral ventricular administration of GLP-1 results in a marked enhancement of the expression of the immediate early responsive transcription factor c-*fos* in neuronal cell bodies located in the ventral medial hypothalamus and a corresponding reduction in the expression of the orexigenic hormones neuropeptide Y and GRH (396, 397). Notably, ablation of the arcuate nucleus and parts of the circumventricular organ by administration of monosodium glutamate to rats abolishes the inhibition of feeding invoked by intracerebral ventricular injection of GLP-1 (402).

Whether in physiological circumstances GLP-1 produced locally in the brain or GLP-1 in the circulation acts on hypothalamus receptors is uncertain. The administration of GLP-1 by the intraperitoneal route is reported to be ineffective in reducing food intake in rats (396). There is some debate about whether the reduction of inhibition of feeding behavior in rats in response to intracerebroventricular GLP-1 is due to satiety or to a food aversion (399, 403, 404). Of additional concern is the observation that GLP-1 receptor null mice lacking a functional GLP-1 receptor display normal feeding behavior, although they are glucose intolerant (339,

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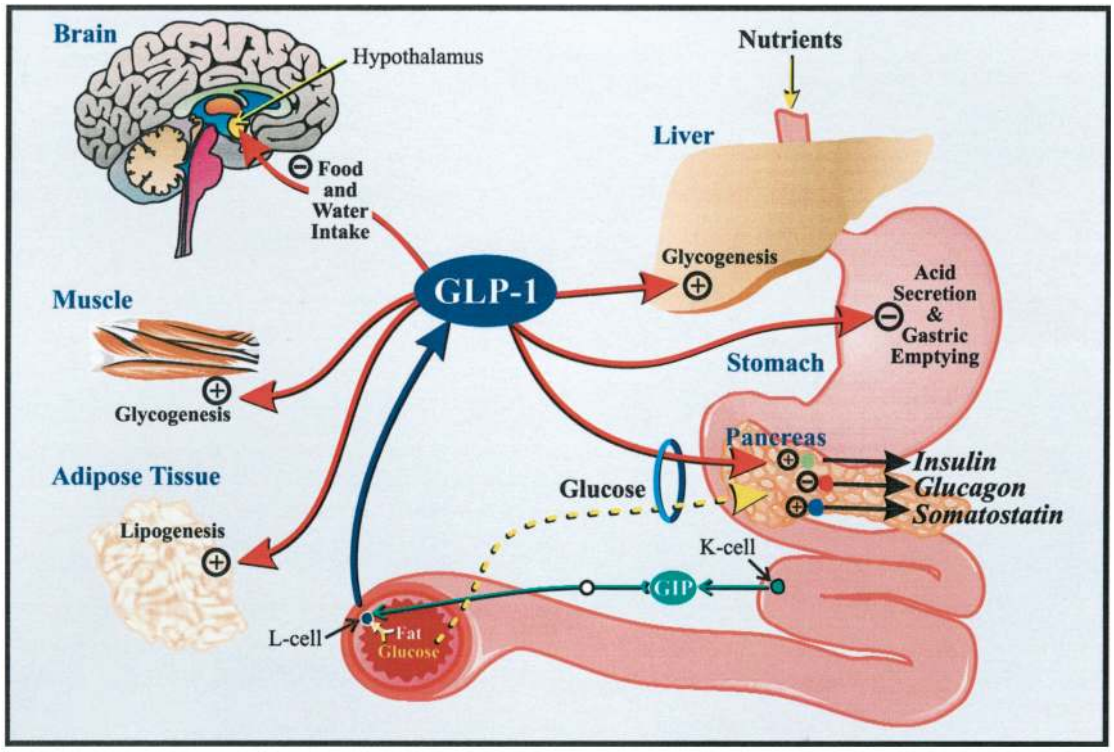

FIG. 15. Summary of GLP-1 actions. The *diagram* summarizes the currently understood targets of GLP-1 actions. In the endocrine pancreas GLP-1 stimulates both insulin and somatostatin secretion in a glucose-dependent manner and inhibits glucagon secretion. However, it is uncertain whether GLP-1 inhibits glucagon secretion by direct actions on α-cells or indirectly by the known paracrine-inhibitory effects of insulin and somatostatin on α-cells. GLP-1 is an effective inhibitor of gastric motility and emptying and curtails food intake by inducing satiety. Direct 'insulinomimetic' actions of GLP-1 on fat, liver, and muscle to induce lipogenesis and glycogenesis have been implied but remain to be definitively established.

405). However, in studies in humans, infusions of GLP-1 for 2, 6, 8, or 48 h appear to result in a reduction in food intake and have been interpreted as a satiety effect and not food aversion (406-409).

