Glucagon-Like Peptide-1 Synthetic Analogs: New Therapeutic Agents for Use in the Treatment of Diabetes Mellitus

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Abstract: Glucagon-like peptide-1-(7-36)-amide (GLP-1) is a potent blood glucose-lowering hormone now under investigation for use as a therapeutic agent in the treatment of type 2 (adult onset) diabetes mellitus. GLP-1 binds with high affinity to G protein-coupled receptors (GPCRs) located on pancreatic β-cells, and it exerts insulinotropic actions that include the stimulation of insulin gene transcription, insulin biosynthesis, and insulin secretion. The beneficial therapeutic action of GLP-1 also includes its ability to act as a growth factor, stimulating formation of new pancreatic islets (neogenesis) while slowing β-cell death (apoptosis). GLP-1 belongs to a large family of structurally-related hormones and neuropeptides that include glucagon, secretin, GIP, PACAP, and VIP. Biosynthesis of GLP-1 occurs in the enteroendocrine L-cells of the distal intestine, and the release of GLP-1 into the systemic circulation accompanies ingestion of a meal. Although GLP-1 is inactivated rapidly by dipeptidyl peptidase IV (DDP-IV), synthetic analogs of GLP-1 exist, and efforts have been directed at engineering these peptides so that they are resistant to enzymatic hydrolysis. Additional modifications of GLP-1 incorporate fatty acylation and drug affinity complex (DAC) technology to improve serum albumin binding, thereby slowing renal clearance of the peptides. NN2211, LY315902, LY307161, and CJC-1131 are GLP-1 synthetic analogs that reproduce many of the biological actions of GLP-1, but with a prolonged duration of action. AC2993 (Exendin-4) is a naturally occurring peptide isolated from the lizard *Heloderma*, and it acts as a high affinity agonist at the GLP-1 receptor. This review summarizes structural features and signal transduction properties of GLP-1 and its cognate β-cell GPCR. The usefulness of synthetic GLP-1 analogs as blood glucose-lowering agents is discussed, and the applicability of GLP-1 as a therapeutic agent for treatment of type 2 diabetes is highlighted.

Keywords: GLP-1, diabetes mellitus, insulin secretion.

A. INTRODUCTION

Glucagon-like peptide-1-(7-36)-amide (GLP-1, also known as t-GLP-1 or GLIP) is an intestinally-derived insulinotropic hormone that has attracted considerable attention by virtue of its proven ability to act as a blood glucose lowering agent. The efficacy with which GLP-1 lowers concentrations of blood glucose in type 2 diabetic subjects (adult onset diabetes mellitus) has prompted clinical investigations whereby the therapeutic value of GLP-1 as an antidiabetogenic agent has been substantiated. Earlier studies revealed that GLP-1 is an effective stimulator of insulin secretion from pancreatic β-cells located in the islets of Langerhans. More recently, it has become appreciated that GLP-1 also exhibits trophic factor-like properties, acting to stimulate β-cell growth and differentiation. These effects of GLP-1 are complemented by its ability to suppress appetite and to delay gastric emptying. Since GLP-1 is a naturally occurring hormone, and because GLP-1 is not reported to exert deleterious side effects, new efforts are currently under way to develop GLP-1 synthetic analogs that exhibit an optimal pharmacokinetic and pharmacodynamic spectrum of action commensurate with their use as therapeutic agents. The intention of this review is to highlight recent advances in this field and to provide an overview of the potential usefulness of GLP-1 and its synthetic analogs for treatment of type 2 diabetes mellitus. For a detailed discussion of previous studies concerning GLP-1, the reader is referred to earlier in-depth reviews of this subject matter [1-9].

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B. GLP-1 AS A BLOOD GLUCOSE-LOWERING AGENT

The blood glucose-lowering hormone GLP-1 possesses insulinotropic properties that indicate its usefulness as a therapeutic agent in the treatment of diabetes mellitus. When administered by intravenous infusion to type 2 diabetic subjects, GLP-1 stimulates pancreatic insulin secretion and blunts the postprandial hyperglycemic excursion that is typically observed following ingestion of a meal [10-12]. Short term or continuous infusion of GLP-1 restores fasting glycemia in type 2 diabetic subjects [13-19], and available evidence indicates that GLP-1 remains an effective blood glucose lowering agent under conditions in which subjects are not responsive to administered sulfonylureas such as tolbutamide, glyburide, or glipizide [20-22]. Antidiabetogenic actions of GLP-1 in type 2 diabetic subjects are manifest as an improvement of pancreatic β-cell function, as measured by a clear restoration of the missing

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first phase component of glucose-dependent insulin secretion [10,15,19,23]. Also observed is an augmentation of second phase insulin secretion [14] and an increased amplitude of pulsatile insulin secretion [24,25]. Simultaneously, levels of fasting blood glucose are reduced (typically by 4-5 mmol/L) [19]. Such beneficial actions of administered GLP-1 may be a consequence of its ability to compensate for a major deficit of endogenous GLP-1 synthesis, secretion, or metabolism in diabetic subjects [26,27]. The long term therapeutic value of GLP-1 is evident given that it is reported to reduce circulating hemoglobin A_{1c} levels by as much as 1.3% [19].

From the standpoint of its usefulness as a blood glucose lowering agent, an attractive pharmacodynamic property of GLP-1 is that it stimulates insulin secretion only under conditions in which the concentration of blood glucose is elevated. As concentrations of blood glucose fall in response to administered GLP-1, the insulin secretagogue action of GLP-1 is self-terminating [10]. Therefore, unlike administered insulin, a natural safeguard exists whereby GLP-1 is less likely to induce hypoglycemia in type 2 diabetic subjects [28,29]. These antidiabetogenic properties of GLP-1 are observed not only with intravenous infusion, but also following subcutaneous administration [19,23,29,30-33,] or oral administration via a buccal tablet [34].

Although not fully understood, there is evidence that the blood glucose lowering action of GLP-1 is also attributable, at least in part, to its ability to suppress pancreatic glucagon secretion [11,12,23,35]. This action of the hormone reduces hepatic glucose output and may explain, at least in part, an ability of GLP-1 to lower levels of blood glucose in type 1 (juvenile-onset) diabetic subjects [11,36]. Interestingly, the ability of GLP-1 to suppress pancreatic glucagon secretion also appears to be glucose-dependent. Under conditions of hypoglycemia, no suppression is observed [37]. Therefore, available evidence suggests that GLP-1 does not impair the ability of glucagon to act as a counter regulatory hormone in support of hepatic glucose output under conditions of hypoglycemia.

It is important to note that GLP-1 is also an inhibitor of gastrointestinal secretion and motility, not only in healthy individuals [38], but in type 2 diabetic subjects as well [21,31,39]. GLP-1 slows gastric emptying and delays nutrient absorption, actions that are likely to play a major role in determining the effectiveness of GLP-1 as a regulator of blood glucose concentration during the time immediately following ingestion of a meal [40]. The inhibition of gastric emptying by GLP-1 appears to reflect its normal physiological role as an intestinal hormone because new evidence exists that it mediates the "ileal brake" phenomenon [41]. Concomitant with this gastrointestinal action, GLP-1 also acts within the central nervous system as a mediator of satiety [42]. Evidence has been presented that GLP-1 suppresses appetite [43,44], an effect of the hormone that appears to involve hypothalamic appetite control centers where GLP-1 is synthesized [45,46] and where GLP-1 receptors are expressed [46,47].

Although controversial, there is limited evidence that GLP-1 exerts an insulinomimetic action to directly facilitate glucose uptake in peripheral tissues such as liver, fat or muscle. Early on it was recognized that GLP-1 increases glucose disposal in type 1 diabetic subjects [11] and healthy

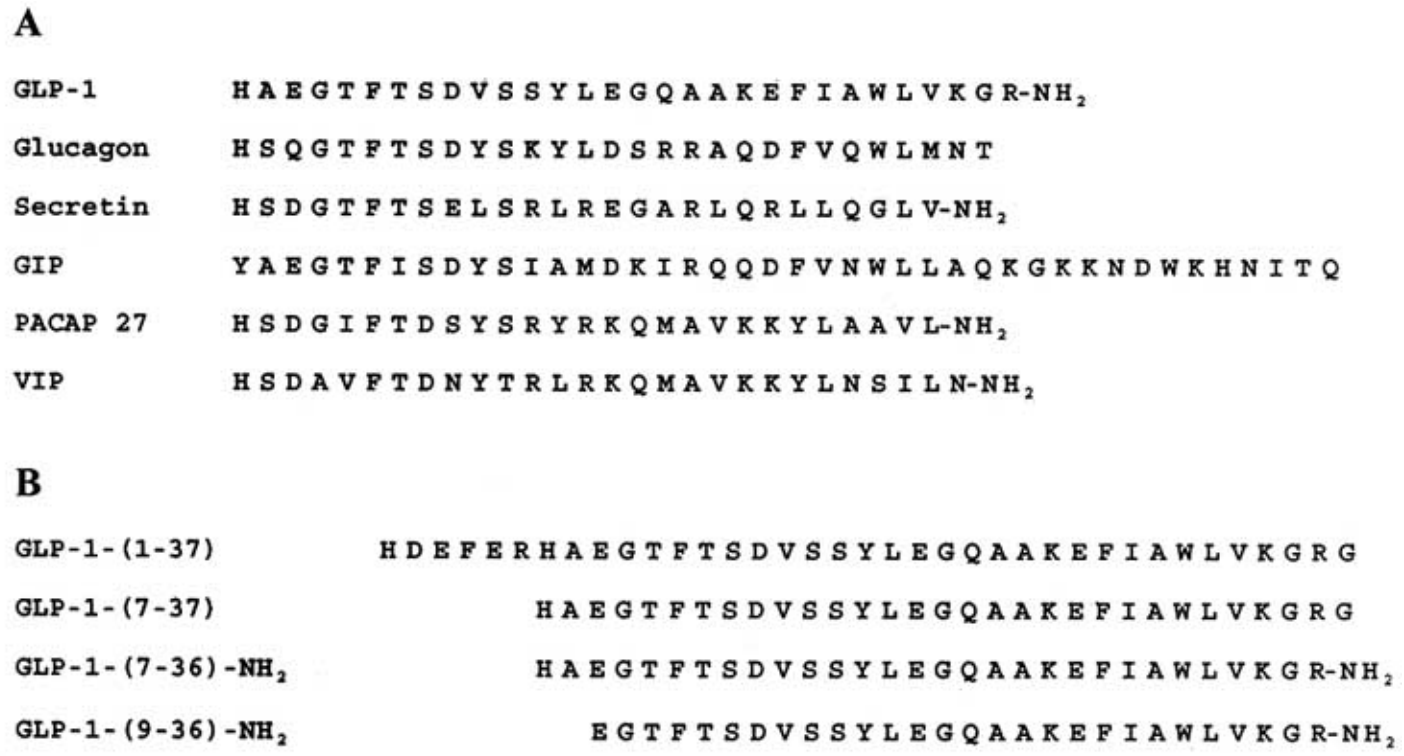

Fig. (1). (A) Alignment of the secretin-like family of peptide hormones including GLP-1-(7-36)-amide. (B) Precursor GLP-1-(1-37) aligned with mature forms of the hormone consisting of GLP-1-(7-37) and GLP-1-(7-36)-amide as well as the immediate degradation product GLP-1-(9-36)-amide.

subject [48], while simultaneously reducing the postprandial glycemic excursion [35,36,49]. Of particular interest is one report that in the OLETF rat model of type 2 diabetes, GLP-1 stimulated uptake of 2-deoxy-D-glucose in skeletal muscle while exerting no effect on hepatic glucose output [50]. Therefore, alterations of glucose disposal in response to GLP-1 may not be explained simply by a suppression of glucagon secretion. This conclusion is supported by a recent report in which GLP-1 was shown to increase insulin sensitivity in depancreatized dogs [51]. Similarly, GLP-1 was reported to increase insulin sensitivity and insulin-mediated glucose uptake in elderly and obese diabetic subjects [52,53]. Despite these observations, no firm conclusion can as yet be made concerning the possible existence of GLP-1 receptors on hepatocytes, adipocytes, or skeletal muscle, and furthermore, such findings have been contradicted by several alternative reports [54-58].

