Inhibition of Dipeptidyl Peptidase-4 (DPP-4) – A Novel Approach to Treat Type 2 Diabetes

Bo Ahrén*

Department of Medicine, Lund University, Lund, and Section of Endocrinology and Diabetology, Lund University Hospital, Lund, Sweden

Abstract: A novel approach for treatment of type 2 diabetes is based on the gut hormone glucagon-like peptide-1 (GLP-1), which is antidiabetic due to its combined action to stimulate insulin secretion, increase beta-cell mass, inhibit glucagon secretion, reduce the rate of gastric emptying and induce satiety. A problem is, however, that the peptide is rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4), resulting in a half-life of active GLP-1 of only approximately 1-2 minutes. To overcome this inconvenient drawback for the treatment of diabetes, two strategies have been successful; one strategy uses DPP-4 resistant GLP-1 receptor agonists whereas the other strategy uses inhibition of DPP-4. Such inhibition will increase the levels of endogenous active GLP-1 and prolong its half-life. The rationale behind the strategy is evident from studies in animals with genetic deletion of DPP-4, which have improved glucose tolerance and increased insulin secretion in response to oral glucose. Furthermore, in experimental animals, different pharmacological DPP-4 inhibitors are antidiabetic. Recently also studies in subjects with type 2 diabetes have shown that prolonged DPP-4 inhibition for up to 1 year is antidiabetogenic because fasting and postprandial glucose as well as HbA_{1c} levels are reduced. This is seen in association with good tolerability and weight neutrality. Hence, DPP-4 inhibition has the potential to be a novel, efficient and tolerable approach to treat type 2 diabetes.

Keywords: DPP-4, GLP-1, insulin secretion, diabetes, treatment.

THE ENZYME DIPEPTIDYL PEPTIDASE-4 (DPP-4)

Expression and localisation of DPP-4 DPP-4 (DPP IV, CD 26, EC 3.4.14.5) was first described in 1996 as an enzyme occurring in homogenates of rat livers as well as in commercially available enzyme preparations having the activity of releasing the dipeptide glycine-proline from gly-pro-2-naphtylamide [1]. Later studies showed that DPP-4 is located to the plasma membrane of hepatocytes around the bile canaliculi and on the bile duct epithelia [2]. The enzyme is expressed in other tissues as well [2]. A particular high expression has been observed in the kidney, where DPP-4 is localized to the glomerular basement membrane and the proximal convoluted tubules [3]. DPP-4 is also expressed in epithelial cells of the pancreatic duct, in brush-border membranes of enterocytes and microvilli of trophoblasts in the placenta, in activated T-helper lymphocytes, in macrophages, in follicular epithelial cells of the thyroid gland, in luteal cells, in fibroblasts in the mammary gland, in synovia and in the skin [2,4-11]. In the nervous system, DPP-4 is expressed in circumventricular organs and lepto-meningeal cells, brain capillary cells and peripheral Schwann cells [12-14]. Thus, the enzyme is widely distributed throughout the body. As reviewed by Mentlein, the DPP-4 activity per gram tissue is highest in the kidney, followed by the lung, adrenal gland, jejunum, liver, parotid gland, spleen and testis [2]. Of particular importance for DPP-4 as a target for treatment of type 2 diabetes is that the enzyme has close contact with the circulation, because it is located on endothelial cells of the blood vessels throughout the body [15] and also circulates as a soluble enzyme [2,16].

*Address correspondence to this author at the Department of Medicine, Lund University, B11 BMC, SE-221 84 LUND, Sweden; Tel: +4646 222 0758; Fax: +4646 222 0757; E-mail: Bo.Ahren@med.lu.se

Structure of DPP-4 DPP-4 is a glycoprotein consisting of 766 amino acids in humans, whereas in rats, DPP-4 consists of 767 amino acids; there is an 85% identity between the human and rat sequences [17]. The gene encoding for the enzyme is located on the long arm of chromosome 2 (2q24.3) and it contains 26 exons [18]. Several transcription factors, such as NF B or AP2, bind to the gene [19]. DPP-4 is a socalled type II integral membrane protein, having a short intracellular N-terminal hydrophilic portion consisting 6 amino acids and a 22 amino acid hydrophobic membrane-spanning domain [20]. The remaining portion of the enzyme, i.e., a 738 amino acid sequence, is located extracellularly. DPP-4 is expressed on cell surfaces as a dimer with two units anchored through their N-terminal ends in the plasma membrane and the C-terminal ends located close to each other extracellularly [21]. The C-terminal, catalytic sites, of the two DPP-4 enzymes located in the dimer form a small pocket thereby having localized high catalytic activity [21].

Catalytic activity of DPP-4 DPP-4 is a protease with the catalytic site occurring in the C-terminal extracellular end of the sequence [21,22]; the action of the enzyme is to cleave oligopeptides after the 2nd amino acid releasing a dipeptide from the oligopeptide. The active site of the enzyme resides in a small five-amino acid region centered around a serine residue in position 630 (G^{628}, W^{629}, S^{630}, Y^{631}, G^{632}) [2,22]; amino acid substitution in this region results in loss of catalytic activity [2]. DPP-4 has restricted substrate specificity. First, its three-dimensional structure makes it

1573-4080/05 $50.00+.00 © 2005 Bentham Science Publishers Ltd.

impossible for the enzyme to act on peptides larger than approximately 80 amino acids [23]; on the other hand, a size exceeding approximately 30 amino acids is required. Second, peptides with proline or alanine as the 2nd amino acid are hydrolyzed by a high efficiency; in fact proline or alanine as the 2nd amino acid may be regarded as almost obligatory [2]. Nevertheless, it has been demonstrated that also peptides with glycine, serine, valine or leucine as the 2nd amino acid may be substrates, albeit with low affinity [24,25]. In contrast, the 1st amino acid in the oligopeptides cleaved by DPP-4 may be any unsubstituted amino acid. Hence, the optimal substrate for DPP-4 is an oligopeptide having 30-80 amino acids in length with proline or alanine as the 2nd amino acid from the N-terminal end.

As has been reviewed by Mentlein, a number of bioactive peptides are substrates for DPP-4, since they meet these requirements [2]. Several of these peptides are neuropeptides, i.e., they are produced in nerves and released as neurotransmitters upon activation of the nerves. Examples of these neuropeptides are substance P, gastrin-releasing peptide (GRP), neuropeptide Y (NPY), and pituitary adenylate cyclase activating polypeptide (PACAP) [23,26-29]. Other substrates of DPP-4 are involved in immune responses, such as macrophage-derived chemokine (MDC), monocyte chemotactive protein (MCP) and regulated-on-activation normal T cell expressed and secreted (RANTES) protein [30-32]. Other DPP-4 substrates are oligopeptides involved in digestion and metabolism, such as enterostatin and insulin-like growth factor-1 (IGF-1) [2,33]. Finally, several gastrointestinal hormones are substrates for DPP-4, such as peptide YY (PYY) [27], glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and glucose-dependent insulinotropic polypeptide (GIP) [34,35]. Hence, DPP-4 is a wide-spread enzyme with activity to cleave the two N-terminal amino acids of a number of biologically active peptides involved in different functions in immunology, gastroenterology and endocrinology.

Physiology of DPP-4 The physiological function of DPP-4 is far from understood. Its distribution to organs having physiological barriers and involved in nutrition or secretion, suggests that the enzyme is of physiological importance for defence and nutritional/digestive functions. DPP-4 may also be regarded as a proteolytic enzyme involved in the inactivation of bioactive peptides, particularly in relation to immunomodulation and glucose homeostasis [2,16]. Another potential function of DPP-4 is a binding function, because a cystein-rich domain of the enzyme apart from the catalytic site binds preferentially to collagen, which may be of importance for its function [36]. Hence, DPP-4 may function both as a protease and a binding protein.

