Insulin production is much greater in response to oral glucose relative to the same quantity of glucose administered intravenously. This so-called ‘incretin effect’ is attributed to signals arising from the gut following ingestion of glucose that potentiate insulin release from pancreatic beta cells. There are two hormones released from endocrine cells lining the intestine during the consumption of glucose that collectively can account for the incretin effect. Both glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted in proportion to the quantity of glucose ingested and potently stimulate insulin release. Notably, the insulinotropic action ceases once glucose levels return to basal. In subjects with type 2 diabetes, the incretin effect is typically impaired or absent, such that oral glucose no longer yields a greater insulin response than intravenous glucose. Interestingly, GIP infusion does not promote insulin secretion in these subjects, whereas GLP-1 retains insulinotropic activity. Therefore, a defect in GIP signaling may contribute to the pathogenesis of type 2 diabetes, whereas GLP-1 may be useful as a therapeutic agent. Indeed GLP-1 has several other anti-diabetic actions, including suppression of glucagon release, reduction of gastric emptying, reduction of food intake, and enhancement of insulin biosynthesis and beta cell mass. The usefulness of exogenous GLP-1 is hampered by rapid dipeptidyl peptidase IV (DPIV) mediated degradation following injection or infusion. Therefore, clinical trials are underway with DPIV-resistant analogs and DPIV inhibitors as novel agents to treat diabetes.

McIntyre et al. demonstrated that intravenous glucose administration resulted in a lower plasma insulin response than when given by intrajejunal infusion, even though lower blood glucose levels were achieved by the later [52]. By matching the intravenous and oral glucose profiles, it was estimated that close to 50% of the insulin response was a result of gut factors [75].

GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP)

GIP is a 42 amino acid peptide that was isolated from extracts of porcine intestine by Brown and colleagues in 1970 [6-8]. It was originally termed gastric inhibitory polypeptide as GIP was demonstrated to inhibit gastric acid and pepsin secretion. A few years later, Dupré et al. demonstrated that porcine GIP infused intravenously in humans in concert with glucose significantly augmented insulin release relative to glucose alone [17]. The insulin response was maintained for the duration of the GIP infusion and was not observed during euglycemic conditions. The glucose-dependent insulinotropic activity of GIP was also observed in rats [71], dogs [73] and in another study in humans [20]. As a result of these observations, it was proposed that the acronym GIP be given the alternate designation, glucose-dependent insulinotropic polypeptide.

GIP is released from gut endocrine cells termed ‘K-cells’ lining the mucosa of the duodenum and jejunum [9]. Circulating levels of GIP increase several fold following ingestion of meals containing glucose or fat [45, 60, 72]. Notably, GIP release in response to oral fat alone is not associated with an increase in plasma insulin levels unless intravenous glucose is also administered [11, 79]. The glucose dependency of GIP-stimulated insulin secretion provides a safeguard against inappropriate insulin release that might otherwise occur during a high fat, low carbohydrate meal. Under these circumstances, GIP is predominantly functioning as an ‘enterogastrone’, or hormone secreted in response to fat or its digestive products in the intestinal lumen that inhibits gastric acid secretion [42].
The insulinotropic activity of GIP appears to result from direct actions of GIP on pancreatic beta-cells, as GIP is insulinotropic in isolated islets [26] and beta-cell lines [2]. The GIP receptor is expressed on beta-cells where GIP binding evokes activation of adenyl cyclase and an increase in cAMP levels [86]. Mice in which the expression of the GIP receptor has been disrupted display impaired glucose tolerance [56]. Similarly, blocking GIP action with antisera to the receptor [46] or to GIP itself [18] results in impaired insulin secretion and hypoglycemia, confirming the importance of GIP in promoting brusk insulin secretion following ingestion of glucose in order to minimize blood glucose excursions. However, these types of studies also revealed that GIP alone is unlikely to fully account for the incretin effect [18, 19].