C. GLP-1 AND GLP-1 RECEPTOR-MEDIATED SIGNAL TRANSDUCTION

In order to appreciate exactly how GLP-1 may exert pancreatic and/or extra-pancreatic effects, it is useful to review current concepts concerning GLP-1 biosynthesis and action. GLP-1 is one member of a large family of structurally-related hormones and neuropeptides that include glucagon, secretin, glucose-dependent insulinotropic polypeptide (GIP), pituitary adenylyl cyclase activating polypeptide (PACAP), and vasoactive intestinal polypeptide (VIP) (Fig. **1A**). GLP-1 is synthesized by enteroendocrine L-cells of the distal intestine, and it is released into the systemic circulation concomitant with a meal. Secretion is stimulated by carbohydrates, fat, hormones, and neural reflexes [59-61]. The intestinal L-cells synthesize and process proglucagon such that the principal post translational end product is GLP-1 rather than glucagon. *In vitro* analyses of GLP-1 biosynthesis have been facilitated by the availability of cell lines (GLUTag, NCI-H716) that exhibit regulated secretion of the hormone [62-64]. The specificity of post translational processing within L-cells distinguishes them from pancreatic α-cells where glucagon is the principal end product. The immature form of GLP-1 is GLP-1-(1-37), and it is thought to be biologically inert (Fig. **1B**). Processing of GLP-1-(1-37) generates the biologically active isopeptides GLP-1-(7-37) and GLP-1-(7-36)-amide, with the amidated peptide representing the fully mature form of GLP-1. Circulating levels of GLP-1 rise to the low picomolar range after ingestion of a meal [65], and GLP-1 is rapidly inactivated by serum dipeptidyl peptidase IV (DDP-IV; EC 3.4.14.5) [66-68]. This generates GLP-1-(9-36)-amide, a degradation product devoid of agonist activity at the GLP-1 receptor (Fig. **1B**). No evidence exists demonstrating a significant difference in pharmacological action when comparing GLP-1-(7-37) and GLP-1-(7-36)-amide.

The GLP-1 receptor cDNA was first identified by use of an expression cloning strategy [69]. Subsequently, it was established that GLP-1 receptors are expressed in pancreatic islets of Langerhans, stomach, lung, heart, kidney, and brain [47]. GLP-1 receptors have also been located to vagal sensory afferent nerve endings constituting a hepatoportal vein glucose sensor [70,71]. This pattern of tissue specific expression of receptors underlies the ability of GLP-1 to act as a stimulus for insulin secretion, to slow gastric emptying, and to suppress appetite. The GLP-1 receptor genomic sequence identifies it as a member of Class II (Family B) heptahelical GPCRs. Receptors of this family are structurally-related and recognize GLP-1, glucagon, GIP, secretin, PACAP, VIP, calcitonin, parathyroid hormone (PTH), and corticotropin releasing factor (CRF).

A common feature of Class II GPCRs is their ability to mediate stimulatory actions of peptide hormones on cAMP production, Ca^{2+} signaling, and exocytosis. This is also true for GLP-1 receptors expressed on pancreatic β-cells. By stimulating production of cAMP, GLP-1 acts as a modulator of the β-cell glucose signaling system (Fig.**2**). The GLP-1 receptor is positively coupled to adenylyl cyclase by heterotrimeric G_s proteins [72]. Evidence also exists for coupling of GLP-1 receptors to G_i and $G_{q/11}$ proteins [73]. GLP-1 receptor occupancy stimulates cAMP production, whereas very little evidence exists for a major effect of GLP-1 on β-cell inositol phosphate production. PKA is a down stream effector of the GLP-1 receptor, and it is activated by an increase of $[cAMP]_i$. Evidence exists for the targeting of PKA to specific subcellular compartments via A-kinase anchoring proteins (AKAPs) [74,75], thereby offering one potential explanation for how the cAMP-dependent actions of GLP-1 may be spatially restricted within the β-cell. An additional target of cAMP action is the newly recognized family of cAMP-binding proteins known as cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs, also referred to as *Epac*) [76-79]. The cAMPGEFs couple cAMP production to the activation of Rap1, a small molecular weight G protein. Novel signaling properties of the GLP-1 receptor include its ability to activate immediate early genes (IEGs) [80], to increase the number of insulin receptors on insulin-secreting cells [81,82], to stimulate mitogen-activated protein kinases (p38 MAPK, ERK), phosphatidylinositol 3-kinase [83], atypical protein kinase C-ζ [84], Ca^{2+}/calmodulin-regulated protein kinase [85], protein kinase B (*Akt*), and hormone-sensitive lipase [86]. It seems likely that at least some of these effects result from activation of *Epac* by GLP-1. Available evidence also indicates an important action of GLP-1 to regulate β-cell ion channel function. GLP-1 has been reported to inhibit ATP-sensitive K^+ channels (K-ATP) [87-89] and to facilitate the opening of voltage-dependent Ca^{2+} channels (VDCCs) [3]. Intracellular Ca^{2+} release channels are also targeted by GLP-1 in a cAMP-dependent manner [74,90,91]. They include the type-2 isoform of ryanodine receptor (RYR-2) Ca^{2+} release channels located on the endoplasmic reticulum.

D. GLP-1 AS A β-CELL GLUCOSE COMPETENCE HORMONE

It is now established that GLP-1 acts a competence hormone in support of glucose-dependent insulin secretion [1,87,92,93]. Under conditions in which β-cells are metabolically compromised, GLP-1 acts to restore the sensitivity of these cells to glucose. Since type 2 diabetes mellitus is a metabolic disorder in which β-cells lose their ability to respond to glucose, the induction of glucose competence by GLP-1 may be of major therapeutic

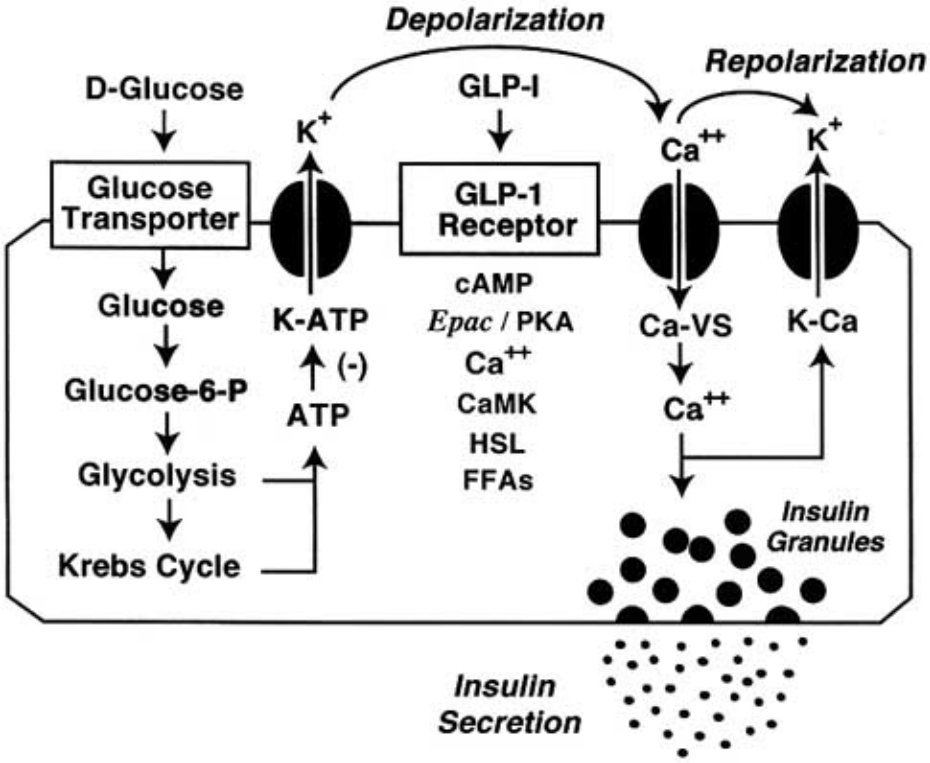

Fig. (2). Illustrated is the role GLP-1 plays as a modulator of the β-cell glucose signaling system. Glucose stimulates insulin secretion, and GLP-1 potentiates the action of glucose by activating multiple signal transduction pathways important to Ca^{2+}-dependent exocytosis. Uptake of glucose is mediated by the type-2 glucose transporter (Glut2) and glucose is converted to glucose-6 phosphate by glucokinase, a type-IV hexokinase that is rate-limiting for β-cell glucose sensing. Aerobic glycolysis generates metabolic coupling factors, one of which is ATP. An increase of the cytosolic ATP/ADP concentration ratio results in the closing of ATP-sensitive K^+ channels (K-ATP), thereby producing membrane depolarization and activation of Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (Ca-VS). Ca^{2+} influx produces an increase of $[Ca^{2+}]_i$ and triggers fusion of insulin secretory granules with the plasma membrane. Repolarization of the membrane is due, in part, to the Ca^{2+}-dependent activation of K^+ channels (K-Ca). GLP-1 modulates this sequence of events via second messengers (cAMP, Ca^{2+}), protein kinase A (PKA), guanine nucleotide exchange factors (*Epac*), Ca^{2+}-calmodulin-regulated protein kinase (CaMK), hormone sensitive lipase (HSL), and lipid metabolites including free fatty acids (FFAs).

importance. Recent studies suggest that glucose competence results from the ability of GLP-1 to act as a glucose-sensitizer. The central locus for this effect appears to be intermediary metabolism where GLP-1 facilitates glucose-dependent mitochondrial ATP production [94]. This key observation provides a clear explanation for why the insulin secretagogue action of GLP-1 in β-cells is entirely glucose-dependent. An initiating event for induction of glucose competence is likely to be the release of Ca^{2+} from intracellular Ca^{2+} stores. This is Ca^{2+}-induced Ca^{2+} release (CICR), and it is stimulated by GLP-1. Available evidence suggests that endoplasmic reticulum-derived Ca^{2+} interacts with cAMP to facilitate mitochondrial oxidative phosphorylation. Intramitochondrial targets of Ca^{2+} include Ca^{2+}-sensitive dehydrogenases, whereas the targets of cAMP may include mitochondrial PKA and *Epac*, although this remains to be determined. Regardless of the precise signal transduction mechanism involved, it is reasonable to hypothesize that the induction of β-cell glucose competence by GLP-1 might contribute to its ability to lower concentrations of blood glucose in type 2 diabetic subjects. Indeed, the restoration of first phase insulin secretion by GLP-1 in diabetic subjects is understandable in view of this hormone's ability to augment glucose-dependent ATP production, thereby closing β-cell K-ATP channels. This action of GLP-1 at K-ATP is analogous to the effect produced by sulfonylureas (tolbutamide, glyburide, glipizide), however it is unique in that it is entirely dependent on metabolism of glucose rather than being the consequence of a direct pharmacological inhibition of K-ATP.