INCRETIN HORMONES

Incretin hormones The idea of inhibiting DPP-4 as a treatment for type 2 diabetes originates from findings that DPP-4 is efficient in inactivating GLP-1. GLP-1 is a gastrointestinal hormone, which is produced mainly in the intestinal L-cells, located in the distal portion of the small intestine [37]. It is an incretin hormone, meaning that it is released following meal intake and potentiates glucose-stimulated insulin secretion [38]. It has also a number of other actions, such as delaying gastric emptying [39] and inhibiting glucagon secretion [40]. Several recent reviews have discussed these effects of GLP-1 [40-42]. Together the effects result in lowering of circulating glucose when administered both to healthy volunteers and to subjects with type 2 diabetes [43-47]. In addition, GLP-1 induces satiety which reduces food intake, and GLP-1 has profound actions of pancreatic beta cell function resulting in increased insulin gene expression and biosynthesis, islet neogenesis and inhibition of pancreatic beta cell apoptosis, leading to increased beta cell mass [for review see 41,48]. Altogether, these actions have prompted development of GLP-1 in the treatment of type 2 diabetes [40,41,49]. GLP-1 is, however, not the only incretin hormone. Also GIP (glucose-dependent insulinotropic peptide), which is produced by the K-cells located mainly in the duodenum, is an incretin hormone [for review see 50]. In fact, GLP-1 and GIP account for the main incretin action in humans, and it has been estimated that the incretin action accounts for approximately 70% of insulin secretion after a meal intake [51].

DPP-4 inactivates GLP-1 and GIP The two incretin hormones, GLP-1 and GIP, have both short circulating half-lives. Already a preliminary report in 1992 suggested that a serum factor was responsible for the rapid inactivation of the hormones [52] and in 1993 it was demonstrated that DPP-4 mediates the inactivation [35]. This was demonstrated by incubating purified DPP-4 and plasma with GLP-1 and GIP, which was found to remove the two N-terminal amino acids of the hormones. Similar finding of degradation of GLP-1 by removal of the N-terminal dipeptide was observed in vivo after administration of the intact GLP-1 [53,54] as well as after studying the metabolism of endogenously released GLP-1 [55-57]. Since the N-terminal ends of GLP-1 and GIP are required for biological activity [58-62], these results show that DPP-4 mediates the inactivation of the hormones. The inactivation of the two hormones by DPP-4 is rapid, which is due to the localization of the enzyme in capillaries close to the intestinal cells where the hormones are produced [63] as well as the high abundance of the enzyme in the vasculature, circulation and liver [2]. This results in a half-life of only 1-2 min for intact GLP-1 of 7 min for intact GIP [56,64,65]. Another consequence of this rapid inactivation of the two hormones is that the active (intact) GLP-1 accounts only for 40% of total GLP-1 in the circulation under fasting conditions and for 60% after food ingestion [55], whereas the corresponding figures for GIP are 30 and 40%, respectively [56]. This has importance when interpreting results from measurements of various forms of the two hormones.

Since the knowledge of the rapid inactivation of GLP-1 by DPP-4 was established in the mid 1990s, the development of GLP-1-based therapy for type 2 diabetes has focused on circumventing this drawback. Two strategies have evolved. One strategy is the development of DPP-4 resistant GLP-1 receptor agonists (GLP-1 mimetics), such as exenatide and liraglutide [for review see 40,41,66,67]. The other strategy is the development of compounds which inhibit DPP-4. This latter idea was summarized by Deacon and Holst in 1998 [68]. The rationale for DPP-4 inhibition as a target for treatment of type 2 diabetes is therefore that the degradation of endogenously released GLP-1 is inhibited. This results in

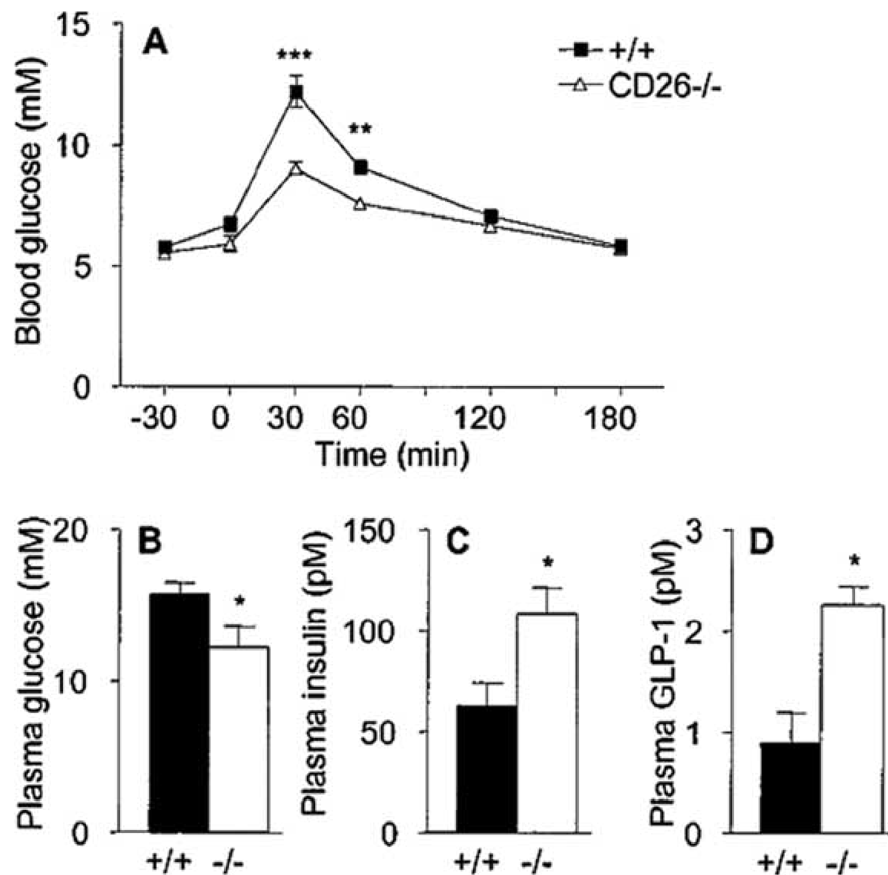

Fig. (1). Enhanced oral glucose tolerance and increased levels of insulin and GLP-1 in DPP-4 knockout mice (CD26-/- mice). (A) Blood glucose concentrations measured at various times before and after oral administration of glucose (2g/kg, given at time 0) to female wild-type or CD26-/- mice, as indicated. n=7. In bottom is shown levels of glucose (B), insulin (C) and intact GLP-1 (D) in plasma from male wild-type (closed bars) and CD26-/- (open bars) mice taken 15 min after oral glucose challenge. n=8. *, PO<0.05; **, P<0.01; ***, P<0.001. The Figure is reproduced from reference [75], published in Proceedings of the National Academy of Sciences, U.S.A., 2000.

increased and prolonged concentrations of the active form of GLP-1, utilizing its efficient antidiabetic actions. It should be emphasized that although DPP-4 inhibition will increase also the concentration of active GIP, this has probably only minor impact on the metabolism in diabetes, since it has been demonstrated that the efficiency of GIP to stimulate insulin secretion is reduced in type 2 diabetes [69] as well as in first degree relatives of patients with type 2 diabetes [70].

