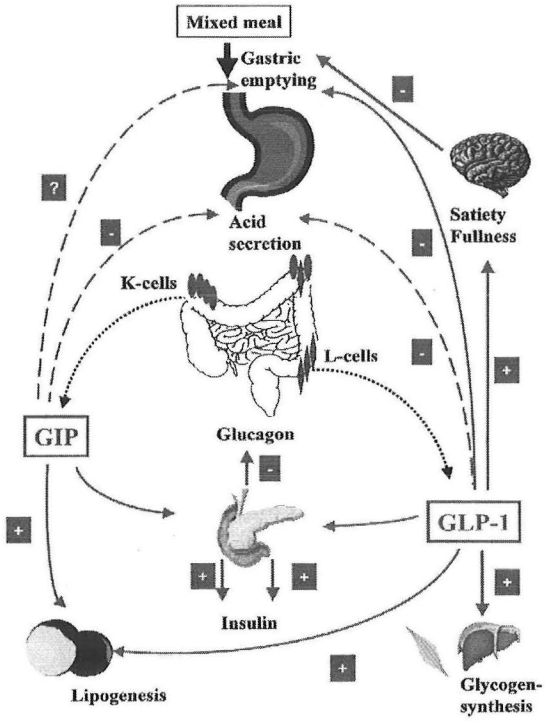
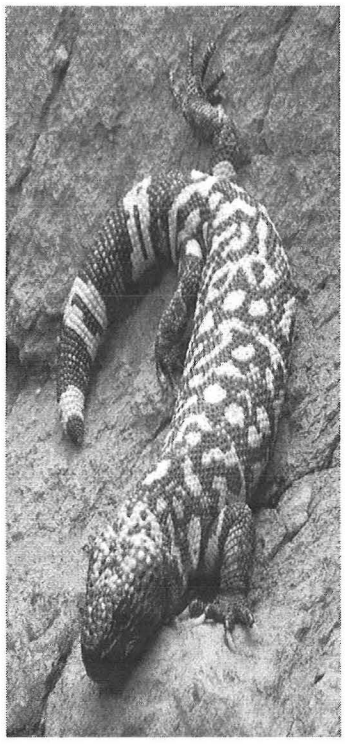
Incretin Hormones and Diabetes Mellitus





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His areas of clinical interest include: treatment algorithms for Type 2 DM, Post-Transplantation Diabetes, insulin pump therapy and therapeutic considerations in the management of Latino populations.

Dr. Mora acknowledges a potential conflict of interest from his participation as a member of Speakers Bureaus for Amylin, Eli Lilly, Merck, Novartis and Novo Nordisk Pharmaceuticals.

There will be no off label uses of pharmacological agents discussed in this presentation.

Introduction

During the last five decades, there has a been an explosion of incidence of diabetes mellitus type 2 (T2DM) around the world, but more marked on the Western Hemisphere societies, with emphasizes in special ethnicities and clearly associated with a similar epidemic of obesity in these individuals.

The understanding of the complex pathophysiology of this disease has advanced dramatically, leading to the elucidation of the natural history of a progressive disorder that involves not only the traditional organs such as liver, pancreas and peripheral tissues (muscle and fat) but also the central nervous system (CNS), other endocrine glands and more recently the Gastrointestinal (GI) System.

Digestion and absorption of nutrients are time-intensive processes needed to protect against malabsorption and metabolic disturbances during the post-prandial period. This includes the fine-tuned interaction of gastrointestinal nutrient transit, endocrine and exocrine secretion, and gut-brain responses like the induction of satiety or the feeling of fullness, bloating or nausea. The interplay between gastrointestinal transit and endocrine pancreatic secretion plays a key role to optimize nutrient digestion and absorption. As the release of insulin and glucagon accounts for metabolization of absorbed nutrients, intestinal braking mechanisms in response to nutrients within the gut lumen regulates the speed of transit of the luminal content to optimize digestion and absorption. About 25 years ago, two concepts have been separately developed describing the mechanism of action for intestinal nutrients to regulate endocrine pancreatic secretion and to induce an intestinal brake: the incretin concept, as part of the complex neuroendocrine regulation of the entero-insular axis and the "ileal brake", that describes the braking of

gastrointestinal transit and inhibition of exocrine pancreatic and gastric secretion triggered by nutrients in the distal intestine. The most potent trigger is fat, with protein and carbohydrate also trigger the ileal brake, and end products of nutrient digestion such as fatty acids are required. This brake initiates a distal-to-proximal intestino-intestinal and intestino-gastric feedback loop that inhibits upper gut motility and secretion, thus preventing nutrient malabsoption. While peptide YY (PYY) co-expressed with GLP-1 and released from L-cells within ileo-colonic junction in parallel to ileal nutrient perfusion is suggested as a humoral mediator of the ileal brake, neural factors may also contribute as extrinsic denervation, naloxone, 5 H-T3 antagonists, and adrenoceptor antagonists blocked the inhibitory effect of the ileal brake.[1]

More recently, there has been a rediscovery of the crosstalk between the many hormones that are secreted in the process of food digestion and absorption and the more traditional glucose control pathways at the pancreas and liver. This new view now includes the existence of endocrine and neural pathways from CNS to GI system and vice versa, that regulate hormonal secretion, GI motility and the control of hunger and satiety.

