Glucagon-like peptide-1 (GLP-1): a gut hormone of potential interest in the treatment of diabetes

Bo Ahrén*

Summary

GLP-1 (glucagon-like peptide-1) is a gut hormone which is released into the blood stream after feeding. Its main action is to stimulate insulin secretion through potentiating the insulinotropic action of glucose. The peptide is encoded in the glucagon gene and expressed mainly in the gut L cells. It exerts its actions through activating specific receptors of the seven transmembraneous domain-G-protein-coupled type with 463 amino acids. Its main signalling mechanism is activation of adenylate cyclase and formation of cyclic AMP. The peptide also increases the cytoplasmic concentration of Ca^2 which is thought to be executed both through a Na^+-dependent uptake of extracellular Ca^{2+} and through release of Ca^{2+} from intracellular Ca^{2+} stores. GLP-1 also inhibits glucagon secretion and inhibits gastric emptying and gastric acid and pancreatic exocrine secretion. Its integrated action on carbohydrate metabolism results in reduction of circulating glucose, and GLP-1 has therefore been suggested as a therapeutic alternative in diabetes. Finally, GLP-1 is also expressed in neurons in the hypothalamus, and may be involved in the regulation of feeding behaviour, since it inhibits food intake. *BioEssays* **20**:642–651, 1998.

Introduction

The gut hormone GLP-1 (glucagon-like peptide-1) is produced in the intestinal endocrine L-cells. It is a C-terminally amidated 30 amino acid peptide, which is processed from proglucagon. It is secreted into the blood stream after a meal intake and it functions as an incretin hormone. The present day knowledge on GLP-1 is the result of biomedical research during the last 50 years. Landmark discoveries include the 1948 demonstration of glucagon-like bioactivity in the gut mucosa[1] and the identification of enteroglucagon cells by the use of glucagon antisera in the early 1970s.[2] In 1983, the structure of the glucagon gene was identified[3] and in 1986, the processing of the proglucagon to various peptides in the pancreas and the gut was established.[4] It was thereby demonstrated that the main hormone produced by the enteroglucagon cells (now called L cells) is GLP-1.[4] In 1987, it was also shown that GLP-1 is released into the circulation during food intake and stimulates insulin secretion in humans, and its function as an incretin factor was established.[5] Furthermore, around 1990, it was demonstrated that GLP-1 inhibits gastric emptying and gastric acid secretion, and its function as an enterogastrone factor was suggested.[6] Its signalling action through activation of adenylate cyclase was demonstrated in 1987,[7] and in 1992, the specific GLP-1 receptor was cloned and found to be of the seven transmembraneous domain G-protein coupled type.[8] A study in 1992 also opened the possibility of using GLP-1 in the treatment of diabetes.[9] Finally, in 1996, it was found that GLP-1 inhibits food intake.[10]

Department of Medicine, Lund University, Malmö, Sweden.
*Correspondence to: Dr. Bo Ahrén, Department of Medicine, Malmö University Hospital, S-205 02 Malmö, Sweden.

Characterization of the GLP-1 producing intestinal L cells

The main site of expression of GLP-1 is in the intestinal L cells,[11] although GLP-1 is also expressed in certain neurons

BioEssays 20:642–651, © 1998 John Wiley & Sons, Inc.

in the hypothalamus[12] and in small quantities in the pancreatic A cells.[13] Although the existence of glucagon-like substances in the gut was known since the 1940s,[1] the gut enteroglucagon cells were identified in the early 1970s by the use of glucagon antisera.[2] These cells, which now are called L cells, are distributed in the mucosal lining of the small and large intestines and are of the open-type endocrine cells with a characteristic slender apical process with a surface exhibiting microvilli directed towards the lumen of the gut.[11] At the base of the cell, typically large secretory granules containing fully processed GLP-1 are located; these granules liberate the hormone when appropriately activated (Fig. 1). The density of these cells increases throughout the small intestine to reach a maximum in the distal ileum. The cells occur also in the colon, and also here their density is charactestically increased distally, to reach a maximum in the rectum.[11]

Expression of the GLP-1 encoding gene

GLP-1 is encoded in the glucagon gene which is 9.4 kb in length, localized to band q36-q37 on chromosome 2 and consists of six exons and five introns.[14] The sequence of the glucagon gene was first identified in the hamster[3] and later demonstrated also in humans.[14,15] It has been shown that it is the same glucagon gene which is expressed in the intestinal L cells and in the pancreatic A cells.[16] In a study on the regulation of the glucagon gene expression on fetal rat intestinal mucosal cells, it was found that elevation of cyclic AMP increases the cellular amount of proglucagon mRNA, suggesting the existence of a cyclic AMP responsive element in the regulatory region of the intestinal glucagon gene.[17] However, more studies are required to elucidate which factors that are of importance for GLP-1 gene expression.

Processing of preproglucagon

As a result of translation of the glucagon mRNA, the 180 amino acid preproglucagon sequence is produced, and after cleavage of an N-terminal signal peptide, the remaining 160 amino acid proglucagon is packaged into secretory granules. Proglucagon then undergoes a series of posttranslational modifications, which are different in the gut and the pancreas.[4,13] In the L cells, GLP-1 is processed in two steps (Fig. 2): first proglucagon$_{72–108}$ is formed, which is then truncated by cleavage of the N-terminal 6 amino acids, removal of the C-terminal glycine, and amidation of the remaining C-terminal residue arginine, resulting in proglucagon$_{78–107amide}$ (=GLP-1), which is the main secretory hormone of the L-cells. Approximately 20% of GLP-1 secreted from the L cells is, however, not amidated, but instead secreted as GLP-1$_{7–37}$, i.e., proglucagon$_{78–108}$, which exerts identical effects as GLP-1.[13] Finally, a recent study showed that already at the time of exocytosis, GLP-1 is metabolized by removal of the two N-terminal amino acids, yielding GLP-1$_{9–36amide}$, which is a GLP-1 antagonist.[18] Taking this into account, GLP-1 seems