DPP-4 KNOCK OUT ANIMALS

The rationale of using DPP-4 inhibition in the treatment of diabetes is supported by results from animals having defective DPP-4 activity or genetic deletion of DPP-4. A substrain of Fischer-344 rats has a missense mutation of the DPP-4 gene in the catalytic region of the enzyme, changing the serine moiety of the vital GWSYR sequence to arginine [71]. Thus, these rats have a defective DPP-4 activity [72], which is accompanied by increased glucose tolerance after oral glucose challenge in association with increased insulin levels [73]. Furthermore, these rats are resistant to development of glucose intolerance after feeding a high-fat diet [74]. These findings are consistent with the view that the released incretin hormones after oral glucose are not inactivated in Fischer-344 rats, due to lack of DPP-4 activity, which enhances insulin secretion thereby improving glucose tolerance. Similar results are seen in mice with a targeted inactivation of the DPP-4 gene resulting in loss of DPP-4 production [75]. As is illustrated in Fig. **1** (reproduced from reference [75]) these knockout mice have increased glucose tolerance after oral glucose challenge (2g/kg) in association with increased insulin levels and increased level of active GLP-1 when compared to wildtype mice [75]. This shows that the oral loading of glucose has increased GLP-1 secretion and GLP-1 in turn has stimulated insulin secretion to reduce glucose, and since the inactivation of GLP-1 in the DPP-4 knockout animals is delayed active GLP-1 levels were augmented with an accompanying enhanced insulin response and improved glucose tolerance. It has also been demonstrated that mice lacking the gene encoding DPP-4 do not increase body weight or circulating insulin when fed a high-fat diet; they therefore seem refractory to the development of obesity and insulin resistance after high-fat diet [76]. Pair-feeding experiments and indirect calorimetry showed that was was due to reduced food intake in combination with increased energy expenditure. These studies in animals lacking DPP-4 activity therefore show that DPP-4 is involved both in the acute regulation of insulin secretion and

glucose homeostasis as well as in long-term regulation of body weight. In general, DPP-4 deficient animals are in good health and have normal neurological, motor and reproductive function. A recent study demonstrated, however, that DPP-4 deficient Fischer-344 rats exhibit reduced stress-induced analgesia [77], suggesting involvement of DPP-4 sensitive peptides in this process.

PHARMACOLOGICAL DPP-4 INHIBITORS

The profound action of DPP-4 to inactivate the antidiabetic hormone GLP-1 in association with findings of improved glucose tolerance in animals lacking DPP-4 activity have led to the assumption that DPP-4 inhibition may be a target for treatment of type 2 diabetes [68]. Since the catalytic domain in DPP-4 seems to reside in the serine residue in position 630 [2,22], serine enzyme inhibitors have been found to be suitable compounds for development into DPP-4 inhibitors. Therefore, efficient DPP-4 inhibitors are serine inhibitors like diisopropyl fluorophosphate, dietyl-4-nitrophenyl phosphate and metal ions, whereas inhibitors of other classes of proteases are ineffective, such as EDTA or leupeptin [2,78]. Other DPP-4 inhibitors are proline-based dipeptide mimetics having a boronic acid, being irreversible inhibitors [79]. Selective compounds exhibiting DPP-4 antagonistic property at micromolar concentrations are the tripeptides isoleucin-prolin-isoleucin (diprotin A) and valine-proline-valine (diprotin B) [80], and substrate analogs acting as inhibitors include proline boronic acid dipeptide inhibitors, dipeptide phosphonates, dipeptide 2-cyanopyrrolidides and aminoacyl pyrrolidides [2,81,82]. There exists also natural compounds with DPP-4 inhibitory action, such as PYY(3-36) and the Tat protein of HIV-1, the common demonimator being a proline in the 3rd position from the N-terminal end [2,83]. Several of these compounds are, however, unsuitable for development as pharmacological agents due to either non-specificity or, as is the case for many compounds, a chemical instability.

Compounds having a greater stability are dipeptides derivatives based on thiazolidide (for example isoleucine thiazolidide and PE32/98) or pyrrolidide (for example 4-aminocyclohexylglycine, valine pyrrolidide and fluoropyrrolidine) [84-89]. Also N-alkylamines based on cyanopyrrolidine have been shown to have great chemical stability [90,91] and recently it was also reported that dipeptide surrogate compounds containing a cyclopropanated prolinenitrile derived from proline are potent DPP-4 inhibitors with high chemical stability [92]. Therefore, there exists today a number of stable DPP-4 inhibitors being reversible inhibitors of the catalytic site of DPP-4.

An important issue in developing DPP-4 inhibitors for the treatment of diabetes is, beside chemical stability, the specificity of inhibition. It has been demonstrated that DPP-4 belongs to a family class of dipeptidyl peptidase enzymes [93]. These different DPPs are called DPP-6, DPP-7, DPP-8, DPP-9, DPP-X, FAP (fibroblast activation protein), APP (acylaminoacyl peptidase), POP (prolyl oligopeptidase), PCP (prolyl carboxypeptidase) and QPP (quiescent cell proline dipeptidase) [93,94]. Since the catalytic regions of these enzymes show similarity to each other, DPP-4 inhibitory compounds might be non-specific. The clinical importance of this is at present unclear. On one hand, a high specificity for DPP-4 would be advantage to avoid inhibition of degradation of substrates for the other DPPs; on the other hand, some of the substrates inactivated by the other DPPs might add to the benefit of non-specific DPP-4 inhibitors. Further studies are required to establish the clinically most valuable and efficient compound. Specificity of the compounds has also relevance for potential adverse events. For example, it has been demonstrated that inhibiton of DPP-8 and DPP-9 is associated with severe side effects in dogs [95]. However, no such adverse events have been observed after inhibition of DPP-4.

DPP-4 INHIBITION IN ANIMAL STUDIES

That DPP-4 inhibition carries a potential benefit in the treatment of type 2 diabetes was initiallt studied using the DPP-4 inhibitor valine-pyrrolidide. Valine-pyrrolidide was thereby been demonstrated to inhibit DPP-4 activity and increase the concentration of the active GLP-1 in association with improved GLP-1-stimulated insulin secretion in pigs [96]. Valine-pyrrolidide, administered through an oral gavage, also improves glucose tolerance and increases insulin secretion in normal mice and in mice rendered insulin resistant by a high-fat diet [97]. That latter study also demonstrated that valine-pyrrolidide markedly increased the active concentration of GLP-1 after intravenous GLP-1 administration, showing that naturally this peptide is avidly degraded in vivo. Also the DPP-4 inhibitor isoleucine-thiazolidide improves glucose tolerance in animal studies. Thus, isoleucin-thiazolidide given orally to rats at a dose which inhibits DPP-4 activity by 70% was associated with augmented concentration of active GLP-1 after duodenal glucose administration in conjunction with an earlier and increased peak of plasma insulin and a more rapid clearance of glucose [85]. Isoleucin-thiazolidide was also found to improve glucose tolerance and augment insulin secretion in association with prolonged half-life of GLP-1 in lean and obese Zucker rats [85]. Similar results were obtained in both rats and mice with the DPP-4 inhibitor, NVP-DPP728. In rats, oral administration of NVP-DPP728 improved glucose tolerance and increased insulin levels both under normal conditions and after induction of insulin resistance by high-fat feeding [74] and in lean and obese fatty Zucker rats [98]. Furthermore, in mice, a long-term study with administration of NVP-DPP728 for 8 weeks showed an almost complete inhibition of DPP-4 activity in association with improved glucose tolerance and insulin secretion after oral glucose [99]. Interestingly, also glucose-stimulated insulin secretion in islets isolated from mice after 8 weeks of treatment with NVP-DPP728 was improved, suggesting that DPP-4 inhibition has upregulated signalling mechanisms in the islets. One such mechanism could be the glucose transporter-4 (GLUT-4), the expression of which was increased in islets after DPP-4 inhibition [99]. Similar findings were obtained from a long-term (3 months) study in obese Zucker rats, using the DPP-4 inhibitor P32/98 [100]. It was found that daily administration of the compound progressively increased glucose tolerance and insulin secretion after oral glucose, and also after 3 months treatment, P32/98 increased the glucose-stimulated insulin secretion from perfused pancreas. This was associated with increased insulin-

mediated glucose uptake in skeletal muscle, as suggestive also of increased insulin sensitivity. This was confirmed in a study using another strain of rats (Vancouver diabetic fatty rats) in which 12 weeks treatment with P32/98 increased both hepatic and peripheral insulin sensitivity as determined by euglycemic hyperinsulinemic clamp with tracer glucose infusion [101]. Glucose tolerance and insulin secretion after oral glucose have also been shown to be augmented by oral administration of two methanoprolinenitrile dipeptide-derived DPP-4 inhibitors in obese Zucker rats [92] as well as after oral administration of the DPP-4-inhibitor, LAF237, in normal and insulin resistant mice [102]. Hence, several studies have demonstrated that pharmacological DPP-4 inhibitors improve glucose tolerance in both normal animals and in different animal models of glucose intolerance or diabetes and that this is executed in association with increased concentration of active GLP-1 and insulin.

