Inhibition of the Activity of Dipeptidyl-Peptidase IV as a Treatment for Type 2 Diabetes

Jens J. Holst and Carolyn F. Deacon

The insulinotropic hormone, glucagon-like peptide 1 (GLP-1), which has been proposed as a new treatment for type 2 diabetes, is metabolized extremely rapidly by the ubiquitous enzyme, dipeptidyl peptidase IV (DPP-IV), resulting in the formation of a metabolite, which may act as an antagonist at the GLP-1 receptor. Because of this, the effects of single injections of GLP-1 are short-lasting, and for full demonstration of its antidiabetogenic effects, continuous intravenous infusion is required. To exploit the therapeutic potential of GLP-1 clinically, we here propose the use of specific inhibitors of DPP-IV. We have demonstrated that the administration of such inhibitors may completely protect exogenous GLP-1 from DPP-IV-mediated degradation, thereby greatly enhancing its insulinotropic effect, and provided evidence that endogenous GLP-1 may be equally protected. Preliminary studies by others in glucose-intol-erant experimental animals have shown that DPP-IV inhibition greatly ameliorates the condition. GLP-1 has multifaceted actions, which include stimulation of insulin gene expression, trophic effects on the β-cells, inhibition of glucagon secretion, promotion of satiety, inhibition of food intake, and slowing of gastric emptying, all of which contribute to normalizing elevated glucose levels. Because of this, we predict that inhibition of DPP-IV, which will elevate the levels of active GLP-1 and reduce the levels of the antagonistic metabolite, may be useful to treat impaired glucose tolerance and perhaps prevent transition to type 2 diabetes. The actions of DPP-IV, other than degradation of GLP-1, particularly in the immune system are discussed, but it is concluded that side effects of inhibition therapy are likely to be mild. Thus, DPP-IV inhibition may be an effective supplement to diet and exercise treatment in attempts to prevent the deterioration of glucose metabolism associated with the Western lifestyle. Diabetes 47:1663–1670, 1998
FIG. 1. Increase in plasma concentrations of total (□) and intact (●) GLP-1 after subtraction of endogenous levels and after subcutaneous administration of GLP-1(7-36) amide (1.5 nmol/kg) in type 2 diabetic subjects (n = 8). *P < 0.05; **P < 0.001.

The purpose of this review is to discuss the potential clinical use of DPP-IV inhibition in view of the evidence available at present. In the following 11 points, the pros and cons of this approach will be discussed.

1. **Side effects of DPP-IV inhibition.** The side effects of DPP-IV inhibition are of paramount importance. DPP-IV is reported to act not only to degrade regulatory peptides with Pro or Ala in position 2 (1), but also to play an important role in the immune system. Thus in addition to its enzymatic actions, DPP-IV, as a membrane-associated molecule on the surface of T-cells (where it is also known as CD26), has a function in the immune system by contributing to T-cell activation and proliferation (16). Here, its role in transduction of activation signals is dependent on its interaction with other membrane-expressed antigens such as CD45 (17). Whether this function of DPP-IV/CD26 is dependent on its enzymatic activity has not yet been conclusively demonstrated. In studies using specific competitive and irreversible inhibitors, which block up to 95% of the enzymatic activity (18) or mutant CD26 molecules devoid of enzymatic activity (19), T-cell activation was unimpaired, suggesting that the enzymatic activity of the molecule was not required. However, another study using DPP-IV inhibitors indicated that the enzymatic activity was involved in the signal transduction cascade (20). Studies using mutant DPP-IV/CD26 molecules have indicated a role for the enzymatic activity in modulating the responsiveness of T-cells (21), while others have indicated that it is important but not essential for its co-stimulatory activity (22), and suggested that DPP-IV/CD26 functions to augment the cellular responses. It therefore appears that the immune functions of DPP-IV are largely
FIG. 2. Plasma concentrations of total (□) and intact (●) GLP-1 in blood sampled from the carotid artery of anesthetized pigs during intravenous infusions of GLP-1(7-36) amide (5 pmol · kg⁻¹ · min⁻¹). Intravenous glucose (0.2 g/kg) was given during 21 min and 30 min of each GLP-1 infusion; a DPP-IV inhibitor (val-pyrrolidide; 300 pmol/kg) was given at 100 min.

notably, a compound with a half-life of 8 days (presumably the half-life of the enzyme) has been described (27). This compound may be particularly suitable for the study of long-term "side" effects.

