The Incretin Approach for Diabetes Treatment

Modulation of Islet Hormone Release by GLP-1 Agonism

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Glucagon-like peptide (GLP)-1 is a gut hormone that stimulates insulin secretion, gene expression, and β-cell growth. Together with the related hormone glucose-dependent insulinotropic polypeptide (GIP), it is responsible for the incretin effect, the augmentation of insulin secretion after oral as opposed to intravenous administration of glucose. Type 2 diabetic patients typically have little or no incretin-mediated augmentation of insulin secretion. This is due to decreased secretion of GLP-1 and loss of the insulinotropic effects of GIP. GLP-1, however, retains insulinotropic effects, and the hormone effectively improves metabolism in patients with type 2 diabetes. Continuous subcutaneous administration greatly improved glucose profiles and lowered body weight and HbA_{1c} levels. Further, free fatty acid levels were lowered, insulin resistance was improved, and β-cell performance was greatly improved. The natural peptide is rapidly degraded by the enzyme dipeptidyl peptidase IV (DPP IV), but resistant analogs as well as inhibitors of DPP IV are now under development, and both approaches have shown remarkable efficacy in experimental and clinical studies. *Diabetes* 53 (Suppl. 3):S197–S204, 2004

THE INCRETIN EFFECT IN TYPE 2 DIABETES

It is now recognized that inadequate secretion of insulin may be a very early element in the development of type 2 diabetes and that its progression is due to declining β-cell function (1–3). The β-cell defect is partly due to loss of β-cells, but the loss, which may amount to 50% in advanced type 2 diabetes (4), does not seem to parallel the dysfunction. This raises the possibility that the dysfunction could at least be partly due to dysregulation. Thus, dysfunction of the autonomic innervation of the islets could be responsible, perhaps particularly with respect to the early insulin response to meal (5,6). Endocrine regulation of islet function could also be involved. Up to two-thirds of the insulin normally secreted in relation to meal intake is thought to be due to the insulinotropic actions of the so-called incretin hormones. The incretin effect is defined as the increased stimulation of insulin secretion elicited by oral as compared with intravenous administration of glucose under similar plasma glucose levels. Indeed, patients with type 2 diabetes have been demonstrated to exhibit an almost total loss of incretin effect (7). Therefore, it could be hypothesized that deficient incretin function plays an essential contributory role in the pathogenesis of type 2 diabetes. Deficient incretin effect could be due to impaired secretion of the incretin hormones as well as to impaired effects on islet function. Furthermore, if a defect is identified, a therapy based on substitution of the defective element might be devised.

THE INCRETIN HORMONES

Today, there is general agreement that the two most important incretin hormones are glucose-dependent insulinotropic polypeptide (GIP), formerly known as gastric inhibitory polypeptide, and glucagon-like peptide (GLP)-1 (8,9). Both are potent insulinotropic hormones released by oral glucose as well as ingestion of mixed meals.

GIP. GIP is a peptide of 42 amino acids belonging to the glucagon-secretin family of peptides, the members of which have pronounced sequence homology, particularly in the NH_2-terminus. It is processed from a 153–amino acid precursor (10), but specific functions for other fragments of the precursor have not been identified. The GIP receptor has been cloned and is related to the receptors for the other members of the glucagon-secretin family. It is expressed in the islets and also in the gut, adipose tissue, heart, pituitary, adrenal cortex, and several regions of the brain (11). GIP is secreted from specific endocrine cells, so-called K cells, with highest density in the duodenum but found in the entire small intestinal mucosa (12). Secretion is stimulated by absorbable carbohydrates and by lipids. GIP secretion is therefore greatly increased in response to meals, resulting in 10- to 20-fold elevations of the plasma concentration (13).

Interaction of GIP with its receptor on the β-cells causes elevation of cAMP levels, which in turn increases the intracellular calcium concentration and enhances exocytosis of insulin-containing granules by a mechanism distal to the elevation of calcium (14).

GLP-1. GLP-1 is a product of the glucagon gene (15). It is expressed not only in pancreatic α-cells but also in the L-cells of the intestinal mucosa, one of the most abundant endocrine cells of the gut (16). Here the primary translation product proglucagon is not cleaved to produce gluca-

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DPP IV, dipeptidyl peptidase IV; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide.