Besides stimulation of insulin secretion, GLP-1 has been shown also to increase pancreatic beta-cell mass by stimulating beta cell neogenesis and inhibit pancreatic beta cell apoptosis, which may executed through activation of the transcription factor PDX-1 [103]. PDX-1 is the pancreatic-duodenal homeobox 1, which is a protein that is critically required for normal pancreas development and for proper differentiation of the endocrine pancreas. In an attempt to examine whether similar action is seen after DPP-4 inhibition, a study was conducted in which Wistar rats were rendered diabetic by means of the beta cell toxic compound streptozotocin and thereafter they were treated for 7 days with the DPP-4 inhibitor, P32/98. It was found that P32/98-treated rats had reduced glucose levels and normalized insulin levels in association with improved glucose tolerance [104]. More importantly, however, it was found that P32/98-treated animals had increased number of small islets and total beta cells, as suggestive of increased beta cell mass [101]. A similar result was obtained in an 8-week study on NVP-DPP728 in mice, where the mean islet size was reduced after DPP-4 inhibition [99]. Hence, it seems as if long-term DPP-4 inhibition has profound effects also on islet morphology, suggesting a stimulation of islet neogenesis.

The increased concentration of active GLP-1 during DPP-4 inhibition might negatively influence the secretion of the incretin hormones through a classical negative feed back loop as was observed in DPP-4 deficient Fischetr-344 rats [105]. This has been experimentally shown in a study in dogs, in which the DPP-4 inhibitor, NVP-DPP728 was given at a dose that reduced the DPP-4 activity by 90%. This was followed by increased levels of active GLP-1, but at the same time the total concentration of active and inactive GLP-1 were reduced, as a sign of inhibited secretion [106]. Similar results were recently also demonstrated in humans [107]. This would imply that a limitation of DPP-4 inhibition is an inhibiton of secretion of the incretin hormones, which however would be operative only when the inhibition actually increases the active concentration of the hormones, i.e., when the compounds have clinical effects.

An important topic in relation to DPP-4 inhibition as a treatment of diabetes is whether the inhibitors would be able to prevent the onset of the disease. Thus, it is supposed that subjects with type 2 diabetes undergo a transition from normal glucose tolerance through a state of impaired glucose tolerance (IGT) to the fully developed type 2 diabetes. Prevention of this transition would therefore reduce the occurrence of the disease. One experimental study in Zucker diabetic fatty (ZDF) rats suggested this to be the case. It was found that 7 days treatment of these rats with the DPP-4 inhibitor, FE999011, before the onset of hyperglycemia, delayed the development of type 2 diabetes by 21 days. This was seen in association with reduced levels of triglycerides and free fatty acids, increased GLP-1 levels and, interestingly, increased expression of GLP-1 receptors in the pancreas [108]. Hence, it seems as if a delay in the onset of type 2 diabetes is feasible after DPP-4 inhibition.

Although it seems clear that improvement of the glycemic condition in animal models by DPP-4 inhibition is seen in association with increased concentration of active GLP-1, it remains to be finally established that this is the sole mechanism of action. A few studies have explored this topic. One study has shown that the improvement of glucose intolerance by valine-pyrrolidide is indeed dependent on DPP-4 activity, since the compound had no effect in mice genetically lacking DPP-4 [76]. Another study has shown that valine-pyrrolidide has no influence on insulin secretion when added to incubated isolated islets [97]. These studies therefore show that valine-pyrrolidide augments glucose tolerance by a DPP-4-dependent prevention of inactivation of hormones in vivo stimulating insulin secretion. Since DPP-4 is known to inactivate several biologically active peptides, prevention of degradation of more peptides than GLP-1 might contribute to the effect. One recent study showed that after valine-pyrollidide, the insulinotropic action of not only GLP-1 and GIP, but also of GRP and PACAP were augmented in mice, suggesting the potential ability of DPP-4 inhibitors to augment insulin secretion by means of several peptides [109]. However, it was recently shown that valine-pyrrolidide, as well as the DPP-4 inhibitors, SYR106124 and LAF237, lack effect on glucose tolerance and insulin secretion in mice with a double incretin receptor knockout, i.e., lacking both GLP-1- and GIP-receptors [110]. This suggests that GLP-1 and GIP are the major peptides contributing to the antidiabetic effect of DPP-4 inhibitors in mice. Further studies are, however, required to establish the detailed molecular basis of the improved glucose metabolism after DPP-4 inhibition.

A few studies have compared the degree of DPP-4 inhibition and improvement of glucose tolerance. It has thus been demonstrated that after oral administration of various DPP-4 inhibitors, DPP-4 activity is reduced by ~100% [99], 70% [85], 65% [86] and 35-60% [92], all along improved glucose tolerance. This might suggest that complete inhibition of DPP-4 activity is not required for achievement improved glucose tolerance. However, more studies are required for comparison of the kinetic of enzyme inhibition versus bioactivity of the various compounds.

DPP-4 INHIBITION IN HUMAN STUDIES

Rationale for use of DPP-4 inhibition in type 2 diabetes. A main rationale for using DPP-4 inhibition in the treatment of type 2 diabetes is that the incretin hormone, GLP-1, has antidiabetic properties due to several important mechanism,

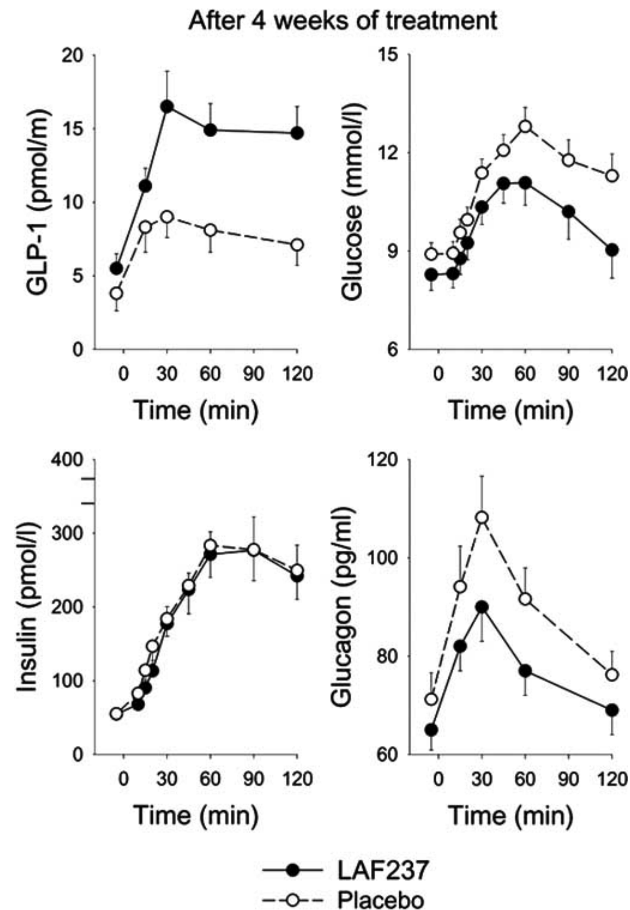

Fig. (2). Active GLP-1, glucose, insulin and glucagon levels before and after intake of breakfast (performed at time 0) after 4 weeks of treatment with placebo (n=19) or the DPP-4 inhibitor, LAF237 (100 mgx1 daily; n=18) in subjects with type 2 diabetes. The Figure is reproduced from reference [113] after permission. Copyright 2004, The Endocrine Society.