As mentioned, a number of other regulatory peptides, including the duodenal incretin hormone, GIP, and two members of the pancreatic polypeptide (PP) family, peptide YY (PYY) and neuropeptide Y (NPY), are also substrates for DPP-IV (but not PP itself) (1,29). No studies have been conducted so far in which the protection of these hormones was determined. In DPP-IV-deficient Fischer rats, GIP levels were reported to be reduced and pancreatic sensitivity to GIP decreased, perhaps as compensatory measures (25). Most likely, levels of intact GIP will increase on DPP-IV inhibition. Elevated levels of GIP may contribute to enhanced glucose tolerance (although presumably not in human type 2 diabetes, see below), and this "side effect" therefore, must be considered expedient.

On digestion with DPP-IV, the 36 amino acid peptides PYY and NPY generate NH₂-terminally truncated 3-36 metabolites (29). NPY is a neuropeptide and probably plays a limited role as a circulating peptide (see below). PYY, however, is a gut hormone, produced in the L-cells, the same cells that produce GLP-1. While GLP-1 stored in the L-cell almost exclusively consists of intact GLP-1 (28), about 40% of stored PYY is accounted for by PYY 336 (30), indicating that part of the truncated form found in plasma (30) is not generated by DPP-IV digestion in the circulation. And in contrast to the metabolite of GLP-1, PYY 3-36 is highly active, retaining full activity toward the Y2 receptors, but losing its effects on Y1 receptors (31). Some of the peripheral actions of PYY, which are mainly related to its functions as one of the hormones of the "ileal brake mechanism" (inhibition of upper gastrointestinal functions elicited by the presence of food in the distal small intestine [32-34]) seem to be mediated via Y2 receptors (35) but Y1 receptors may also be involved (36). Thus, the extent to which inhibition of DPP-IV increases the ratio of intact-truncated PYY in the circulation is difficult to predict, but will, if it occurs, cause a change toward activation of more Y1 and fewer Y2 receptors. However, the consequences of this change are also difficult to predict. Presumably, PYY-mediated regulation of gastrointestinal functions will be marginally affected, but perhaps other, mainly Y1 receptor-regulated
functions such as blood flow regulation, could be affected (34). In initial human studies of DPP-IV inhibition, careful blood pressure control will be required.

The hypothalamic peptide, growth hormone-releasing hormone (GRH), which is structurally related to GLP-1, is also a substrate for DPP-IV and is inactivated by digestion (37). However, DPP-IV-resistant analogs of GRH are rapidly degraded by enzymes other than DPP-IV (38), and it is uncertain whether inhibition of DPP-IV will affect the actions of GRH released to the pituitary portal circulation.

2. Long-term efficacy of the compound. Indeed, the very fact that the DPP-IV-deficient Fischer rats (25) seem completely unaffected might suggest that compensatory mechanisms may take over in DPP-IV-deficient animals. Similarly, other routes of GLP-1 degradation might be uncovered or induced during continuing DPP-IV inhibition. Thus, first, will GLP-1 degradation remain inhibited on long-term inhibitor administration? Second, will the effects of DPP-IV inhibition themselves show tachyphylaxis (i.e., will there be tachyphylaxis to the effects of an increased level of intact GLP-1)?