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gon like in the islets but to release from its COOH-terminal part the two glucagon-like peptides GLP-1 and GLP-2 (17), both showing ~50% sequence homology with glucagon. GLP-1 secretion is stimulated by the presence of nutrients in the lumen of the gut (9), and its secretion throughout the day is highly correlated to the release of insulin (18). GLP-1 is one of the most potent insulin-releasing substances known, exceeding that of GIP (19). Like GIP it interacts with a G protein–coupled receptor on the β-cells, which causes accumulation of cAMP, and most if not all of the subsequent effects seem to be secondary to this (14).

Which is the most important incretin hormone? The incretin function of GIP, first suggested by Dupre et al. (20), was confirmed in detailed clamp studies by Andersen et al. (21) and has been probed in immunoneutralization studies (22) and, more recently, in studies employing a fragment, GIP (7-30)amide, which turns out to be a GIP receptor antagonist (23). Both treatments reduced insulin responses to oral glucose and impaired glucose tolerance. Mice with a targeted deletion of the GIP receptor gene become glucose intolerant (24,25). Using the mimicry approach, in which endogenous concentrations are mimicked by intravenous infusion, it could be shown that the elevated GIP concentrations elicited by oral glucose can completely account for the accompanying augmented insulin release (26). The glucose intolerance of the GIP receptor knockout mice is not severe, but these mice may compensate by hypersecretion of other insulinotropic factors. Nevertheless, it is evident that GIP is not the only incretin hormone. Immunoneutralization experiments clearly showed that intestinal extracts contain potent insulinotropic agents in addition to GIP (27). Also, investigations by Lauritsen and colleagues (28,29) in patients with resections of different parts of the small intestine, or in celiac disease, showed that the incretin effect does not correlate to GIP secretion, and that the distal small intestine releases an additional incretin. In all probability, this additional incretin is GLP-1. Like GIP, it is strongly insulinotropic in mimicry experiments (30), and antagonists of the GLP-1 receptor show that GLP-1 is responsible for a substantial part of the insulin response to oral glucose (31,32). In agreement with these observations, mice with a targeted deletion of the GLP-1 receptor become glucose intolerant and may develop fasting hyperglycemia (33). GIP secretion and pancreatic sensitivity to GIP are augmented in these knockout mice (34), suggesting that acute ablation of the GLP-1 activity could have even more extensive effects.

Thus, the evidence for an important incretin function for both hormones is quite strong. However, GIP has been reported to be insulinotropic only at elevated glucose levels and to be less potent than GLP-1. GLP-1 on the other hand circulates in much lower (up to 10-fold) concentrations than GIP. Vilsboll et al. (35), therefore, recently reinvestigated the insulinotropic effects of the two hormones infused at rates that would result in physiological elevations of their concentration; glucose concentrations were clamped at fasting or slightly elevated levels in order to mimic the prandial situation. At their physiological postprandial concentrations, the two hormones had similar and highly significant insulinotropic effects at fasting glucose as well as at 6 mmol/l, whereas at 7 mmol/l GLP-1 was somewhat more effective. Thus, both hormones normally contribute to the incretin effect in humans from the beginning of the meal (because increases in their concentrations are seen already after 5–10 min). Together, the two hormones appear to act in an additive manner. Thus, when GIP and GLP-1 infusions (which separately provided similar insulin responses) were combined, the resulting response amounted to approximately the sum of the individual responses (36). Recently, mice with a double knockout of the GIP and GLP-1 receptors have been generated (37,38). The results obtained in these animals are consistent with an additive effect of the two hormones on glucose tolerance. In none of the studies did the double knockout animals develop fasting hyperglycemia, and their insulin sensitivity was normal. Interestingly, the potent GLP-1 receptor agonist exendin-4 (AGONISTS OF THE GLP-1 RECEPTOR: EXENDIN 4), which in control animals profoundly lowered blood glucose, had no effect in the double knockouts, suggesting that it acts exclusively via incretin receptors. Furthermore, valine pyrrolidide and SYR 106124, inhibitors of dipeptidyl peptidase IV (DPP IV) (see below), the enzyme responsible for the initial metabolism of GLP-1 and GIP, which lower blood glucose and improve glucose tolerance in both GLP-1 and GIP single receptor knockout mice, had no effects in the double knockouts, suggesting that the effects of these inhibitors are exerted mainly via enhancing GIP and GLP-1 survival, without important involvement of other substrates.