E. THE INSULIN SECRETAGOGUE ACTION OF GLP-1

GLP-1 stimulates pancreatic insulin secretion under conditions in which β-cells are exposed to concentrations of glucose typical of the postprandial state (> 7.5 mM). Insulin secretion occurs in a pulsatile manner, and GLP-1 increases the amplitude of each pulse without changing the pulse frequency [24,25,95]. These effects of GLP-1 are accompanied by increased oscillatory electrical activity of the

islets due to the generation of action potentials [96]. Evidence exists indicating that GLP-1 facilitates both the triggering (first phase) and augmentation (second phase) pathways of glucose-dependent insulin secretion [3]. Stimulatory effects of GLP-1 on insulin secretion appear to be mediated primarily by cAMP [3]. New findings indicate that GLP-1 and cAMP target insulin granule-associated proteins including *Epac* [76] and Rim2 (77), thereby increasing the likelihood that a readily releasable pool of secretory granules will undergo exocytosis in response to an increase of intracellular Ca^{2+} concentration. Simultaneously, GLP-1 appears to stimulate refilling of the readily releasable pool, an effect attributed to the mobilization of a reserve pool of secretory granules [97]. Such direct effects of GLP-1 on the exocytotic secretory apparatus are complimented by the ability of GLP-1 to increase β-cell electrical activity, thereby facilitating influx of Ca^{2+} through VDCCs [87]. Simultaneously, GLP-1 mobilizes Ca^{2+} from intracellular Ca^{2+} stores via CICR, thereby amplifying the exocytosis triggered by Ca^{2+} influx [98]. The Ca^{2+} stores mobilized by GLP-1 appear to correspond to those that are sensitive to ryanodine, caffeine, and thapsigargin [75,98]. Indeed, evidence has been presented that β-cell RYR Ca^{2+} release channels are important intermediaries linking GLP-1-stimulated cAMP production to Ca^{2+} mobilization and the initiation of Ca^{2+}-dependent insulin granule exocytosis [98].

F. STIMULATION OF INSULIN GENE EXPRESSION BY GLP-1

GLP-1 stimulates insulin gene expression by virtue of its ability to increase transcription of the insulin gene while simultaneously stabilizing preproinsulin mRNA [99-103]. GLP-1 also increases translational biosynthesis of proinsulin. These effects of GLP-1 resemble the previously described action of glucose to increase β-cell insulin content. The ability of GLP-1 to stimulate insulin gene transcription has been studied in isolated islets of Langerhans and in a variety of insulinoma cell lines. In general, GLP-1 exerts multiple stimulatory influences on insulin gene promoter activity, as expected given that it activates more than one signal transduction pathway. These signaling pathways converge at the promoter to regulate the function of transcription factors that interact with specific response elements (Fig.**3**). Although a conventional cAMP signaling mechanism involving PKA, CREB, and the insulin gene cAMP response element (CRE) has been suggested to play a significant role in this effect, it now appears more likely that actions of GLP-1 at the CRE are PKA-independent. This conclusion is based on studies of the INS-1 insulin-secreting cell line where it was demonstrated that pharmacological inhibitors of PKA (H-89, KT 5720) failed to block stimulatory actions of GLP-1 at a luciferase reporter incorporating -410 bp of the rat insulin I gene promoter (RIP1-Luc). Interestingly, the action of GLP-1 was shown to be blocked by the serine/threonine protein kinase inhibitor Ro 31-8220, by transfection of INS-1 cells with a dominant negative isoform of CREB (A-CREB), or by introduction of inactivating mutations at the CRE. On the basis of these observations, it was suggested that stimulatory actions of GLP-1 at the CRE are mediated by basic region leucine zipper (bZIP) transcription factor related in structure to CREB, and that the transactivation function of such bZIPs might be upregulated by an Ro 31-8220-sensitive MAPK-activated kinase such as RSK and/or MSK.

Evidence has also been presented that the pancreatic/duodenal homeodomain transcription factor PDX-1 mediates stimulatory actions of GLP-1 at A-elements of the insulin gene promoter. PDX-1 translocates to the nucleus in response to GLP-1, an effect mediated by PKA [104]. Levels of PDX-1 mRNA are increased by GLP-1 [105,106], and binding of PDX-1 to A1 elements of the rat insulin I and II gene promoters is facilitated. The transactivation function of PDX-1 is stimulated by GLP-1 [107], and GLP-1 also stimulates a luciferase reporter incorporating synthetic multimerized E2/A4/A3 elements of RIP1. Given that the A-elements of the insulin gene promoter are established to be mediators of glucose insulinotropic action, they appear to be a locus at which nutrient metabolism and hormonal signal transduction interact.

Recently, it has been suggested that NFAT transcription factors (nuclear factor of activated T-cells) mediate stimulatory effects of GLP-1 on insulin gene transcription [108]. Three NFAT binding sites have been identified in the rat insulin I gene promoter (RIP1), and it was suggested that GLP-1 facilitates binding of NFAT to the promoter in a

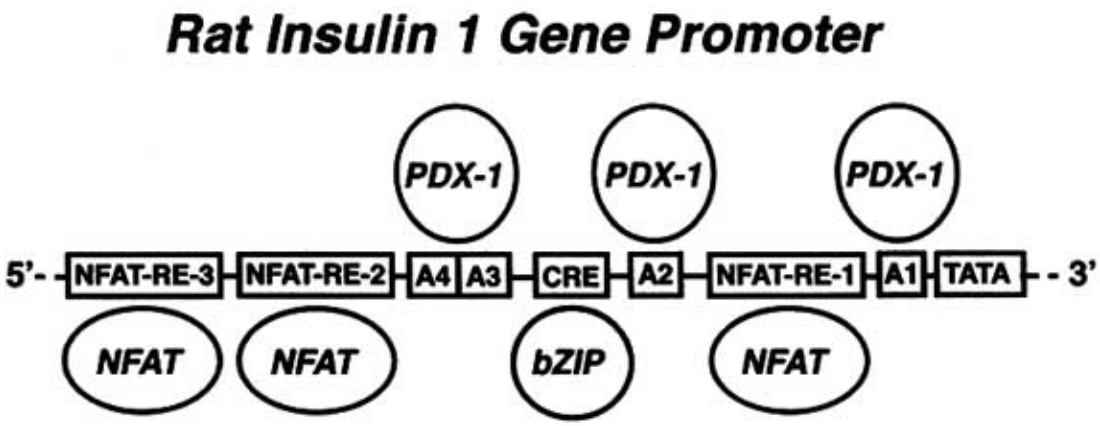

Fig. (3). Response elements and transcription factors that are targeted by GLP-1 for increased insulin gene transcription. Within the first -400 bp of the promoter are found 3 predicted response elements for NFAT (NFAT-RE-1-3). Also present are A4/A3 elements that bind the homeodomain transcription factor PDX-1. A non-palindromic cyclic AMP response element (CRE) mediates stimulatory effects of GLP-1 via basic region leucine zipper (bZIP) transcription factors related in structure to CREB.

cAMP/PKA and Ca^{2+}-dependent manner. Analogous to the previously described stimulatory effect of cAMP and Ca^{2+} at the glucagon gene promoter [109], it was suggested that the cAMP and Ca^{2+}-dependence of GLP-1 action at RIP1 is explained by activation of protein phosphatase 2B (PP2B, calcineurin). In this scheme, PP2B-mediated dephosphorylation of NFAT leads to its nuclear translocation and subsequent stimulation of insulin gene transcription.

G. NEWLY RECOGNIZED GROWTH FACTOR-LIKE EFFECTS OF GLP-1

Substantial evidence now exists that GLP-1 acts as a β-cell growth factor with neogenic [106,110], mitogenic [83,84,111,112] and anti-apoptotic activities [112-114]. Administration of GLP-1 or the GLP-1 analog Exendin-4 to rats increases β-cell mass in a partial pancreatectomy model of type 2 diabetes [110]. This effect has also been observed in rats using the streptozotocin model of diabetes [115], the Goto-Kakizaki model of genetic diabetes [116], or in glucose-intolerant rats of advanced age [117]. Similarly, Exendin-4 and the long lasting GLP-1 derivative NN2211 increase β-cell mass in *db/db* diabetic mice [112,118]. In each of these animal models it has been suggested that the increase of β-cell mass results from the increased differentiation of β-cells from precursor pancreatic ductal stem cells (neogenesis). Surprisingly, the proliferative capacity of preexisting β-cells appears to be stimulated, thereby explaining the mitogenic action of GLP-1. These effects of GLP-1 and Exendin-4 are associated with increased expression of PDX-1, and in fact, the stimulation of PDX-1 expression may be a pivotal event underlying the differentiation of ductal stem cells to β-cells [106,117-123]. Neogenesis in response to GLP-1 or Exendin-4 has been studied *in vitro* using primary cultures of progenitor β-cells. Evidence was presented that GLP-1 and Exendin-4 induce the differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells [122,124]. Findings similar to these were also obtained using primary cultures of rat pancreatic ductal cells [125]. Recently, it has been suggested that GLP-1 acts not only as a growth factor in support of β-cell neogenesis, but also as a morphogen for embryonic development of the pancreas [124,126]. It may be concluded that the *in vivo* therapeutic actions of GLP-1 may include its ability to stimulate pancreatic growth and differentiation in human diabetic subjects where β-cell mass is insufficient to compensate for insulin resistance.

