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HEALTH AND DISEASE

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Preface

In recent years, the research on Dipeptidyl Aminopeptidases has led from basic science to first concepts of pharmaceutical applications. The complex role of these enzymes in physiological processes as well as in the pathophysiology of diseases such as diabetes mellitus, rheumatoid arthritis, autoimmune disease, transplant rejections and cytokine-mediated abortions has become evident and provides a rationale for therapeutic interventions by a modulation of their respective enzymatic activity.

The *International Conference on Dipeptidyl Aminopeptidases*, held in Berlin, Germany, in September 2002, was initiated in an attempt to bridge basic science, results of clinical studies and strategies for therapeutic applications. Experts in the field presented a state-of-the-art view on the various aspects of Dipeptidyl Aminopeptidases, thus contributing to a sound basis for decisions on research and therapeutic strategies. The conference, fueled by the presentation of the crystallographic structure of DPP IV by Dr. D. Webb, was concluded by a panel discussion on perspectives and limitations of DPP IV inhibitors.

Selected authors have shared their opinion on basic science, clinical applications and therapeutic interventions with the audience, and they extend their view to the readers of this book. By doing so, they have generously supported us in our attempt to provide a comprehensive overview over Dipeptidyl Aminopeptidases at a time when the therapeutic implications of enzyme inhibitors are imminent, demanding a critical risk assessment based on a detailed understanding of the complex function that these enzymes appear to exert.

A conference on Dipeptidyl Aminopeptidases hosted by a Department of Internal Medicine/Psychosomatics with an interest in these enzymes may be

surprising, but reflects the current state of research on these enzymes, with a growing understanding of specific clinical implications and therapeutic options. A glance at a preliminary list of substrates of DPP IV (Table 1) may help to understand such interest: DPP IV-mediated turnover of peptide hormones in various areas of relevance for the body-mind relationship must thrill every one interested in functional thinking. Especially a psychosomaticist in psychoanalytical tradition is reminded of the expectation of Freud, the founder of 20th century’s mainstream psychosomatics, that one day every psychological event will be understood from the underlying physiological and biochemical processes. The contributions to our conference and to this book nurture the idea that research on DPP IV is an example for a step towards a biochemical, or even molecular founded psychosomatics.

Table 1. Examples of substrates for DPP IV activity

Substrate	Conceivable implications of DPP IV activity
Growth hormone releasing factor	Nutritional Control ▪ hunger ▪ satiety
Glucagon-like peptide 1 (GLP-1)	
Glucagon-like peptide 2 (GLP-2)	
Gastric inhibitory peptide (GIP)	
Gastrin-releasing peptide (GRP)	
Procolipase	
Enterostatin	
Pancreatic Polypeptide	
Trypsinogen	
Neuropeptide Y (NPY)	
SDF-1 α (stromal-cell-derived factor-1 α)	Immunomodulation: Mechanisms of defence
Lymphotoxin (aa 1-5)	
Eotaxin	
TNF- α	
IP-10	
Monocyte chemotactic protein (MCP-2)	
RANTES	
CLIP (ACTH fragment 18-39)	
Endomorphin-1	Mood: ▪ elation ▪ dysphoria ▪ anxiety ▪ anger ▪ pain
β -Casomorphin	
Kentsin	
Morphiceptin	
Substance P	
Tyr-MIF-1	

For reviews cf. ^{1,2}

Although hypothetical at this stage, an integrative concept as presented in Figure 1 may help to appreciate consequences of changes in DPP IV activity or of DPP IV inhibitors. The potential of such inhibitors for novel treatment approaches of type II diabetes has served as a spearhead in the search for

substances with inhibitory activity, and continues to fuel research on structure and function of DPP IV as well as on the growing number of DPP IV-like enzymes. The targeted modulation of these enzymes in distinct sites of the organism represents a pharmacodynamic and pharmacokinetic challenge for the development of novel drugs and prodrugs. Given the complexity of the biological processes affected by inhibitors of Dipeptidyl aminopeptidases, their clinical use cannot be fully appreciated yet.