There are at least two mechanisms by which GLP-1 may gain access to the appetite control centers located in the hypothalamus: local production of GLP-1 within the brain and uptake of intestinally derived GLP-1 in the circulation. Compelling experimental evidence has been presented in support of both mechanisms, and they are not mutually exclusive. The proglucagon gene is expressed in the nucleus of the solitary tract, which is the nucleus of the vagus nerve that regulates the autonomic functions of the gut. Furthermore, proglucagon produced in the nucleus tractus solitarius is processed to GLPs (179). Injection of the retrograde tracer FluoroGold (Fluorochrome International, Englewood, CO) into the nucleus of the solitary tract showed that the caudal neurons containing GLP-1 project to the paraventricular nucleus (179). Thus, an attractive mechanism for the exertion of GLP-1 actions to inhibit feeding behavior would be the activation of GLP-1 production in the nucleus tractus solitarius via afferent enervation from the vagus nerve. Oral nutrients would then signal to the brain through the autonomic nervous system. It is tempting to speculate that this may constitute a prandial satiety signal generated during feeding, a signal to cease food consumption because enough has already been consumed. However, if an axonal transport of GLP-1 from the hindbrain to the hypothalamus is required, it may not be rapid enough to account for meal-induced satiety (20-30 min).

Perhaps the more plausible mechanism is the uptake by brain of GLP-1 in the circulation released from the intestines in response to a meal. Remarkably, ^{125}I-labeled GLP-1 injected into rats localizes to the subfornical organ and the area postrema of the brain within 5 min after the injection (410). These regions of the circumventricular organ are known sites where blood-borne macromolecules can pass across the blood-brain barrier. The satiety-inducing obesity hormone leptin in the circulation is believed to gain access to the satiety centers in the hypothalamus via the circumventricular organ that contains a high concentration of leptin receptors, so called short-form receptors that have high affinity for leptin, but are defective in their signal transduction (411-414). The model proposed for leptin transport into the brain is that the receptors extract leptin from the plasma and transport the leptin into the hypothalamus. Thus, in analogy with the mechanism of transport of leptin from the circulation to the brain, it seems reasonable to propose that GLP-1 released into the circulation in response to meals is similarly transported to the brain. The timing of GLP-1 release after a meal (15-30 min) and the demonstrated rapid uptake of GLP-1 by the circumventricular organ (<5 min) would be consistent with the development of satiety invoked by GLP-1 during the course of a meal.

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G. Liver, skeletal muscle, and fat

There are numerous reports of high-affinity (nM) GLP-1 binding sites and physiological actions on liver, skeletal muscle, and fat cells (415-436). The actions of GLP-1 on these tissues are anabolic, *i.e.*, glycogenic and lipogenic. These actions are the opposite of glucagon, which are catabolic, *i.e.*, glycogenolytic and lipolytic. A further paradoxical and as yet unexplained circumstance is that there is no reliable or reproducible evidence that the known GLP-1 receptor is expressed in liver, muscle, or fat (334). In fact, when examined, GLP-1 evidently suppresses cAMP formation in adipocytes and myocytes (425, 428, 436). The known GLP-1 receptor is coupled to Gs and the activation of adenylyl cyclase. Thus, one is led to the conclusion that if a GLP-1 receptor truly exists on hepatocytes, myocytes, and adipocytes, it must be different from the known, cloned, and characterized GLP-1 receptor. At least two possibilities arise to explain the existence of a second GLP-1 receptor. One possible explanation is that there is a second yet unidentified gene locus encoding a second GLP-1 receptor. The second possible explanation is that an altered, perhaps alternatively spliced, receptor of one or more of the GLP-1/glucagon-related members of the superfamily of glucagon-related peptide receptors is responsible. In this regard, it is worth noting that a new gene family of receptor-interactive proteins has been identified only recently (437). These proteins, RAMPs (receptor activity-modifying proteins) appear to interact at the cytoplasmic face with G protein-coupled receptors to alter ligand selectivity and binding affinities. For example, the calcitonin gene-related peptide receptor (CGRP-R) has been shown to interact with either one of two isoforms of RAMP, RAMP1 or RAMP2. In the presence of RAMP1, the receptor selectively binds CGRP, and, in the presence of RAMP2, binding selectivity switches markedly to adreno medullin, a peptide hormone related in structure to CGRP (437). Further, the CGRP-R is in the same G protein-coupled receptor subgroup as the receptors for GLP-1, glucagon, PACAP, and vasoactive intestinal peptide. Thus, it is tempting to speculate that the apparent peripheral actions of GLP-1 on liver, skeletal muscle, and adipose tissue to promote glucose uptake and utilization by insulin-independent mechanisms are mediated by one of the receptors in the glucagon-related family, perhaps via interactions with tissue-specific isoforms of the RAMP family of proteins.