**GLUCAGON-LIKE PEPTIDE-1 (GLP-1)**

During the cloning of the anglerfish preproglucagon in the early 1980’s, it was noted that in addition to glucagon, the precursor molecule contained a glucagon-related peptide (GRP) that bore a strong homology to the sequence of GIP [48, 50]. This led the investigators to suggest that the GRP might be an intestinal incretin hormone. This notion was supported by the investigators observation that similar preproglucagon mRNAs were expressed in the anglerfish pancreas and intestine [49]. Mammalian preproglucagon were subsequently cloned and the pancreatic and intestinal GRPs were termed glucagon-like peptides (GLPs) [4, 47]. It was determined that while glucagon is the primary bioactive peptide formed in pancreatic alpha-cells, post-translational processing of proglucagon in intestinal “L-cells” liberates a 30 amino acid peptide, GLP-1 [15, 57] which soon thereafter in 1987 was demonstrated to be a potent insulinotropic hormone in rats [58], pigs [35] and humans [44]. Like that of GIP, the plasma level of GLP-1 increases following ingestion of meals containing fat or glucose [22, 28, 44, 69] and the insulinotropic activity of GLP-1 is glucose-dependent [87]. Therefore, GLP-1 fulfills the role of an incretin hormone. Studies in which intravenous glucose is supplemented with physiological concentrations of GIP and GLP-1 indicate that collectively, these two hormones can fully account for the incretin effect in humans [63].

The receptor for GLP-1 is present on pancreatic beta-cells, and like the GIP receptor is coupled to activation of adenylate cyclase [83]. As predicted, mice in which the GLP-1 receptor is disrupted have impaired glucose tolerance [81]. However, these animals also frequently display fasting hyperglycemia, indicating that the physiological importance of GLP-1 extends beyond its role as an incretin hormone [81]. This notion is supported by studies with GIP and GLP-1 antagonists in which acute disruption of GLP-1 action, but not GIP, increases the blood glucose excursion and decreases the insulin response to intraperitoneal glucose [3]. Thus while the role of GIP in glucose homeostasis appears restricted to its incretin action, GLP-1 plays an essential role in regulating glycemia independent of enteral nutrient ingestion [3]. GLP-1 infusion reduces plasma glucagon levels and humans infused with a GLP-1 antagonist in the euglycemic state have significantly increased plasma glucagon levels, in spite of unaltered plasma insulin, causing blood glucose levels to rise [80]. Thus basal GLP-1 is important for reducing glucagon production, perhaps mediated by the inhibitory hormone somatostatin [33].

It is becoming increasingly evident that GLP-1 has several additional actions that help to reduce blood glucose excursions. Patients infused with GLP-1 report a feeling of satiety suggesting a role for GLP-1 in the regulation of food intake. Thus GLP-1 infusion dose-dependently reduces spontaneous energy intake in humans [24, 31], and repeated administration to rats yields weight loss [12, 53]. The mechanism appears to require GLP-1 signaling pathways in hypothalamic satiety centers [85]. At higher pharmacological doses subjects can experience gastrointestinal discomfort and nausea — a result of the actions of GLP-1 to reduce gastric emptying. When administered at physiological doses, the ability of GLP-1 to reduce gastric emptying persists, such that a physiological function as an “ileal brake” has been ascribed to GLP-1 [65]. Therefore, as a result of decreased food intake, decreased gastric emptying, and reduced glucagon concentrations, GLP-1 infusion in humans during a meal can reduce the glucose excursion during a meal with no change or even a reduction in the meal-related insulin responses [65, 88]. These findings have led some to speculate that under normal conditions, in contrast to GIP, GLP-1 may play only a minor role in the incretin effect [62]. Nevertheless, its potent insulinotropic actions during hyperglycemia suggest therapeutic potential for GLP-1 in the treatment of diabetes. Moreover, unlike the sulfonylurea class of insulin secretagogues, GLP-1 stimulates insulin biosynthesis [16] and increases beta cell mass by stimulating neogenesis and proliferation, promoting differentiation and inhibiting apoptosis [14]. Therefore, GLP-1 presents several significant advantages over conventional diabetes therapies.