These questions cannot be answered presently, but must be investigated in long-term studies of DPP-IV inhibition. Again, such studies could be conducted in experimental animals using the available inhibitors such as val-pyrrolidide. With respect to tachyphylaxis to GLP-1, this important question has been addressed in a few studies of GLP-1 administration. In two studies in which GLP-1 was infused continuously for 7 days to patients with type 2 diabetes, there was no sign of tachyphylaxis with respect to its effects on glucose metabolism (39,40), and in a recent study the antidiabetic effect of a GLP-1 analog delivered by engineered cells transplanted into glucose-intolerant mice was preserved for the duration of the experiment (1 month) (41). However, no studies have addressed the question directly. In addition, there is evidence that the gastrointestinal effects of GLP-1 (see below) may show tachyphylaxis (42). It must be borne in mind, however, that development of tachyphylaxis may depend on the dosage scheme. The continued presence of elevated levels of (active) GLP-1 might promote tachyphylaxis as opposed to discontinuous therapy, although the relatively small increases in GLP-1 levels that may be obtained by DPP-IV inhibition may be less prone to cause tachyphylaxis.

3. Is it possible to protect endogenous GLP-1 from degradation? This essential question has not been addressed so far. DPP-IV-mediated degradation of GLP-1 is extensive, reflecting the widespread distribution of the enzyme, and occurs not only in plasma but also in numerous tissues, with, for example, the liver being one of the major sites for inactivation of the circulating peptide (9). In a recent study, we showed that, although GLP-1 in the gut is stored entirely in the intact form, 50% of the newly secreted peptide released from isolated perfused preparations of pig ileum was already degraded by the time it reached the local venous drainage (28). This degradation could be completely prevented by intraluminal or intravascular val-pyrrolidide (28).

In our study of administration of the same compound to pigs in vivo (12), we found that degradation of GLP-1, secreted in the basal state, was greatly reduced (determined by comparison of levels of intact and total [intact + metabolite] GLP-1 with and without inhibitor; Fig. 2). However, the consequences for GLP-1 secretion in relation to meals have not been investigated. Our prediction is that it will be possible to protect endogenous GLP-1 extensively from degradation.

4. Is full protection of endogenous GLP-1 enough to have a significant effect in type 2 diabetes? In our original study (3), levels of total GLP-1 increased from ~12.6 to 22.3 pmol/l postprandially. The levels of intact GLP-1 increased from 3.3 to 9.9 pmol/l; the difference was reasonably accounted for by the concentration of the metabolite. With a DPP-IV inhibitor, it could be predicted that all of the 12.6 and 22.3 pmol/l would occur as intact, biologically active peptide, a two- to fourfold increase. In addition, there would be no antagonist (9-36 amide) to antagonize the actions of GLP-1. In our experiments conducted in patients with type 2 diabetes, full normalization of blood glucose levels was obtained during intravenous infusion of GLP-1 at a rate of 1.2 pmol \cdot kg^{-1} \cdot min^{-1} (43). This infusion rate increases levels of intact GLP-1 to 15–20 pmol/l (7), and on top of this, there is a concentration of 80–100 pmol/l of the antagonistic metabolite. Thus, one would predict that DPP-IV inhibition might produce plasma levels of intact GLP-1 that would be large enough to significantly and unopposedly affect the target organs for GLP-1. The effect would be largest postprandially, but inspection of the 24-h profile for plasma GLP-1 (44) reveals that although there are clear meal-related increases, GLP levels remain elevated throughout the day, once the digestive processes are initiated by breakfast ingestion. In addition, there is evidence that even fasting subjects may have a small but significant secretion of GLP-1 (3) and studies by Toft-Nielsen et al. (45), in which it was shown that the basal levels could actually be significantly suppressed by somatostatin infusion). The preliminary studies (13–15) cited earlier reveal that administration of DPP-IV inhibitors to glucose-intolerant rodents does in fact improve glucose tolerance (but, clearly, in these studies the responsible mechanism could not be deduced). Our prediction is that DPP-IV inhibition will have a significant effect.