INCRETINS IN TYPE 2 DIABETES

Given that GIP and GLP-1 together are responsible for the incretin effect in healthy subjects, it is possible to analyze the incretin defect in patients with type 2 diabetes. Theoretically, the defect could be due to impaired secretion or accelerated metabolism of the incretin hormones; alternatively, the effect of the hormones could be compromised.

There are many publications on the secretion of GIP in type 2 diabetes, and both increased, normal, and decreased secretion have been reported (39). In a recent study in patients with type 2 diabetes covering a wide clinical spectrum of the disease, Toft-Nielsen et al. (40), using a highly specific COOH-terminal GIP assay, found near-normal fasting levels and meal responses with no correlations between metabolic parameters and GIP responses. In the same study, a very significant impairment of GLP-1 secretion was observed. By multiple regression analysis, the impairment was found related to impaired β-cell function. In a previous study in a small group of identical twins discordant for type 2 diabetes, the GLP-1 response was lower in the diabetic twin (41), whereas in first-degree relatives of diabetic individuals, the 24-h GLP-1 profiles were normal (42), probably indicating that impaired secretion is a consequence rather than a cause of diabetes. GLP-1 is metabolized extremely rapidly by the ubiquitous enzyme DPP IV, which cleaves off two amino acid residues from the NH_2-terminus and renders the metabolite [designated GLP-1 (9-36)amide] inactive (43). This occurs with such rapidity that a steady state is never obtained. In fact, only ~10–15% of the hormone reaches the systemic circulation and thereby the pancreas in intact biologically active form. In agreement with this, the circulating concentrations of the intact hormone are much lower than those of the metabolite, but in patients with

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type 2 diabetes during meal intake, intact hormone concentrations are lower than in control subjects (44). Differences in elimination cannot explain this (45), which must therefore be due to decreased secretion. Physiologically, the extensive and even local degradation of GLP-1 suggests that its main activities are exerted locally before the peptide is degraded. There is indeed substantial evidence that the peptide normally acts via activation of sensory neurons, which then via central reflexes influence central and gastrointestinal functions, including insulin secretion (46–48).

The complex mechanism of action of the hormone makes it difficult to estimate the impact of decreased L-cell secretion for β-cell function, because with intravenous infusion one can only mimic the part of its actions that is exerted via the endocrine route. Activation of the sensory neural pathway probably requires higher concentrations, as probably found locally around nerve endings in the intestine. Impaired secretion of GLP-1, therefore, is likely to contribute significantly to the impaired incretin effect in type 2 diabetes.

Regarding the effect of the hormones, a dramatic difference emerges. Nauck et al. (19) studied the effects of intravenous infusions of GIP and GLP-1 in moderate type 2 diabetic patients and matched control subjects and found that the insulinotropic effect of GIP was almost lost in patients, whereas the insulin response to GLP-1 was similar to control. Similar results were obtained by Elahi et al. (49). In a recent study of advanced type 2 diabetic patients (50), GIP infusion was incapable of enhancing second-phase insulin secretion (and glucose turnover) during hyperglycemic clamp, whereas with GLP-1 the insulin response to hyperglycemia was completely restored. Although supraphysiological GLP-1 concentrations in these studies restored the glucose responsiveness of β-cells, this does not imply that the β-cell sensitivity to GLP-1 is normal. On the contrary, as shown by Kjems et al. (51), β-cell sensitivity may be considerably impaired, and this may further aggravate the impact of impaired GLP-1 secretion in type 2 diabetic patients.

THE INCRETIN APPROACH IN THE TREATMENT OF TYPE 2 DIABETES

Based on the two defects identified, the decreased secretion of GLP-1 and the loss of second phase stimulation of insulin secretion by GIP, it could be hypothesized that GLP-1 (but not GIP) could be used for diabetes treatment as a substitution therapy. Indeed, GLP-1 administration has been shown to be highly effective in the treatment of type 2 diabetes, causing marked improvements in glycemic profile, insulin sensitivity, and β-cell performance, in addition to reducing weight (52). As discussed above, the hormone is metabolized rapidly by DPP IV, and the native peptide therefore cannot be used clinically. Instead, resistant analogs of the hormone (or agonists of the GLP-1 receptor) are currently developed, along with DPP IV inhibitors demonstrated to protect the endogenous hormone and enhance its activity. Agonists currently in clinical development include both albumin-bound analogs of GLP-1 and exendin-4, a lizard peptide. Clinical studies with exendin have been carried out for >6 months and indicated efficacy in patients inadequately treated with oral antidiabetic agents. Orally active DPP IV inhibitors, suitable for once-daily administration, have demonstrated similar efficacy. Diabetes therapy based on GLP-1 receptor activation, therefore, seems very promising. Obviously, it will not be possible to evaluate the drawbacks or benefits of the individual agents until the results of extensive clinical trials are available. However, on the basis of the already available data, it is possible to discuss apparent strengths and weaknesses of the various approaches.