**IMPAIRED INCRETIN EFFECT IN DIABETES**

In subjects with type 2 diabetes, there is a blunted or absent incretin effect — glucose induced insulin secretion is no longer enhanced when glucose is provided...
orally versus intravenously [66] (fig. 1). Furthermore, an impairment of glucose-induced insulin secretion characterizes pre-diabetic subjects with “impaired glucose tolerance”, and thus an impaired incretin effect may not only characterize type 2 diabetes, but also predispose the onset of diabetes [25]. These observations suggest abnormalities in GIP or GLP-1 secretion or action could be involved in the pathogenesis of diabetes. There does not appear to be any consistent impairment of GIP secretion in subjects with type 2 diabetes. However, multiple studies have determined that the insulinotropic action of GIP is markedly reduced in diabetic subjects, compared to healthy subjects [36, 43]. Notably, in some studies, preserved insulinotropic activity of GLP-1 has been observed in the same diabetic subjects that fail to respond to GIP [21, 64]. Therefore, a defect in GIP action may contribute the reduction in insulin secretion that characterizes subjects with impaired glucose tolerance and type 2 diabetes. In a rat model of obesity and diabetes, the Vancouver Diabetic Fatty Zucker rat, impaired GIP action may be attributed to reduced expression of GIP receptor in pancreatic islets [51]. Whether humans with diabetes also have a defect in islet GIP receptor expression remains to be determined.

The GLP-1 response to meals is perhaps 20% lower in patients with type 2 diabetes relative to healthy subjects [84], which is probably insufficient to account for the impaired insulin secretion. Furthermore, in contrast to GIP, the insulinotropic action to exogenous GLP-1 is typically retained in subjects with type 2 diabetes [21, 64]. Indeed infusion of GLP-1 in patients with type 2 diabetes virtually normalizes both fasting and meal-induced excursions in blood glucose [30, 78, 90]. Given the glucose-dependent nature of GLP-1 that eliminates the risk of hypoglycemia, and additional complementary anti-diabetic actions of GLP-1, there is a great deal of interest in using GLP-1 as a novel strategy to treat type 2 diabetes.

DEGRADATION OF GIP AND GLP-1

Given that type 2 diabetes is characterized by a faulty incretin effect, incretin therapy would appear to be a rational approach to improve glucose homeostasis. While continuous infusions of GLP-1 are very effective in this regard [30, 78, 90], limited success has been achieved by injection [29, 67]. It is likely that the poor results of injection are attributable to the rapid degradation of the incretins. Within minutes of secretion or injection, both GIP and GLP-1 undergo proteolytic cleavage by the enzyme dipeptidyl peptidase IV (DPIV) which removes an amino-terminal dipeptide, resulting in non-insulinotropic fragments [13, 39]. The enzyme is widely expressed, including the very capillaries into which the hormones are normally secreted [32]. Thus there are typically much greater concentrations of the inactive fragments relative to the intact peptides. As a result of the activity of dipeptidyl peptidase IV, achieving biologically effective incretin levels requires maintained infusion, which is not very practical from a therapeutic perspective. Therefore, various attempts have been made to protect the insulinotropic forms of the incretins sufficiently to normalize blood glucose levels.

Figure 1: Blunted incretin effect in subjects with type 2 diabetes. Venous immunoreactive insulin levels (IR-Insulin) for 14 diabetic patients and 8 age- and weight matched metabolically healthy control subjects after an oral glucose load (50g/400ml; filled circles) and during “isoglycemic” intravenous glucose infusion (open circles). Asterisks denote significant difference (p<0.05) to the respective value after the oral load. Adapted from Nauck et al. [66], copyright Springer-Verlag.
GIP ANALOGS

Despite the fact that several studies have demonstrated an absence of insulinotropic activity of GIP in subjects with type 2 diabetes, it remains possible that elevated pharmacological levels might still be therapeutic. The lack of effects of GIP on GI motility may facilitate the use of higher GIP concentrations than GLP-1, which is limited by gastrointestinal actions. Thus analogs of GIP have been generated in which there are substitution, deletion, or modification of residues in the amino terminus that retain insulinotropic activity yet resist cleavage, and thereby inactivation, by DPIV [27]. Such analogs may be more amenable to single mealtime injections than native GIP and thus more practical than prolonged infusions. Subcutaneous injection of one such analog, [D-Ala (2)] GIP, was effective at reducing the glycemic excursion during an oral glucose tolerance test in DPIV-resistant Zucker rats via augmented insulin secretion [34]. These findings indicate that it is possible to overcome GIP resistance in diabetic models and thus studies with DPIV-resistant GIP analogs in diabetic humans are warranted.