In the past 5 years and for several more to come, many pharmaceutical agents will become part of our therapeutic armamentarium, that are based on this new understanding that will improve our capacity to control this disorder but at the same time, will complicate the development of practical treatment approaches for T2DM that can be implemented by both the general practitioner as well as the specialist.[2]

Evolution of Gastrointestinal Endocrinology

In 1902, William M. Bayliss and Ernest H. Starling published their paper " The mechanism of pancreatic secretion" given birth to the gastrointestinal endocrinology. The

authors had shown that acid extracts of intestinal mucosa contained a factor that stimulated via the blood stream the exocrine secretion of the pancreas and named this factor secretin. Their finding revolutionized physiology because at this time it was believed that solely nerves controlled the functions of the body. In his famous four "Cronian Lectures: on the chemical correlation of the functions of the body, Starling introduced the word "hormone" for chemical factors which influenced via the blood stream the function of a distant organ. He exemplified this with his experiments on the effect of secretin on pancreatic exocrine secretion. It is believed that Starling had already considered the possibility that the duodenum does also supply a chemical excitant for the internal secretion of the pancreas. Not until the discovery of insulin by Banting and Best in 1921, a systematic search for a gut hormone influencing carbohydrate metabolism took place. Unfortunately, the inconsistent literature on the existence of a gut hormone regulating glucose metabolism led to the premature condemnation of the incretin concept and no further research was conducted for 25 years. Progress in peptide chemistry and the development of modern methods for purification and sequencing, as well as highly specific and sensitive radioimmunoassay (RIA) initiated interest in intestinal factors enhancing insulin secretion. It started with the proof that an oral glucose load induced a significantly greater insulin response (measured by RIA) than an intravenous glucose injection even in the case of higher blood glucose levels during the intravenous glucose load. Elrick et al from Denver and McIntyre et al from London communicated this finding simultaneously and independently in 1964. It was estimated that 50% of the insulin secreted after an oral glucose load was released by gastrointestinal factors and Unger and Eisentraut coined for this system the term "enteroinsular axis".

Since several groups demonstrated that the plasma insulin levels increased after the injection of an extract of intestinal mucosa, all known gastrointestinal hormones were considered either alone or in combination as possible incretin candidates. However, careful investigations revealed that none of the hormones known at that time, qualified for incretin effects per the following criteria:

(1) The hormone must be released from gut endocrine cells after ingestion of nutrients, especially of glucose.

(2) The circulating hormone must stimulate insulin secretion in a concentration which is easily achieved after ingestion of a nutrient

(3) The hormone releases insulin only at elevated glucose levels (glucose dependence)

It was not until 1970, when John C. Brown from Vancouver isolated and sequenced a new peptide that he named gastric inhibitory polypeptide (GIP) because of its inhibitory effect on gastric acid secretion. Later he demonstrated the insulinotropic action of GIP in man and suggested to read the acronyms as "glucose dependent insulinotropic polypeptide".

Work for the next 15 years in many laboratories elucidated the role of GIP in pathophysiology but a role in pathogenesis or treatment of diabetes mellitus could not be established. It was not until 1985, when cloning the preproglucagon, the GLP-1 that demonstrated strong insulinotropic effects and gave birth to new diabetes therapy based on the incretin concept, identified a new peptide.[3]

Gut hormones were among the first described true hormones, typically initially in terms of their functions and it was not until recently that many of their structures were defined.

There are classified into gut peptide families and many will share many structural and functional characteristics.[4]

Discovery of GIP and GLP-1

Almost 30 years ago, Moore et all first reported on the antidiabetogenic effect of an extract of duodenal mucous membranes. The authors proposed a stimulation of pancreatic secretion to be mediated by this extract. However, it took another 60 years until the establishment of an immunoassay for insulin allowed Dupre and Beck to show an insulinotropic effect of intestinal mucous extracts in normal subjects. In contrast, no stimulation of insulin release could be observed in juvenile-onset diabetic subjects. Before this insulinotropic effect of a duodenal mucous extract had been observed, an inhibitory influence on gastric acid secretion was demonstrated. Therefore in 1930, Kosaka and Lim proposed the term "enterogastrone" based on their observations, that gastric acid secretion and gastric emptying could be inhibited by intravenous infused extracts of intestinal mucosa. Further purification of such extracts that were devoid of cholezystokinin-pancreozyme (CCK-PZ) activity confirmed the presence of other intestinal hormones with inhibitory effect on gastric acid secretion. Based on these effects, the name "Gastric Inhibitory Peptide" was proposed by Brown in 1971 followed by the report the complete amino acid sequence of this peptide. Since hypersecretion of GIP following oral glucose was observed in type 2 diabetic patients, it was hypothesized that a diminished responsiveness of insulin secretion towards GIP might take part in the development of type 2 diabetes. Along with this hypothesis, a reduced insulinotropic effect of GIP was described after the intravenous administration of the peptide in type 2 diabetic patients. The ingestion of carbohydrate and lipid rich meal has been shown to be