Diabetes treatment with native GLP-1. How effective are agonists of the GLP-1 receptor for diabetes treatment? It has been demonstrated repeatedly that intravenous infusion of GLP-1 at doses of 1–1.2 pmol · kg^{-1} · min^{-1} can normalize fasting plasma glucose in type 2 diabetic individuals, even in long-term insulin-treated patients with poor residual β-cell capacity (53). In a detailed study of a large group of patients, Toft-Nielsen et al. (54) found that GLP-1 infusion had the largest glucose-lowering effect in the patients with the highest fasting plasma glucose values; on the other hand, normalization required many hours and euglycemia was not reached even after 7 h (but glucose levels were still declining). It can be hypothesized that in patients with very little residual β-cell capacity, GLP-1 infusion will not be able to induce euglycemia in the fed state. However, virtually complete normalization of plasma glucose during infusion of GLP-1 overnight and during subsequent meals was reported by Rachman et al. (55); possibly these patients had a milder disease than those of Toft-Nielsen's study. The most important set of data in this respect was presented by Larsen et al. (56–58). They infused GLP-1 continuously to a group of type 2 diabetic patients for 7 days at four dose rates: 4, 8, 16, and 24 ng · kg^{-1} · min^{-1} (4 ng · kg^{-1} · min^{-1}corresponds to app. 1.2 pmol · kg^{-1} · min^{-1}). The two highest doses were poorly tolerated and were discontinued. This actually defines the therapeutic window for native GLP-1. There was a pronounced clinical effect of both the 4-ng and 8-ng infusion rates and no side effects. The full effect on glycemia was obtained after a few hours, and there was no further improvement during the subsequent 7 days. However, blood glucose levels were not completely normalized, with fasting and intermeal levels of ~7 mmol/l. In this group of patients, therefore, it appears that GLP-1 at the highest tolerated dose did not acutely reestablish euglycemia. Zander et al. (52) used portable insulin pumps to deliver 4.8 pmol · kg^{-1} · min^{-1} GLP-1 continuously for 6 weeks in a group of uncontrolled, obese type 2 diabetic individuals. No changes were observed in the saline-treated group, whereas in the GLP-1 group fasting and average plasma glucose concentrations were lowered by ~5 mmol/l, HbA_{1c} decreased by 1.2%, free fatty acids were significantly lowered, and the patients had a significant and gradual weight loss of ~2 kg. In addition, insulin sensitivity almost doubled, and insulin secretion capacity (measured using a 30 mmol/l glucose clamp + arginine) greatly improved. There were no significant side effects. In spite of the pronounced hypoglycemic effect (5 mmol/l), however, normal glucose values were not obtained (fasting levels ~10 mmol/l, somewhat lower toward the evening). This could be due the fact that the dose selected was not necessarily maximal (note that the dose was not associated with side effects). Further studies using the

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same technique indicated that a higher infusion rate might be more effective (59). Also, subcutaneous infusion may not be optimal; very high levels of the metabolite GLP-1 (9-36)amide are generated during the infusion, and although this metabolite has been reported to have insulin-independent glucose-lowering properties (60), it also acts as a GLP-1 receptor antagonist (61). From these studies, it can be concluded that subacute GLP-1 treatment has not yet been documented to induce euglycemia in typical type 2 diabetic subjects. It would be unreasonable to expect anything else from the GLP-1 receptor agonist described below as long as additional effects of these have not been identified.