GLP-1 ANALOGS

While the effect of exogenous GIP appears attenuated in subjects with type 2 diabetes, GLP-1 retains potent insulinotropic activity. In addition, GLP-1 acutely suppresses glucagon secretion and slows gastric emptying, such that GLP-1 given by continuous infusion or multiple injections is able to dramatically improve postprandial glucose homeostasis in subjects with type 2 diabetes (fig. 2). Therefore, there has been a great deal of interest by pharmaceutical companies in developing novel therapeutics based upon GLP-1. Since the utility of native GLP-1 is restricted by the rapid DPIV-mediated degradation, much of the effort has been directed towards the development of DPIV-resistant GLP-1 analogs that retain biological activity for hours, rather than minutes. LY315902 (Eli Lilly & Company) is a GLP-1 analog that retains similar pharmacological properties of native GLP-1, yet is DPIV resistant as a result of an N-terminal modification and has a circulating half-life of several hours due to the addition of an aliphatic octanoic acid moiety which promotes binding with serum albumin [10]. The biological activity of this compound has been reported in rats [61], but not yet in humans. NN2211 (liraglutide; Novo Nordisk A/S) is another acylated GLP-1 derivative that attains prolonged action as a result of albumin binding, metabolic stability, and slow release from the injection site. Bedtime administration of NN2211 in the form of a single subcutaneous injection (10µg/kg) significantly reduced fasting and postprandial (~ 12hr post administration) glucose levels in subjects with type 2 diabetes [37]. While GLP-1 analogs such as LY315902 and NN2211 may be suitable for once per day administration, others are developing longer-lasting sustained release formulations. Albugon (Human Genome Sciences), a DPIV resistant form of GLP-1 fused to human serum albumin, has a circulating half life in monkeys of ~ 3 days [5]. Remarkably, this large GLP-1 fusion protein retains biological activity and is able to restore near-normal glycemic control over a 24 hour period in diabetic rodent models. CJC-1131 (Conjuchem) is a DPIV resistant “GLP-1 analog” that conjugates to albumin in vivo resulting in a half life in rats of 18 hours [40]. Clinical trials with this and the other GLP-1 analogs will continue to carefully assess safety and efficacy, both as monotherapies and in combination with other glucose-lowering agents.

Amylin Pharmaceuticals in partnership with Eli Lilly is pursuing a naturally occurring GLP-1 agonist, Exendin-4, a 39 amino acid peptide isolated from the salivary gland venom of the lizard Heloderma suspectum [68]. Exendin-4 has 53% amino acid identity with mammalian GLP-1, binds with high affinity to the GLP-1 receptor, and in most bioassays has the same actions as GLP-1. Notably, the N-terminal sequence of exendin-4 is resistant to degradation by DPIV. Therefore, it has a much longer plasma half-life than GLP-1 and as a result is more potent. In clinical trials involving patients with type 2 diabetes, synthetic exendin-4 (exenatide) administered once or twice per day was found to reduce both fasting and postprandial glucose excursions [41]. Mild transient headache, nausea and vomiting were the main adverse events. In a phase II study in patients not attaining HbA1c goals ≤ 8% with oral sulfonylureas and/or metformin, 28 days of exenatide (0.08µg/kg, 2 or 3 times daily) reduced HbA1c by ~ 0.9% compared to baseline [23]. This is quite remarkable considering that HbA1c reflects glycosia over a period of 2 to 3 months. Almost 1/3 of the subjects in this trial reported nausea, but this declined to 13% by the end of the 28 day treatment period. Antibodies to exenatide were detected in 19% of the subjects but there was no correlation with the glycemic response. Amylin is working with Alkermes, Inc. to develop a sustained release formulation of exenatide (exenatide LAR) that might allow once-a-month administration to treat type 2 diabetes. Future studies will be required to determine if gastrointestinal actions of exenin and immune responses limit the therapeutic potential of exendin-based compounds.
DIPEPTIDYL PEPTIDASE IV (DPIV) INHIBITORS