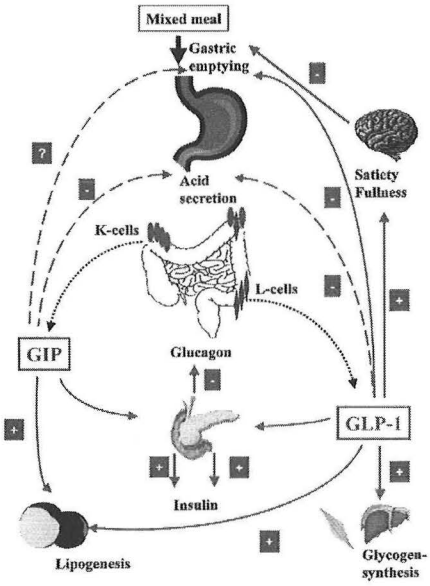
the main stimulant for the secretion of GIP. However, the mediation of GIP secretion following meal ingestion reaches peak concentrations already 15-30 minutes after the intake, long before the substrates ingested are present in the gut. Therefore, an involvement of the vagus nerve in the stimulation of GIP secretion as well as GLP-1 has been implicated.[5]

In cultured preadipocytes, incubation with GIP dose-dependently stimulates lipoprotein lipase activity and this effect is unique since it is not share by GLP-1. In addition, GIP has been shown to induce fatty acid incorporation into adipose tissue in epididymal fat pads in animals. Nauck et al studied the effects of physiological doses of synthetic human GIP alone, and in co-infusion with human GLP-1 in humans. In this study, neither GIP nor GLP-1 inhibited gastric acid secretion under physiological conditions. While GLP-1 is known to be a potent inhibitor of gastric emptying, GIP seems to act in an opposite way, leading to accelerated emptying of the stomach.

In terms of the endocrine secretion effects of GIP, the studies concluded that the effect of endogenously released GIP is an important mechanism of postprandial insulin secretion with very little role in the fasting state. In normal subjects, GIP is responsible of approximately 60% of the incretin effect. As well as GLP-1, GIP stimulates beta cell proliferation. It is clear that in contrast with GLP-1, the insulinotropic effect of GIP is markedly reduced in type 2 diabetic patients and paradoxically may increase glucagon levels.

The reduced response of insulin secretion to the administration of exogenous GIP characterizes Type 2 DM, so the question arises whether the loss of the GIP effect

represents a specific phenomenon that might be involved in the pathogenesis of type 2 DM or whether it is the result of an impaired beta cell function in more general terms. In addition, the molecular defect underlying the loss of GIP effect in type 2 diabetes remains unclear. Considering the preserved insulinotropic activity of the GLP-1, that shares most of its signaling pathways with GIP, it is conceivable that the reduced insulinotropic effect of GIP is due to a specific defect. In summary, in type 2 DM the GLP-1 secretion is defective but the effect is sustained, but the GIP secretion is normal but the effect is lost. This defect may have a hereditary component since there have been some studies of first-degree relatives of type 2 diabetic individuals who showed partial loss of response to GIP. Unfortunately, these individuals were not at a higher risk of developing type 2 DM. Finally, since the insulinotropic effect of GIP is lost in type 2 DM the application of the peptide in the treatment of this condition does not seem to display any advantage over GLP-1.



GIP and GLP-1 are known as the two most important insulinotropic gut hormones.

GIP is a 42 amino acid peptide produced predominantly in duodenal K cell in the proximal small intestine. GIP has also been localized to the central nervous system, where it may play a role in control of cell survival. The predominant stimulus for GIP secretion is nutrient intake; circulating levels of GIP are low in the fasted state and rise within minutes of food ingestion. As GIP contains an alanine at position 2, it is an excellent substrate for dipeptidyl peptidase-4, an essential enzyme regulating the degradation for both GIP and LGP-1. In contrast, GLP-1 is produced in enteroendocrine cells in the distal small bowel and colon. Plasma levels of GIP-1 also rise rapidly within minutes of food intake, hence it seems likely that both neural and endocrine factor promote GLP-1 secretion from distal L cells, well before digested nutrients traverse the small bowel to make direct contact with enteroendocrine L cells. Proglucagon is processed to glicentin, oxyntomodulin, GLP-1 and GLP-2 in gut L cells, via processing that requires prohormone convertase-1. GLP-1 also contain an alanine in position 2 and are rapidly degraded by DPP-1 and it has been shown that a substantial proportion of GLP-1 in the portal and systemic circulation has already been cleaved by DPP-4 and also rapidly cleared by the kidney[6].

GLP-1 production is at the L cells located in the ileum and colon and the K cell primarily located in the duodenum. secretes GIP[7].

The human GLP-1 (GLP-1R) receptor is a 463 amino acid G protein-coupled receptor widely expressed in pancreatic islets, kidney, lung, heart and multiple regions of the peripheral and central nervous system. Within islets, the GLP-1R is predominantly localized to Beta cells. Engagement of the GLP-1R stimulates cyclic AMP formation and activation of downstream pathways coupled to protein kinase A and cAMP regulated

guanine nucleotide exchange factors. GLP-1R agonists promote cyclic AMP response element binding protein (CREB) phosphorylation and also regulate CREB activity through glucose-dependent stimulation of the cytoplasmic to nuclear translocation of TORC2, a CREB coactivator. GLP-1R activation is also couple to increased intracellular calcium, inhibition of voltage-dependent K currents and activation of immediate early gene expression through effects of Erk1/2, protein kinase C and phosphatidylinositol 3-kinase (PI3K). GLP-1R agonist promote expansion of islet mass in association with increased Pdx-1 and also promote preservation and expansion of beta cell mass through inhibition of apoptotic pathways such as caspase-3. GIP has also shown to exert proliferative and antiapoptotic actions on islet beta cells.