Agonists of the GLP-1 receptor: exendin 4. The best-studied GLP-1 receptor activator is exendin 4. Its intrinsic potency toward the GLP-1 receptor seems to be similar to that of GLP-1. It may have effects in addition to those elicited by activation of the GLP-1 receptor (62), but studies in mice with GLP-1 receptor deletion do not support this (38). In vivo, however, it is much more potent than GLP-1 due to slower degradation. Amino acid no. 2 is substituted with Gly (while GLP-1 has Ala), and because of this, exendin 4 is resistant to DPP IV (63). However, this does not in itself prolong the survival of the molecule in the body very much (from a half-life of 2 min to ~4 min) (63), but exendin is also eliminated much more slowly than the GLP-1 metabolite (or position 2–substituted analogs) mainly because of a slower renal elimination with a clearance of 1.8 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} similar to the normal glomerular filtration rate (64). Otherwise, exendin 4 appears to act in humans in a manner identical to GLP-1 (64). The clinical usefulness of exendin 4 was evaluated in a proof-of-concept phase 2 study (65). Exendin 4 (now named AC2993 or exenatide) was injected subcutaneously two to three times daily for 4 weeks in patients already treated with metformin, sulfonylurea, or both. In all groups, there was a reduction in HbA_{1c} of 0.7–1.1%. The most common adverse effect was transient mild to moderate nausea. Mild hypoglycemia was reported in about a third of the patients also treated with sulfonylurea. This finding was not substantiated by measurements but could reflect a partial uncoupling of the glucose dependency of the insulinotropic actions of GLP-1 by sulfonylurea (66). On the other hand, the incidences also illustrate the potency of this combination (66). Recently, in phase 3 studies, exenatide was given twice daily initially in doses of 5 μg for 1 month and subsequently at 5 or 10 μg per injection for 5 months (see Amylin Corporation's Web site [67]). This approach apparently reduced the tendency to cause initial nausea. Mild hypoglycemia was noted in 35% of the patients also treated with sulfonylurea. The average drop in HbA_{1c} was 1% from >8%, and values <7% were observed in 40–46% (at 10 μg). A most interesting finding was significant weight loss, also observed in an open-label study (68), where a gradual weight loss was seen over a period of 26 weeks. It can be concluded that exenatide provides considerable additional glycemic control, even in patients inadequately treated with oral antidiabetic agents, and also causes weight loss, which can be predicted to provide further improvements of metabolism (69). It should be noted that twice-daily injection of exenatide does not provide full 24-h exposure of the GLP-1 receptors, considered important for full antidiabetic effect of at least intravenously administered GLP-1 (58), and this may explain the apparently less conspicuous effect on fasting plasma glucose (a drop of only 1 mmol/l over 26 weeks) with exenatide compared with native GLP-1 given continuously. A more pronounced and gradual fall in HbA_{1c} was observed, but the decline flattened out during the last 3 months. This may indicate that mean 24-h plasma glucose was lowered relatively more than fasting plasma glucose. HbA_{1c} levels after 1 year of treatment have been reported (67) and appear not to have declined further over the last half year. Again, this may reflect the lack of 24-h exposure with the current treatment regimen. In agreement with this, Amylin has announced that a slow-release formulation is in development. It should also be noted that exenatide has not been clinically evaluated in monotherapy, but only as an adjunct to existing therapies, where native GLP-1 has been shown to exert additive or even synergistic effects.

Albumin-bound GLP-1 derivatives. Another approach has been to bind a GLP-1 analog to albumin in order to exploit the slow elimination kinetics of this molecule. NovoNordisk developed an acylated derivative of GLP-1, which binds noncovalently to albumin; Conjuchem created an analog of GLP-1, which after injection establishes a covalent bond with albumin; and Human Genome Sciences generated a fusion protein consisting of a DPP IV–resistant GLP-1 analog covalently bound to human albumin.