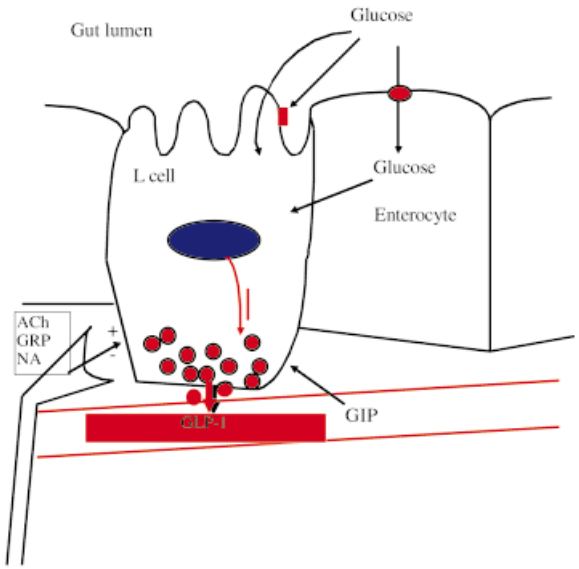

Figure 1. Schematic model of an intestinal L cell with its microvilli protruding into the gut lumen and its close anatomical relationship with neighboring enterocytes. The L cells are polarized with secretory granules, containing the processed proglucagon with GLP-1, located to the base closely to the blood vessels. The L cells may be activated by absorbed nutrients (symbolized by glucose) either, although unlikely, through a direct action on the L cells (via chemoreceptors or through absorption) or, more likely, indirectly through absorbtion by the enterocytes, by GIP or other hormones arriving through the blood stream, or through nerves within the gut wall. These nerves might be cholinergic or peptidergic (stimulatory action on L cells) or adrenergic (inhibitory), Ach (=acetyl choline), GRP (=gastrin releasing peptide), and NA (=noradrenaline) represent neurotransmitters.

to be secreted as GLP-1$_{7–36amide}$ (~ 40%), GLP-1$_{9–36amide}$ (~ 40%), and GLP-1$_{7–37}$ (~ 20%).

In the pancreatic A cells, the processing of proglucagon is different than in the L cells. Thus, in these A cells, glucagon is the main product and GLP-1 is produced only in minimal amount.[13] The different posttranslational processing of proglucagon in the gut versus in the pancreas implies that different enzymes responsible for the processing are involved in the two cell types. Although not finally established, there is experimental evidence that the enzyme PC2 (proconvertase 2) is involved in the processing of proglucagon in the pancreatic A cells, whereas PC3 (proconvertase 3) and probably an additional yet unidentified enzyme are involved in the processing of proglucagon in the intestinal L cells.[19]

Structure of GLP-1

The amino acid sequence of GLP-1 is identical in all mammalian species examined sofar.[20] It consists of 30 amino acids

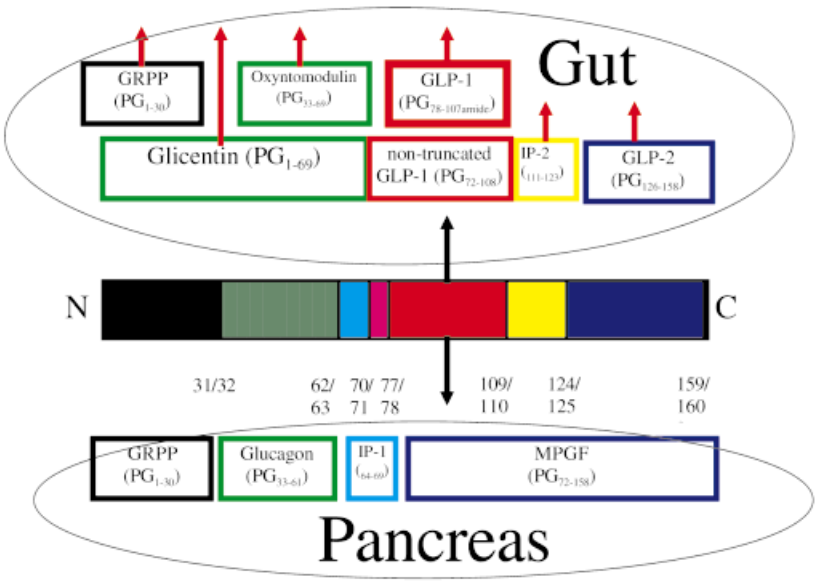

Figure 2. Processing of the proglucagon molecule.[13] Proglucagon contains 7 dibasic pairs of amino acids which are preferential sites for cleavage during the processing, as indicated by numbered amino acids. The processing of the promolecule is different in the intestinal L cells (shown above the proglucagon sequence) versus in the pancreatic A cells (shown below the proglucagon sequence). In the L cells, the proglucagon$_{1–69}$ (=PG$_{1–69}$) sequence is finally secreted as glicentin (60–80%), GRPP (glicentin related polypeptide; 20–40%), and oxyntomodulin (20–40%), whereas the PG$_{78–108}$ sequence is secreted as GLP-1 (=GLP-1$_{7–36amide}$; 80%, of which half is rapidly, probably already at the time of exocytosis, metabolized to GLP-1$_{9–36amide}$) and GLP-1$_{7–37}$ (20%, not shown in Fig.). Red arrows show secreted products from the gut. In the A cells, GRPP, glucagon, and IP-1 (intervening peptide 1) are secreted, and of the MPGF sequence, ≈70% is secreted as MPGF, ≈25% as GLP-1, ≈5% as GLP-1$_{7–37}$, and ≈30% as GLP-2.

with the C-terminal arginine moiety being amidated and 14 of the 30 amino acids being identical to those in glucagon (Fig. 3). Magnetic resonance imaging technique has shown that GLP-1 in a micelle takes the form of a two helical structure (amino acids 7–14 and 18–29, respectively), which is similar to that of glucagon.[21] Studies on the active site of the molecule have revealed that both the C-terminal and the N-terminal ends of the peptide are of importance for activity, since removal of the N-terminal histidine or the three C-terminal amino acids results in complete loss of activity.[22]

Secretion of GLP-1

In the intestinal L-cells, GLP-1 is stored in the secretory granules, and released into the blood stream when the cells are activated. The most important stimulus for secretion of GLP-1 is ingestion of a mixed meal, which increases circulating GLP-1 within 15 minutes.[5] Also oral ingestion of glucose releases GLP-1.[5,23,24] The stimulus for secretion seems to be nutrients reaching the small intestine, since the secretion of GLP-1 correlates to the gastric emptying rate.[24] Although L cells are equipped with luminally directed chemosensors, a study demonstrating that nonabsorbable sugars (sucrose and 2-deoxy-glucose) do not stimulate GLP-1 secretion from the perfused dog ileum suggests that nutrients need to be absorbed to stimulate GLP-1 secretion.[25] Since glucose absorption is executed by the sodium-glucose-cotransporter (SGLT-1), it is of interest that this transporter does not seem to exist in the L-cells, but only in the neighbouring enterocytes.[26] Hence, it is possible that the main stimulus for GLP-1 secretion is a paracrine activation by neighboring enterocytes after absorption of nutrients.

Also hormones and neurotransmitters affect GLP-1 secretion from the intestine. Thus, it has been shown in rat L cells, that GLP-1 secretion is stimulated by GIP (gastric inhibitory polypeptide), GRP (gastrin releasing peptide), and the muscarinic agonist, bethanechol, but inhibited by somatostatin.[27] Furthermore, in the pig ileum, GLP-1 secretion is stimulated by GRP, substance P and neurokinin A, whereas the secretion is inhibited by adrenergic nerve activation.[13] Hence, whereas the main regulator of GLP-1 secretion is nutrient absorption, the secretion seems to be modulated by stimulatory and inhibitory influences by other gut hormones and by the enteric nervous system. However, more studies are required to establish the integrated action of various factors on GLP-1 secretion.