An important determinant of GLP-1 action on control of postprandial glucose is deceleration of the rate of gastric emptying, which occurs within minutes of pharmacological exogenous GLP-1R agonist administration. The mechanisms by which GLP-1 inhibits gastric emptying appear complex and involve communication with the central and peripheral nervous system, especially through ascending vagal afferents for the GLP-1R. Figure 2

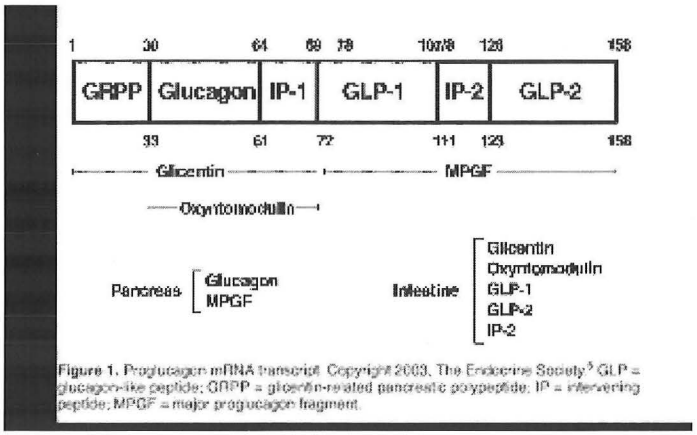


Figure 1. Proglucagon mRNA transcript. Copyright 2003, The Endocrine Society.[3] GLP = glucagon-like peptide; GRPP = glicentin-related pancreatic polypeptide; IP = intervening peptide; MPGF = major proglucagon fragment.

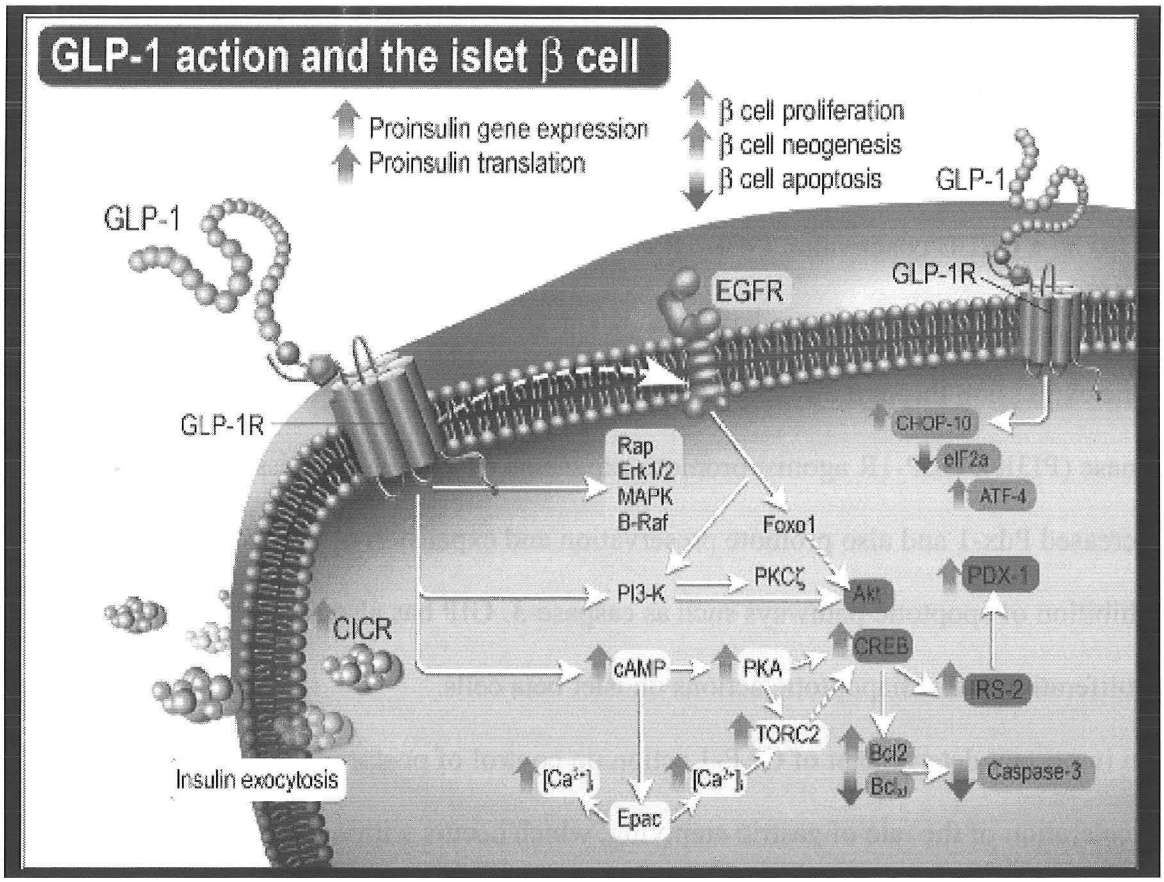


Figure 3, adapted from reference[8]

No reports exist of genetic mutations in patients with T2DM or evidence those patients with MODY have a genetic linkage to defects in the GLP-1 receptor gene. In patients with T2DM or impaired GT, there are modest but significant reductions in meal – stimulated circulating levels of GLP-1. GLP-1 is rapidly secreted by the L cells of the intestine in response to food ingestion in humans, by both neural and hormonal signaling initiated by exposing the proximal gastrointestinal tract to ingested nutrients, as well as by subsequent direct contact of those nutrients as the are exposed to the L cells in the distal jejunum and ileum, particularly in response to a mixed meal or a meal high in fat and complex carbohydrates.