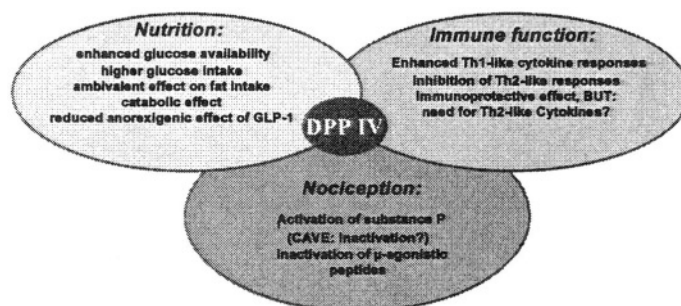


Figure 1. Pleiotropic involvement of DPP IV activity (modified after ¹⁾)

At this stage, it may be helpful to label these novel and potent substances *Peptide modulators*, denominating a process rather than a clinical indication and thus avoiding a premature narrowing on a single disease entity. Furthermore, the risk assessment in the exploitation of inhibitory substances should take potential side effects such as an impact on hunger and satiety, pain perception or immunomodulation into account.

The Berlin Conference has continued a series of similar conferences on this topic; a further continuation in 2004 was announced by Uwe Lendeckel and Dirk Reinhold from Magdeburg, members of a team that pioneered in the research on Dipeptidyl Aminopeptidases. We are convinced that major advancements justifying further conferences can be expected in the next future.

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Implementation of GLP-1 Based Therapy of Type 2 Diabetes Mellitus Using DPP-IV Inhibitors

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SUMMARY

GLP-1 is a peptide hormone from the intestinal mucosa. It is secreted in response to meal ingestion and normally functions in the so-called ileal brake i. e. inhibition of upper gastrointestinal motility and secretion when nutrients are present in the distal small intestine. It also induces satiety and promotes tissue deposition of ingested glucose by stimulating insulin secretion. Thus, it is an essential incretin hormone. In addition, the hormone has been demonstrated to promote insulin biosynthesis and insulin gene expression and to have trophic effects on the beta cells. The trophic effects include proliferation of existing beta cells, maturation of new cells from duct progenitor cells and inhibition of apoptosis. Furthermore glucagon secretion is inhibited. Because of these effects, the hormone effectively improves metabolism in patients with type 2 diabetes mellitus. However, continuous administration of the peptide is necessary because of an exceptionally rapid rate of degradation catalyzed by the enzyme dipeptidyl peptidase IV. With inhibitors of this enzyme, it is possible to protect the endogenous hormone and thereby elevate both fasting and postprandial levels of the active hormone. This leads to enhanced insulin secretion and glucose turnover. But will DPP-IV inhibition enhance all effects of the endogenous peptide? The mode of action of GLP-1 is complex involving also interactions with sensory

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neurons and the central nervous system, where a DPP-IV mediated degradation does not seem to occur. Therefore, it is as yet uncertain whether DPP-IV inhibitors will affect gastrointestinal motility, appetite and food intake. Even the effects of GLP-1 effects on the pancreatic islets may be partly neurally mediated and therefore uninfluenced by DPP-IV inhibition.

1. WHAT IS GLP-1 (GLUCAGON-LIKE PEPTIDE-1)?

GLP-1 is a product of the glucagon gene¹. This gene is expressed not only in the pancreatic alpha-cells, but also in the L-cells of the intestinal mucosa, one of the most abundant endocrine cells of the gut². Here, the primary translation product, proglucagon, is cleaved, not to produce glucagon as in the islets, but to release from its C-terminal part the two glucagon-like peptides GLP-1 and GLP-2³, which show about 50 % sequence homology with glucagon. In spite of the structural homology, GLP-2 shares few of the biological actions of GLP-1, but rather acts as a regulator of adaptive growth in the gut^{4,5}. GLP-1 secretion is stimulated by the presence of nutrients in the lumen of the gut (but additional neural or endocrine mechanisms may also operate)⁶, and the secretion of GLP-1 throughout the day is highly correlated to the release of insulin⁷. This is particularly evident in patients with reactive hyperglycemia after gastric operations and accelerated gastric emptying of meals. These patients have grossly exaggerated GLP-1 responses to test meals, which correlate with the gastric emptying rate as well as with postprandial insulin levels⁸. Indeed, it has been shown that a pronounced reactive hyperglycaemia can be provoked in healthy subjects, if the meal-induced glucose excursions and exaggerated GLP-1 responses of the gastrectomized patients are mimicked by intravenous infusions of glucose and GLP-1⁹. In healthy subjects, GLP-1 levels are elevated 2-4 fold in response to ingestion of each meal⁷, but also in the interdigestive state there appears to be a certain basal secretion. Thus, fasting levels of GLP-1 may be significantly suppressed during infusions of high doses of somatostatin¹⁰, a peptide shown to exert a pronounced constraining effect on GLP-1 secretion¹¹.