Circulation of GLP-1

The fasting circulatory level of GLP-1 is approximately 5–15 pmol/l, and this level is increased to approximately 20–30 pmol/l after ingestion of a meal.[5,23,24] Following its secretion into the blood, GLP-1 is rapidly and extensively removed from the circulation. Two main routes of elimination of GLP-1 have been described. A first route is enzymatic removal of the two N-terminal amino acids (histidine and alanin), yielding des-His^1-Ala^2-GLP-1 or GLP-1$_{9–36amide}$, by the enzyme dipeptidyl peptidase IV (DPPIV).[28] A second route of elimination is through extraction by the kidney, implying that subjects with kidney failure have elevated circulating levels of GLP-1.[29] A

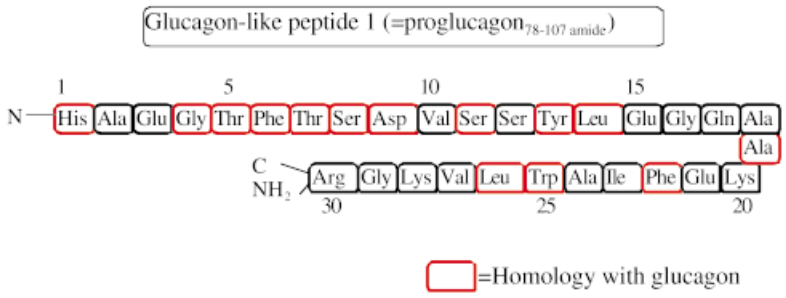

Figure 3. Amino acid sequence of GLP-1. Amino acids surrounded by red line are homologous to glucagon.

theoretical possibility for a third route of elimination of GLP-1 from the circulation is inactivation at the site of its receptors. However, whether such receptor-linked inactivation significantly contributes to the elimination of GLP-1 from the circulation is at present not known. The efficient removal of GLP-1 from the circulation results in a short circulating time of the peptide. Thus, the calculated half life after intravenous administration of GLP-1 has been found to be as short as 4 minutes.[5]

The optimal antisera to be used for radioimmunoassay determination of GLP-1 have been discussed by Holst.[13] C-terminally directed assays, which are the most common, measure both GLP-1 and the metabolite GLP-$1_{9–36amide}$, whereas N-terminally directed assays measure specifically the biologically active GLP-1. If an assay uses two antisera measuring both the C- and N-terminal portions of the peptide, both the secretory rate of GLP-1 as well as the biological active form of GLP-1 will be determined.

Characterization of GLP-1 receptors

The first demonstration of GLP-1 receptors were performed in membranes from insulin producing RINm5F cells, where iodinated GLP-1 is bound to a single class of binding sites and selectively displaced by GLP-1.[30] Later work characterized the receptor molecule by isolating cDNA clones from a rat pancreatic islet library by expression cloning strategy.[8] It was thereby demonstrated that the GLP-1 receptor consists of 463 amino acids and belongs to the superfamily of seven transmembraneous spanning G-protein-coupled receptors, which also includes receptors for glucagon, GIP, VIP (vasoactive intestinal polypeptide), secretin, PACAP (pituitary adenylate cyclase activating polypeptide), and PTH (parathyroid hormone).[8] However, these other peptides do not bind to the GLP-1 receptors, which therefore are specific for the peptide. Interestingly, two peptides isolated from the venom of a Gila monster, the *Heloderma suspectum*, called exendin-4 and exendin-(9–39), exhibit high homology to GLP-1, and have been shown to bind to the GLP-1 receptors with high affinity.[31] Since exendin-4 is a agonist and exendin-(9–39) an antagonist to GLP-1 at the receptors,[31] these peptides are tools for examining the functional role of GLP-1.

In the seven transmembraneous domain type of receptors, the extracellular surface (i.e., the extracellular N-terminal end and the exterior portion of the seven transmembraneous domains) is of importance for binding of the ligand, i.e., for the specificity of the receptors, whereas the intracellular surface of the receptor is of importance for recognition and activation of G-proteins which are of importance for further signalling mechanisms.[32] Following synthesis, the receptor is finally localized in the membrane, and after ligand-receptor interaction, GLP-1 is internalized into the cells.[33]

Identical GLP-1 receptors as in rat islets have been cloned from human islets,[31] and, the receptor is also expressed in the hypothalamus.[34] Expression is also evident in the lung and likely to occur in the liver, in skeletal muscles and in the kidney, although final evidence for this has yet to be presented.[20] In humans, the GLP-1 receptor gene is localized to the long arm of chromosome 6.[35] Alteration in receptor expression might be one mode to the regulation of GLP-1 activity. One study in cultured rat islets has thereby shown that expression of the GLP-1 receptor is inhibited by dexamethasone and slightly increased by glucose, whereas elevation of cyclic AMP appears to be without effect.[36] In contrast, other studies in insulin producing RINm5F cells have shown that agents raising cyclic AMP reduces GLP-1 receptor expression, which would suggest a desensitization mechanism.[20] Hence, more studies are required to establish the regulation of GLP-1 receptor expression.

Effects of GLP-1

GLP-1 exerts a number of actions when administered experimentally in different systems. Its main function is related to regulation of islet function, regulation of gastric motility and gastric acid and pancreatic enzyme secretion, and regulation of food intake.

Stimulation of insulin secretion

GLP-1 potently stimulates insulin secretion in vivo and in vitro in a variety of species including humans[5,37–41] and stimulates

proinsulin gene expression and synthesis.[7] The insulinotropic action is glucose dependent, and requires a glucose threshold of approximately 3 mmol/l.[13,41] This implies that the insulinotropic action of GLP-1 is self-limiting, since after reduction of circulating glucose by the raising insulin concentration, the insulin secretory response of GLP-1 vanishes. Since stimulation of insulin secretion and gene expression is seen also in isolated islets and in insulin producing cells,[7,39] the action is explained by a direct effect on the islet B cells, which are known to express the GLP-1 receptors.[8,31] Furthermore, in parallel with its stimulation of insulin secretion, GLP-1 stimulates also the secretion of IAPP (islet amyloid polypeptide), which is expressed together with insulin in the B cell granules.[42]