The effect of GLP-1 in beta cell differentiation involves the transcription factors pancreatic-duodenal homeobox factor 1 (PDX-1) and hepatocyte nuclear factor 3 (HNF3beta) and appears to be mediated by mitogen activated protein (MAP) kinases including extracellular signal related kinase (ERK) and an isoform of protein kinase C. In contrast to the primary role of cAMP in the acute stimulation of insulin secretion, GLP-1 mediated beta cell growth is likely activated with the PI 3-kinase pathway as a proximal signaling step. In addition, it has recently been proposed that one of the mechanism by which GLP-1 promotes growth of beta cells is through activation the epidermal growth factor receptor.

Finally, there is now evidence that GLP-1 and GIP inhibit beta cell apoptosis, another action that would promote expansion of beta cell mass. Exendin-4 decreases cytokine-induced apoptosis in isolated murine beta cell and streptozotocin-induced apoptosis in intact mice. Moreover, mice with targeted deletion of the GLP-1 receptor gene have higher rates of beta cell apoptosis than control mice.

Since mice with a targeted deletion of the GIP receptor have slightly increased islet area while mice with a GLP-1 receptor knockout have only slightly decreased islet size, signaling by the incretins does not appear to be essential for the beta cell and islet development. More likely, GIP and GLP-1 regulate islet cell mass in response to external challenges. It is possible that his function serves as part of the homeostatic system to match insulin production with chronic nutrient demands. Indeed, support for this view can be drawn from recent studies implicating GIP and GLP-1 signaling in the compensatory beta cell hypertrophy to insulin resistance following chronic high fat feeding.

The most compelling clinical question raised by the recent finding of incretin effects on beta cell mass is whether this action has utility for the treatment of diabetes. There is currently evidence indicating that persons with T2DM have diminished beta cell mass that contributes to the insufficient insulin secretion that is central to the pathogenesis of this condition. There are no therapies currently available to address decreased islet mass. Studies in rodents, however, indicate that even transient or intermittent treatment with GLP-1 receptor agonists improves glucose tolerance in part through augmentation of beta cell mass. If chronic administration of GLP-1 or even GIP, is shown to stimulate the expansion of beta cells in the islets of patients with diabetes, through some combination of neogenesis, proliferation and decreased apoptosis, it would provide a novel and powerful therapy. Such an action could alter the natural progression of diabetes and conceivably have potential as a preventive therapy.

Applied pharmacology of the GI Endocrine system

Amylin and Pramlintide

Amylin is a naturally occurring 37 amino-acid peptide that is normally cosecreted in equimolar amounts with insulin from the pancreatic beta cells. Amylin secretion has been shown to be delayed and diminished in more advanced cases of type 2 diabetes and markedly reduced to absent in people who have type 1 diabetes. In addition, amylin secretion is gestational diabetes is also impaired and characterized by inappropriately exaggerated secretion during pregnancy followed by impaired post stimulatory secretion in the puerperium compared with pregnant women who do not have gestational diabetes. Though initially presumed to be related to the islet amyloid in the pathogenesis and

progression of type 2 diabetes, the physiologic role of amylin in postprandial glycemic control is now fairly well established and different from the amyloid.

Among the identified metabolic effects of amylin and its synthetic analog, pramlitide, are: 1) suppression of endogenous glucagon production, especially in the postprandial state 2) consequent reduction of postprandial hepatic glucose production 3) reduction in gastric emptying time 4) centrally-mediated induction of satiety and 5) reduction in postprandial glucose levels.

Because of the innate tendency of the amylin compound to aggregate and adhere to surfaces and its instability in solution, it is difficult to store, mass manufacture and formulate as a pharmaceutical much of which has been resolved with the discovery of the amylin analog, pramlintide.

The long-term efficacy of pramlintide as an adjunct to insulin has been established in subjects with type 1 and type 2 diabetes. In cohorts, the addition of amylin to insulin resulted in significant reductions in hemoglobin Ac (between 0.6-0.9%) with mean weight loss of around 2 kg., without concomitant increases in insulin doses or frequency of hypoglycemic episodes. These findings have been sustained over 2 years in uncontrolled open-label extension studies. Pramlintide has also been demonstrated for reducing postprandial glycemic surges even in subjects already being treated with regular insulin, insulin lispro and in one study insulin pump.[9]

Discovery of GLP-1 receptor agonist and GLP-1 analog

Proof of concept for the feasibility of using native GLP-1 for therapeutic purposes was obtained in a 6-week study of patients with type 2 diabetes. GLP-1 delivered via

continuous subcutaneous infusion significantly lowered both fasting and postprandial glucose, in association with a 1.3% reduction in HbA1c.