5. What is the rationale for treating type 2 diabetes with increased availability of GLP-1? Or, in other words, why is it that the GLP-1 these patients produce cannot help their β-cells keep up insulin production? The answer to this question is twofold. First, there is now evidence that the secretion of GLP-1 is impaired in type 2 diabetes (M.-B. Toft-Nielsen, S. Madsbad, J.J.H., unpublished studies of meal-induced GLP-1 secretion in 55 patients with type 2 diabetes). The initial meal-induced increase seems to be the same, but the duration of the increase is markedly shorter. However, the deficient response does not seem to be responsible for diabetes, but rather to be a consequence of diabetes. This is because GLP-1 secretion is only slightly impaired in patients with impaired glucose tolerance and with a high probability for transition to overt diabetes (J. Lindqvist, J. Pignon, J.J.H., S. Efendic, unpublished observations) (if the GLP-1 deficiency had been a primary cause, one would have expected a similar impairment of secretion in prediabetes); indeed, in identical twins discordant for type 2 diabetes (at the time of investigation), GLP-1 secretion was impaired in the diabetic but less so in the glucose-tolerant twin (47). Thus, the decreased postprandial GLP-1 response in type 2 diabetes may aggravate the disease but does not cause the disease. More importantly, however, it seems that an important and perhaps primary defect in type 2 diabetes may be an impaired incretin function (i.e., little augmentation of insulin secretion after oral as compared with
increased insulin-mediated glucose disposal. As noted above, the other incretin hormone, GIP, may also be protected, and its effects are therefore enhanced; in addition, elevated levels of the "ideal-brake" hormone, PYY, may dampen postprandial glucose excursions. However, in this context, lack of specificity of DPP-IV inhibition must be considered expedient.

7. Is the compound intrinsically safe? With this, we think of the toxicology of the compound (i.e., side effects apart from those due to DPP-IV inhibition). We will have to consider that the patients likely to benefit from a DPP-IV inhibitor will have to take the drug every day for several decades of their lifetime. Any side effect will probably seriously limit the usefulness of the compound. On the other hand, if a nontoxic compound can be developed, it is likely to have a vast applicability. In view of the fact that efficient and apparently nontoxic inhibitors already exist, such compounds would seem easily to develop. In fact, a nontoxic, orally active compound with reasonable pharmacokinetics (see below) might actually make one of the greatest dreams of the diabetologist to come true: it might prevent the transition from impaired glucose tolerance to overt type 2 diabetes. This is because elevated levels of active GLP-1 would be expected to restore the (mild) incretin deficiency (discussed above) and normalize completely glucose levels (as shown in the first animal studies). The lowered glucose levels would then remove the demand on the β-cell thereby reducing insulin secretion, which together would lead to normalization of insulin sensitivity. All of these plus perhaps specific direct effects of GLP-1 might result in promoted growth and survival of the β-cells (55–57). It could be envisaged that the drug could be given to patients discovered by population screening in the 40–70 age-group, having borderline elevations of fasting blood glucose (perhaps as low as 5.9 mmol/l). Oral glucose tolerance tests may also be carried out on a screening basis, leading to the identification of individuals with impaired glucose tolerance and perhaps a family history of type 2 diabetes. Since the treatment can be predicted to have little effect unless blood glucose is truly elevated (GLP-1 has very little effect in subjects with normal glucose levels regardless of dose because its actions are glucose dependent [58]) and because it should be nontoxic, overtreatment would be expected to have inconspicuous negative effects (except perhaps for hypothetically causing an increased tendency to postprandial reactive hypoglycemia, see below). On the contrary, treatment might still have beneficial effects on body weight (see below).