The increase of β-cell mass observed in response to long term (days) treatment with GLP-1 is preceded by a short term (hrs) action of the hormone. This short term effect is referred to as "functional maturation" whereby immature β-cells become competent to secrete insulin in response to glucose. Although functional maturation has been described in studies of fetal human and pig islet-like cell clusters [127,128], it is not clear at the present time whether this phenomenon results from immediate (i.e., within seconds) effects of GLP-1 on intermediary metabolism (as is the case for the induction of Glucose Competence), or alternatively, major alterations in the level of expression of key signaling molecules subserving β-cell glucose signaling (Glut2 transporter, glucokinase, K-ATP channels).

To investigate the mitogenic activity of GLP-1, Buteau and co-workers [129] studied INS-1 insulin-secreting cells that serve as a model system for analyses of β-cell signal transduction. It was reported that the proliferation of INS-1 cells in response to GLP-1 results from "transactivation" of the EGF receptor. In this signaling event, GLP-1 activates c-Src and promotes the release of a soluble EGF receptor ligand (betacelluline), thereby initiating a cascade of protein kinase-mediated phosphorylation reactions catalyzed by PI-3K, atypical PKC-ζ, and PKB, among others [112,129]. These findings are of interest because they suggest a possible usefulness of GLP-1 as a stimulus for the *in vitro* expansion of progenitor β-cells prior to induction of differentiation. Such a strategy might allow large numbers of β-cells to be derived in cell cultures, thereby providing a source of insulin-secreting cells for use in transplantation.

The beneficial action of GLP-1 extends to its ability to slow β-cell apoptosis and to protect against stress. For example, GLP-1 and Exendin-4 protect against β-cell apoptosis resulting from the treatment of mice with streptozotocin [114]. Similarly, both GLP-1 and Exendin-4 protect against cytokine-induced cell death in preparations of rat β-cells [114]. The importance of such findings is emphasized by a recent report demonstrating that the protective action of GLP-1 is evident in Zucker diabetic *fa/fa* rats, a genetic model of obesity-related type 2 diabetes [113]. It is also of interest that GLP-1 appears to exert a cytoprotective action not only in β-cells, but also in neurons [130]. Such findings extend on previous studies demonstrating that PACAP, a neuropeptide related in structure to GLP-1, exerts a neuroprotective effect within the brain [131]. On the basis of these observations, it may be speculated that one unexpected and beneficial therapeutic action of GLP-1 may be its ability to protect against β-cell apoptosis associated with autoimmune destruction (type 1 diabetes) or β-cell exhaustion (type 2 diabetes).

In conclusion, the above summarized actions of GLP-1 are understandable if there exists a compensatory mechanism whereby biosynthesis of GLP-1 is stimulated under conditions in which β-cells are stressed. Evidence that this is in fact the case is provided by one recent report demonstrating that the biosynthesis of GLP-1 is stimulated in rats made diabetic by treatment with streptozotocin [132]. In this animal model of diabetes, increased biosynthesis of GLP-1 occurs secondary to the increased expression of a prohormone converting enzyme (PC1/3) that processes proglucagon to generate GLP-1. Evidently, accelerated biosynthesis of GLP-1 is an evolutionary adaptation to stress, thereby allowing an organism to match intra-islet levels of GLP-1 to the prevailing need for β-cell mitogenesis, neogenesis, and differentiation. Summarized in (Fig.**4**) are the multiple signaling pathways by which GLP-1 influences growth, differentiation, neogenesis, survival, insulin biosynthesis, and insulin secretion in β-cells.

H. GLP-1 SYNTHETIC ANALOGS OBTAINED BY CHEMICAL MODIFICATION

Clinical studies have demonstrated that the blood glucose lowering action of GLP-1 is transient owing to the short plasma half life (1.5-5.0 min) of the peptide. Sustained

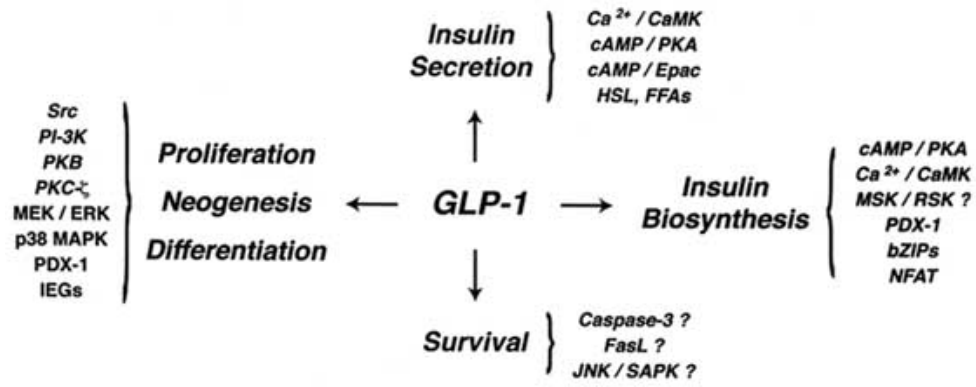

Fig. (4). Summary of the biological actions of GLP-1 in β-cells. Illustrated are the second messengers, kinases, and transcription factors that mediate insulinotropic effects of GLP-1 on insulin biosynthesis and secretion. Also illustrated are the growth factor-like actions of GLP-1 that are important to proliferation, neogenesis, differentiation, and survival of β-cells.

lowering of blood glucose concentration is only observed with continuous infusion, as demonstrated in studies in which GLP-1 was administered by intravenous infusion over a 24 hr time course [22]. Clearly, this mode of administration is not compatible with general use by the public. To address this short coming, efforts have been directed to engineer long lasting analogs of GLP-1 that are synthetic peptides and which exhibit insulinotropic and blood glucose lowering activities *in vivo*. These peptides include NN2211, LY315902, and CJC-1131 (Fig.**5**). The two principal strategies rely on conferring DPP IV resistance and reduced plasma clearance of the peptides. With intravenous administration of GLP-1, the half-life of circulating GLP-1 is only 1.5-5.0 min in humans (Table 1). In contrast, the half-life of Exendin-4, NN2211, and CJC-1131 when administered intravenously is 26 min, 8 hr, and 18 hr, respectively (Table 1). The duration of action of GLP-1 and its synthetic analogs is dramatically extended by use of subcutaneous administration (Table 1).

Resistance to DPP IV has been achieved by amino acid substitutions introduced at or near the NH_2-terminus to prevent enzymatic conversion of GLP-1-(7-36)-amide to GLP-1-(9-36)-amide. Typically, these substitutions have been introduced at position 8 of GLP-1 such that the alanine residue is converted to alpha-aminoisobutyric acid, glycine, serine, or threonine [133]. Such compounds retain their ability to bind to the GLP-1 receptor, are capable of stimulating pancreatic insulin secretion [133,134], and correct fasting hyperglycemia in diabetic mice [135]. Modifications introduced at the NH_2-terminus histidine residue have also been studied. For example, NH_2-terminal glycation to generate His(7)-glucitol-GLP-1 was shown to

Primary Sequences

GLP-1-(7-37)	H A E G T F T S D V S S Y L E G Q A A -Lys26- E F I A W L V -Lys34- G R G
NN2211	H A E G T F T S D V S S Y L E G Q A A -Lys26*- E F I A W L V -Arg34- G R G
CJC-1131	H A E G T F T S D V S S Y L E G Q A A -Lys26- E F I A W L V -Lys34- G R-Lys37*
LY315902	des-H A E G T F T S D V S S Y L E G Q A A -Arg26- E F I A W L V -Lys34*- G R G
AC2933	H G E G T F T S D L S K Q M E E E A V -Arg20- L F I E W L K N G G P S S G A P P P S

Modified Lysine Residues

NN2211	Lys26-(*N*-ε-(γ-Glu(*N*-α-hexadecanoyl)))
CJC-1131	Lys37[2-[2-[2-maleimidopropionamido(ethoxy)ethoxy]acetamide
LY315902	Lys34-(octanoyl)

Fig. (5). (*Top*) Alignment of GLP-1-(7-37) and related synthetic analogs including NN2211, LY315902, CJC-1131, and AC2933 (exendin-4). The lysine residue at position 26 of NN2211 or at position 34 of LY315902 is modified by acylation to generate hexadecanoyl or octanoyl side chains, respectively. In NN2211 lysine residue 34 of GLP-1 is substituted by arginine. In LY315902 the histidine residue at the N-terminus does not contain an amino group (des-His), whereas the lysine residue at position 26 is substituted by arginine. CJC-1131 contains a D-alanine substituted for L-alanine that is normally found at position 8 of GLP-1. It also contains an additional lysine residue that extends from the arginine at position 36 of GLP-1. The lysine contains a reactive chemical linker at its ε amino group. (*Bottom*) Indicated are lysine residues (*) that are modified as shown.

Table 1. Reported Half-Life Of GLP-1 and Its Analogs *In Vivo*

Analog	Modifications	Species	Route of Administration	Half Life	Refs. Cited
GLP-1		Humans	S/C	1 - 1.5 hrs	[30]
GLP-1		Humans	I/V	1.5 - 5 min	[30]
GLP-1		Pigs	S/C	1 hrs	[140]
Exendin-4 AC2933	See Fig. 5 for structure	Humans	I/V	26 min	[156]
NN2211	Lys34Arg substitution; Lys26 modification with fatty acid moiety	Rats	S/C	4 hrs	[118]
NN2211		Pigs	S/C	14 hrs	[140]
NN2211		Humans	S/C	12 hrs	[142]
NN2211		Humans	S/C	10 hrs	[163]
NN2211		Humans	I/V	8 hrs	[118]
CJC-1131	L-Ala8 to D-Ala8 substitution; addition of the Lys37 modified with attached chemical linker	Rats	I/V	18 hrs	[164]
LY315902	Lys26Arg substitution; Lys34 modification with fatty acid moiety; desamidation His7	Dogs	S/C	3 - 6 hrs	[146]
LY307161SR	N/A	Dogs	S/C	18 hrs	[165]

Abbreviations: S/C - subcutaneous; I/V - intravenous; N/A - no available data

generate a peptide that exhibited DPP IV resistance, insulin secretagogue activity, and which lowered concentrations of blood glucose in rats [136]. Additional NH_2-terminus modifications that have been studied include N-methylation, α-methylation, des amidation, and imidazole lactic acid substitution [137]. These compounds retain their ability to bind to the GLP-1 receptor, and are also reported to stimulate cAMP production in an insulinoma cell line, thereby suggesting a possible *in vivo* insulinotropic activity that has yet to be fully evaluated. Another novel strategy utilized the insertion of a 6-aminohexanoic acid moiety between the His(7) and Ala(8) positions of GLP-1 to generate GLP-1 Aha(8) [138]. This compound stimulated cAMP production, insulin secretion, and lowered blood glucose concentration in Zucker *fa/fa* rats. Finally, it has been reported that substitution of L-alanine with D-alanine at position 8 of GLP-1 generates a DPP IV resistant peptide with insulinotropic and blood glucose lowering activities in *db/db* mice [139].