The numerous reports of anabolic actions of GLP-1 on liver, muscle, and fat have prompted the design and execution of several studies *in vivo* in dogs and humans to identify possible direct actions of GLP-1 on glucose uptake independent of its insulinotropic action. Initial studies by Gutniak *et al.* (239) using the artificial pancreas in studies of both type 1 and type 2 diabetic subjects strongly suggested that GLP-1 stimulated peripheral uptake of glucose independently of insulin actions. In subsequent studies, GLP-1 was found to enhance glucose disappearance, in part, by increasing glucose disposal independently of changes in insulin (422, 438). However, most subsequent studies using sophisticated glucose clamp technologies have failed to detect insulin-like effects of GLP-1 on peripheral tissues (439-441). These studies, however, have been done in normal (nondiabetic) subjects. Therefore, it remains possible that the insulin-like actions of GLP-1 are more detectable in diabetic subjects with dysregulated glucose homeostasis and reduced insulin sensitivity. Although in one study GLP-1 had no effect on insulin sensitivity in subjects with type 2 diabetes (442), the analysis has been questioned (443). Recently, GLP-1 was demonstrated to potentiate insulin action during a hyperinsulinemic clamp in moderately hyperglycemic depancreatized dogs (443). This was due to GLP-1's effect of enhancing insulin-stimulated glucose utilization, while there was no effect of GLP-1 on the insulin-induced suppression of glucose production. Notably, GLP-1 had no effect in the presence of low insulin, suggesting GLP-1 has no insulin-independent actions in this model (443). It remains to be determined whether this insulin-potentiating effect of GLP-1 can also be shown in subjects with type 2 diabetes. Finally, whole-body glucose utilization is similar in wild-type and GLP-1 receptor $-/-$ mice under both basal and hyperinsulinemic conditions (340). The experimental evidence that GLP-1, or derivatives thereof, have anabolic actions on peripheral tissues, *e.g.*, liver, muscle, and fat, independent of actions of insulin, is conflicting and inconclusive at the present time.

H. Pituitary, hypothalamus, and thyroid

Several experimental findings suggest that GLP-1 activates hormone secretion from the anterior pituitary gland, where GLP-1 receptors have been detected (444). GLP-1 is reported to stimulate cAMP formation and TSH release from a cultured TSH-producing cell line derived from mouse pituitary thyrotropes as well as dispersed rat anterior pituitary cells (445). Similarly, GLP-1 stimulated LHRH release from cultured GTI-7 neuronal cells and intracerebroventricular injection of GLP-1 in rats resulted in a prompt increase in plasma LH levels (446). In human subjects administered GLP-1, plasma ACTH levels increased, suggesting a stimulatory effect of GLP-1 on pituitary corticotrophs (440). GLP-1 receptors are expressed in the rat C cell lines, CA77 and 6/23, and in the normal rat thyroid, where GLP-1 stimulates calcitonin release (447, 448).

I. Cardiovascular system

The administration of GLP-1 to rats results in increases in arterial blood pressure and heart rate (449, 450). These effects of GLP-1 appear not to be mediated through catecholamines. Although GLP-1 receptors have been detected in heart (334), the actions of GLP-1 on the cardiovascular system have been attributed to actions of GLP-1 receptors in the nucleus tractus solitarius, which is involved in the central control of cardiovascular function (449).

J. GLP-2

GLP-2 is cosecreted with GLP-1 from intestinal L cells. Until recently, there were no clear physiological functions attributable to GLP-2. However, there were hints that a product of the intestinal proglucagon gene may function in in-

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testinal adaptation. First, after intestinal resection, injury, or inflammation, there is a rapid and sustained increase in the abundance of proglucagon mRNA in residual ileum, accompanied by increases in plasma levels of proglucagon-derived peptides (289, 451-453). These observations suggested that proglucagon-derived peptides are possible modulators of adaptive bowel growth. Second, two patients with gross mucosal hypertrophy resulting from endocrine tumors were identified (454, 455). In one case, the abnormalities, which also included altered intestinal motility and absorptive function, disappeared after resection of the tumor located in the kidney (454). Glucagon-like immunoreactivity was extracted from this tumor, which resembled the intestinal form (enteroglucagon) as opposed to pancreatic glucagon (456). In the other case, an islet cell carcinoma of the α-cell type was identified. This patient had features characteristic of the pancreatic glucagonoma syndrome but also had large villi in the proximal duodenum (455). Tissue was not available to extract glucagon-like immunoreactive species for analysis.