The insulinotropic action of GLP-1 seems to be one of its main physiological function. Hence, GLP-1 is thought to act as a socalled *incretin*, which is defined as a gut hormone released during food intake and potentiating glucose-stimulated insulin secretion. Several gastrointestinal hormones have during the recent decades been suggested to be important incretins. Evidence that GLP-1 is, perhaps in conjunction with GIP, the main incretin is that its potentiating action on glucose-stimulated insulin secretion is exerted at circulating concentrations which occur postprandially,[5] that the GLP-1 receptor antagonist, exendin$_{9-39}$, inhibits insulin secretion following intestinal glucose administration in rats,[43] and that GLP-1 receptor knock-out mice have glucose intolerance after oral glucose.[44]

Effects on other islet hormones

GLP-1 also inhibits glucagon secretion[5,9,38,40,45] and stimulates somatostatin secretion,[20,45] but is without influence on the secretion of PP (pancreatic polypeptide).[46] The inhibitory influence on glucagon secretion was previously thought to be indirectly mediated, since glucagon producing cells were thought not to express GLP-1 receptors.[47] However, recent studies have demonstrated expression of GLP-1 receptors also in glucagon secreting A cells.[48] It is therefore possible that the inhibition by GLP-1 of glucagon is executed both through a direct action on the A cells and indirectly through the release of insulin, since insulin inhibits glucagon secretion. It is also possible that GLP-1 inhibits glucagon secretion through an indirect action mediated by somatostatin secretion, since insulin somatostatin secreting cells express GLP-1 receptors.[49]

Effects on carbohydrate metabolism

GLP-1 has been shown to reduce circulating glucose in both humans and animals.[43,50] This might be due to the increased insulin/glucagon ratio (due to stimulation of insulin secretion and inhibition of glucagon secretion), which would inhibit liver glucose production and thereby reduce the circulating levels of glucose. Glucose kinetic studies with tracer technique have also demonstrated both in humans[51] and rats[40] that GLP-1 reduces hepatic glucose production. Consequently, after preventing the islet actions of GLP-1 by means of somatostatin, it has also been shown that the inhibition of liver glucose delivery is prevented.[51] However, a peripheral, islet-independent, mechanism might still contribute to the glucose reducing effect of GLP-1, since it has been shown that GLP-1 increases the insulin-independent glucose-dependent glucose uptake, the socalled glucose effectiveness, which occurs without any effect on insulin sensitivity[52] and an increased clearance of glucose induced by GLP-1 has been inferred from results of the glucose tracer studies, the molecular mechanism of which is not known at present.[51] On the other hand, insulin sensitivity seems not to be altered by GLP-1 as verified by studies with the euglycemic, hyperinsulinemic clamp technique in both healthy subjects and in subjects with diabetes.[53,54] Therefore, GLP-1 seems to reduce circulating glucose mainly by increasing the circulating insulin to glucagon ratio, although contribution by peripheral, insulin-independent, mechanisms can not be excluded.

Inhibition of gastric emptying and gastric acid and pancreatic exocrine secretion

GLP-1 has been demonstrated to inhibit gastric emptying and to inhibit gastric acid and pancreatic enzyme secretion both basally and after stimulation by mixed meal and pentagastrin in humans.[6,55] It has been argued that these effects are centrally mediated, since GLP-1 does not affect gastric acid secretion in vagotomized subjects.[56] Since these effects are exerted at levels of GLP-1 which circulate postprandially,[6,55] it has been argued that GLP-1 physiologically functions as an *enterogastrone* mediating the socalled *ileal brake*, i.e., the endocrine inhibition of secretion and motility in the proximal gastrointestinal tract and pancreas which is induced by nutrients in the ileum.[57] However, more studies are required to examine the potential role of GLP-1 as an enterogastrone.

Inhibition of food intake

A third proposed physiological function of GLP-1 is its central action to inhibit food intake. Background for this hypothesis is that GLP-1 is expressed in neurons in the brain.[12] These nerves originate in the brain stem, mainly in the solitary tract, where they receive afferent information from the gatrointestinal tract, and they project on the hypothalamus.[13,20] Furthermore, GLP-1 receptors have been localized to these areas in the hypothalamus, mainly to nuclei of importance for the regulation of food intake.[13,20,34] Moreover, GLP-1 inhibits food intake when administered in the third cerebral ventricle in rats,[10,58] and this action is inhibited by the GLP-1 receptor antagonist, exendin$_{9-39}$.[58] It may therefore be hypothesized that afferent nerves activated by food intake liberate neuronal, central, GLP-1 to inhibit food intake. The mechanisms responsible for the activation of these afferent nerves are not

known and more studies are also required to examine the mechanism of this action of GLP-1, considering the complex nature of the regulation of food intake. Whether also enteral GLP-1 is involved in this process by activating the afferent nerves liberating central GLP-1 is an intriguing possibility. It should be emphasised that it is not entirely excluded that also circulating GLP-1 might have central effects, since central GLP-1 receptors are localized also in the area postrema and in the subfornical organ at locations which are accessible from the systemic circulation.[59]

Physiology of GLP-1

Figure 4 presents a proposed model of integrated GLP-1 physiology. The model suggests that enteral GLP-1 is released from the gut and from hypothalamic nuclei following food digestion. GLP-1 then exerts a variety of effects. First, it contributes to the postprandial insulin secretion *(incretin)* and the inhibition of gastric emptying and gastric acid secretion seen late after food intake *(enterogastrone)*. Furthermore, GLP-1 also stimulates somatostatin secretion and inhibits glucagon secretion. Finally, mainly through central neuronal, GLP-1 inhibits food intake. The model suggests that GLP-1 is an important gut hormone adapting the organism to the ingested food: it is responding to food digestion and functions as an incretin to help storing the ingested nutrients, as an enterogastrone to inhibit proximal processes digesting the food, and as part of the gut-brain axis to inhibit food intake.

To study the physiology of GLP-1, mice with a targeted disruption of the GLP-1 receptor gene were generated.[44] It was found that blood glucose levels were elevated after oral glucose ingestion, consistent with the function of GLP-1 as an *incretin*. It was interestingly also demonstrated that the glucose tolerance after intraperitoneal administration of glucose was impaired, suggesting that GLP-1 might be involved also in the regulation of insulin secretion independent from food digestion. In contrast, although the GLP-1 receptor disrupted mice did not respond with reduction in food intake following central GLP-1 administration, they had a normal baseline food intake and body weight, suggesting that the food intake is redundantly regulated by a variety of factors and therefore that the GLP-1 receptors are not of vital importance for regulation of food intake.[44]