This new principle of diabetes treatment should be viewed under the perspective that ~50% of patients with type 2 diabetes have irreparable complications at the time of diagnosis. It is generally assumed that the complications are due to long-standing disturbances of glucose metabolism. If it were possible to improve glucose metabolism at an earlier stage, it might be possible to reduce the prevalence of complications. In addition, the existence of an efficient treatment of early impaired glucose metabolism with few or no side effects would be an incentive for general practitioners to try to identify such cases among their patients at a much earlier stage. Thus with DPP-IV inhibitor, it may be possible to prevent or delay type 2 diabetes and its complications.
8. Gastrointestinal and satiating effects. GLP-1 is known to potently inhibit gastrointestinal motility and secretion (the "ileal brake" effect [33]). Thus, GLP-1 is a hormone signaling the presence of an abundance of nutrients in the distal small intestine, and which acts to limit further transfer to and digestion of foodstuff in the intestine until the load already present has been absorbed. Possibly, these effects will be enhanced during chronic therapy with a DPP-IV inhibitor. To what extent that this is good or bad cannot be settled presently. Judging from experience with infusion of GLP-1 into diabetic patients, the gastrointestinal effects will be to even out meal-induced glucose excursions (39,40,59,60). GLP-1 secretion is greatly exaggerated in patients with accelerated gastric emptying (61,62), and it has been shown recently that the exaggerated secretion is sufficient to explain reactive hypoglycemia in these patients (63). Thus it must be considered whether or not DPP-IV inhibition may be associated with an increased risk of reactive hypoglycemia. In insulin-resistant patients, the risk is likely to be small (it has proven difficult to bring about reactive hypoglycemia with GLP-1 in diabetic patients, in contrast to subjects with normal glucose tolerance and insulin sensitivity (T. Vilsbøll, T. Krarup, J.J.H., unpublished observations), but in subjects with faster-than-average gastric emptying and normal insulin sensitivity, this risk must be taken into account.

GLP-1 has also been shown to promote satiety and mildly reduce caloric intake in humans (64), including obese subjects (65). Presumably, GLP-1 functions as one of the physiological intestinal satiety signals (33). To what extent that the satiating effect is related to its effects on gastrointestinal motility is not known. Because of this effect, it may be predicted that DPP-IV inhibition would reduce food intake. Of course, this is a desirable effect, given the fact that obesity is both a causative and an aggravating factor for type 2 diabetes, insulin resistance, and glucose intolerance. It is impossible at present to predict how effective DPP-IV inhibition will be in this respect. High doses of exogenous GLP-1 can provoke nausea and even vomiting (39,66). These dose results in levels of intact GLP-1 that greatly exceed those that result from DPP-IV inhibition. As mentioned above, the latter levels may be obtained with the usual "therapeutic" infusion rates of ~1.2 pmol·kg⁻¹·min⁻¹. Such infusion rates, even when extended for 7 continuous days, have never been noted to have side effects (39,52). Ill effects because of the satiating actions would, therefore, not be expected.

9. Effects on GLP-2 secretion. Like GLP-1, glucagon-like peptide-2 (GLP-2) is a product of intestinal processing of proglucagon, and is secreted in parallel with GLP-1 (33). Until recently, its biological function was not known, but studies by Drucker et al. (67) suggest that it acts as an intestinal growth factor. Further studies revealed that both endogenous and exogenous GLP-2 are substrates for DPP-IV (68). Accordingly, in recent (unpublished) studies in our laboratory, the growth-promoting effect on the small intestinal mucosa of subcutaneous injections of GLP-2 was greatly enhanced by the simultaneous administration of the specific DPP-IV inhibitor, val-pyrrolidine, and, similarly, a DPP-IV-resistant analog was shown to be much more effective than natural GLP-2 in rats (68). In human plasma, about half of the circulating GLP-2 represents intact GLP-2, and the remaining half represents the truncated metabolite GLP-2 (3-33) (69). Similar figures apply to intravenously infused GLP-2. Thus DPP-IV inhibition is likely to cause a lesser increase in the concentration of active GLP-2 than that observed for GLP-1. Nevertheless, DPP-IV inhibition may have effects on intestinal proliferation. It should be recalled that the growth-promoting effect of the intestinal proglucagon-derived peptides was suspected because of a report of a tumor that apparently secreted such peptides and was associated with villous hypertrophy and gross intestinal stasis (70). Subsequently, the growth-promoting effect of GLP-2 was discovered because a transplantable tumor secreting proglucagon products caused intestinal proliferation (67). Therefore, the appearance of the intestinal mucosa must be monitored in long-term studies of DPP-IV inhibition.