and that these actions remain in subjects with diabetes [40-47] in combination with the profound importance of DPP-4 to inactive GLP-1 [52-57,68]. Hence, DPP-4 inhibition would augment the concentration of endogenously released GLP-1 in association with prolonged half-life. Furthermore, subjects with type 2 diabetes have reduced circulating concentrations of active GLP-1 after meal ingestion [111]. Hence, DPP-4 inhibition by preventing the inactivation of GLP-1 will tend to normalize the reduced GLP-1 levels in type 2 diabetes. Finally, another important rationale for DPP-4 inhibition is that by preventing the inactivation of GLP-1, the long-term actions of the hormone on beta cell neogenesis and apoptosis may be instituted [48], which might lead to a disease modification by preserving long-term beta-cell function in subjects with type 2 diabetes.

Anti-diabetic action. After establishing the rationale behind the concept of inhibiting DPP-4 in the treatment of type 2 diabetes and after the encouraging results in animal studies, it was of great interest that DPP-4 inhibition improves glucose tolerance also in humans. A first, proof-of-concept, study in humans examined the DPP-4 inhibitor, NVP-DPP728 in a 4 week study in diet-controlled subjects with type 2 diabetes in comparison with placebo [112]. A total of 93 patients were included in the study, which had two administration regimes of the compound (150mgx2 and 100mgx3). The patients were mildly diabetic having a baseline HbA_{1c} of 7.4% and baseline glucose of 8.5 mmol/l. It was found that the 4 week treatment with NVP-DPP728 was associated with reduced fasting glucose (by ~1 mmol/l), prandial glucose (by ~1.2 mmol/l) and 24h glucose (by ~1.0 mmol/l). Although not an efficacy parameter due to the short duration of the study, also HbA_{1c} was reduced, to 6.9%. A follow-up study using the DPP-4 inhibitor LAF237, which can be given once daily, arrived at the same conclusion. In this study, patients with dietary-controlled type 2 diabetes were treated with LAF237 at 100 mg daily for 4 weeks. It was found that compared to placebo, LAF237 reduced fasting glucose by ~0.7 mmol/l, 4 h prandial glucose excursion by ~1.4 mmol/l and mean 24h glucose by ~0.9 mmol/l in association with a significantly reduced HbA_{1c} levels [113]. After the 4 weeks of treatment, a standard meal breakfast was given and active GLP-1, glucose, insulin and glucagon were determined (Fig. **2**). It was found that the concentration of active GLP-1 increased in response to the breakfast, and this response was augmented by more than 100% in patients treated with LAF237, showing the efficiency of DPP-4 inhibition to prevent the inactivation of endogenously released hormone. Furthermore, the glucose tolerance after breakfast ingestion was augmented in patients treated with LAF237, as evident by reduced prandial glucose level. This was associated with reduced glucagon response to the breakfast. When relating the change in glucagon to change in glucose, it was found that the reduction in glucagon correlated to the improved glucose tolerance, suggesting an important mechanism through inhibited glucagon secretion. Finally, as also is seen in Fig. **2**, the insulin response to breakfast ingestion was not altered by LAF237. This probably represents a stimulation of insulin secretion by LAF237, since the glucose stimulus for insulin secretion was reduced; hence another stimulus has augmented the insulin response to glucose, most likely the active GLP-1. Thus, these 4 weeks studies showed good and promising effects of DPP-4 inhibition in subjects with type 2 diabetes.

Recently, long-term studies have been undertaken with DPP-4 inhibition. In one study, the DPP-4 inhibitor, LAF237 (50mgx1), was given to subjects with type 2 diabetes already treated with metformin. The study had an initial 12 weeks phase with a further 40 week extension phase. The study population consisted of patients with type 2 diabetes with a mean HbA_{1c} level of 7.8%. During the 12 week core study, HbA_{1c} levels were significantly reduced in subjects given LAF237 but not significantly altered in the placebo group. The placebo-related reduction in HbA_{1c} in subjects given LAF237 was 0.7%, and, similarly, fasting glucose was reduced (by 1.2 mmol/l) [114]. During the 40 week extension period, HbA_{1c} increased in the placebo-treated group (which was treated with metformin), but remained stable in the group given LAF237 plus metformin, resulting reduction in HbA_{1c} after 1 year by 1.1% in this group compared with the group treated with placebo plus metformin. This shows that DPP-4 inhibition is efficient in substantially reducing HbA_{1c} levels on a long-term basis and

the results provide evidence for a potential prevention of deterioration of the disease. Another study has examined the 12 week efficiency of LAF237 (25 mgx2) given as monotherapy in subjects with type 2 diabetes [115]. Again, a reduction in HbA_{1c} was observed: by 1.2% in subjects having a mean baseline HbA_{1c} levels of 8.5%. Also in studies using other DPP-4 inhibitors, such as P93/01 [116] and MK-0431 [117], improved glucose tolerance has been observed in humans. Hence, several different DPP-4 inhibitors have shown efficiency in humans.

Tolerability. The clinical studies undertaken sofar have shown that DPP-4 inhibition in human is highly tolerable and safe. Hence, only few adverse events have been reported. For example, in the 1-year study with LAF237 at 50 mgx1 [114], the overall incidence of adverse events was similar in the group given LAF237 compared with the group given placebo and only one serious adverse event (peripheral edema) was suspected to be drug related. There has been no notable changes in haematology, biochemistry or ECG suspected to be drug-related. Hence, during a long-term period of 1 year, DPP-4 inhibition appears to be well tolerated [114]. A potential concern with the use of DPP-4 inhibitors have been that the enzyme is involved in the inactivation of bioactive peptides involved in the immune system [2]. This could potentially affect the immune function. The initial study showed that subjects treated with NVP-DPP728 had nasopharyngitis in a higher frequency than placebo-treated subjects [99]. However, in later studies with other DPP-4 inhibitors, this adverse event has not been seen and, similarly, no clinical sign of altered immune function has been observed [113,114]. Therefore, although detailed studies on the immune system during treatment with DPP-4 inhibition has not yet been undertaken, there is no indication of altered immune function during the treatment.

DPP-4 is also responsible for the inactivation of various neuropeptides, such as neuropeptide Y and substance P [2], and, therefore, prevention of their inactivation may be of importance during DPP-4 inhibition. However, again no evidence of effects or adverse events mediated by this potential action of DPP-4 inhibition has been seen in the clinical trials. The lack of such effect might be explained by the fact that most of these bioactive peptides have other inactivation systems apart from DPP-4 and, therefore, DPP-4 inhibition will not cause a complete prevention of their inactivation. Also, the clinically used DPP-4 inhibition has not been associated with a 24h 100% DPP-4 inhibition for a long period of time, and, therefore, the bioactive peptide may be inactivated by DPP-4 also during DPP-4 inhibition, particularly during night time when the inhibitors are given during day time. However, more studies are required to examine the potential action of clinically used DPP-4 inhibition of the inactivation of the many bioactive peptides being substrates for the enzyme. Finally, an important aspect of DPP-4 inhibition is that hypoglycemia is very rarely observed during treatment, which is expected from the glucose-dependency of the incretin actions.