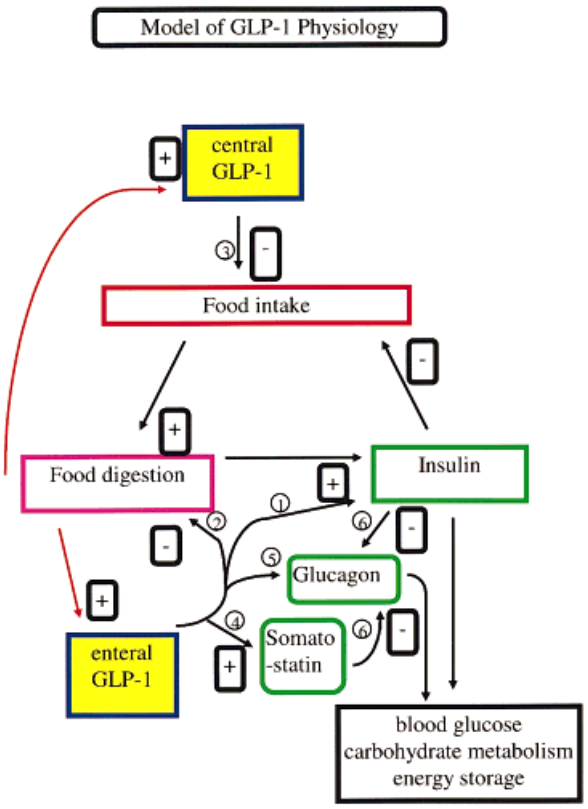

Figure 4. Model for physiology of GLP-1, GLP-1 is released from the gut and hypothalamic neurons following food digestion (red arrows). GLP-1 then exerts three main functions: stimulation of insulin secretion (①, *incretin action)*, inhibition of food digestion (②, i.e., gastric motility, gastric acid secretion, and pancreatic enzyme secretion, *enterogastrone action*), and inhibition of food intake (③), In addition, GLP-1 stimulates somatostatin (④) and inhibits glucagon secretion, the latter effect both through a direct action on the pancreatic A cells (⑤) and indirectly through insulin and somatostatin (⑥). As a net effect, GLP-1 influenoes carbohydrate metabolism and energy storage with a pronounced action to lower circulating glucose.

Signal transduction of GLP-1

The signalling mechanisms induced by activation of the GLP-1 receptors have been most deeply characterized in insulin producing cells. Under normal conditions, the main insulin secretagogue is glucose and several transduction mechanisms are involved in insulin secretion stimulated by glucose. First, glucose is metabolised which increases the cytosolic ratio of ATP/ADP. This depolarises the plasma membrane through closure of ATP-regulated K^+-channels, which causes opening of voltage-sensitive Ca^{2+}-channels and uptake of extracellular Ca^{2+} to raise the cytosolic concentration of Ca^{2+}. Second, glucose induces phosphoinositide hydrolysis, which yields production of diacyl glycerol, activating protein kinase C, and inositol-1,4,5-trisphophate (IP_3), liberating Ca^{2+} from intracellular storage sites. Finally, glucose activates adenylate cyclase, which yields production of cyclic AMP and activation of protein kinase A. These second messengers (cytosolic Ca^{2+}, protein kinase C, and protein kinase A) then activate the exocytotic machinery which releases insulin.

GLP-1 stimulates insulin secretion by potentiating the insulinotropic action of glucose, implying that the action of GLP-1 is glucose-dependent.[13,41] Hence, glucose is permissive for the insulinotropic action of GLP-1, which is explained by GLP-1 potentiating the efficiency of the signaling pathways initiated by glucose. Several of the pathways initiated by glucose have thereby been shown to be potentiated by GLP-1. A main signaling mechanism initiated by GLP-1 receptors is activation of adenylate cyclase which induces formation of cyclic AMP.[7,22] This in turn activates protein kinase A, which therefore is thought to be of major importance for the insulinotropic action of GLP-1. This has been verified by studies showing that pharmacological inhibition of protein kinase A activity impairs the insulinotropic action of GLP-1.[60] However, GLP-1 has also been shown to increase the cytosolic concentration of Ca^{2+}.[61,62] This action is largely inhibited by omission of extracellular Ca^{2+} from the medium or by introducing specific inhibitors of the plasma membrane Ca^{2+} channels, showing that the raised cytosolic Ca^{2+} by GLP-1 is mediated by opening of Ca^{2+} channels and uptake of extracellular Ca^{2+}. This action might be mediated by cyclic AMP activating a nonselective cation channel causing depolarisation through uptake of Na^{+}.[60] Consequently, omission of Na^{+} from the extracellular medium abolishes both the increase in cytosolic Ca^{2} and the stimulation of insulin secretion induced by GLP-1.[61] In addition, GLP-1 has been suggested to promote the closure of the ATP-regulated K^{+}-channels by glucose.[63] The mechanism underlying this effect is still not established. The increase in cytosolic Ca^{2+} induced by GLP-1 has also been shown to be due to liberation of Ca^{2+} from intracellular stores through a Ca^{2+}-induced Ca^{2+} liberation; through the raised cytosolic Ca^{2+} a further raise is induced by release of Ca^{2+} from the intracellular stores by a process inhibited by ryanodine.[64] It should be emphasised that the signalling action of GLP-1 differs between different experimental systems, mainly explained by the use of different insulin producing cells.[62] Interpretations therefore have to be undertaken cautiously.

In contrast to these actions on adenylate cyclase and Ca^{2+}-channels, GLP-1 does not seem to induce phosphoinositide hydrolysis or to potentiate cellular glucose metabolism or glucose utilization.[39,65] Figure 5 shows a proposed model of signalling mechanisms for the potentiating action by GLP-1 on insulin secretion in insulin producing cells, suggesting 1) that GLP-1 activates adenylate cyclase to form cyclic AMP, which activates protein kinase A and enhances the uptake of extracellular Ca^{2+}; 2) that the increased cytosolic Ca^{2+} further liberates Ca^{2+} through a Ca^{2+}-induced Ca^{2+}-liberation; 3) that Na^{+} uptake is of importance for Ca^{2+} uptake, and 4) that the increased cytosolic Ca^{2+} in conjunction with the activated protein kinase A stimulates the translocation and exocytosis of insulin-containing secretory granules.

Pathophysiology of GLP-1

Diabetes

Since GLP-1 is a main incretin factor and a potent insulinotropic hormone, it could be speculated that GLP-1 is involved in the pathogenesis of type 2 diabetes, the hallmark of which is islet dysfunction with impaired insulin secretion. However, the increase in circulating GLP-1 following oral glucose intake is not altered in subjects with impaired glucose tolerance, which precedes the development of diabetes.[23] Furthermore, the insulinotropic action of GLP-1 is preserved in diabetes.[53,66] These findings thus argue against involvement of the peptide in the pathogenesis of type 2 diabetes. In contrast, one study has shown impaired GLP-1 secretion in response to oral glucose in subjects with type 2 diabetes, in spite of normal fasting GLP-1 levels, which could suggest involvement of GLP-1 in the metabolic perturbations of fully developed diabetes.[67]

Dumping syndrome

In the dumping syndrome, GLP-1 has been thought to be pathophysiologically important. Thus, if food reaches the distal intestine very rapidly, for example after gastrectomy, a fast liberation of a large amount of GLP-1 may occur, which might raise GLP-1 levels to 300 pmol/l.[24] This might cause reactive hyperinsulinemia and hypoglycemia.