Midchain modifications of GLP-1 have also been investigated with the expectation that such substitutions might improve the duration of action, potency, or efficacy. These expectations are based on the belief that the overall conformation of the GLP-1 peptide is strongly influenced by the midchain tertiary structure. Analogs investigated include those with multiple alanine substitutions introduced between positions 14 and 22 of GLP-1, some of which provided modest increases of biological activity [139]. Modifications introduced close to or immediately at the GLP-1 C-terminus include amino acid substitutions, fatty acid acylations, and introduction of reactive groups by drug affinity complex (DAC) chemistry. In NN2211 [118, 140-145] an Arg substitution is introduced at Lys(34). In LY315902 [146] the Arg for Lys substitution occurs at position 26 and a des His(7) modification is also introduced at the peptide's NH_2-terminus. Both NN2211 and LY315902 incorporate fatty acid acylations. In NN2211 a hexadecanoic acid (C16) is introduced as a side chain on Lys(26), whereas in LY315902 the side chain is comprised of octanoic acid (C8) at Lys(34). Fatty acylation confers to each peptide an ability to non-covalently bind via hydrophobic interactions with serum albumin, thereby slowing renal clearance and dramatically extending the circulating half-life from 4-5 min to 10 hours or more [140,146]. Similarly, a new method by which to extend the half life of GLP-1 is to use DAC chemistry. CJC-1131 is a peptide that contains a C-terminal reactive group that confers covalent binding to serum albumin, and which was recently demonstrated to lower blood glucose, delay gastric emptying, and reduce appetite when tested in dogs, rats, and mice [147-149]. Surprisingly, GLP-1 is able to bind to its receptor and to exert agonist activity when covalently bound to albumin in this manner. Finally, an additional GLP-1 analog with a prolonged duration of action is LY307161 SR. It is administered as a sustained release formulation and is effective as a blood glucose lowering agent in type 2 diabetic subjects [150,151]. However, the structure of LY307161 SR is as yet undisclosed.

I. EXENDIN-4: A NATURALLY OCCURRING GLP-1 ANALOG

Long lasting synthetic analogs of GLP-1 are not restricted to peptides incorporating modifications introduced by chemical engineering. A naturally occurring DPP IV

Table 2. Reported Actions Of Exendin-4 *In Vivo*

Experimental Model	Treatment Modality	Plasma Insulin	Plasma Glucose	Other Effects	Refs. Cited
db/db Mice	Single Dose Acute	Increase (fasting)	Decrease (fasting)	---------	[157]
ob/ob Mice	Single Dose Acute			---------	**[157]**
Diabetic Rhesus Monkeys	Single Dose Acute	--------	Decrease (fasting)	---------	[157]
ZDF rats	Single Dose Chronic	--------	---------	HbA1c Decrease BW Decrease	[157]
db/db Mice	Single Dose Chronic	Increase (fasting)	Decrease (fasting)	HbA1c Decrease	[166]
Partial Pancrea-tectomized Rats	Single Dose Chronic	--------	Decrease (postprandial)	β-cell Mass Increase	[110]
ZDF Rats	Single Dose Chronic	Increase (fasting & IPGTT)	Decrease (fasting)	HbA1c Decrease BW Decrease	[167]
STZ-Treated Newborn Rats	Single Dose Chronic	--------	Decrease (fasting)	β-cell Mass Increase	[115]
Healthy Volunteers	Single Dose Acute	NE (fasting) Increase (postprandial)	Decrease (fasting & postprandial)	Caloric Intake Decrease	[156]
Type 2 Diabetic Patients	Single Dose Acute	Increase (fasting & HGC)	--------	--------	[157]
Type 2 Diabetic Patients	Single Dose Chronic	--------	Decrease	HbA1c Decrease	[168]

Abbreviations: HbA1c - hemoglobin A_{1c}; BW - body weight; IPGTT - intraperitoneal glucose tolerance test; IVGTT - intravenous glucose tolerance test; OGTT - oral glucose tolerance test; NE - no effect; HGC - hyperglycemic clamp; EGC - euglycemic clamp; STZ - streptozocin

resistant GLP-1 analog with potent blood glucose lowering properties was originally identified in extracts of Gila monster lizard (*Heloderma suspectum*) salivary glands, and it is known as Exendin-4 because it was demonstrated to interact with receptors located on exocrine (EX-endin) and endocrine (ex-END-in) tissues of the pancreas [152,153]. The specific interactions of Exendin-4 (an agonist form) and Exendin-(9-39) (an antagonist form) with the GLP-1 receptor was demonstrated [154], and more recently it has become appreciated that Exendin-4 recapitulates many if not all of the biological actions of GLP-1 [155]. These GLP-1-like actions of Exendin-4 are understandable because it retains within its primary sequence amino acid residues at positions His7, Gly10, Phe12, Thr13, Ser14, Asp15, Phe28, and Ile29 that are critical to receptor binding and receptor stimulation [72,156].

Table 3. Reported Actions Of NN2211 *In Vivo*

Experimental Model	Treatment Modality	Plasma Insulin	Plasma Glucose	Other Effects	Refs. Cited
ob/ob Mice	Multiple Dose Acute	Increase (fasting)	Decrease (fasting)	Food Intake Decrease	[118]
db/db Mice	Single Dose Chronic	Increase (fasting)	Decrease (fasting)	β-cell Mass Increase	[118]
Glucose-Intolerant Pigs	Single Dose Acute	Increase (HGC)	---------	Glucagon Decrease (HGC)	[143]
Glucose-Intolerant Pigs	Single Dose Chronic	Increase (OGTT)	---------	Gastric Emptying Decrease	[143]
Diet Induced Obese Rats	Single Dose Chronic	Increase (fasting)	Decrease (fasting & OGTT)	BW Decrease	[145]
Healthy Volunteers	Multiple Dose Escalation	NE	Decrease (postprandial)	Dose-Dependent Side Effects Increase	[142]
Healthy Volunteers	Multiple Dose Escalation	Increase (IVGTT)	NE	Glucagon NE	[169]
Type 2 Diabetic Patients	Single Dose Acute	Increase (fasting & postprandial)	Decrease (fasting & postprandial)	Glucagon Decrease (postprandial)	[163]

Table 4. Reported Actions Of LY307161 *In Vivo*

Experimental Model	Treatment Modality	Plasma Insulin	Plasma Glucose	Other Effects	Refs. Cited
Dogs	Single Dose Acute	Increase (HGC)	---------	---------	[165]
Dogs	Single Dose Acute	NE (EGC)	---------	---------	[165]
Type 2 Diabetic Patients	Single Dose Chronic	---------	Decrease (fasting)	BW Decrease	[151]
Type 2 Diabetic Patients	Single Dose Chronic	---------	Decrease (fasting & postprandial)	---------	[150]

Remarkably, acute dose-response studies in *db/db* and *ob/ob* mice demonstrate that Exendin-4 is approximately 5,500 fold more potent as a blood glucose lowering agent than GLP-1 when comparing the amounts of peptides administered intraperitoneally [157]. This extreme potency of Exendin-4 does not reflect a major increase in its affinity for the GLP-1 receptor, but instead most likely reflects the increased concentrations of Exendin-4 achieved in the blood in the absence of significant DPP IV hydrolysis. When administered to healthy individuals or type 2 diabetic subjects, Exendin-4 reduces fasting and postprandial glucose concentrations [158,159]. AC2993 is synthetic Exendin-4 (Fig.**5**), and it is under clinical investigation for use as a blood glucose lowering agent [160-162]. Exendin-4 is also reported to suppress appetite while simultaneously slowing gastric emptying [158]. This has prompted interest in the possible use of AC2993 as an anti-obesity agent. Finally, the reported ability of Exendin-4 to promote β-cell neogenesis, growth, and differentiation [110] suggests a possible usefulness of AC2993 as a stimulus for pancreatic regeneration.

J. SUMMARY OF THE THERAPEUTIC POTENTIAL OF GLP-1 SYNTHETIC ANALOGS

Tables 2-5 summarize the reported actions of Exendin-4, NN2211, LY315902, and CJC-1131 when tested *in vivo*.

Table 5. Reported Actions Of CJC-1131 *In Vivo*

Experimental Model	Treatment Modality	Plasma Insulin	Plasma Glucose	Other Effects	Refs. Cited
Dogs Rats	Single Dose Chronic & Escalating Dose	---------	---------	BW Decrease Food Intake Decrease	[147]
db/db Mice	Single Dose Acute	---------	Decrease (OGTT)	---------	[148]
GLP-1R$^{-/-}$ Mice	Single Dose Acute	NE (glucose- stimulated)	NE	---------	[148]
db/db Mice	Single Dose Chronic	NE (glucose-stimulated)	Decrease (OGTT & IPGTT)	Increase Pro insulin mRNA	[148]
Wistar Rats CD1 Mice	Single Dose Acute	Increase (OGTT & IPGTT)	Decrease (OGTT & IPGTT)	---------	[149]
C57BL/6 Mice	Single Dose Chronic	----------	Decrease (fasting & postprandial)	---------	[149]
C57BL/6 Mice	Single Dose Acute	----------	Decrease (OGTT)	---------	[170]
db/db Mice	Single Dose Acute	----------	Decrease (fasting & OGTT)	---------	[170]
C57BL/6 Mice	Twice Daily Chronic	----------	Decrease (fasting,OGTT, IPGTT)	BW Decrease Increase: Islet Area, Islet Number, Rates of Islet Cell Replication	[170]
db/db Mice	Twice Daily Chronic	----------	Decrease (fasting,OGTT, IPGTT)	Increase: Proinsulin mRNA, Islet Area, Islet Number, Rates of Islet Cell Replication	[170]

Listed are the experimental models, treatment modalities, effects of the analogs on plasma insulin and plasma glucose, as well as a variety of other effects including alterations of HbA_{1C}, body weight, plasma glucagon, food intake, gastric emptying, β-cell mass, and pancreatic insulin content. As is the case for GLP-1, administration of Exendin-4, NN2211, LY315902, and CJC-1131 is found to lower concentrations of blood glucose in healthy individuals as well as type 2 diabetic patients. These effects are associated with an increase of plasma insulin and a decrease of plasma glucagon only when levels of blood glucose are elevated, as does occur in the postprandial state or under conditions of chronic hyperglycemia. These effects of the GLP-1 analogs contrast with the lack of efficacy of the closely related incretin hormone GIP which is not an effective blood glucose lowering agent in type 2 diabetic subjects [10,30].