GLP-1 therapy was well tolerated, and associated with reduced levels of free fatty acids, improved insulin sensitivity and a 1.9 kg reduction in body weight. As the native GLP-1 undergoes rapids enzymatic inactivation by DPP-4, the efficacy of degradation resistant GLP-1R agonist suitable for once or twice daily administration has been examined.

Exendin-4 is a naturally occurring 39 amino acid GLP-1 receptor agonist originally isolated from the venom of the Heloderma suspectum. Exendin-4 is encoded by a distinct gene in the lizard, which also contains 2 genes for proglucagon however a gene for exendin-4 has not been yet detected in mammalian species. Exendin-4 is not the GLP-1 of mammals; it has its own GLP-1, which is much closer to mammalian GLP-1.

Alignment of native GLP-1 and exendin-4 amino acid sequences demonstrates 53% amino acid identity, and exendin-4 is a highly potent GLP-1R agonist both in vitro and in vivo. Exendin-4 contains a glycine residue at position 2, thereby conferring resistance to cleavage by DPP-4.[10]

Exendin-4 mimics all of the glucose-lowering actions of GLP-1, yet is several orders of magnitude more potent that native GLP-1 following parenteral administration, due to its enhanced pharmacokinetic profile. Studies have been conducted of exenatide against placebo, and in combination with metformin and or sulfonylureas, which represent the current labeling of the medication.

Although anti-exenatide antibodies have been detected in, as much as 49% of the patients treated, the presence or absence of these antibodies did not correlate with the therapeutic response of the subjects.

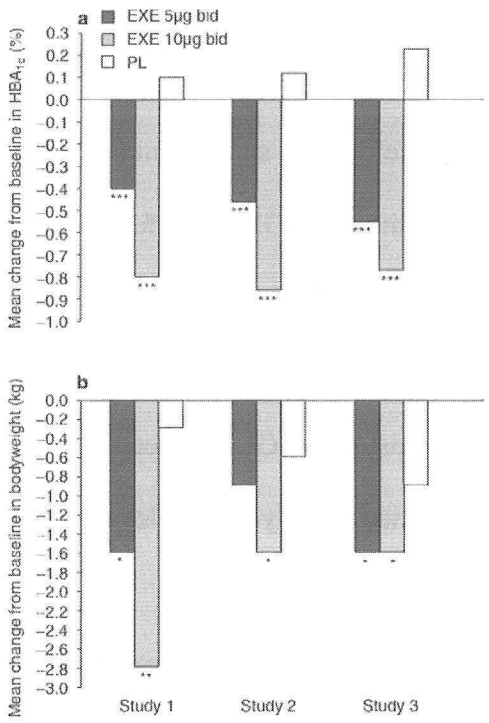
Exenatide:

Exenetide is a naturally occurring 39-aminoacid GLP-1 agonist isolated from the salivary gland venom of the lizard Heloderma suspectum (Gila monster). This peptide has 53% amino acid similarity to mammalian GLP-1, effectively binds to the GLP-1 receptor and is highly resistant to DPP-IV.

Exenatide was not created by sequential modification of the structure of GLP-1, therefore is not an analog but an agonist of the GLP-1 receptor. Unlike GLP-1, which contains an alanine at position 2, exenatide has glycine molecule in that position and therefore is not a substrate of DPP-IV.[2]

Exenetide suppresses glucagons secretion, slows gastric emptying, reduces food intake and promotes beta cell proliferation and neogenesis from precursor cells. Exenetide does not enhance insulin activity in noThe long term efficacy of subcutaneous exenatide in adults with type 2 diabetes and suboptimal glycemic control despite treatment with metformin, sulfonylurea or metformin plus a sulfonylurea was examined in three large 30-week randomized, triple blind placebo controlled, multicenter, phase III trials. Mean hemoglobin A1c was 8.2-8.5% with the primary efficacy endpoint in all three studies was the change from baseline in HbA1c.[11]

Exenatide has also been compared with insulin glargine as adjunctive therapy for patients with type diabetes not controlled on oral agents and the results showed that exenatide was associated with a greater reduction in postprandial glucose whereas insulin glargine was more effective at lowering fasting glucose. Exenatide, however, was associated with a mean 2.3 kg weight loss, whereas patients treated with insulin glargine gained of 1.8 kg.



Post hoc completer analyses revealed that the beneficial effects of exenatide on HbA1c and bodyweight were maintained for up to 82 weeks. At week 82, reductions from baseline in HbA1c in patients who received exenatide 10 ucg twice daily for the entire 82 weeks compared with those who received placebo for weeks 0-30 were 1.2% versus 1.3% in patients receiving metformin, 1.5% versus 1/3% in patients receiving a sulfonylurea and 1.0% versus 1.2% in patients receiving metformin plus a sulfonylurea. Among patients with an HbA1c of >7% at baseline, 62%, 65% and 39% of exenatide 10 ucg twice daily recipients had achieved an HbA1c of $\leq$7% at week 82.