Endocrine tumors

GLP-1 has also been shown to be expressed in gastroenteropancreatic endocrine tumors, although increased circulating levels of GLP-1 are rarely seen in subjects with those tumors.[68] One interesting feature of the GLP-1 positive tumors is that they seem to exhibit a lower rate of distant metastases and a higher rate of curative resections.[68] The relevance of this finding for tumor biology remains to be established, however.

GLP-1 and treatment of diabetes

The pattern of effects of GLP-1 suggests that it may be used in the treatment of diabetes. Thus, GLP-1 stimulates insulin secretion and inhibits glucagon secretion, delays gastric emptying and exerts, perhaps, a peripheral stimulatory action of glucose uptake. It has therefore during the 1990s been a great deal of interest to develop GLP-1 as a therapeutic modality in diabetes. Intravenous or subcutaneous GLP-1 administration has thus shown that GLP-1 exerts antidiabetogenic action by reducing basal and postprandial glucose concentrations in subjects with type 1 or type 2 diabetes.[9,49,53,66,69,70] In type 2 diabetic subjects, it has also been shown that GLP-1 increases the sensitivity of the B cells for glucose, which is a main defect associated with diabetes.[53] The antidiabetogenic action of the peptide has also been shown to persist during a 7 day study period, suggesting that

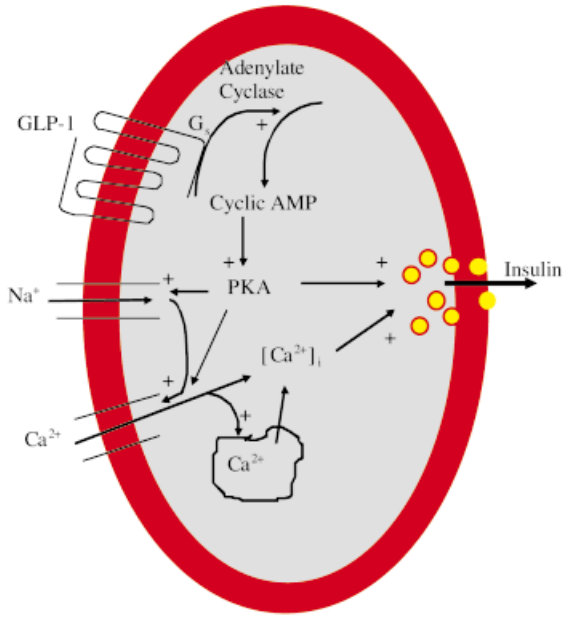

Figure 5. Model for signaling mechanism of GLP-1 in insulin producing cells, GLP-1 activates receptors of the seven transmembrane domain G-protein coupled type. Through activation of adenylate cyclase, cyclic AMP is formed which activates protein kinase A (PKA), PKA increases the uptake of Ca^{2+}, probably partially through a Na^{+} dependent mechanism. The Ca^{2+} uptake increases cytosolic concentration of Ca^{2+} which together with activated PKA stimulates exocytosis of insulin-containing secretory granules, Ca^{2+} also activates Ca^{2+}-dependent release of Ca^{2+} from stores. It has to be emphasized that the insulinotropic action of GLP-1 requires glucose. Thus, GLP-1 is not a secretagogue per se but requires the permissive action of glucose. For simplicity, the action of glucose is not illustrated in Figure.

tachyphylaxis or down regulation of receptors do not occur under these conditions.[71]

One advantage of GLP-1 in comparison with established therapeutic regimens is that the risk of reactive hypoglycemia is small, since the insulinotropic action of GLP-1 is self-limited, due to its glucose dependency, and vanishes when the glucose level is reduced. GLP-1 is also well tolerated, which has been shown in a dose-response study in which adverse effects (nausea, vomiting, chills) were seen only at very high dose levels.[72] A main problem for future studies is that GLP-1 is shortlived; the half life after intravenous administration is only 4 minutes[5] and after subcutaneous administration it is 30–60 minutes.[71] Therefore, attempts are required to prolong its duration, like slow release forms or concomitant administration of inhibitors of its degradation. A second problem is that GLP-1 has to be given as intravenous or subcutaneous administration. To optimise its therapeutic potential, it is therefore of importance to examine other modes of administration. In one such attempt, we have in Malmö used a buccal tablet of 400 µg GLP-1. We found that a sufficient concentration of circulating GLP-1 was achieved after administration of this tablet both in healthy volunteers[73] and in subjects with diabetes.[74] Therefore, this mode of treatment might be developed further for GLP-1 in the treatment of diabetes.

Hence, preliminary, and at present only short-term experiments, have suggested that GLP-1 might be a new therapeutic mode for the treatment of diabetes. However, several problems remain and have to be overcome. These problems include that long-term effects have not been established, that the optimal mode of administration is to be defined, that its entire sequence seems required for agonistic action, making production expensive, and that at present, we lack information on optimal timing and dosing of GLP-1 in relation to meals. Therefore, more studies are warranted to further exploit this potential new treatment modality for diabetes.

Concluding remarks

In only a few years time, a large amount of data has accumulated regarding GLP-1. Thus, GLP-1 has been shown to be the main hormone of the L cells in the gut and to be the main physiological *incretin* hormone and the peptide is probably also a main *enterogastrone*. Furthermore, the importance of cyclic AMP for insulin secretion has been underscored by studies on GLP-1 and, moreover, the importance of cyclic AMP as its second messenger has been established. Finally, GLP-1 represents an interesting attempt of using a physiological gastrointestinal hormone in the potential treatment of diabetes. Hence, GLP-1 represents a peptide which is of interest both for the basic and the clinical researcher.

Acknowledgments

Quoted own studies have been supported by the Swedish Medical Research Council (grant no 14X-6834), Ernhold Lundström, Albert Påhlsson and Novo Nordic Foundations, Swedish Diabetes Association, Malmö University Hospital, and the Faculty of Medicine, Lund University. The study with the buccal tablet was supported by Thera Tech Inc., Salt Lake City, Utah.

References

1 Sutherland S, deDuve C (1948) Origin and distribution of the hyperglycemic glycogenolytic factor of the pancreas *J Biol Chem* 175:663–674.
2 Polak JM, Bloom S, Coulling IU, Pearse AGE (1971) Immunofluorescent localization of enteroglucagon cells in the gastrointestinal tract of the dog. *Gut* 12:311–318.
3 Bell GI, Santerre RF, Mullenbach GT (1983) Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 302:716–718.
4 Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF (1986) Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 261:11880–11889.