CONCLUSIONS

Prevention of the degradation of GLP-1 by means of DPP-4 is a potential novel treatment of type 2 diabetes. The concept has received experimental support from animal studies and, most importantly, clinical support from studies in subjects with type 2 diabetes up to 1 year. DPP-4 inhibition has thereby been proven efficient and shown good tolerability. This new class of antidiabetic compounds is therefore, together with the GLP-1 receptor agonists, most promising for future treatment of type 2 diabetes. What is now required is further long-term studies using different subgroups of patients, such as newly diagnosed patients versus patients with an advanced disease, and patients without prior pharmacological treatment of the disease versus patients with long-standing pharmacological treatment. These studies should concentrate on efficiency, durability, safety and tolerability of the compounds. They should also concentrate on possible combination of DPP-4 inhibition with other modes of treatment, such as insulin sensitizers (thiazolidindiones), inhibitors of hepatic glucose production (metformin), insulin secretagogues (sulfonylureas) and insulin.

ACKNOWLEDGEMENTS

For support of own studies on DPP-4, the author is grateful to Swedish Research Council (Grant 6834), The Swedish Diabetes Association, Region Skane, Albert Påhlsson Foundation, Lund University Hospital Research Funds, the Faculty of Medicine, Lund University and Novartis Pharmaceuticals, Basel, Switzerland.

REFERENCES

[1] Hopsu-Havu, V.K.; Glenner, G.G. *Histochemie* **1966**, *7*, 197-201.
[2] Mentlein, R. *Regul. Pept.* **1999**, *85*, 9-24.
[3] Kettman, U.; Humbel, B.; Holzhausen, H.J. *Acta Histochem.* **1992**, *92*, 225-227.
[4] Heymann, E.; Mentlein, R.; Nausch, I.; Erlanson-Albertsson, C.; Yoshimoto, T.; Feller, A..C. *Biomed. Biophys. Acta* **1986**, *45*, 575-584.
[5] Mentlein, R.; Heymann, E.; Scholz, W.; Feller, A.C.; Flad, H.D. *Cell Immunol.* **1984**, *89*, 11-19.
[6] Jackman, H.L.; Tan, F.L.; Schraufnagel, D.; Dragovic, T.; Dezso, B.; Becker, R.P.; Edrös, E.G. *Am. J. Resp. Cell Mol. Biol.* **1995**, *13*, 196-204.
[7] Sahara, N.; Fukasawa, K.; Harada, M.; Suzuki, K. *Acta Histochem. Cytochem.* **1981**, *14*, 581-587.
[8] Fujiwara, H.; Maeda, M.; Imai, K.; Fukuoka, M.; Yasuda, K.; Takakura, K. *J. Clin. Endocrinol. Metab.* **1992**, *75*, 1352-1357.
[9] Atherton, A.J.; Monaghan, OP.; Warburton, M.J.; Robertson, D.; Kenny, A.J.; Gusterson, B.A. *Int. J. Cancer* **1992**, *50*, 15-19.
[10] Bathon, J.M.; Proud, D.; Mizutani, S.; Ward, P.E. *J. Clin. Invest.* **1992**, *90*, 981-991.
[11] Raynaud, F.; Bauvois, B.; Gerbaud, O.; Evainbrion, D. *J. Cell Physiol*. **1992**, *151*, 378-385.
[12] Mitro, A.; Lojda, Z. *Histochemistry* **1988**, *88*, 645-646.
[13] Bernstein, H.G.; Schön, E.; Ansorge, S.; Röse, I.; Dorn, A. *Int. J. Devel. Neurosci.* **1987**, *5*, 237-242.
[14] Haninec, P.; Grim, M. *Int. J. Devel. Neurosci.* **1990**, *8*, 175-180.
[15] Lojda, Z. *Histochemistry* **1979**, *59*, 153-166.
[16] Lambeir, A.M.; Durinx, C.; Scharpe, S.; De Meester, I. Crit. *Rev. Clin. Lab. Sci.* **2003**, *40*, 209-294.
[17] Misumi, Y.; Hayashi, Y.; Arakawam, F.; Ikehara, Y. *Biochim. Biophys. Acta* **1992**, *1131*, 333-336.
[18] Abbott, C.A.; Baker, E.; Sutherland, G.R.; McCaughan, G.W. *Immunogenetics* **1994**, *40*, 331-338.
[19] Böhm, S.K.; Gum, J.R.; Erickson, R.H.; Hicks, J.W.; Kim, Y.S. *Biochem. J.* **1995**, *311*, 835-843.
[20] Hong, W.; Doyle, D. *J. Cell Biol.* **1990**, *111*, 323-338.
[21] Ludwig, K.; Yan, S.; Fan, H.; Reutter, W.; Bottcher, C. *Biochem. Biophys. Res. Commun.* **2003**, *304*, 73-77.