More recently, marked proliferation of intestinal epithelium was observed in mice bearing subcutaneous proglucagon-producing tumors (457). These mice demonstrated elevated levels of several proglucagon-derived peptides (glicentin, oxyntomodulin, glucagon, GLP-1, and GLP-2). Drucker and colleagues (457) identified GLP-2 as the specific proglucagon-derived product that functions as a small intestinal growth factor *in vivo*. Mice injected with GLP-2 demonstrated crypt cell proliferation and increased bowel weight and villus growth within 4 days of initiation of GLP-2 administration (457). In contrast, GLP-1 had no significant effect on these parameters. Subsequent *in vivo* studies indicate that GLP-2 regulates both cell proliferation and apoptosis and promotes intestinal growth after both short- and long-term administration (458-460). The increased GLP-2 production observed in diabetic rats suggests a role for GLP-2 in diabetes-associated bowel growth (461). There is clear therapeutic potential for such an epithelial growth factor, as has been recently demonstrated. GLP-2 treatment normalized small intestinal mass (which was otherwise reduced) after total parenteral nutrition (462). In mice with dextran sulfate-induced colitis, GLP-2 treatment significantly increased colon length, crypt depth, and mucosal area and integrity, collectively resulting in reduced weight loss (463). Furthermore, GLP-2 administration suppressed the inflammatory response (463). Whether GLP-2 has equivalent beneficial actions on inflammation and destruction of the intestinal epithelial mucosa in human disease awaits clinical trials.

In addition to these trophic actions, it also appears that GLP-2 affects functional aspects of intestinal epithelium. Activities of duodenal maltase, sucrase, lactase, glutamyl transpeptidase, and DPP IV were increased after GLP-2 treatment, accompanied by increased absorption of leucine plus triolein (464). In these studies, GLP-2 treatment did not alter glucose or maltose absorption (464). However, Cheeseman *et al.* (465, 466) noted increased trafficking of the sodium-dependent glucose transporter (SGLT-1) and increased jejunal basolateral membrane glucose transport in rats. Like GLP-1, GLP-2 may also operate as a hormonal transmitter of the so-called 'ileal brake' effect. GLP-2 dose-dependently inhibited centrally induced antral motility in pigs (467). The mechanisms of GLP-2 action will be more fully understood since the identification of the GLP-2 receptor has just recently been reported (468).

X. GLP Receptors

A. Structure

Before the cloning of the GLP-1 receptor (GLP-1R) in 1992, (469), specific receptors for GLP-1 were detected on tumor-derived β- and δ-cell lines (345, 354, 470-475), rat islets (355), rat lung membranes (387, 388, 476), rat gastric glands (477), and in rat brain (174, 387, 478). A cDNA for the GLP-1R was eventually isolated by transient expression of a rat pancreatic islet cDNA library into COS cells, screened by binding of radiolabeled GLP-1 (469). Subsequently, a human pancreatic GLP-1 receptor that shares approximately 90% homology at the amino acid level with the rat receptor was cloned (77, 479, 480). The gene for the human GLP-1 receptor is localized to chromosome 6p21 (481). The identified receptor is a member of the seven membrane-spanning, G protein-coupled family of receptors, including glucagon (482), VIP (483), secretin (484), GIP (485), PACAP (486), GHF (487), calcitonin (488), and PTH (489). The identity of the amino acid sequence between these receptor proteins ranges between 27 and 49%, while the sequence identity to receptors of other subfamilies of G protein-coupled receptors is less than 10%. The receptor consists of 463 amino acids containing eight hydrophobic segments. The N-terminal hydrophobic segment is probably a signal sequence, whereas the others are membrane-spanning hydrophobic motifs. Ligand-binding analyses of the recombinant receptors expressed in and assembled on the surface of β-cells or heterologous cells show that the selectivity for the binding of GLP-1 is approximately 1 nM, whereas all of the other peptides of the glucagon superfamily bind poorly or not at all with the exception of glucagon, which is a weak, full agonist with a binding affinity of 100- to 1,000-fold less that that of GLP-1 (490, 491). Exendin-4, a 39-amino acid peptide isolated from venom of the lizard *Heloderma suspectum* (Gila monster) (492), is structurally related to GLP-1 and is a potent agonist exhibiting a similar binding affinity to the GLP-1 receptor (76, 77). In the lizard, different genes encode GLP-1 and exendin, and it is unlikely that a mammalian exendin exists (79, 80). The amino terminally truncated form of exendin (exendin 9-39), is a potent antagonist of GLP-1 capable of inhibiting GLP-1 binding and resultant cAMP formation (76, 77). Exendin 9-39 has therefore been used extensively to antagonize actions of GLP-1 both *in vitro* (391, 445, 493, 494) and *in vivo* (256, 336-338, 353, 384, 394, 396, 398, 400, 450, 495-497). Exendin 9-39 may not be completely specific for the GLP-1 receptor, however, as this peptide also displaces GIP binding from its receptor and inhibits cAMP generation by GIP, albeit only when used in the micromolar range (498, 499). Recently, other truncated forms of exendin that are more potent antagonists of GLP-1 than exendin 9-39 have been generated (92). It has not been reported whether or not these peptides also interact with the GIP receptor.