With the demonstration that DPIV is the principle enzyme responsible for the degradation of both GIP and GLP-1 in vitro [55] and in vivo [39, 70], it was proposed that inhibition of DPIV might be a means to enhance the incretin effect [70]. An attractive feature for testing this approach to treat diabetes was the availability of orally active inhibitors of DPIV. In 1998, Pederson and colleagues demonstrated that acute inhibition of endogenous DPIV by the specific inhibitor Ile-thiazolidide in obese Zucker rats nearly normalized glucose tolerance with enhanced insulin secretion attributable to increased circulating half-lives of GIP and GLP-1 [74]. Subsequent studies examined the effect of DPIV inhibitors administered to Zucker rats for several weeks and noted that improvements in glucose tolerance could be sustained [76], with improved hepatic and peripheral insulin sensitivity [77], such that the onset of diabetes could be delayed [82]. Several pharmaceutical companies have entered into development of DPIV inhibitors to treat diabetes. A Novartis compound (NVP DPP728) was tested in 93 patients with type 2 diabetes (mean fasting blood glucose 8.5mM, HbA1c 7.4%) over 4 weeks and found to reduce mean 24-hour glucose levels by 1.0mM and HbA1c to 6.9% [1] (fig. 3). The treatment was well tolerated, although there was one confirmed episode of hypoglycemia, 4 subjects with symptoms of nasopharyngitis and 5 that experienced pruritus. Confounding the interpretation of these findings is the fact that DPIV regulates the biological activity of dozens of substrates in addition to the incretins, including several cytokines, hematopoietic growth factors, neuropeptides and hormones [54]. Therefore, ongoing clinical trials will need to carefully assess whether any symptoms arising are specific to the drug used or a result of DPIV inhibition in general. Rats deficient in DPIV appear to have good general health and normal neurological and motor functions, but exhibit various behavioral differences, including a reduced stress-induced analgesia [38]. These animals also appear to develop compensatory down-regulation of incretin hormone production and/or sensitivity [72]. It will be of interest to determine if the therapeutic effect of long-term DPIV inhibition in humans wanes over time as a result of the same phenomenon.

CONCLUSION

As results from clinical trials continue to reveal the therapeutic potential of incretin mimetics, there is increasing optimism that these compounds may successfully develop into a novel approach to treat diabetes. It appears that these compounds are likely to have several significant advantages over currently available therapies, such as the glucose-dependent nature of their actions and complementary effects of reducing food intake and gastric emptying, suppressing glucagon production, and increasing insulin biosynthesis and beta cell mass. Thus incretin mimetics have the potential to completely restore normoglycemic control and thereby reduce or even eliminate diabetic complications, even in subjects who currently fail to respond to conventional oral anti-hyperglycemia medicines. There are currently efforts to optimize dosing and mode of delivery as well as explore incretin mimetic combination therapy with existing diabetes drugs. The ability to deliver DPIV inhibitors orally to augment endogenous incretin action is appealing, but the lack of specificity warrants careful clinical trials to examine the full spectrum of actions and safety of the compounds. Clinical studies over the next few years will reveal if incretins will come to the rescue for patients suffering from diabetes.

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Figure 3: Inhibition of DPIV improves glucose control over a 4-week study period in subjects with type 2 diabetes. Fasting blood glucose and HbA1c values during the 4-week run-in period and after 4 weeks of treatment (left) and the 4-hour glycemic response to breakfast ingestion (450kcal) before and after the 4-week treatment period with placebo (open circles, n=32) or DPIV inhibitor at 100mg t.i.d. (triangles, n=30) or 150mg b.i.d. (filled circles, n=30) in subjects with type 2 diabetes (means±SEM). From Ahren et al. [1], copyright © 2002 American Diabetes Association. From Diabetes Care, vol. 25, 2002; 869-879. Reprinted with permission from The American Diabetes Association.

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