[22] Ogata, S.; Misumi, Y.; Tsuji, E.; Takami, N.; Oda, K.; Ikehara, Y. *Biochemistry* **1992**, *31*, 2582-2587.
[23] Naush, I.; Mentlein, R.; Heymann, E. *Biol. Chem. Hoppe-Seyler* **1990**, *371*, 1113-1118.
[24] Bongers, J.; Lambros, T.; Ahmad, M.; Heimer, E.P. *Biochem. J.* **1995**, *311*,835-843.
[25] Hong, W.; Doyle, D. *J. Cell Biol.* **1990**, *111*, 323-328.
[26] Kato, T.; Nagatsu, T.; Fukasawa, K.; Harada, M.; Nagatsu, I.; Sakakuibara, S. *Biochim. Biophys. Acta* **1978**, *525*, 417-422.
[27] Mentlein, R.; Gallwitz, B.; Schmidt, W.E. *Regul. Pept.* **1993**, *49*, 133-144.
[28] Zhu, L.; Tamvakopoulos, C.; Xie, D.; Dragovic, J.; Shen, X.; Fenyk-Melody, J.E.; Schmidt, K.; Bagchi, A.; Griffin, P.R.; Thornberry, N.A.; Sinha Roy, R. *J. Biol. Chem*. **2003**, *278*, 22418-22423.
[29] Lambeir, A.M.; Durinx, C.; Proost, P.; van Damme, J.; Scharpé, S.; De Meester, I. *FEBS Lett* **2001**, *507*, 327-330.
[30] Oravecz, T.; Pall, M.; Rodriquez, G.; Gorrell, M.D.; Ditto, M.;Nguyen, N.Y.; Boykins, R.; Unsworth, E.; Norccross, M.A. *J. Exp. Med.* **1997**, *186*, 1865-1872.
[31] Proost, P.; DeMeester, U.; Schols, D.; Struyf, S.; Lambeir, A.M.; Wuyts, A.; Opdenakker, G.; De Clerq, E.; Scharpe, S.; Van Damme, J. *J. Biol. Chem.* **1998**, *273*, 7222-7227.
[32] Proost, P.; Struyf, S.; Schols, D.; Opdenakker, G.; Sozzani, S.; Allavena, P.; Mantovani, A.; Augustyns, K.; Bal, G.; Haemers, A.; Lambeir, A.M.; Scharpe, S.; Van Damme, J. DeMeester, U. *J. Biol. Chem.* **1999**, *274*, 3988-3993.
[33] Bouras, M.; Huneau, J.F.; Luengo, C.; Erlanson-Albertsson, C.; Tomé, D. *Peptides* **1995**, *16*, 399-405.
[34] Drucker, D.J.; Shi, Q.; Crivici, A.; Sumner-Smith, M.; Tavers, W.; Hill, M.; DeForest, L.; Cooper, S.; Brubaker, P.L. *Nature Biotechnol.* **1997**, *15*, 673-677.
[35] Mentlein, R.; Gallwitz, B.; Schmidt, W.E. *Eur. J. Biochem.* **1993**, *214*, 829-835.
[36] Löster, K.; Zeilinger, K.; Reutter, W. *Biochem. Biophys. Res. Commun.* **1995**, *217*, 341-348.
[37] Damholt, A.B.; Buchan, S.M.; Holst, J.J.; Kofod, H. *Endocrinology* **1999**, *140*, 4800-4808.
[38] Kreymann, G.; Ghatei, M.A.; Bloom, S.R. *Lancet* **1987,** *2*, 1300-1304.
[39] Nauck, M.A.; Niedereichholz, U: Ettler, R; Holst, J.J.; Ørskov, C.; Ritzel, R.; Schmiegel, W.H. *Am. J. Physiol.* **1997**, *273*, E981-E988.
[40] Ahrén, B. *BioEssays* **1998**, *20*, 642-651.
[41] Drucker, D.J. *Mol. Endocrinol.* **2003**, *17*, 161-171.
[42] Holst, J.J.; Gromada, J. *Am. J. Physiol.* **2004**, *287*, E199-E206.
[43] Nathan, D.M.; Schreiber, E.; Fogel, H.; Mojsov, S.; Habener, J.F. *Diabetes Care* **1992**, *15*, 270-276.
[44] Rachman, J.; Gribble, F.M.; Bartow, B.A.; Levy, J.E.; Buchanan, K.D.; Turner, R.C. *Diabetes* **1996**, *45*, 1524-1530.
[45] Gutniak, M.K.; Larsson, H.; Heiber, S.J.; Juneskans, O.T.; Holst, J.J.; Ahrén, B. Potential therapeutic levels of glucagon-like peptide-1 achieved in humans by a buccal tablet. *Diabetes Care* **1996**, *19*, 843-848.
[46] Gutniak, M.K.; Larsson, H.; Sanders, S.W.; Juneskans, O.; Holst, J.J.; Ahrén, B. GLP-1 tablet in type 2 diabetes in fasting and postprandial conditions. *Diabetes Care* **1997**, *20*, 1874-1879.
[47] Zander, M.; Madsbad, S.; Lysgaard Madsen, J.; Holst, J.J. *Lancet* **2002**, *359*, 824-830.
[48] Egan, J.M.; Bulotta, A.; Hui, H.; Perfetti, R. *Diabetes Metab. Res. Rev.* **2003**, *19*, 115-123.
[49] Ahrén, B. *Current Diabetes Report* **2003**, *3*, 365-372.
[50] Meier, J.J.; Nauck, M.A.; Schmidt, W.E.; Gallwitz, B. *Regul. Pept.* **2002**, *107*, 1-13.
[51] Nauck, M.; Stockman, F.; Ebert, R.; Creutzfeldt, W. *Diabetologia* **1986**, *29*, 46-52.
[52] Bruckley, D.I.; Lundquist, P. *Regul. Pept.* **1992**, *40*, 117.
[53] Deacon, C.F.; Nauck, M.A.; Toft-Nielsen, M.; Pridal, L.; Willms, B.; Holst, J.J. *Diabetes* **1995**, *44*, 1126-1131.
[54] Kieffer, T.J.; McIntosh, C.H.; Pederson, R.A. *Endocrinology* **1995**, *136*, 3585-3596.
[55] Deacon, C.F.; Johnsen, A.H.; Holst, J.J. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 952-957.
[56] Deacon, C.F.; Nauck, M.A.; Meier, J.; Hucking, K.; Holst, J.J. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 3575-3581.
[57] Deacon, C.F.; Wamberg, S.; Bie, P.; Hughes, T.E.; Holst, J.J. *J. Endocrinol.* **2002**, *172*, 355-362.
[58] Jörnvall, H.; Carlquist, M.; Kwauk, S.; Otte, S.C.; McIntosh, C.H.; Brown, J.C.; Mutt, V. *FEBS Lett.* **1981**, *123*, 205-210.
[59] Adelhorst, K.; Hedegaard, B.B.; Knudsen, L.B.; Kirk, O. *J. Biol. Chem.* **1994**, *269*, 6275-6278.
[60] Rolin, B.; Deacon, C.F.; Carr, R.D.; Ahrén, B. *Eur. J. Pharmacol.* **2004**, *494*, 283-288.
[61] Knudsen, L.B.; Pridal, L. *Eur. J. Pharmacol.* **1996**, *318*, 429-435.
[62] Dvahl, T.P.; Paty, B.W.; Fuller, B.D.; Prigeon, R.L.; D´Alessio, D.A. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 1772-1779
[63] Hansen, L.; Deacon, C.F.; Ørskov, C.; Holst, J.J. *Endocrinology* **1999**, *140*, 5356-5363.
[64] Vilsbøll, T.; Agersø, H.; Krarup, T.; Holst, J.J. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 220-224.
[65] Meier, J.J.; Nauck, M.A.; Kranz, D.; Holst, J.J.; Deacon, C.F.; Gaeckler, D.; Schmidt, W.E.; Gallwitz, B. *Diabetes* **2004**, *53*, 654-662.
[66] Rachman, J. *Diabet. Med.* **2004**, *21*, suppl. 1, 18-20.
[67] Drucker, D.J. *Diabetes Care* **2003**, *26*, 2929-2940.
[68] Holst, J.J.; Deacon, C.F. *Diabetes* **1998**, *47*, 1663-1670
[69] Nauck, M.A.; Heimesaat, M.M.; Ørskov, C.; Holst, J.J.; Ebert, R.; Creutzfeldt, W. *J. Clin. Invest.* **1996**, *91*, 301-307.
[70] Meier, J.J.; Hucking, K.; Holst, J.J.; Deacon, C.F.; Schmiegel, W.H.; Nauck, M.A. *Diabetes* **2001**, *50*: 2497-2504.
[71] Fujiwara, T.; Tsuji, E.; Misumii, Y.; Takami, N.; Ikehara, Y. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 776-784.
[72] Watanabe, Y.; Kojima, T.; Fujimoto, Y. *Experentia* **1987**, *43*, 400-401.
[73] Nagakura, T.; Yasuda, N.; Yamazaki, K.; Ikuta, H.; Yoshikawa, S.; Asano, O.; Tanaka, I. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 501-506.
[74] Mitani, H.; Takimoto, M.; Hughes, T.E.; Kimura, M. *Jpn. J. Pharmacol.* **2002**, *88*, 442-450.
[75] Marguet, D.; Baggio, L.; Kobayashi, T.; Bernard, A.M.; Pierres, M.; Nielsen, P.F.; Ribel, U.; Watanabe, T.; Drucker, D.J.; Wagtmann, N. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6874-6879.
[76] Conarello, S.L.; Li, Z.; Ronan, J.; Roy, R.S.; Zhu, L.; Jiang, G.; Liu, F.; Woods, J.; Zycband, E.; Moller, D.E.; Thornberry, N.A.; Zhang, B.B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *27*, 100: 6825-6830.
[77] Karl, T.; Hoffman, T.; Pabst, R.; von Horsten, S. *Physiol. Behav.* **2003**, *80*, 123-134.
[78] Püschel, G.; Mentlein, R.; Heymann, E. *Eur. J. Biochem.* **1982**, *126*, 359-365.
[79] Coutts, S.J.; Kelly, T.A.; Snow, R.J.; Kennedy, C.A.; Barton, R.W.; Adams, J.; Krolikowski, D.A.; Freeman, D.M.; Campbell, S.J.; Ksiazek, J.F.; Bachovchin, W. *J. Med. Chem.* **1996**, *39*, 2087-2094.
[80] Rahfeld, J.; Schierhorn, M.; Hartrodt, B.; Neubert, K.; Heins, J. *Biochim. Biophys. Acta* **1991**, *1076*, 314-316.
[81] Demuth, H.U.; Heins, J. In: Austin, R.G. Dipeptidyl Peptidase IV (CD 26) in Metabolism and the Immuno Response, **1995**, pp. 1-35.
[82] Ashworth, D.M.; Atrash, B.; Baker, G.R.; Baxter, A.J.; Jenkins, P.D.; Jones, D.M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163-1166.
[83] Wrengerm S.; Reinhold, D.; Hoffman, T.; Kraft, M.; Frank, R.; Faust, J.; Neubert, K.; Ansorge, S. *FEBS Lett.* **1996**, *383*, 145-149.
[84] Sorbera, L.A.; Revel, L.; Castagner, J. *Drugs Future* **2001**, *26*, 859-864.
[85] Pauly, R.P.; Demuth, H.U.; Rosche, F.; Schmidt, J.; White, H.A.; Lynn, F.; McIntosh, C.H.S.; Pederson, R.A. *Metabolism* **1999**, *48*, 385-389.
[86] Pederson, S.A.; White, H.A.; Schlenzig, D.; Pauly, R.P.; McIntosh, C.H.S.; Demuth, H.U. *Diabetes* **1998**, *47*, 1253-1258.
[87] Parmee, E.R.; He, J.; Masracchio, A.; Edmondson, S.D.; Colwell, L.; Eiermann, G.; Freeney, W.P.; Habulihaz, B.; He, H.; Kilburn, R.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R.A.; Petrov, A.; DiSalvo, J.; Wu, J.K.; Thornberry, N.A.; Weber, A.E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 43-46.
[88] Ashton, W.T.; Dong, H.; Sisco, R.M.; Doss, G.A.; Leiting, B.; Patel, R.A.; Wu, J.K.; Marsillo, F.; Thornberry, N.A.; Weber, A.E. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 859-863.
[89] Caldwell, C.G.; Chen, P.; He, J.; Parmee, E.R.; Leiting, B.; Marsilio, F.; Patel, R.A.; Wu, J.K.; Eiermann, G.J.; Petrov, A.; He, H.; Lyons, K.A.; Thornberry, N.A.; Weber, A.E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1265-1268.
[90] Hughes, T.E.; Mone, M.D.; Russell, M.E.; Weldon, S.C.; Villhauer, E.B. *Biochemistry* **1999**, *38*, 11597-11603.