5 Kreyman B, Ghatei MA, Williams G, Bloom SR (1987) Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* 2:1300–1303.
6 Wettergren A, Scholdager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ (1993) Truncated GLP-1 (proglucagon 72–107 amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 38:665–673.
7 Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF (1987): Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in rat islet cell line. *Proc Natl Acad Sci USA* 84:3434–3438.
8 Thorens B (1992) Expression cloning of the pancreatic beta cell receptor of the gluco-incretin hormone glucagon-like peptide I. *Proc Natl Acad Sci USA* 89:8641–8645.
9 Gutniak M, Ørskov C, Holst JJ, Ahrén B, Efendic S (1992) Antidiabetogenic effect of glucagon-like peptide-1(7–36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316–1322.
10 Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379: 69–72.
11 Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B (1992) Glucagon-like peptide 1 cells in the gastrointestinal tract and pancreas of rat, pig, and man. *Eur J Clin Invest* 22:283–291.
12 Jin SL, Han VK, Simmons JG, Towle AC, Lauderm JM, Lund PK (1988) Distribution of glucagon-like peptide I (GLP I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J Comp Neurol* 271:519–532.
13 Holst JJ (1997) Enteroglucagon. *Annu Rev Physiol* 57:257–271.
14 White JW, Saunders GF (1986) Structure of the human glucagon gene, *Nucleic Acids Res* 14:4719–4730.
15 Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC (1983) Exon duplication and divergence in the human preproglucagon gene, *Nature* 304:368–371.
16 Novak U, Wilks A, Buell G, McEwen S (1987) Identical mRNA for preproglucagon in pancreas and gut. *Eur J Biochem* 164:553–558.
17 Drucker DJ, Brubaker PL (1989) Proglucagon gene expression is regulated by a cyclic AMP-dependent pathway in rat intestine. *Proc Natl Acad Sci USA* 86:3953–3957.
18 Holst JJ, Hansen L, Deacon CF (1997) The GLP-1 receptor antagonist, GLP-1 (9–36amide), is a primary product of the intestinal L-cell. *Diabetologia* 40:suppl 1, A27.
19 Rouillé Y, Martin S, Steiner DF (1995) Differential processing of proglucagon by the subtilisin-like prohormone convertases PC2 and PC3 to generate either glucagon or glucagon-like peptide. *J Biol Chem* 270:26488–26496.
20 Göke B, Göke R, Fehmann HC, Bode HP (1996) Physiology and pathophysiology of GLP-1, In: Fehmann HC, Göke B (eds): *The Insulinotropic Gut Hormone Glucagon-Like Peptide-1, Frontiers in Diabetes*. Basel: Karger, pp. 275–309.
21 Thornton K, Gorenstein DG (1994) Structure of glucagon-like peptide (7–36)amide in a dodecylphosphocholine micelle as determined by 2D NMR. *Biochemistry* 33:3532–3539.
22 Gefel D, Hendrick GK, Mojsov S, Habener JF, Weir GC (1990) Glucagon-like peptide-I analogs: effects on insulin secretion and adenosine 3′5′-monophosphate formation. *Endocrinology* 126:2164–2168.
23 Ahrén B, Larsson H, Holst JJ (1997) Reduced gastric inhibitory polypeptide but normal glucagon-like peptide 1 response to oral glucose in postmenopausal women with impaired glucose tolerance. *Eur J Endocrinol* 137:127–131.
24 Miholic J, Ørskov C, Holst JJ, Kotzer H, Meyer HJ (1991) Emptying of the gastric substitute, glucagon-like peptide-1 (GLP-1), and reactive hypoglycemia after total gastrectomy. *Dig Dis Sci* 36:1361–1370.
25 Sugiyama K, Manaka H, Kato T, Yamatani K, Tominaga M, Sasaki H (1994) Stimulation of truncated glucagon-like peptide-1 release from the isolated perfused canine ileum by glucose absorption. *Digestion* 55:24–28.
26 Manaka H, Sugiyama K, Hama K, Yamatani K, Sasaki H (1997) Glucose absorption and GLP-1 secretion. *Diabetologia* 40:suppl 1: A129.
27 Brubaker PL (1991) Regulation of intestinal proglucagon-derived peptide secretion by intestinal regulatory peptides. *Endocrinology* 128:3175–3182.
28 Mentlein R, Gallwitz B, Schmidt W (1993) Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1 (7–36)amide, peptide histidine methionine and is responsible for their degradation in serum. *Eur J Biochem* 214:829–835.
29 Ørskov C, Andreasen J, Holst JJ (1992) All products of proglucagon are elevated in plasma from uremic patients. *J Clin Endocrinol Metab* 74:379–384.
30 Göke R, Conlon JM (1988) Receptors for glucagon-like peptide-1(7–36)amide on rat insulinoma-derived cells. *J Endocrinol* 116:357–362.
31 Thorens B, Porret A, Bühler L, Deng SP, Morel P, Widmann C (1993) Cloning and functional expression of the human islet GLP-1 receptor, Demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor. *Diabetes* 42:1678–1682.
32 Wess J (1997) G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. *FASEB J* 11:346–354.
33 Göke R, Richter G, Göke B, Trautmann M, Arnold R (1989) Internalization of glucagon-like peptide-1(7–36)amide in rat insulinoma cells. *Res Exp Med* 189:257–264.
34 Alvarez E, Roncero I, Chowen JA, Thorens B, Blazquez E (1996) Gene expression of the rat glucagon-like peptide-1 (GLP-1) receptor in rat brain. *J Neurochem* 66:920–927.
35 Stoffel M, Espinos III R, Le Beau MM, Bell GI (1993) Human glucagon-like peptide-1 receptor gene, Localization to chromosome band 6p21 by fluorescence in situ hybridization and linkage of a highly polymorphic simple tandem repeat DNA polymorphism to other markers on chromosome 6. *Diabetes* 42:1215–1218.
36 Abrahamsen N, Nishimura E (1995) Regulation of glucagon and glucagon-like peptide-1 receptor messenger ribonucleic acid expression in cultured rat pancreatic islets by glucose, cyclic adenosine 3′5′-monophosphate, and glucocorticoids. *Endocrinology* 135:1572–1578.
37 Holst JJ, Ørskov AC, Schwartz TW, Nielsen OV (1987) Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 211:169–174.
38 Fridolf T, Böttcher G, Sundler F, Ahrén B (1991) GLP-1 and GLP-$1_{(7–36)}$ amide: Influences on basal and stimulated insulin and glucagon secretion in the mouse. *Pancreas* 6:208–215.
39 Fridolf T, Ahrén B (1991) GLP-$1_{(7–36)}$ amide stimulates insulin secretion in rat islets: studies on the mode of action. *Diabet Res* 16:185–191.
40 van Dijk G, Lindskog S, Holst JJ, Steffens AB, Ahrén B (1996) Effects of glucagon-like peptide-I on glucose turnover in rats. *Am J Physiol* 270:E1015–E1021.
41 Ahrén B (1995) Insulinotropic action of truncated glucagon-like peptide-1 in mice. *Acta Physiol Scand* 153:205–206.
42 Ahrén B, Gutniak M (1997) No correlation between insulin and islet amyloid polypeptide (IAPP) after stimulation with glucagon-like peptide-1 (GLP-1) in type 2 diabetes. *Eur J Endocrinol* 137:643–649.
43 Kolligs F, Fehmann HC, Göke R, Göke B (1995) Reduction of the incretin effect in rats by the glucagon-like peptide-1 receptor antagonist exendin (9–39) amide. *Diabetes* 44:16–19.
44 Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ (1996) Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Med* 2:1254–1258.
45 Ørskov C, Holst JJ, Nielsen OV (1988) Effect of truncated glucagon-like peptide-1 (proglucagon 78–107amide) on endocrine secretion from pig pancreas, antrum and stomach. *Endocrinology* 123:2009–2013.
46 Ahrén B, Gingerich RL, Havel PJ (1995) Effects of cholecystokinin and glucagon-like peptide 1 on the secretion of pancreatic polypeptide in mice. *Regul Pept* 59:297–302.
47 Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z, Pipeleers D, Gremlich S, Thorens B, Schuit F (1996) Expresion and functional activity of glucagon, glucagon-like peptide I and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. *Diabetes* 45:257–261.
48 Heller RS, Kieffer TJ, Habener JF (1997) Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing α-cells of the rat endocrine pancreas. *Diabetes* 46:785–791.
49 Fehmann HC, Habener JF (1991) Functional receptors for the insulinotropic hormone glucagon-like peptide-1(7–37) on a somatostatin secreting cell line. *FEBS Lett* 279:335–340.
50 Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W (1993) Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in Type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744.
51 Larsson H, Holst JJ, Ahrén B (1997) Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. *Acta Physiol Scand* 160:413–422.
52 D'Alessio D, Kahn SE, Leusner C, Ensinck JW (1994) Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Endocrinol Metab* 93:2263–2266.
53 Ørskov L, Holst JJ, Møller J, Ørskov C, Møller N, Alberti KGMM, Schmitz O (1996) GLP-1 does not acutely affect insulin sensitivity in healthy man. *Diabetologia* 39:1227–1232.
54 Ahrén B, Larsson H, Holst JJ (1997) Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:473–478.
55 O'Hallorran DJ, Nikou GC, Kreymann B, Ghatei MA, Bloom SR (1990) Glucagon-like peptide-1 (7–36)-NH_2: a physiological inhibitor of gastric acid secretion in man. *J Endocrinol* 126:169–173.
56 Wettergren A, Wøjdemann M, Meisner S, Stadil F, Holst JJ (1997) The inhibitory effect of glucagon-like peptide-1 7–36amide on gastric acid secretion in man depends on an intact vagal innervation. *Gut* 40:597–601.
57 Layer P, Holst JJ (1993) GLP-1: a humoral mediator of the ileal brake in humans? *Digestion* 54:385–386.
58 Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Møller M, Sheikh SP (1996) Central administration of GLP-1-(7–36) amide inhibits food and water intake in rats. *Am J Physiol* 271:R848–R856.
59 Ørskov C, Poulsen SS, Møller M, Holst JJ (1996) Glucagon-like peptide-1 receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide 1. *Diabetes* 45:832–835.
60 Holz GG, Leech CA, Habener JF (1995) Activation of a cAMP-regulated Ca^{2+}-signaling pathway in pancreatic beta-cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem* 270:17749–17757.
61 Fridolf T, Ahrén B (1993) Effects of glucagon-like peptide-1(7–36)amide on the cytoplasmic calcium concentration in rat islet cells. *Mol Cell Endocrinol* 96:85–90.