CONCLUSION

Although the future of GLP-1 synthetic analogs appears bright, several hurdles remain to be overcome. Appropriate routes of administration that do not require the use of injectable formulations are highly desirable. Possible alternative routes of administration include slow release formulations based on the transdermal absorption of peptides from a cutaneous patch or via an inhalational route. Uncertainty also exists as to the potential usefulness of these synthetic peptides in combination drug therapy. Possible approaches might take into account the combined administration of GLP-1-based synthetic peptides along with insulin sensitizers or secretagogues such as metformin, thiazolidinediones, or sulfonylureas. One exciting prospect that remains to be validated is the possible usefulness of GLP-1-based synthetic peptides not only as insulinotropic agents for use in the treatment of type 2 diabetes, but also as insulinomimetic agents active in type 1 diabetic subjects. Finally, it should be noted that there is the potential to develop small molecular weight non-peptide-based therapeutic agents with agonist activity at the GLP-1 receptor.

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REFERENCES

[1] Holz, G.G.; Habener, J.F. *Trends Biochem. Sci.,* **1992,** *17,* 388-93.
[2] Holst, J.J. *Annu. Rev. Physiol.,* **1997,** *59,* 257-71.
[3] Gromada, J.; Holst, J.J.; Rorsman, P. *Pflugers Arch.,* **1998,** *435,* 583-94.
[4] Ahren, B. *Bioessays,* **1998,** *20,* 642-51.
[5] Nauck, M.A. *Acta Diabetol.,* **1998,** *35,* 117-29.
[6] Kieffer, T.J.; Habener, J.F. *Endocr. Rev.,* **1999,** *20,* 876-913.
[7] Doyle, M.E.; Egan, J.M. *Recent Prog. Horm. Res.,* **2001,** *56,* 377-99 .
[8] Habener, J.F. Current Opinion In Endocrinology & Diabetes, **2001,** 8, 74-81.
[9] Drucker, D.J. *Gastroenterology,* **2002,** *122,* 531-44.
[10] Nathan, D.M.; Schreiber, E.; Fogel, H.; Mojsov, S.; Habener, J.F. *Diabetes Care*, **1992,** *15*, 270-6.
[11] Gutniak, M.K.; Orskov, C.; Holst, J.J.; Ahren, B.; Efendic, S. *N. Engl. J. Med.*, **1992,** *326,* 1316-22.
[12] Nauck, M.A.; Heimesaat, M.M.; Orskov, C.; Holst, J.J.; Ebert, R.; Creutzfeldt, W. *J. Clin. Invest*., **1993a,** *91,* 301-7.
[13] Nauck, M.A.; Kleine, N.; Orskov, C.; Holst, J.J.; Willms, B.; Creutzfeldt, W. *Diabetologia*, **1993b,** *36,* 741-4.
[14] Rachman, J.; Gribble, F.M.; Barrow, B.A.; Levy, J.C.; Buchanan, K.D.; Turner, R.C. *Diabetes*, **1996,** *45,* 1524-30.
[15] Rachman, J.; Barrow, B.A.; Levy, J.C.; Turner, R,C. *Diabetologia*, **1997,** *40,* 205-11.
[16] Willms, B.; Idowu, K.; Holst, J.J.; Creutzfeldt, W.; Nauck, M.A. *Exp. Clin. Endocrinol. Diabetes*, **1998,** *106,* 103-7.
[17] Nauck, M.A.; Weber, I.; Bach, I.; Richter, S.; Orskov, C.; Holst, J.J.; Schmiegel, W. *Diabet. Med.*, **1998,** *11,* 973-45.
[18] Toft-Nielsen, M.B.; Madsbad, S.; Holst, J.J. *J. Clin. Endocrinol. Metab.,* **2001,** *86,* 3853-60.
[19] Zander, M.; Madsbad, S.; Madsen, J.L.; Holst, J.J. *Lancet,* **2002,** *359,* 824-30.
[20] Nauck, M.A.; Sauerwald, A.; Ritzel, R.; Holst, J.J.; Schmiegel, W. *Diabetes Care*, **1998**, *21,* 1925-31.
[21] Schirra, Leicht, P.; Hildenbrand, P.; Beglinger, C.; Arnold, R.; Goke, B.; Katschinski, M. *J. Endocrinol.,* **1998,** *156,* 177-86.
[22] Larsen, J.; Hylleberg, B.; Ng, K.; Damsbo, P. *Diabetes Care*, **2001,** *24*, 1416-21.
[23] Todd, J.F.; Edwards, C.M.; Ghatei, M.A.; Mather, H.M.; Bloom, S.R. *Clin. Sci. (Lond),* **1998,** *95,* 325-9. .
[24] Ritzel, R.; Schulte, M.; Porksen, N.; Nauck, M.S.; Holst, J.J.; Juhl, C.; Marz, W.; Schmitz, O.; Shmiegel, W.H.; Nauck, M.A. *Diabetes*, **2001,** *50*, 776-84.
[25] Juhl, C.B.; Schmitz, O.; Pincus, S.; Holst, J.J.; Veldhuis, J.; Porksen, N. *Diabetologia*, **2000,** *43*, 583-8.
[26] Lugari, R.; Dell'Anna, C.; Ugolotti, D.; Dei Cas, A.; Barilli, A.L.; Zandomeneghi, R.; Marani, B.; Iotti, M.; Orlandini, A.; Gnudi, A. *Horm. Metab. Res.,* **2000,** *32,* 424-8.
[27] Vilsboll, T.; Krarup, T.; Deacon, C.F.; Madsbad, S.; Holst, J.J. *Diabetes,* **2001,** *50*, 609-13.
[28] Qualmann, C.; Nauck, M.A.; Holst, J.J.; Orskov, C.; Creutzfeldt, W. *Acta Diabetol.,* **1995,** *32,* 13-6.
[29] Vilsboll, T.; Krarup, T.; Madsbad, S.; Holst, J.J. *Diabet. Med.,* **2001,** *18*, 144-9.
[30] Gutniak, M.K.; Linde, B.; Holst, J.J.; Efendic, S. *Diabetes Care*, **1994,** *17*, 1039-44.
[31] Nauck, M.A.; Wollschlager, D.; Werner, J.; Holst, J.J.; Orskov, C.; Creutzfeldt, W.; Willms, B. *Diabetologia*, **1996,** *39,* 1546-53.
[32] Todd, J.F.; Wilding, J.P.; Edwards, C.M.; Khan, F.A.; Ghatei, M.A.; Bloom, S.R. *Eur. J. Clin. Invest.,* **1997,** *27,* 533-6. .
[33] Edwards, C.M.; Todd, J.F.; Ghatei, M.A.; Bloom, S.R. *Clin. Sci. (Lond)*, **1998,** *95,* 719-24.
[34] Gutniak, M.K.; Larsson, H.; Sanders, S.W.;. Juneskans, O.; Holst, J.J.; Ahren, B. *Diabetes Care*, **1997,** *20,* 1874-9.
[35] Creutzfeldt, W.O.; Kleine, N.; Willms, B.; Orskov, C.; Holst, J.J.; Nauck, M.A. *Diabetes Care,* **1996,** *19,* 580-6.
[36] Dupre, J.; Behme, M.T.; Hramiak, I.M.; McFarlane, P.; Williamson, M.P.; Zabel, P.; McDonald, T.J. *Diabetes,* **1995,** *44,* 626-30.
[37] Nauck, M.A.; Heimesaat, M.M.; Behle, K.; Holst, J.J.; Nauck, M.S.; Ritzel, R.; Hufner, M.; Schmiegel, W.H. *J. Clin. Endocrinol. Metab.*, **2002,** *87,*1239-46.
[38] Naslund, E.; Bogefors, J.; Skogar, S.; Gryback, P.; Jacobsson, H.; Holst, J.J.; Hellstrom, P.M. *Am. J. Physiol.,* **1999,** *277(3 Pt 2),* R910-6.
[39] Willms, B.; Werner, J.; Holst, J.J.; Orskov, C.; Creutzfeldt, W.; Nauck, M.A. *J. Clin. Endocrinol. Metab.*, **1996,** *81,* 327-32.
[40] Gutniak, M.K.; Svartberg, J.;. Hellstrom, P.M.; Holst, J.J.; Ahren, N.; Ahren, B. *J. Intern. Med.*, **2001,** *250,* 81-7.
[41] Nauck, M.A. *Diabetologia,* **1999,** *42,* 373-9.
[42] Gutzwiller, J.P.; Drewe, J.; Goke, B.; Schmidt, H.; Rohrer, B.; Lareida, J.; Beglinger, C. *Am. J. Physiol.,* **1999,** *276(Pt 2),* R1541-4.
[43] Turton, M.D.; O'Shea, D.; Gunn, I.; Beak, S.A.; Edwards, C.M.; Meeran, K.; Choi, S.J.; Taylor, G.M.; Heath, M.M.; Lambert, P.D.; Wilding, J.P.; Smith, D.M.; Ghatei, M.A.; Herbert, J.; Bloom, S.R. *Nature,* **1996,** *379,* 69-72.
[44] Toft-Nielsen, M.B.; Madsbad, S.; Holst, J.J. *Diabetes Care,* **1999,** *22,* 1137-43.
[45] Larsen, P.J.; Tang-Christensen, M.; Holst, J.J.; Orskov, C. *Neuroscience,* **1997,** *77,* 257-70.

[46] Merchenthaler, I.; Lane, M.; Shughrue, P. *J. Comp. Nuerol.,* **1999,** *403,* 261-80.

[47] Bullock, B.P.; Heller, R.S.; Habener, J.F. *Endocrinology*, **1996,** *137,* 2968-78.

[48] D'Alessio, D.A.; Kahn, S.E.; Leusner, C.R.; Ensinck, J.W. *J. Clin. Invest.,* **1994,** *93,* 2263-6.

[49] Gutniak, M.K.; Juntti-Berggren, L.; Hellstrom, P.M.; Guenifi, A.; Holst, J.J.; Efendic, S. *Diabetes Care*, **1996,** *19*, 857-63.

[50] Muzino, A.; Kuwajima, M.; Ishida, K.; Noma, Y.; Murakami, T.; Tateishi, K.; Sato, I.; Shima, K. *Metabolism,* **1997,** *46,* 745-9.

[51] Sandhu, H.; Wiesenthal, S.R.; MacDonald, P.E.; McCall, R.H.; Tchipashvili, V.; Rashid, S.; Satkunarajan, M.; Irwin, D.M.; Shi, Z.O.; Brubaker, P.L.; Vranic, M.; Efendic, S.; Giacca, A. *Diabetes,* **1999,** *48,* 1045-53.

[52] Meneilly, G.S.; McIntosh, C.H.; Pederson, R.A.; Habener, J.F.; Gingerich, R.; Egan, J.M.; Finegood, D.T.; Elahi, D. *Diabetes Care,* **2001,** *24,* 1951-6.

[53] Egan, J.M.; Meneilly, G.S.; Habener, J.F.; Elahi, D. *J. Clin. Endocrinol. Metab.,* **2002,** *87,* 3768-73.