The biological effects of GLP-1 comprise not only an effect on insulin secreting cells, but also on other cells of the islets as well as effects on several extrapancreatic sites.

Insulinotropic effects

Firstly, GLP-1 is one of the most potent insulinotropic substances known, with half maximal effective concentrations for its effects on the beta cells around 10 pmol/l¹². Its insulinotropic effect is strictly glucose dependent and there is no effect on insulin secretion at glucose concentrations below approximately 4.5 mmol/l¹³. GLP-1's insulinotropic activity is exerted via interaction with a specific receptor located on the cell membrane of the β -cells^{14;15}. Binding of GLP-1 to the receptor causes activation - via a stimulatory G-protein - of adenylate cyclase resulting in the formation of cAMP^{16;17}. There is agreement that all of the actions of GLP-1 are secondary to the formation of cAMP. Subsequent activation of protein kinase A leads to a plethora of events including altered ion channel activity, intracellular calcium²⁺ handling and enhanced exocytosis of insulin containing granules¹⁷. As mentioned a certain level of glucose must be present for GLP-1 to have any effect on insulin secretion. In addition, GLP-1 potentiates strongly the insulinotropic actions of glucose itself. Conversely, it seems that GLP-1 (or perhaps any hormone that can causes sufficient cAMP accumulation in the β -cells?) is required for glucose to exert its activity. Thus, in experiments in single beta cells, neither glucose nor GLP-1 alone affect intracellular calcium levels or membrane potential, whereas together they bring about a strong activation^{18;19}. In other words, GLP-1 conveys "glucose competence" to the β -cells¹⁸. The clinical implication of the dependence on blood glucose concentrations at or above normal fasting glucose levels is, of course, that GLP-1 is incapable of causing profound hypoglycaemia²⁰. The reactive hypoglycaemia alluded to above⁹, is due to an extremely exaggerated release of insulin, which, due to the rather slow inactivation time of insulin, may lead to a short-lasting overshoot of insulin's hypoglycaemic effects extending beyond the stage where insulin secretion (and the GLP-1 effect) has ceased because of falling glucose concentrations.

Secondly, GLP-1 stimulates all steps of insulin biosynthesis as well as insulin gene transcription²¹, thereby providing continued and augmented supplies of insulin for secretion. In addition, it upregulates the genes for the cellular machinery involved in insulin secretion, such as the glucokinase and GLUT-2 genes²².

And finally and most importantly, GLP-1 has been shown to have trophic effects on β -cells. Not only does it stimulate β -cell proliferation^{23;24}, it also enhances the differentiation of new β -cells from progenitor cells in the pancreatic duct epithelium²⁵. A proliferation was also induced in aging glucose-intolerant rats with a resulting improvement of glucose tolerance²⁶. This indicates that GLP-1 may be capable of providing new β cells in

individuals with an insufficient number of functioning cells²⁶ such as 2DM patients (although it is not yet established to what extent this process occurs in humans).

Most recently, but of no less interest, GLP-1 has been shown to be capable of inhibiting both cytokine and fatty acid induced apoptosis in beta cells^{27,28}. Since the normal number of beta cells is maintained in a balance between apoptosis and proliferation²⁹, this observation is of considerable interest, and also raises the possibility that GLP-1 could be useful in conditions with beta cell apoptosis. Thus a GLP-1 analogue, NN 2211 (NovoNordisk, Denmark), has been demonstrated to markedly reduce and delay the development of diabetes in ZDF rats³⁰, the diabetes of which is known to occur as apoptosis reduces the number of functioning beta cells³¹.