Several structure/function studies have been performed to determine which regions of the GLP-1 receptor are critical

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for binding specificity, signal transduction, and receptor regulation/desensitization. One approach has been the generation of chimeric receptors. The human glucagon and GLP-1 receptors are quite similar (47% amino acid identity), yet glucagon binds to the glucagon receptor with a dissociation constant (K_d) that is approximately 1000-fold lower than the K_d for glucagon binding to the GLP-1 receptor. The generation of chimeric glucagon/GLP-1 receptors revealed that noncontiguous domains within the membrane proximal half of the amino-terminal extension, the first extracellular loop, and the third, fourth, and sixth transmembrane domains within the glucagon receptor are important for high-affinity glucagon binding (500). The substitution of as few as four residues in the N-terminal extracellular domain of the GLP-1 receptor with the analogous region of the glucagon receptor results in a 50-fold decrease in selectivity of this receptor for GLP-1 over glucagon (501). Similarly, chimeric GLP-1/GIP receptors indicate the N-terminal domain of the GIP receptor acts as a ligand-specific binding domain (502). Indeed, the isolated, solubilized N-terminal region of the GLP-1 receptor competes for GLP-1 binding with the intact wild-type receptor, emphasizing the significance of this region of the GLP-1 receptor for the binding of ligand (503). Even a single amino acid substitution within the N-terminal extracellular domain (substitution of tryptophan at either position 39, 72, 91, 110, or 120 by alanine) abrogates GLP-1 binding, indicating the importance of a positive charge and imidazole ring at these positions (504, 505).

B. Signaling

Shortly after the identification of GLP-1, it was recognized that the actions of GLP-1 are mediated, at least in part, through adenylate cyclase. Activation of the cAMP signal transduction pathway by GLP-1 was first observed in rat brain (392) and insulinoma cells (73); however, it was unclear whether these effects were mediated by a specific receptor for GLP-1 (73). High-affinity binding sites [Michaelis-Menten constant (K_m) = 1 nM] for GLP-1 and activation of cAMP signal transduction in insulinoma cell lines was found in 1988 (470-472), suggesting that the hormone acts through specific receptors located on the surface of pancreatic β-cells that are coupled to the stimulatory G protein (Gs). Specific determinants for the efficient coupling of the GLP-1 receptor to adenylyl cyclase are located mainly in the predicted junction of the fifth transmembrane helix and the third intracellular loop (506, 507). However, single substitutions in the first intracellular loop results in reduced GLP-1-mediated stimulation of cAMP without altering receptor expression (507, 508).

Within β-cells, cAMP potentiates glucose-induced closure of ATP-sensitive K^+(K-ATP) channels (341), thereby generating cellular depolarization, activation of voltage-dependent Ca^{2+} channels (VDCCs), and influx of Ca^{2+}. GLP-1 may also increase intracellular calcium concentration ($[Ca^{2+}]_i$) by mobilizing Ca^{2+} from intracellular stores, both by activation of a phospholipase C pathway and cAMP-dependent Ca^{2+}-induced Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores (509-515).The GLP-1-induced rise of $[Ca^{2+}]_i$ serves as an important trigger for exocytosis of insulin. GLP-1 also exerts a direct stimulatory influence on the entry of Ca^{2+} through dihydropyridine-sensitive (L-type) VDCCs (516) and stimulates the opening of Ca^{2+}-activated nonselective cation channels (NSCCs) that are permeant to Ca^{2+} as well as Na^+ (517-519). The NSCCs may play a critical role in regulating the membrane potential of β-cells (see Fig. 14). GLP-1-mediated activation of an inward, nonselective cation current together with a decrease in the activity of K-ATP channels results in membrane depolarization, activation of voltage-dependent calcium channels, and stimulation of insulin secretion. The increase in intracellular $[Na^+]$ resulting from the influx of Na^+ through NSCCs and efflux of K^+ through K-ATP channels is then corrected by activity of the Na:K-ATPase.

The activation of the cAMP/PKA pathway by GLP-1 also appears to enhance insulin secretion at a distal site, beyond the elevation of $[Ca^{2+}]_i$ in stimulus secretion coupling (520, 521). Thus GLP-1 appears to potentiate insulin release by increasing the effectiveness of the K-ATP channel-independent action of glucose (520). Notably, activation of the cAMP/PKA pathway in β-cells by GLP-1 augments Ca^{2+}-stimulated insulin release only in the presence of glucose (516, 520, 522). Collectively, these functions of GLP-1 render otherwise unresponsive β-cells responsive to glucose – the glucose competence concept (341).

C. Distribution

The GLP-1 receptor has a wide distribution of tissues, having been located in brain, lung, pancreatic islets, stomach, hypothalamus, heart, intestine, and kidney (174, 334, 335, 354, 388, 395, 475-478, 523-527). Although most reports indicate that liver, muscle, and adipose tissues do not express the known GLP-1 receptor, investigators have repeatedly reported binding and *in vitro* and *in vivo* effects (mainly glucogenesis) of GLP-1 in these tissues (415-436). Some *in vitro* studies indicate that GLP-1 decreases intracellular cAMP levels in adipocytes and myocytes, a response opposite to that observed in pancreatic β-cells in response to the same peptide (425, 428, 436). The effects of GLP-1 on skeletal muscle are reported not to be mediated by the cAMP pathway (415, 430). Finally, others have disputed the action of GLP-1 on these peripheral tissues (528-530). However, the existence of isotypes of the known GLP-1 receptor or of a yet unidentified GLP-1 receptor coded by a separate gene may be suspected.