10. Effects on GLP-1 in the central nervous system. Fully processed GLP-1 is produced in neurons of the nucleus of the solitary tract in the brainstem (71). Fibers from these neurons impinge on cell bodies in the paraventricular nucleus and the arcuate nucleus, some of which may produce corticotropin-releasing hormone (72). Intracerebroventricular administration of GLP-1 causes inhibition of food intake (73,74). It is not known whether brain GLP-1 is metabolized by DPP-IV; indeed, only a limited number of studies have examined whether DPP-IV itself is present in the brain. In the rodent brain, DPP-IV has been demonstrated in the capillary endothelium of the choroid plexus (75), in the median eminence (76), on cerebellar and spinal cord astrocytes, and in nerve perineurium (77). Conversely, other studies have failed to show its presence in microvessels of the subformical organ or in blood-brain barrier microvessels (78), in capillary endothelium (77), or in the ependyma of the choroid plexus (75). Yet other studies showed both a developmental pattern and a differentiation between immunohistochemical and histochemical findings: DPP-IV immunoreactivity was widespread in the rat fetal brain, in neural cells, and numerous capillary endothelial cells, but disappeared postnatally, except for some capillary endothelial cells; however, DPP-IV activity was not demonstrated at any time (79). Variant DPP-IV isoforms lacking catalytic activity have been shown to have widespread distribution in both the bovine and rat central nervous system (80,81). The question of whether an enzymatically active DPP-IV molecule is present in the brain, particularly at sites where it would have access to brain GLP-1, remains to be resolved. If it is, then it becomes an issue of importance whether or not a future inhibitor of DPP-IV crosses the blood-brain barrier. If brain GLP-1 is metabolized by the enzyme, an inhibitor that crosses the barrier may enhance the inhibitory effect of brain GLP-1 on food intake. If not, it is, perhaps, desirable that a future compound does not cross the barrier. This discussion also applies to the question of DPP-IV-mediated truncation of NPY, a neuropeptide with a widespread distribution in the brain. Like the PYY metabolite, NPY 3-36 is a selective Y2 receptor agonist (82).
as a preventive medication in patients with impaired glucose tolerance, it may be that intermittent therapy is sufficient. By intermittent administration, any tachyphylaxis that might develop to the drug or to the augmented peptide levels, might simultaneously be relieved. Perhaps a drug that could be given orally once a day and still secure adequate inhibition of the enzyme throughout the daytime would be preferable.

CONCLUSIONS
Clearly, many questions are yet unanswered. Given the particular theoretical potential for a DPP-IV inhibitor to ameliorate parts of the metabolic syndrome (obesity, insulin resistance, impaired glucose tolerance) and perhaps to prevent transition from impaired glucose tolerance to overt type 2 diabetes, it is of particular importance that a future inhibitor is nontoxic. If a nontoxic drug with the desired pharmacokinetics and pharmacodynamics can be developed, it would be expected to have a major therapeutic impact.

Note Added in Proof: Improved glucose tolerance by DPP-IV inhibition in Zucker fatty rats was reported by Pederson et al. after the submission of this article (Pederson RA, White HA, Schlenzig D, Pauly RP, McIntosh CHS, Demuth H-U: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. Diabetes 47:1253–1258, 1998).

REFERENCES
40. Larsen J, Dansbo P: GLP-1 must be present continuously in order to obtain a good glycemic control in NIDDM (Abstract). Diabetes 46 (Suppl. 1):180A, 1997