The NovoNordisk compound NN2211 consists of native GLP-1, in which a C16 acyl chain is attached via a glutamoyl spacer to lysine residue no. 26 (while Lys34 was substituted by Arg). The compound shows slow release from the subcutaneous injection site and binds to albumin, which renders the molecule resistant to DPP IV and allows at least the bound fraction to escape renal elimination. This has resulted in a half-life in healthy and type 2 diabetic subjects of 10–12 h following a single subcutaneous injection (70) and thereby adequate 24-h exposure after single injection. In addition, in chronic treatment the large postinjection concentration excursions caused by less long-lived analogs, which may be associated with side effects such as nausea, are likely to be avoided (71). The analog itself is equipotent with GLP-1 at the cloned human GLP-1 receptor. Because of its extensive half-life, the analog has proven suitable for studies in rodents, in which native GLP-1 barely has effects because of extensive metabolism. Thus, in rats with β-cell deficiencies, NN2211 (now also called Liraglutide) had marked antihyperglycemic effects and greatly delayed development of diabetes. There were also significant effects on β-cell mass and food intake (72). Similar effects were observed in diabetic *ob/ob* and *db/db* mice (73). NN2211 also induced lasting and reversible weight loss in both normal and obese rats (74). This analog, like GLP-1 or exendin, inhibited fatty acid– and cytokine-induced apoptosis in primary β-cells (75,76). In clinical studies, NN2211 effectively reduced fasting as well as meal-related (12 h postinjection) glycemia by modifying insulin secretion, delaying gastric emptying, and suppressing prandial glucagon secretion (70). In a blinded phase 2 study involving 3 months of daily injections (77), ascending doses of NN2211 in monotherapy were com-

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pared with glimepiride. It was concluded that the compound improved glycemic control comparably to glimepiride. Weight was maintained with a tendency to decrease, and the risk of hypoglycemia was very low. Antibodies against NN2211 could not be detected. This analog clearly possesses favorable pharmacokinetic properties. The clinical results obtained so far are derived from studies in patients with very mild diabetes. The glucose-lowering effect was maximal after 1 week, and there was no further lowering over the next ~3 months.

Other agonists. The Conjuchem compound CJC-1131 is composed of a D-Ala8–substituted GLP-1 molecule with a linker and a reactive moiety attached to the COOH-terminus. After injection in vivo, this molecule conjugates covalently to Lys34 of albumin and thereby acquires the half-life of albumin. The CJC-1131–albumin conjugate binds to the GLP-1 receptor and activates cAMP with a potency similar to that of GLP-1 (78). It lowered blood glucose in wild-type mice but not in mice with a GLP-1 receptor knockout. Blood glucose levels were lower in CJC-1131–treated *db/db* mice even 1 week after discontinuation of the compound. It also increased pancreatic insulin mRNA levels and markers of β-cell proliferation (78). In recent studies in human volunteers, elimination half-lives of 9–14 days were found (79), and in studies presented at the International Diabetes Federation Congress in 2003, the compound was reported to have dose-dependent effects on glycemia lasting at least 48 h and up to 8 days in some patients. Phase 2 clinical studies are currently ongoing. The compound is theoretically of considerable interest, because it seems to demonstrate *1*) that molecules as large as albumin can get access to the relevant GLP-1 receptors and activate them and *2*) that they can do so presumably without intracellular internalization. The very long half-life of the molecule suggests that it might be useful for administration once weekly. The company claims that the conjugate is not antigenic. The apparent retention of high biological activity in spite of the presence of the albumin moiety contrasts strikingly to the NN 2211 compound, the intrinsic activity of which is clearly lowered by albumin binding (by ~2–3 orders of magnitude), suggesting that the site of attachment to albumin is of importance and that the linker position and length in 2211 could be suboptimal.

Agonists and β-cell survival. The long-term results obtained with GLP-1 analogs raise the question whether long-term GLP-1 treatment in humans is truly associated with a β-cell protective effect. Such an effect should have translated into a gradual lowering of fasting plasma glucose concentrations, which has not been observed. On the other hand, therapy for 3 months (NN 2211) may not be sufficient, and regarding exenatide, its pharmacokinetics are probably suboptimal. It must also be taken into account that for exenatide, control group results have not been reported (possibility of impairment?).