Inhibition of glucagon secretion

In addition to its effects on the β -cells, GLP-1 also strongly inhibits glucagon secretion³². The mechanism by which this occurs is unclear. Both inhibition via stimulated insulin release and via stimulated somatostatin secretion have been suggested¹², but a direct effect on the glucagon cells which seem to express GLP-1 receptors^{33,34} is also possible. It is of considerable clinical importance that the inhibition exhibits glucose dependency. Thus, in a recent study in healthy volunteers, glucagon secretion was studied under the conditions of a stepwise, hyperinsulinemic, hypoglycaemic clamp, with and without additional infusion of GLP-1. At basal glycaemia and at the earliest steps of the clamp, glucagon secretion was clearly suppressed during GLP-1 infusion compared to controls, but at lower glucose levels glucagon secretion was stimulated by hypoglycaemia and to identical levels in the two groups³⁵. Thus it is unlikely that a GLP-1-based treatment will impede the glucagon-mediated defence against hypoglycemia in the clinical setting.

The importance of the inhibition of glucagon secretion at hyperglycaemic levels, i.e. for diabetes treatment is perhaps best illustrated in studies of GLP-1 infusion in patients with type 1 (insulin-dependent) diabetes and no residual β -cell secretory capacity³⁶. In these patients, GLP-1 retains substantial glucose lowering activity, in spite of undetectable C-peptide responses (i. e. there is no insulin effect), while glucagon secretion is strongly inhibited. We assume that the glucose-lowering effect is a consequence of the inhibition of glucagon secretion, since we have shown that the glucose lowering effect of GLP-1 is probably mainly due to inhibition of hepatic glucose production as a result of its combined action on the secretion of the two pancreatic glucoregulatory hormones³⁷.

Effects on the gastrointestinal tract

Further important effects of GLP-1 include inhibition of gastrointestinal secretion and motility, notably gastric emptying^{38;39}.

A considerable amount of recent evidence seems to indicate that, physiologically, one of the main functions of GLP-1 is to act as one of the hormones of the so-called "ileal brake", an endocrine mechanism by which the presence of nutrients in the distal small intestine causes inhibition of upper gastrointestinal motor and secretory activity⁴⁰. Thus, if the ileum is perfused luminally with solutions containing lipids or carbohydrates in amounts corresponding to what has been called physiological malabsorption, GLP-1 secretion is markedly stimulated. At the same time gastric and pancreatic secretion is inhibited⁴¹. If the plasma concentrations observed in these experiments are reproduced by intravenous infusion of exogenous GLP-1, gastric motility and secretion as well as pancreatic secretion are markedly inhibited⁴². Physiologically, it may be that these effects of GLP-1 are more important than the insulinotropic activity. Thus, when GLP-1 is infused intravenously during ingestion of a meal, the insulin responses are diminished dose-dependently, rather than being enhanced³⁹. At the same time gastric emptying is being progressively retarded, so that the explanation of the reduced insulin secretion is the reduced gastric emptying of and reduced subsequent absorption of insulinotropic nutrients; recall, that GLP-1

physiological role of GLP-1 may be to adjust the delivery of chyme to the digestive and absorptive capacity of the gut by retarding propulsion and digestion of the gastric contents^{39;42;43}.

The inhibitory effect of GLP-1 on gastric emptying is desirable in patients with diabetes because the slower gastric emptying rate reduces postprandial glucose excursions. This is evident from the use of another potent gastric inhibitor, amylin, with similar effects on gastric emptying for diabetes treatment⁴⁴. Amylin reduces postprandial glucose excursions sufficiently to lower haemoglobin A1c levels significantly in patients with diabetes mellitus.

Effects on appetite and food intake

Nutrients in the ileum are thought to have a satiating effect, curtailing food intake⁴⁵. Does GLP-1 play a physiological role as a satiating agent? Indeed, recent studies have shown that infusions of slightly supraphysiological amounts of GLP-1 significantly enhance satiety and reduce food intake in normal subjects^{46;47}. The effect on food intake and

satiety is preserved in obese subjects also⁴⁸ as well as in obese subjects with type 2 diabetes^{49;50}.