62 Ahrén B, Simonsson E, Karlsson S (1996) Glucagon-like peptide-$1_{(7-36)}$amide and cytoplasmic calcium in insulin producing cells. *Acta Physiol Scand* 157:333–341.
63 Holz GG, Kühtreiber WM, Habener JF (1993) Pancreatic β-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-I(7–37). *Nature* 361:362–365.
64 Gromada J, Dissing S, Bokvist K, Renström E, Frøkjär-Jensen J, Wulff BS, Rorsman P (1995) Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting βTC3-cells by enhancement of intracellular calcium mobilization. *Diabetes* 44:767–774.
65 Zawalich WS, Zawalich KC, Rasmussen H (1993) Influence of glucagon-like petide-1 on β-cell responsiveness. *Regul Pept* 44:277–283.
66 Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W (1993) Preserved incretin activity of GLP-1 (7–36amide) but not of synthetic human GIP in patients with type 2 diabetes mellitus. *J Clin Invest* 91:301–307.
67 Vaag AA, Holst JJ, Völund A, Beck-Nielsen HB (1996) Gut incretin hormones in identical twins discordant for non-insulin diabetes mellitus (NIDDM) - evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. *Eur J Endocrinol* 135:425–432.
68 Eissele R, Göke R, Weichhardt U, Ferhmann HC, Arnold R, Göke B (1994) Glucagon-like peptide 1 immunoreactivity in gastroentero-pancreatic endocrine tumors: a light- and electron-microscopic study. *Cell Tissue Res* 276:571–580.
69 Nathan DM, Schreiber E, Mojsov S, Habener JF (1992) Insulinotropic action of glucagonlike peptide-1-(7–37) in diabetic and nondiabetic subjects. *Diabetes Care* 15:270–276.
70 Rachman J, Barrow BA, Levy JC, Turner RC (1997) Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* 40:205–211.
71 Juntti-Berggren L, Pigon J, Karpe F, Hamsten A, Gutniak M, Vignati L, Efendic S (1996) The antidiabetogenic effect of GLP-1 is maintained during a 7-day treatment period and improves diabetic dyslipoproteinemia in NIDDM patients. *Diabetes Care* 19:1200–1206.
72 Ritzel R, Ørskov C, Holst JJ, Nauck MA (1995) Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1(7–36 amide) after subcutaneous injection in healthy volunteers, Dose-response relationships. *Diabetologia* 38:720–725.
73 Gutniak MK, Larsson H, Heiber SJ, Juneskans OT, Holst JJ, Ahrén B (1996) Potential therapeutic levels of glucagon-like peptide-1 achieved in humans by a buccal tablet. *Diabetes Care* 19:843–848.
74 Gutniak MK, Larsson H, Saunders SW, Juneskans O, Holst JJ, Ahrén B (1997) GLP-1 tablet in NIDDM in fasting and postprandial conditions. *Diabetes Care* 20:1874–1879.