[54] Orskov, L.; Holst, J.J.; Moller, J.; Orskov, C.; Moller, N.; Alberti, K.G.; Schmitz, O. *Diabetologia,* **1996,** *39,* 1227-32.

[55] Ryan, A.S.; Egan, J.M.; Habener, J.F.; Elahi, D. *J. Clin. Endocrinol. Metab.,* **1998,** *83,* 2399-404.

[56] Freyse, E.J.; Knospe, S.; Becher, T.; El Hag, O.; Goke B.; Fischer, U. *Metabolism,* **1999,** *48,* 134-7.

[57] Vella, A.; Shah, P.; Basu, R.; Basu, A.; Camilleri, M.; Schwenk, F.W.; Holst, J.J.; Rizza, R.A. *Diabetes*, **2001,** *50*, 565-72.

[58] Vella, A.; Shah, P.; Reed, A.S.; Adkins, A.S.; Basu, R.; Rizza, R.A. *Diabetologia*, **2002,** *45*, 1410-5.

[59] Anini, Y.; Hansotia, T.; Brubaker, P.L. *Endocrinology,* **2002,** *143,* 2420-6.

[60] Rocca, A.S.; Brubaker, P.L. *Endocrinology*, **1999,** *140,* 1687-94.

[61] Brubaker, P.L. . *Endocrinology,* **1991,** *128, 3175*-82.

[62] Drucker, D.J.; Jin, T.; Asa S.L.; Young, T.A.; Brubaker, P.L. *Mol. Endocrinol.,* **1994,** *8,* 1646-55.

[63] Reimann, F.; Gribble, F.M. *Diabetes,* **2002,** *51,* 2757-63.

[64] Reimer, R.A.; Darimont, C.; Gremlich, S.; Nicolas-Metral, V.; Ruegg, U.T.; Mace, K. *Endocrinology*, **2001,** *142,* 4522-8.

[65] Orskov, C.; Rabenhoj, L.; Wettergren, A.; Kofod, H.; Holst, J.J. *Diabetes*, **1994**, *43,* 535-9.

[66] Mentlein, R.; Gallwitz, B.; Schmidt, W.E. *Eur. J. Biochem.,* **1993**, *214*, 829-35.

[67] Kieffer, T.J.; McIntosh, C.H.; Pederson, R.A. *Endocrinology,* **1995**, *136,* 3585-96.

[68] Deacon, C.F.; Johnsen, A.H.; Holst, J.J. *J. Clin. Endocrinol. Metab.,* **1995,** *80, 952*-7.

[69] Thorens, B. *Proc. Natl. Acad. Sci. U.S.A.*, **1992**, *89*, 8641-5.

[70] Burcelin, R.; Da Costa, A.; Drucker, D.; Thorens, B. *Diabetes*, **2001,** *50*, 1720-8.

[71] Nishizawa, M.; Nakabayashi, H.; Kawai, K.; Ito, T.; Kawakami, S.; Nakagawa, A.; Niijima, A.; Uchida, K. *J. Auton. Nerv. Syst.,* **2000,** *80,* 14-21.

[72] Holz, G.G.; Leech, C.A.; Habener, J.F. *Biochimie*, **2000,** *82*, 915-26.

[73] Montrose-Rafizadeh, C.; Avdonin, P.; Garant, M.J.; Rodgers, B.D.; Kole, S.; Yang, H.; Levine, M.A.; Schwindinger, W.; Bernier, M. *Endocrinology,* **1999,** *140,* 1132-40.

[74] Lester, L.B.; Langeberg, L.K.; Scott, J.D. *Proc. Natl. Acad. Sci. USA*, **1997**, *94,* 14942-7.

[75] Fraser, I.D.; Tavalin, S.J.; Lester, L.B.; Langeberg, L.K.; Westphal, A.M.; Dean, R.A.; Marrion, N.V.; Scott, J.D. *EMBO J.,* **1998**, *17,* 2261-72.

[76] Leech, C.A.; Holz, G.G.; Chepurny, O.; Habener, J.F. *Biochem. Biophys. Res. Commun.,* **2000,** *278*, 44-7.

[77] Kang, G.; Chepurny, O.G.; Holz, G.G. *J. Physiol.,* **2001,** *536(Pt 2)*, 375-85.

[78] Ozaki, N.; Shibasaki, T.; Kashima, Y.; Miki, T.; Takahashi, T.; Ueno, H.; Sunaga, Y.; Yano, H.; Matsuura, Y.; Iwanaga, T.; Takai, Y.; Seino, S. *Nat. Cell Biol.,* **2000,** *2,* 805-11.

[79] Kashima, Y.; Miki, T.; Shibasaki, T.; Ozaki, N.; Miyazaki, M.; Yano, H.; Seino, S. *J. Biol. Chem.,* **2001**, *276*, 46046-53.

[80] Susini, S.; Roche, E.; Prentki, M.; Schlegel, W. *FASEB J.,* **1998**, *12*, 1173-82.

[81] Jehle, P.M.; Jehle, D.; Fussganger, R.D.; Adler, G. *Exp. Clin. Endocrinol. Diabetes.,* **1995**, *103 Suppl 2,* 31-6.

[82] Ebinger, M.; Jehle, D.R.; Fussgaenger, R.D.; Fehmann, H.C.; Jehle, P.M. *Am. J. Physiol. Endocrinol. Metab.,* **2000,** *279*, E88-94.

[83] Buteau, J.; Roduit, R.; Susini, S.; Prentki, M. *Diabetologia*, **1999,** *42,* 856-64.

[84] Buteau, J.; Foisy, S.; Rhodes, C.J.; Carpenter, L.; Biden, T.J.; Prentki, M. *Diabetes,* **2001,** *50,* 2237-43.

[85] Ding, W.G.; Kitasato, H.; Matsuura, H. *Exp. Physiol.,* **2001,** *86*, 331-9.

[86] Yaney, G.C.; Civelek, V.N.; Richard, A.M.; Dillon, J.S.; Deeney, J.T.; Hamilton, J.A.; Korchak, H.M.; Tornheim, K.; Corkey, B.E.; Boyd, A.E. 3rd. *Diabetes*, **2001,** *50*, 56-62.

[87] Holz, G.G. 4th.; Kuhtreiber, W.M,; Habener, J.F. *Nature*, **1993**, *361,* 362-5.

[88] Suga, S.; Kanno, T.; Ogawa, Y.; Takeo, T.; Kamimura, N.; Wakui, M. *Pflugers Arch*., **2000,** *440*, 566-72.

[89] Light, P.E.; Manning Fox, J.E.; Riedel, M.J.; Wheeler, M.B. *Mol. Endocrinol.,* **2002,** *16,* 2135-44.

[90] Sasaki, SH; Nakagaki, I.; Kondo, H.; Hori, S. *Pflugers Arch.,* **2002**, *445,* 342-51.

[91] Holz, G.G.; Leech, C.A.; Heller, R.S.; Castonguay, M.; Habener, J.F. *J. Biol. Chem.,* **1999,** *274,* 14147-56.

[92] Byrne, M.M.; Gliem, K.; Wank, U.; Arnold, R.; Katschinski, M.; Polonsky, K.S.; Goke, B. *Diabetes,* **1998**, *47*, 1259-65.

[93] Dachicourt, N.; Serradas, P.; Bailbe, D.; Kergoat, M.; Doare, L.; Portha, B. *J. Endocrinol.,* **1997**, *155*, 369-76.

[94] Tsuboi, T.; da Silva Xavier, G.; Holz, G.G.; Jouaville, L.S.; Thomas, A.P.; Rutter, G.A. *Biochem. J*., **2003,** *369*, 287-99.

[95] Porksen, N.; Grofte, B.; Nyholm, B.; Holst, J.J.; Pincus, S.M.; Veldhuis, J,D.; Schmitz, O.; Butler, P.C. *Diabetes*, **1998**, *47*, 45-9.

[96] Fernandez, J.; Valdeolmillos, M. *Diabetes*, **1999**, *48*, 754-7.

[97] Renstrom, E.; Eliasson, L.; Rorsman, P. *J. Physiol.,* **1997,** *502(Pt 1),* 105-18. .

[98] Kang, G.; Holz, G.G. *J. Physiol.,* **2003,** *546(Pt 1),* 175-89.

[99] Wang, Y.; Egan, J.M.; Raygada, M.; Nadiv, O.; Roth, J.; Montrose-Rafizadeh, C. *Endocrinology*, **1995,** *136,* 4910-7.

[100] Fehmann, H.C.; Habener, J.F. *Endocrinology,* **1992,** *130,* 159-66.

[101] Skoglund, G.; Hussain, M.A.; Holz, G.G. *Diabetes,* **2000,** *49*, 1156-64.

[102] Chepurny, O.G.; Holz, G.G. *Cell Tissue Res.,* **2002,** *307,*191-201.

[103] Chepurny, O.G.; Hussain, M.A.; Holz, G.G. *Endocrinology,* **2002,** *143,* 2303-13.

[104] Wang, X.; Zhou, J.; Doyle, M.E.; Egan, J.M. *Endocrinology*, **2001,** *142*, 1820-7.

[105] Wang, X.; Cahill, C.M.; Pineyro, M.A.; Zhou, J.; Doyle, M.E.; Egan, J.M. *Endocrinology,* **1999**, *140,* 4904-7.

[106] Stoffers, D.A.; Kieffer, T.J.; Hussain, M.A.; Drucker, D.J.; Bonner-Weir, S.; Habener, J.F.; Egan, J.M. *Diabetes*, **2000,** *49*, 741-8.

[107] Hussain, M.A.; Habener, J.F. *Biochem. Biophys. Res. Commun.,* **2000,** *274*, 616-9.

[108] Lawrence, M.C.; Bhatt, H.S.; Easom, R.A. *Diabetes,* **2002,** *51,* 691-8.

[109] Furstenau, U.; Schwaninger M.; Blume, R.; Jendrusch, E. M.; Knepel, W. *J. Biol. Chem.,* **1999**, *274*, 5851-60.

[110] Xu, G.; Stoffers, D.A.; Habener, J.F.; Bonner-Weir, S.; *Diabetes*, **1999,** *48*, 2270-6.

[111] Edvell, A.; Lindstrom, P. *Endocrinology,* **1999,** *140,* 778-83.

[112] Wang, Q.; Brubaker, P.L. *Diabetologia,* **2002,** *45,* 1263-73.

[113] Farilla, L.; Hui, H.; Bertolotto, C.; Kang, E.; Bulotta, A.; Di Mario, U.; Perfetti, R. *Endocrinology*, **2002,** *143*, 4397-408.

[114] Li, Y.; Hansotia, T.; Yusta, B.; Ris, F.; Halban, P.A.; Drucker, D.J. *J. Biol. Chem*., **2003,** *278,* 471-8.