INHIBITION OF GLP-1 DEGRADATION: DPP IV INHIBITORS

The use of inhibitors of DPP IV was suggested on the background of the extreme DPP IV–mediated degradation of GLP-1 in patients with diabetes (80,81). It was demonstrated that with available inhibitors, it was possible to completely protect exogenous and endogenous GLP-1 from degradation and thereby greatly enhance its insulinotropic activity (82). Numerous subsequent studies have indicated that administration of orally active DPP IV inhibitors markedly improve metabolism in animal models of glucose intolerance. For example, in mice rendered glucose intolerant by high-fat diets, the inhibitor valine pyrrolidide almost doubled the plasma levels of intact bioactive GLP-1, augmented insulin secretion, and virtually normalized the considerably impaired glucose tolerance (83). Using the Probiodrug inhibitor, P3298, Pospisilik et al. (84) reported sustained improvements over 12 weeks in fasting glucose, glucose tolerance, insulin sensitivity, and β-cell responsiveness to glucose in diabetic Zucker fatty rats. Using the Ferring inhibitor FE 999011, Sudre et al. (85) demonstrated that chronic inhibition of DPP IV markedly delayed development of diabetes in the Zucker diabetic fatty rat. It is of considerable interest that rats with a mutation in the DPP IV gene, resulting in inactivation of the catalytic subunit of the enzyme, do not develop glucose intolerance with aging (86), while a DPP IV inhibitor (DPP728) improved glucose tolerance in aging control rats. Similarly, mice lacking DPP IV, unlike wild-type animals, are refractory to the development of obesity and hyperinsulinemia upon high-fat feeding and protected against streptozotocin-induced loss of β-cell mass and hyperglycemia (87). These effects seem to be due to elevated levels of GLP-1, and importantly indicate that absence of enzyme activity does not result in an untoward phenotype.

Proof of concept of the use of DPP IV inhibitors for diabetes treatment was obtained by Ahren et al. (88) using the Novartis DP728 inhibitor. Administered two to three times daily for 4 weeks, the inhibitor caused significant lowering of fasting as well as postprandial plasma glucose and significantly lowered HbA_{1c}. There were very few and mild side effects. Novartis has subsequently introduced another inhibitor, LAF 237, which has a longer duration of action and which is, therefore, suitable for once-daily administration. This compound was demonstrated to have, given as a single 100-mg dose, very similar effects to DP 728 administered two to three times daily (89). Notably, there were no significant side effects as noted with DP 728, which had been suspected to be possible consequences of DPP IV inhibition. There are many other substrates for DPP IV than GLP-1 (90), but it seems that the extreme degradation of GLP-1 makes it a preferential target, so that significant protection of GLP-1 may be accomplished without significantly compromising other functions of DPP IV. Most recently Novartis has released data from their Web site (91) derived from clinical studies with LAF237 showing dose-related improvements of HbA_{1c} levels when the drug was administered for 12 weeks, including a significant reduction of 0.68% from a baseline of 7.7% at a dose of 100 mg daily but lowering by 1.2% from a baseline of 8.5%. Added onto metformin, LAF 237 at 100 mg for 3 months caused an HbA_{1c} lowering of 0.82% from a baseline of 7.8%. These clinical effects must be considered highly encouraging. Judging from LAF 237 (and apparently similar results have been obtained with other inhibitors), it appears possible to maintain considerable inhibition of DPP IV activity throughout 24 h without

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causing side effects. Some DPP IV inhibitors clearly have side effects (e.g., valine pyrrolidide), including gastrointestinal bleeding in rats and dogs, cardiovascular effects, and also pruritis and nasopharyngitis observed with DP728. Such side effects are likely to be compound related and perhaps due to interference with other enzymes of the DPP family (e.g., DPP 8 and 9). In agreement with the phenotype of the mutant rats and mice, strong specific inhibition of DPP IV appears not to be intrinsically associated with untoward side effects.

The results obtained in long-term studies are striking (although only available as Web site reports from the company) and suggest that the efficacy of DPP IV inhibition increases with time. Similar observations were reported in a 12-week study in rats (84). On the other hand, it also seems clear that the inhibitors do not affect appetite and food intake and therefore do not lower body weight. This may be because, physiologically, the GLP-1 effect on these parameters is exerted before the molecule is degraded by DPP IV, as discussed above (46). It was expected that DPP IV inhibitors would be particularly suitable for mild cases of diabetes and for diabetes prevention, and early clinical experiments suggested that DPP IV inhibition would not be very effective in advanced type 2 diabetes. This could be due to the impaired secretion of GLP-1 in advanced diabetes (40) in combination with the poor insulin secretory capacity and sensitivity in such patients and their decreased sensitivity to GLP-1 (51). In addition, it has been demonstrated that DPP IV inhibition lowers the rate of secretion of GLP-1 (and GIP) in spite of elevating the plasma concentrations of intact GLP-1 (and GIP) (92) probably because of the existence of a negative feedback loop set in motion by the elevated levels of the intact peptide. It is also unclear to what extent DPP IV inhibition may be associated with β-cell protection (although a positive report with the ProBiodrug compound has appeared [93]).

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