The efficacy of adjunctive therapy with exenatide was compared with that of insulin glargine in a 26-week, randomized, multicenter, phase III study in patients with type 2 diabetes and suboptimal glycemic control despite receiving metformin plus a sulfonylurea. Patients received subcutaneous exenatide 5 ucg twice daily for 4 weeks followed by 10 ucg twice daily or insulin glargine once daily, titrated to a target fasting

glucose level of < 100 mg/dl. At baseline, HbA1c was 8.2% in exenatide recipients and 8.3% in insulin glargine recipients. Adjunctive therapy with exenatide improved glycemic control to a similar extent as insulin, at 26 weeks reductions were -1.0% vs. -1.1 and a similar proportion of patients achieved an HbA1c of $\leq$7% (48% vs. 46%). The change in bodyweight favored exenatide over insulin glargine recipients (-2.3 vs. +1.8 kg; $p<0.001$). Safety was designated a primary endpoint in the three placebo-controlled phase III trials and was also examined in the trial comparing exenatide with insulin glargine. Subcutaneous exenatide was generally well tolerated. The most frequent treatment-emergent adverse events in exenatide recipients were of mild-to-moderate severity and gastrointestinal in nature. Excluding hypoglycemia, the most commonly occurring treatment treatment-emergent adverse events in the placebo-controlled trials include nausea, vomiting, diarrhea, feeling jittery, dizziness and headache.

The incidence of nausea and hypoglycemia peaked in the initial weeks of treatment and then decreased over time. A post hoc analysis revealed that the weight loss seen in exenatide recipients was unlikely to be due to nausea. Gradual dose escalation has been shown to attenuate nausea in exenatide recipients.

Hypoglycemia occurred rarely in patients receiving exenatide 5 or 10 ucg twice daily plus metformin, with an incidence similar to that in placebo plus metformin recipients. A higher incidence of hypoglycemia occurred in patients receiving exenatide 5 or 10 ucg twice daily plus a sulfonylurea and patients receiving exenatide plus metformin plus sulfonylurea versus placebo.

Exenatide can reduce the absorption of orally administered drugs, such as digoxin,,lovastatin, lisinopril and acetaminophen.[2]

Anti-exenatide antibodies, with are of unknown clinical relevance, were present in 41-49% of treatment recipients at week 30. Antibodies were generally of low titer and did not appear to be predictive of glycemic control or adverse events.

A long acting version of exenatide was developed using a polylactide-glycolide microsphere suspension that appears to control glucose for weeks after a single injection.

Liraglutide:

Liraglutide is a synthetic acylated derivative of GLP-1 that has agonist activity at the GLP-1 receptors.

Liraglutide is a fatty acylated GLP-1 molecule that exhibits a prolonged pharmacokinetic profile after a single injection due to noncovalent association with albumin at the same time of mimicking all the actions of the native GLP-1. [12]

Subcutaneous dosing of liraglutide has been shown to reduce food intake and body weight, increase insulin secretion, inhibit glucagons secretion, decrease gastric emptying, reduce blood glucose in a dose-dependent manner and increase the proportion of pancreatic beta cells in mice.

DPP IV inhibitors:

DPP IV is the founding member of a family of DPP activity and/or structure homologue (DASH) proteins, enzymes that are unified by their common postproline cleaving serine dipeptidyl peptidase mechanism. Other members of this family include quiescent cell proline dipeptidase (QPP), DPP8, DDP9, fibroblast activation protein, attractin, and others. Except for DPP-IV, the functions of these enzymes are unknown. Nonetheless, base on their preference for cleavage of certain amino acid, there is a importance consideration of the consequences of inhibiting including immune cell proliferation,

cytokine production and induction of transforming growth factor Beta secretion. Therefore the clinical development of these drugs is linked to their degree of selectivity over other DASH family proteins required for an optimal safety profile.[13]

DPPIV is a pleiotropic enzyme that usually inactivates a variety of peptide hormones, neuropeptides and chemokines. Furthermore, it acts as a binding protein for fibronectin and adenosine deaminase, and is a co-stimulator of T cell activation. In addition to its effects on incretin hormones, DPPIV also prolongs the action of the hormones peptide YY, growth hormone releasing hormone, neuropeptide Y, substance P and chemokines such as stromal cell derived factor 1 (CXCL12) and macrophage-derived chemokine (CCL22). Potential side effects resulting from the prolongation of action of these messengers include neurogenic inflammation (substance P, neuropeptide Y), increases in blood pressure, enhanced general inflammation and allergic reactions (chemokines). However, such side effects have not been observed in preclinical animal or clinical human studies.

Interestingly, metformin has been shown to effectively reduce in vitro the activity of DPP-IV in plasma of type 2 diabetes subjects.[14]

It has been demonstrated that with available inhibitors it was possible to completely protect exogenous and endogenous GLP-1 from the DPP IV mediated degradation and thereby to enhance greatly its insulinotropic activity. Numerous subsequent studies have indicated that administration of orally active inhibitors markedly improve metabolism and glucose regulation in animal models.

The most important potential of the treatment with DPPIV inhibitors should be found in their oral availability and lack of side effects. This means that they can be offered to

subjects at risk for developing diabetes for example: persons with IGT with genetic disposition, obese subjects and subjects with mild diabetes.