D. Regulation

GLP-1 receptor mRNA levels in insulinoma cells are down-regulated during incubation with agents that increase cAMP levels, including GLP-1 itself (531) and by activation of protein kinase C (532). However, another study using rat islets failed to detect any change in GLP-1 receptor mRNA levels after alterations in intracellular cAMP levels but found a significant reduction after incubation with glucocorticoid (dexamethasone) (533). A small but significant decrease in GLP-1 receptor mRNA levels was detected when rat islets were cultured in high (20 mM) glucose, whereas the expression of glucagon receptor mRNA increased (533). In MIN6

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cells, both glucagon and GLP-1 receptor genes showed higher expression levels when the cells were cultured under conditions of high glucose (22 mM) compared with low glucose (0.7 mM) (527). These disparate observations indicate the need to use caution when extrapolating observations from tumor-derived β-cell lines to native β-cells. The promoter region of the human GLP-1 receptor gene has been cloned. It appears to be positively regulated by *cis*-acting enhancing elements (including three Sp1 binding sites) and negatively regulated by more distal elements in a cell- and tissue-specific manner (534-536). The identification of the regulatory region of the GLP-1 receptor gene should allow for an analysis of the mechanisms of regulation of the GLP-1 receptor expression at the transcriptional level.

At the protein level, regulation of GLP-1 receptor expression has been extensively studied in transfected fibroblasts and insulinomas. In these cells, the GLP-1 receptor is susceptible to rapid, reversible homologous and heterologous (by activated protein kinase C) desensitization. Desensitization occurs within 5 min of the binding of GLP-1 to its receptor and is reversed after 10-20 min after the removal of the ligand (474, 531, 537-539). Both homologous and heterologous desensitization of the GLP-1 receptor correlate with phosphorylation of the cytoplasmic tail of the receptor at serine doublets 431/432, 441/442, 444/445, and 451/452 (539, 540). Phosphorylation is also involved in the mechanisms that regulate internalization of the receptor (539). These properties of the GLP-1 receptor must be an important consideration, given the development of treatment strategies of diabetes with GLP-1 or analogs. Thus far, however, there is no evidence that the GLP-1 receptor undergoes desensitization in *in vivo* studies.

E. GLP-2

Recently, high-affinity rat and human GLP-2 receptors were cloned, which share 82% similarity (468). The receptor is approximately 550 amino acids long, has seven predicted transmembrane domains, and clearly belongs to the GLP-1/glucagon/GIP receptor gene family. The gene encoding the human GLP-2 receptor was mapped to chromosome 17p13.3. Competition binding studies with a stable cell line expressing the rat GLP-2 receptor revealed both a high- (K_i = 0.06 nM) and low-affinity site (K_i = 259 nM) (468). K_i values determined for GLP-1, glucagon, and GIP peptides were 928, 500, and 765 nM, respectively. The receptor is functionally coupled to cAMP production with an IC_{50} = 0.58 nM. No cAMP response in cells transiently expressing the rat GLP-2 receptor was observed with 10 nM GLP-1, glucagon, GIP, PTH, secretin, PACAP, VIP, GRF, CRF, or exendin-3. GLP-2 also stimulated both cAMP accumulation and cell proliferation in baby hamster kidney cells expressing a transfected rat GLP-2 receptor (541). However, 8-bromo-cAMP alone did not promote cell proliferation in these cells, suggesting that the GLP-2 receptor may be coupled to activation of mitogenic signaling in heterologous cell types independent of PKA via as yet unidentified downstream mediators (541). Rat GLP-2 receptor RNA levels are highest in jejunum, followed by duodenum, ileum, colon, and stomach, concordant with the reported functional responses after GLP-2 administration (468). In addition, the cloned GLP-2 receptor is expressed in the hypothalamus, raising the possibility that the intestinotrophic hormone has as yet undescribed roles in the central nervous system.

XI. Pathophysiology of GLP-1

There is very little known thus far about whether a deficiency or an excess of GLP-1 production results in or contributes to the pathophysiology of disease. Probably the most established example is a role for excess secretion of GLP-1 in the promotion of hyperinsulinemia in subjects with dumping syndrome (72, 246, 542, 543). The dumping syndrome is most pronounced in individuals who have undergone partial gastrectomy and gastro-jejunal shunt surgery to bypass the duodenum because of severe peptic ulcer disease. The abnormal rapid delivery of oral nutrients into the jejunum elicits a marked increase in plasma GLP-1 levels, up to 10- to 20-fold above levels seen in normal subjects after a meal. Plasma insulin levels are correspondingly high during the early phases in the fall in blood glucose levels. Fortunately, the actions of GLP-1 on pancreatic β-cells are glucose dependent, and as the blood glucose levels fall below normal levels, the insulinotropic actions of GLP-1 are attenuated. This may, in part, explain why a patient with an endocrine tumor that resulted in elevated plasma levels of GLP-1 remained normoglycemic (544).