In addition to the effects of peripherally administered GLP-1, direct injections of GLP-1 into the cerebral ventricles also inhibit food intake^{51;52}. Here the peptide presumably interacts with GLP-1 receptors that are expressed at numerous locations in the brain, but in particular in the hypothalamus around the paraventricular nucleus and the arcuatus^{53;54}. The cerebral GLP-1 receptors are likely to be targets for GLP-1 released from nerve fibers ascending from cell bodies in the nucleus of the solitary tract in the brain stem, in which the proglucagon gene is expressed and in which proglucagon is processed in the same way as in the gut⁵⁵. The question arises whether these neurones are linked to meal-induced satiety. Rinaman et al⁵⁶ analysed the neurons of the nucleus of the brain stem that were activated by various procedures designed to model enteroceptive stress (lithium chloride administration, CCK injection, lipopolysaccharide) and observed C-fos expression in cell bodies that also stained for GLP-1, whereas neurons showing c-fos expression after meal ingestion were distinct from the GLP-1 neurons. In further experiments, they administered the GLP-1 receptor antagonist, exendin 9-39, intracerebroventricularly to rats given lithium chloride as above, and found that the antagonist could completely reverse the anorexigenic effect of systemic lithium chloride⁵⁷. Thus, it seems clear that GLP-1 from brain stem functions as a mediator of the anorexic effects of enteroceptive stress, whereas its role in meal-induced satiety is less clear.

The effect of GLP-1 on appetite and food intake would support attempts at weight reduction in 2DM patients and, if effective, would be considered most desirable. It should be noted though that GLP-1 receptor knock out mice do not become obese⁵⁸, but this may reflect the redundancy of the appetite regulating mechanisms rather than ineffectiveness of the signal.

1.1 Effect of GLP-1 in type 2 diabetes

Taken together all of these effects render GLP-1 unusually attractive as a therapeutic agent. The full hypoglycaemic effect of GLP-1 in type 2 diabetic patients was first demonstrated by Nauck et al⁵⁹ who were able to completely normalize plasma glucose levels by a 4-hour iv GLP-1 infusion in patients admitted to hospital for initiation of insulin treatment because of secondary failure of oral antidiabetic agents. These patients had high haemoglobin A1c levels and fasting plasma glucose levels of approximately 13 mmol/l indicating that GLP-1 would be effective even in patients with limited

insulin reserve. Recently, Zander et al⁶⁰ used continuous subcutaneous infusion of GLP-1 for 6 weeks in order to explore in a subchronic design the potential of GLP-1 in diabetes treatment. After a 3 weeks wash-out period 20 patients were allocated to continuous infusion of either saline or GLP-1 at a rate of 4.8 pmol/kg/min using Minimed^R pumps. The patients were evaluated before, after 1 week and after 6 weeks of treatment. No changes were observed in the saline treated group, whereas in the GLP-1 group fasting and average plasma glucose concentrations were lowered by approximately 5 mmol/l, haemoglobin A1c decreased by 1.2 %, free fatty acids were significantly lowered, and the patients had a significant weight loss of approximately 2 kg. In addition, insulin sensitivity as determined by a hyperinsulinaemic euglycaemic clamp almost doubled, and insulin secretion capacity (measured using a 30 mmol/l glucose clamp + arginine) greatly improved. There was no significant difference between results obtained after one and 6 weeks treatment, but there was a tendency towards further improvement of plasma glucose as well as insulin secretion. There were very few side effects and no differences between saline and GLP-1 treated patients in this respect. Thus, GLP-1 seems to live up to all the expectations as a therapeutic agent for type 2 diabetes. The burning question therefore is how its therapeutic potential can be utilised in clinical praxis.

1.2 Limitation of the effects of GLP-1 because of enzymatic degradation

It spite of the impressive effects of intravenous or subcutaneous infusions of GLP-1, it turned out that simple subcutaneous injections of GLP-1 only lead to small lowering of blood glucose and a short-lived stimulation of insulin secretion⁶¹. The reason is that GLP-1 is degraded extremely rapidly after subcutaneous injection. In fact, less than 10 % of the peptide survives in intact bioactive form⁶². The rapid initial degradation is due to the ubiquitously expressed enzyme, dipeptidyl-peptidase IV (DPP-IV)⁶³, which cleaves off the two N-terminal amino acid residues from GLP-1 whereby the molecule not only is inactivated, but actually may be turned into an antagonist at the GLP-1 receptor⁶⁴. Physiologically this is an interesting process with important implications for the normal functions of GLP-1. Using isolated perfused preparations of porcine small intestine Hansen et al⁶⁵ were able to show that only about 1/3 of the GLP-1 that is secreted from the gut leaves the gut in the intact biologically active form; the remainder is constituted by GLP-1 9-36amide. Immunohistochemical studies showed that