[115] Tourrel, C.; Bailbe, D.; Meile, M.J.; Kergoat, M.; Portha, B. *Diabetes*, **2001,** *50,* 1562-70.

[116] Tourrel, C.; Bailbe, D.; Lacorne, M.; Meile, M.J.; Kergoat, M.; Portha, B. *Diabetes*, **2002**, *51*, 1443-52.

[117] Perfetti, R.; Zhou, J.; Doyle, M.E.; Egan, J.M. *Endocrinology*, **2000,** *141*, 4600-5.

[118] Rolin, B.; Larsen, M.O.; Gotfredsen, C.F.; Deacon, C.F.; Carr, R.D.; Wilken, M.; Knudsen, L.B. *Am. J. Physiol. Endocrinol. Metab.*, **2002,** *283*, E745-52.

[119] Hui, H.; Wright, C.; Perfetti, R. *Diabetes*, **2001,** *50*, 785-96.

[120] Zhou, J.; Wang, X.; Pineyro, M.A.; Egan, J.M. *Diabetes,* **1999,** *48,* 2358-66.

[121] Zhou, J.; Pineyro, M.A.; Wang, X.; Doyle, M.E.; Egan, J.M. *J. Cell. Physiol.* **2002** *192,* 304-14.
[122] Movassat, J.; Beattie, G.M.; Lopez, A.D.; Hayek, A. *J. Clin. Endocrinol. Metab.,* **2002,** *87,* 4775-81.
[123] de la Tour, D.; Halvorsen, T.; Demeterco, C.; Tyrberg, B.; Itkin-Ansari, P.; Loy, M.; Yoo, S.J.; Hao, E.; Bossie, S.; Levine, F. *Mol. Endocrinol*., **2001,** *15*, 476-83.
[124] Abraham, E.J.; Leech, C.A.; Lin, J.C.; Zulewski, H.; Habener, J.F. *Endocrinology*, **2002,** *143*, 3152-61.
[125] Bulotta, A.; Hui, H.; Anastasi, E.; Bertolotto, C.; Boros, L.G.; Di Mario, U.; Perfetti, R. *J. Mol. Endocrinol.,* **2002,** *29,* 347-60. .
[126] Wilson M.E.; Kalamaras, J.A.; German, M.S. *Mech. Dev.,* **2002,** *115,* 171-6.
[127] Otonkoski, T.; Hayek, A. *J. Clin. Endocrinol. Metab.,* **1995**, *80*, 3779-83.
[128] Hardikar, A.A.; Wang, X.Y.; Williams, L.J.; Kwok, J.; Wong, R.; Yao, M.; Tuch, B.E. *Endocrinology*, **2002,** *143*, 3505-14.
[129] Buteau, J.; Foisy, S.; Joly, E.; Prentki, M. *Diabetes* **2003,** *52,* 124-32.
[130] Perry, T.; Haughey, N.J.; Mattson, M.P.; Egan, J.M.; Greig, N.H. *J. Pharmacol. Exp. Ther.,* **2002**, *302*, 881-8.
[131] Vaudry, D.; Pamantung, T.F.; Basille, M.; Rouselle, C.; Fournier, A.; Vaudry, H.; Beauvillian, J.C.; Gonzalez B.J. *Eur. J. Neurosci.,* **2002,** *15,* 1451-60.
[132] Nie, Y.; Nakashima, M.; Brubaker, P.L.; Li, Q.L.; Perfetti, R.; Jansen, E.; Zambre, Y.; Pipeleers, D.; Friedman, T.C. *J. Clin. Invest.,* **2000,** *105,* 955-65.
[133] Deacon, C.F.; Knudsen, L.B.; Madsen, K.; Wiberg, F.C.; Jacobsen, O.; Holst, J.J. *Diabetologia,* **1998,** *41,* 271-8.
[134] Ritzel, U.; Leonhardt, U.; Ottleben, M.; Ruhmann, A.; Eckart, K.; Spiess, J.; Ramadori, G. *J. Endocrinol.,* **1998,** *159,* 93-102.
[135] Burcelin, R.; Dolci, W.; Thorens, B. *Metabolism,* **1999,** *48,* 252-8.
[136] O'Harte, F.P.; Mooney, M.H.; Lawlor, A.; Flatt, P.R. *Biochim. Biophys. Acta,* **2000,** *1474,* 13-22.
[137] Gallwitz, B.; Ropeter, T.; Morys-Wortmann, C.; Mentlein, R.; Siegel, E.G.; Schmidt, W.E. *Regul. Pept.,* **2000,** *86,* 103-11.
[138] Doyle, M.E.; Greig, N.H.; Holloway H.W.; Betkey, J.A.; Bernier, M.; Egan, J.M. *Endocrinology,* **2001,** *142,* 4462-8.
[139] Xiao, Q,; Giguere, J.; Parisien, M., Jeng, W.; St-Pierre, S.A.; Brubaker, P.L, Wheeler, M.B. *Biochemistry,* **2001,** *40,* 2860-9.
[140] Knudsen, L.B.; Nielsen, P.F.; Huusfeldt, P.O.; Johansen, N.L.; Madsen, K.; Pedersen, F.Z.; Thogersen, H.; Wilken, M.; Agerso, H. *J. Med. Chem.,* **2000,** *43,* 1664-9.
[141] Larsen, P.J.; Fledelius, C.; Knudsen, L.B.; Tang-Christensen, M. *Diabetes,* **2001,** *50, 2*350-9.
[142] Agerso, H.; Jensen, L.B.; Elbrond, B.; Rolan, P.; Zdravkovic, M. *Diabetologia,* **2002,** *45,* 195-202. .
[143] Ribel, U.; Larsen, M.O.; Rolin, B.; Carr, R.D.; Wilken, M.; Sturis, J.; Westergaard, L.; Deacon, C.F.; Knudsen, L.B. *Eur. J. Pharmacol.*, **2002**, *451*, 217-25.
[144] Matthews, D.; Madsbad, S.; Schmitz, O.; Langendorf, K.W.; Jakobsen, G. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 339-OR.
[145] Benthem, L.; Wei, L.; Langer, K.Z.; Knudsen, L.B. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 1382-P.
[146] Chou, J.Z.; Place, G.D.; Waters, D.G.; Kirkwood, J.A.; Bowsher, R.R. *J. Pharm. Science,* **1997,** *86,* 768-773. .
[147] Lawrence, B.; Wen, S.Y.; Jette, L.; Thibaudeau, K.; Castaigne, J.-P. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 340-OR.
[148] Kim, J.-G.; Baggio, L.L.; Drucker, D.J. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 1391-P.
[149] Thibaudeau, K.; Smith, D.C.; Jette, L.; Castaigne, J.-P.; Bridon, D.P.; Kim, J.-G.; Baggio, L.L.; Drucker, D.J. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 417-P.
[150] Trautmann, M.E.; Kapitza, C.; Mace, K.F.; Patterson, B.; Hompesche, M.; Heise, T. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 545-P.
[151] Kapitza, C.; Trautmann, M.E.; Heise, T.; Heinemann, L.; Patterson, B. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 341-OR.
[152] Eng, J.; Kleinman, W.A.; Singh, L.; Raufman, J.P. *J. Biol. Chem.,* **1992,** *267,* 7402-5.
[153] Raufman, J.P.; Singh, L.; Singh, G.; Eng, J. *J. Biol. Chem.,* **1992,** *267,* 21432-7.
[154] Goke, R.; Fehmann, H.C.; Linn, T.; Schmidt, H.; Krause, M.; Eng, J.; Goke, B. *J. Biol. Chem.,* **1993,** *268,* 19650-5.
[155] Drucker, D.J.; *Diabetes,* **1998,** *47, 1*59-69.
[156] Adelhorst, K.; Hedegaard, B.; Knudsen, L. B.; Kirk, O. *J. Biol. Chem.,* **1994,** *269*, 6275-78.
[157] Young, A.A; Gedulin, B.R.; Bhavsar, S.; Jodka, C.; Hansen, B.; Denaro, M. *Diabetes,* **1999,** *48,* 1026-34. .
[158] Edwards, C.M.; Stanley, S.A.; Davis, R.; Brynes, A.E.; Frost, G.S.; Seal, L.J.; Ghatei, M.A.; Bloom, S.R. *Am. J. Physiol. Endocrinol. Metab.,* **2001,** *281*, E155-61.
[159] Egan, J.M.; Clocquet, A.R.; Elahi, D. *J. Clin. Endocrinol. Metab*., **2002,** *87,*1282-90.
[160] Fineman, M.S.; Bicsak, T.; Shen, L.; Taylor, K.; Gaines, I.; Varns, A.; Kim, D.; Baron, A. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 343-OR.
[161] Taylor, K.; Kim, D.; Bicsak, T.; Heintz, S.; Varns, A.; Aisporna, M.; Fineman, M.S.; Baron, A. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 344-OR.
[162] Kim, D.; Taylor, K.; Bicsak, T.; Wang, Y.; Aisporna, M.; Heintz, S.; Fineman, M.S.; Baron, A. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 421-P.
[163] Juhl, C.B.; Hollingdal, M.; Sturis, J.; Jakobsen, G.; Agerso, H.; Veldhuis, J.; Porksen, N.; Schmitz, O. *Diabetes,* **2002,** *51,* 424-9.
[164] Bridon, D.P.; Thibaudeau, K.; Larcheveque, B.P.; Pham, K.; Robitaille, M.F.; Drucker, D.J.; Leger, R.; Castaigne, J.-P. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 378-P. .
[165] Myers, S.; Cox, A.; Irwin, L.; Compton, J.; Mace K.F. *62nd ADA Scientific Sessions, San Francisco, CA,* **2002,** Abstr. 1398-P. .
[166] Greig, N.H.; Holloway, H.W.; De Ore, K.A.; Jani, D.; Wang, Y.; Zhou, J.; Garant, . M.J.; Egan, J.M. *Diabetologia,* **1999,** *42,* 45-50.
[167] Szayna, M.; Doyle, M.E.; Betkey, J.A.; Holloway, H.N.; Spencer, R.G.S.; Greig, N.H.; Egan, J.M. *Endocrinology,* **2000,** *141,* 1936-41.
[168] Egan, J.M.; Meneilly, G.S.; Elahi, D. *Am. J. Physiol. Endocrinol. Metab.* (in press).
[169] Elbrond, B.; Jakobsen, G.; Larsen, S.; Agerso, H.; Jensen, L.B.; Rolan, P.; Sturis, J.; Hatorp, V.; Zdravkovic, M. *Diabetes Care,* **2002,** *25,* 1398-404.
[170] Kim, J.G.; Baggio, L.L.; Bridon, D.P.; Castaigne, J.P.; Robitaille, M.F.; Jette, L.; Benquet, C.; Drucker, D.J. *Diabetes,* **2003,** *52,* 751-9.

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