A role for over- or underproduction of GLP-1 in obesity and diabetes mellitus remains controversial. In patients with non-insulin-dependent diabetes mellitus, insulin release is no longer stimulated more by an oral as compared with isoglycemic intravenous glucose, suggesting the loss of incretin stimulation (545-547). Postprandial GLP-1 secretion in response to oral carbohydrate was considerably attenuated in obese subjects and in diabetic twins (548, 549). However, there have been reports of elevated levels of GLP-1 in obese and diabetic patients (550-553), raising the possibility that β-cell insensitivity to GLP-1 exists. Although mice with a null mutation in the GLP-1 receptor exhibit glucose intolerance (339), genetic studies have shown that the GLP-1 receptor locus (6p21) is not included in chromosomal loci carrying susceptibility for diabetes (481, 554, 555). Finally, the GLP-1 response to oral glucose was not altered in postmenopausal women with impaired glucose tolerance (556). At this time it seems reasonable to conclude that obesity and diabetes are not strongly associated with dysregulation of GLP-1 production or secretion.

XII. GLP-1 as a Potential Treatment for Diabetes Mellitus

The prevalence of diabetes mellitus is increasing dramatically in populations of the world. Diabetes develops as a consequence of either an absolute deficiency of insulin production (type 1) or as a relative deficiency of the pancreas to produce insulin in amounts sufficient to meet the body's needs (type 2). Unlike type 1 diabetes in which the β-cells are destroyed by autoimmune processes, in type 2 diabetes the pancreatic β-cells remain intact but fail to produce and se-

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crete insulin in response to elevations in plasma glucose. Thus the β-cells of individuals with type 2 diabetes are capable of producing insulin but are dysregulated in their response to plasma glucose levels. In many patients with diabetes, insulin resistance ultimately compromises the ability of the β-cell to maintain an increased level of insulin biosynthesis and secretion over a prolonged period of time, eventually resulting in worsening of hyperglycemia and accompanying β-cell failure. Although most type 2 diabetics require daily injections of insulin, in some individuals the β-cells can be prompted to respond to glucose by exposure to the sulfonylurea-derived oral hypoglycemic agents. These agents act by binding to the sulfonylurea receptor subunit of the K-ATP channel, resulting in channel closure and depolarization of the cell (557). In pancreatic β-cells that express K-ATP channels, their closure results in insulin secretion. However, similar channels are also located in cardiac and vascular smooth muscle cells (558). In cardiac myocytes, inhibition of K-ATP channels by sulfonylureas prevents ischemic preconditioning, an endogenous cardioprotective mechanism that protects the heart from lethal injury (559). As a result, sulfonylurea treatment could contribute to the risk of myocardial ischemia and infarction. An additional potential side effect is hypoglycemia, as the actions of sulfonylureas are not glucose-dependent (560). Many patients also become refractory to the actions of sulfonylureas and therefore ultimately require insulin injections. Although the sulfonylureas effectively stimulate insulin secretion by their actions on the ATP-sensitive potassium channels on β-cells, they do not stimulate the production of insulin or the transcription of the insulin gene; consequently, exhaustion of insulin stores may occur. This circumstance may explain, in part, the development of tachyphylaxis to these drugs. Although the sulfonylureas and other oral agents such as biguanides [*e.g.*, metformin (Sigma, St Louis, MO) which promotes glucose utilization and reduces hepatic glucose production] and thiazolidinediones [*e.g.*, troglitazone (Parke-Davis, Ann Arbor, MI) which enhances cellular insulin action on glucose and lipid metabolism] have been highly successful in controlling blood sugar levels in many diabetic individuals, a search for even more efficacious drugs has continued (561, 562). In this regard, GLP-1 holds considerable promise (563). In theory, there are several attractive features of GLP-1 that would make it a particularly effective treatment for diabetes. The fact that GLP-1 induces both secretion and production of insulin, and that its activities are mainly glucose dependent, indicates that GLP-1 may have unique advantages over sulfonylurea drugs in the treatment of diabetes. Additionally, GLP-1 lowers glucagon concentrations, slows gastric emptying, reduces food intake, and may enhance insulin sensitivity and stimulate β-cell neogenesis. Therefore, in many aspects, GLP-1 opposes the diabetic phenotype.