DPP-IV is localised to the endothelium of the capillaries of the villi. Newly released GLP-1 therefore gets degraded upon entry into the capillaries. The implications of this is that GLP-1 must have exerted most of its actions before it enters the villous capillary, i. e. in the lamina propria. The hypothesis is that here GLP-1 interacts with sensory afferent neurons and that, therefore, most of its effects are exerted in a reflex like manner. A number of studies support this notion⁶⁶, including studies involving deafferentation⁶⁷, and studies in which impulse activity were detected in the vagal trunks as well as vagal pancreatic efferents after GLP-1 administration in rats⁶⁸ and in studies where reflex activity was inhibited by ganglionic blockers, which also abolished GLP-1 induced insulin secretion⁶⁹. In a series of studies of gastric acid secretion in man, Wettergren et al similarly demonstrated that GLP-1 inhibited vagally stimulated secretion but was ineffective after vagotomy^{70,71}. In conclusion, it seems that, physiologically, an important part of the activity of endogenous GLP-1 is exerted via interaction with sensory nerve fibres relaying in the brain and modulating efferent probably mainly vagal fibres that regulate gastrointestinal secretion and motility as well as pancreatic endocrine secretion. During large meals with large GLP-1 responses larger amounts of intact GLP-1 may reach the islets and other tissues via the circulation and contribute to stimulation of insulin secretion. In fact, it has been shown that the amount of intact GLP-1 that reaches the circulation postprandially may be particularly low in type 2 diabetic subjects⁷².

2. CIRCUMVENTING THE METABOLISM OF GLP-1 USING DPP-IV INHIBITORS

The extensive and rapid degradation of GLP-1 in the body implies that the peptide cannot be immediately employed for clinical treatment of type 2 diabetes. A number of strategies have, therefore, been explored including development of small molecule agonists for the GLP-1 receptor; development of DPP-IV resistant analogues; and finally inhibition of DPP-IV, in analogy with the use of angiotensin converting enzyme inhibitors for the treatment of hypertension^{63,73}. The degradation occurs in the circulation at such a high rates that steady state cannot be established. In this situation the usual parameters of elimination $T_{1/2}$ and metabolic clearance rate have no meaning, but calculated as apparent values the half-life is around 1-1½ min and the clearance rate exceeds cardiac output by a factor of 2^{74,75}.

It was also demonstrated that with available inhibitors it was possible to completely protect exogenous and endogenous GLP-1 from DPP-IV mediated degradation and thereby to enhance greatly its insulinotropic activity⁷⁶. Numerous subsequent studies have indicated that administration of orally active DPP-IV inhibitors markedly improve metabolism and glucose regulation 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 undegraded bioactive GLP-1, augmented insulin secretion and virtually normalised the otherwise considerably impaired glucose tolerance⁷⁷. Using another inhibitor, Pospisilik et al⁷⁸ reported sustained improvements in fasting glucose, glucose tolerance, insulin sensitivity, and β -cell responsiveness to glucose in Zucker fatty rats (a specific Vancouver strain), and finally, in a human study over 4 weeks, very significant improvements of metabolic control resulted from two or three times daily administration of an inhibitor developed by Novartis⁷⁹. There are many other substrates for DPP-IV than GLP-1⁸⁰, but it seems that the extreme degradation of GLP-1 makes it a preferential target, so that significant protection of GLP-1 can be accomplished without significantly compromising the other functions of DPP-IV. It should be noted that Fisher rats with mutations in the catalytic part of DPP-IV have no malfunctions⁸¹. Recently, mice with a targeted deletion of the gene encoding DPP-IV (CD 26) have been generated, and except for showing greater than normal glucose tolerance, these mice also seem to be normal⁸⁴.

3. GLP-1 OR DPP-IV INHIBITORS?

Although the potential of DPP-IV inhibitors in diabetes treatment is amply supported by experimental evidence, it is worthwhile to consider to what extent treatment with GLP-1 (analogues) and DPP-IV inhibitors will be similar and also whether the two treatments are applicable to identical or separate segments of the diabetic population.