Delayed gastric emptying, nausea and vomiting are GLP-1 related side effects. These are seen with high, non-physiological concentrations (above about 60 pmol/l in human plasma), which are only achieved after exogenous administration of the incretin. DPPIV inhibition raises the proportion of active GLP-1 rather than the total of GLP-1 concentration (up to 30 pmol/l after a meal). In this way, an inhibition supports the physiological role of GLP-1 without producing the GLP-1 concentrations that induce the GLP-1 related side effects.[15]

In order to explain the insulinotropic effects of DPPIV inhibitors, mechanisms other than those that prevent GLP-1 degradation in the circulation have to be taken into account. The stabilization of GLP-1 is an important component but also other insulinotropic hormones and neuropeptides undergo stabilization as well.

Both short and long term (probably adaptive) insulinotropic effects are observed during DPPIV inhibition. In OGTT's following a single administration, these agents show clear and immediate effects in normal and diabetic animals. Long term diabetes treatment with these inhibitors results in further improvement. For example, glucose tolerance in diabetic patients was higher after the administration of DPPIV inhibitors for 4 weeks than it was initially.

Very recently (second week of October 2006), the FDA approved the drug sitagliptin (Januvia) for the use in T2DM as monotherapy after a trial of lifestyle modifications or as adjunctive therapy in combination with metformin or thiazolinediones agents.

The usual dose is 100 mg/d per day, with a very good safety and adverse effect profile.

It can be used in patients with renal insufficiency adjusting the dose using either the creatinine clearance or the values of serum creatinine. This drug appears to be safe to use with comorbidites such as congestive heart failure and mild to moderate liver insufficiency, which becomes an important new tool for the treatment of T2DM. Another agent, vildagliptin (Galvus) is awaiting approval expected for the month of November 2006 with similar indications and profile.

Future directions:

An unequivocal hypoglycemic action of GLP-1 in Type 1 Diabetes was demonstrated in the studies of intravenous infusion of the peptide in subjects with type 1 DM in the hyperglycemic post-absorptive state. Under these conditions, without administration of insulin, parenteral infusion of GLP-1 drove blood glucose levels towards the normal range, and this was associated with inhibition of glucagon secretion. It is important to recognize that, in order to interpret the results correctly, the effect of GLP-1 on gastric emptying should be taken into account.

An interesting clinical scenario is the use of GLP-1 agonists on recent onset type-1 DM in whom it well known that there is a substantial recovery of beta cell function through the early months of treatment with insulin, with or without immunotherapy.

This issue of potential clinical importance in the setting of development of interventions to preserve endogenous insulin secretion in recent-onset Type 1 DM.[16]

Another possible important application of these drugs in the recipients of whole pancreas grafts or of isolated islets, where potential effects on pancreatic beta cells might be discernible and clinically important.

Continuous subcutaneous infusion can be a way of providing a constant supply of GLP-1 using commercially available insulin pumps. In one such study, a reduction of HbA1c of 1.2%, free fatty acids and weight loss of approximately 2 kg.[17]

Given their related, but distinct, modes of action, GLP-1 agonists and DPPIV inhibitors appear to complement one another, suggesting that a combination of two agents may be of benefit.

Because DPPIV inhibition primarily supports the physiological functions of endogenous GLP-1 and other insulinotropic hormones, it can be anticipated that such inhibitors will be of particular interest in early forms of T2DM. Protective effects on beta cells would be of great value in these patients and might partly restore their impaired insulin secretion.

Due to the fact the levels of GLP-1 are lower than normal in obese individuals (as in T2DM), treatment with DPPIV inhibitors may restore endogenous active GLP-1 to normal levels, thereby affording excellent therapeutic effects. In addition, if elevated GLP-1 levels result in increased beta cell mass in the long term, treatment with these agents would offer great potential of T2DM.

It appears that in an animal model, orthotopic transplantation of the duodeno-pancreas preserved a normal entero-insular axis of endocrine and exocrine pancreatic functions. [7]

The GLP-1 receptor is a G protein-coupled receptor and is a distinct member of the glucagons-secretin receptor superfamily that has been shown to function by causing intracellular calcium influx in addition to upregulating cAMP and interestingly elevated cAMP has been shown to protect against apoptosis in several cell lines including heart muscle. Bose et al, using both isolated perfused rat heart and whole animal models found

that a GLP-1 infusion in combination with a DPPIV inhibitor before an ischemic challenge, protected both models from myocardial infarction, apparently via activating multiple pro-survival kinases.[18]

Furthermore, pharmacological inhibition of cAMP pathways abolished the protective mechanism and it appears is independent of the augmentation (incretin effect) of insulin levels.[18]

Conclusions:

Diabetes Mellitus type 2 has developed into a very complex heterogenous group of disorders, with a progressive natural history of insulin secretion failure, that despite the introduction of many agents over the past 10 years, still cannot be easily controlled.

The intricate interations of multiple pathophysiological pathways including the Central and Peripheral Nervous Systems, the Gastroinstestinal Tract and the Endocrine organs make it a difficult to task to achieve good metabolic control and even further down, the promise of cure and prevention.

This review is aimed to understand the current state of knowledge of the entero-insular axis and to explore the therapeutical opportunities available with the newly introduced agents.

The world is watching with interest as the United States starts with full force into the incretin hormone field and awaits the dream of regression of the disease in the near future.

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