In practice, the administration of GLP-1 to type 2 diabetic subjects effectively lowers blood glucose levels when given either by intravenous, subcutaneous, or oral buccal routes (239, 245, 277, 342, 349, 377, 385, 442, 564-575). GLP-1 infusions are also effective in reducing blood glucose in insulin-deprived states, including type 1 diabetics (239, 576-579). These actions are perhaps attributable to increased glucose disposition in peripheral tissues, reduced gastric emptying, and reduced hepatic glucose output, possibly secondary to a reduction in glucagon concentrations. Most noteworthy is that improved glycemic control is achieved in diabetic subjects with the subcutaneous administration of GLP-1 for 1 (573) or 3 weeks (570, 580). These studies are encouraging in that GLP-1 remained effective throughout the studies and no indications of tachyphylaxis were observed. The administration of GLP-1 via a buccal tablet also effectively lowers blood glucose levels in diabetic subjects (564). A potential drawback in GLP-1 as an effective therapy for diabetes is that the half-life of the hormone is very short. The half-life of the bioactive form of the peptide *in vivo* is in the range of 1-2 min (322). As discussed earlier, the ubiquitous enzyme DPP IV cleaves the histidine-alanine dipeptide from the amino terminus of GLP-1, thereby eliminating its biological activities (319, 321, 322). Thus, the development of longer acting, DPP IV-resistant forms of GLP-1 (328, 581, 582) may be required to improve the therapeutic potential of GLP-1 for the treatment of diabetes. A prolongation of the effectiveness of GLP-1 can also be achieved by the coadministration of inhibitors of DPP IV (326, 327). Such a strategy was recently shown to produce a more rapid clearance of blood glucose after an oral glucose challenge in normal (583) and obese Zucker rats (327). Thus, to prolong the duration of action of GLP-1 and thereby to enhance the therapeutic effectiveness of the hormone, strategies may involve the design of GLP-1 isoforms resistant to DPP IV or the coadministration of DPP IV inhibitors with GLP-1. Another possibility is the use of the GLP-1 receptor agonist exendin-4, which is more resistant to degradation *in vivo*. Thus, exendin-4 has a longer duration of action than GLP-1, is far more potent, and effectively lowers plasma glucose concentrations in obese diabetic *ob/ob* and *db/db* mice, fatty Zucker rats, and diabetic rhesus monkeys (584). The potential usefulness for an exendin-like molecule in the treatment of humans with diabetes awaits further studies.

XIII. Future Directions

It seems clear that the discovery of GLPs has kindled considerable interest in understanding the physiological role and actions of these new gut and brain hormones. In particular, the available evidence strongly suggests that GLP-1 is involved in the regulation of nutrient metabolism. GLP-1 reduces gastric emptying, stimulates insulin secretion and production, and may stimulate the neogenesis of pancreatic β-cells and promote satiety. GLP-2, on the other hand, has recently been shown to have intestinotrophic activity in rodents, and may also function as a hormonal ileal brake.

Many questions regarding the expression, actions, and physiological importance of the GLPs remain unanswered and are yet to be explored. The complex nature of the post-translational processing of the GLPs from proglucagon raises interesting questions as to why GLP-1 is processed from proglucagon to yield at least four isopeptides, consisting of the N-terminal extended and truncated forms. Why is the C terminus either amidated or glycine extended? The concerted production of the four isopeptides of GLP-1 seems to

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suggest that they may have distinct biological activities. So far, the amino-terminal extended forms of GLP-1, GLP-1(1-37), and GLP-1(1-36)amide have no known biological activities, and the activities of the truncated hormones GLP-1(7-37) and GLP-1(7-36)amide are indistinguishable. Another enigma is how GLPs can exert biological actions when DPP IV cleaves and evidently inactivates both so rapidly. Is it possible that the truncated products have as yet unknown unique biological functions? An additional unexplained phenomenon is why GLP-1 is released so soon after a meal stimulus, presumably by vagal and enteric nerve reflexes or the indirect stimulation by GIP, and yet luminal nutrients stimulate GLP-1 release late after a meal. Why is there this apparent biphasic secretory response of GLP-1? Questions remain to be answered regarding whether actions of GLP-1 in the hypothalamus are on satiety or food aversion. Why and what are the purposes of apparent GLP-1 receptors in liver, muscle, fat, heart, and kidney? What is the role of GLP-1 actions on receptors in the anterior pituitary gland? Finally, a puzzling dilemma is how is it that the peptide exendin 4, produced exclusively in the venom glands of the Gila monster lizard, has such a potent GLP-1-like effect in mammals. Is there a yet unidentified mammalian exendin and a mammalian exendin receptor? There are many questions to be answered with regard to the functions of GLPs in the regulation of human physiology. The finding that GLP-like peptides exist in mammals, fish, birds, amphibians, and reptiles seems to suggest that the structural components of GLPs have been designed to be important for the regulation of metabolism. Given the clear therapeutic potential of GLP-1 and GLP-2, it will be interesting to determine whether these hormones or their derivatives will eventually have a role in the treatment of human disease.

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