The foremost question is efficacy. Will DPP-IV inhibition be as efficacious as GLP-1 analogues? The question involves consideration of the circulating levels of intact GLP-1 obtained after inhibitor administration. During continuous intravenous infusion of GLP-1 only 20 % of the hormone survives in intact form⁶². One would therefore expect an up to five-fold elevation of circulating levels of intact GLP-1 during effective inhibitor

treatment. Alternatively, since it has been demonstrated that DPP-IV inhibition may completely protect the hormone from N-terminal truncation, the levels of intact GLP-1 might approach the levels of "total GLP-1" (intact + metabolite) observed without inhibition⁷⁶. If true, it should be possible to elevate the concentrations of intact GLP-1 to 20-30 pmol/L, which is similar to concentrations obtained in diabetic patients during infusion of GLP-1 at a rate that results in complete normalisation of (fasting) hyperglycaemia⁶². However, recent investigations have shown, that the protection of GLP-1 during DPP-IV inhibition causes a down regulation of the secretion of GLP-1 (and of the other incretin hormone, GIP (glucose dependent insulintropic polypeptide)), so that meal stimulated levels of intact GLP-1 do increase during inhibitor treatment, but not to the levels obtained without inhibitor treatment⁸³. Possibly, a negative feed-back cycle involving increased paracrine somatostatin secretion from D-cells in the vicinity of the GLP-1 secreting L-cells is responsible for the lower meal-induced secretion¹¹. This negative feed-back cycle obviously limits the increases in intact GLP-1 levels one can expect during inhibitor treatment, and, in turn, the insulintropic and blood glucose lowering effects of DPP-IV inhibitors.

Another question is to what extent inhibitor treatment will result in inhibition of gastric motility, appetite and food intake. As discussed above, these effects of GLP-1 apparently involve activation of sensory afferent nerve fibres in the lamina propria of the gut wall⁶⁵, and this interaction is likely to occur *before* GLP-1 enters the capillaries and gets degraded. These actions of *endogenous* GLP-1, therefore, are unlikely to be affected by DPP-IV inhibition. In agreement with this concept, 8 weeks of extensive DPP-IV inhibition did not result in changes in body weight in glucose intolerant rats⁸⁴. DPP-IV inhibition in Zucker Diabetic Fatty rats for 3 months resulted in slightly lower weight gain than in control animals but no change in food intake⁷⁸. The reason for the difference is difficult to unravel in these leptin receptor deficient animals, which during treatment exhibited significantly improved glycaemic control. Similar findings were made by Sudre et al⁸⁵, who noted that no anorectic effects of the inhibitor employed could be demonstrated in fully developed ZDF rats.

As discussed above, even GLP-1 induced enhancement of insulin secretion may involve reflex pathways⁶⁹. It cannot be excluded, therefore, that it is mainly the endocrine actions of GLP-1 (as opposed to the neurally mediated) that are enhanced by DPP-IV inhibition. Interestingly, this may also indicate that DPP-IV inhibition will not result in the nausea and vomiting that results from administration of large amounts of GLP-1, and which may involve interaction with the central nervous system^{56;86}.

Taken together these limitations possibly indicate that DPP-IV inhibition will be less efficacious than administration of GLP-1 analogues, a notion that finds some support from the clinical studies reported by Ahrén et al⁷⁹, where DPP-IV inhibition certainly improved glycaemic control in type 2 diabetic patients, but not to the extent observed with continuous administration of GLP-1^{87;88}.

The most important potential of the treatment with DPP-IV inhibitors⁷³ 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, e.g. persons with IGT, with genetic disposition, obese subjects and subjects with mild diabetes. In these subjects it can be expected, because of the amplification of the GLP-1 effects, that glucose tolerance will be improved and that the beta cells may be protected; and the long term benefits may turn out to be prevention of progression of disease and prevention of complications as indicated in the studies of ZDF rats alluded to above. Thus, DPP-IV inhibitors may be particularly useful for the *treatment of prediabetes*, presently considered a necessity if an explosive development of the type 2 diabetes epidemic is to be prevented.

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