[91] Villhauer, E.B.; Brinkman, J.A.; Naderi, G.B.; Burkey, B.F.; Dunning, B.E.; Prasad, K.; Mangold, B.L.; Russell, M.E.; Hughes, T.E. *J. Med. Chem.* **2003**, *46*, 2774-2789.
[92] Magnin, D.R.; Robl, J.A.; Sulsky, R.B.; Augeri, D.J.; Huang, Y.; Simpkins, L.M.; Taunk, P.C.; Betebenner, D.A.; Robertson, J.G.; Abboa-Offei, B.E.; Wang, A.; Cap, M.; Xin, L.; Sitkoff, D.F.; Malley, M.F.; Gougoutas, J.Z.; Khanna, A.; Huang, Q.; Han, S.P.; Parker, R.A.; Hamann, L.G. *J. Med. Chem.* **2004**, *47*, 2587-2598.
[93] Rosenblum, J.S., Kozarich, J.W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 496-504.
[94] Caldwyll, C.G.; Chen, P.; He, J.; Parmee, E.R.; Leiting, B.; Marsilio, F.; Patel, R.A.; Wu, J.K.; Eiermann, G.J.; Petrov, A.; He, H.; Lyons, K.A.; Thornberry, N.A.; Weber, A.E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1265-1268.
[95] Lankas, G.; Leiting, B.; Roy, R.S.; Eiermann, G.; Biftu, T.; Kim, D.; Ok, H.; Weber, A.; Thornberry, NA. *Diabetes* **2004**, *53*, suppl 1, A2.
[96] Deacon, C.F.; Hughes, T.E.; Holst, J.J. *Diabetes* **1998,** *47*, 764-769.
[97] Ahrén, B.; Holst, J.J.; Mårtensson, H.; Balkan, B. *Eur. J. Pharmacol.* **2000**, *404*: 239-245.
[98] Balkan, B.; Kwasnik, L.; Miserendineo, R.; Holst, J.J.; Li, X. *Diabetologia* **1999**, *42*, 1324-1331.
[99] Reimer, M.K.; Holst, J.J.; Ahrén, B. *Eur. J. Endocrinol.* **2002**, *146*, 717-727.
[100] Pospisilik, J.A.; Stafford, S.G.; Demuth, H.U.; Brownsey, R.; Parkhouse, W.; Finegood, D.T.; McIntosh, C.H.; Pederson, R.A. *Diabetes* **2002**, *51*, 943-950.
[101] Pospisilik, J.A.; Stafford, S.G.; Demuth, H.U.; McIntosh, C.H.; Pederson, R.A. *Diabetes* **2002**, *51*, 2677-2683.
[102] Sörhede Winzell, M.; Ahrén B. *Diabetes* **2004**, in press.
[103] Hui, H.; Perfetti, R. *Eur. J. Endocrinol.* **2002**, *146*, 129-141.
[104] Pederson, R.A.; Kieffer, T.J.; Pauly, R.; Kofod, H.; Kwong, J.; McIntosh, C.H. *Metabolism* **1996**, *45*, 1335-1341.
[105] Pospisilik, J.A.; Martin, J.; Doty, T.; Ehses, J.A.; Pamir, N.; Lynn, F.C.; Piteau, S.; Demuth, H.U.; McIntosh, C.H.; Pederson, R.A. *Diabetes* **2003**, *52*, 741-750.
[106] Deacon, C.F.; Wamberg, S.; Bie, P.; Hughes, T.E.; Holst, J.J. *J. Endocrinol.* **2002**, *172*, 355-362.
[107] El-Ouaghlidi, A.; Rehring, E.; Holst, J.J.; Schweizer, A.; Holmes, D.; Nauck, M.A. *Diabetologia* **2004**, *47*, A284
[108] Sudre, B.; Broqua, P.; White, R.B.; Ashworth, D.; Evans D.M.; Haigh, R.; Junien, J.L.; Aube, M.L. *Diabetes* **2002**, *51*, 1461-1469.
[109] Ahrén, B.; Hughes, T.E. *Diabetes* **2004**, *53*, suppl. 2: A339
[110] Hansotia, T.; Baggio, L.L.; Delmeire, D.; Hinke, S.A.; Yamada, Y.; Tsukiyama, K.; Seino, Y.; Holst, J.J.; Schuit, F.; Drucker, D.J. *Diabetes* **2004**, *53*, 1326-1335.
[111] Vilsbøll, T.; Krarup, T.; Deacon, C.F.; Madsbad, S.; Holst, J.J. *Diabetes* **2001**, *50*, 609-613.
[112] Ahrén, B.; Siumonsson, E.; Larsson, H.; Landin-Olsson, M.; Torgeirsson, H.; Jansson, P.A.; Sandqvist, M.; Båvenholm, P.; Efendic, S.; Eriksson, J.W.; Dickinson, S.; Holmes, D. *Diabetes Care* **2002**, *25*, 869-875.
[113] Ahrén, B.; Landin-Olsson, M.; Jansson, P.A.; Svensson, M.; Holmes, D.; Schweizer, A. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2078-2084.
[114] Ahrén, B.; Gomis, R.; Standl, E.; Mills, D.; Schweizer, A. *Diabetes Care* **2004**, in press
[115] Pratley, R.; Galbreath, E. *Diabetes* **2004**, A83.
[116] Heins, J.; Glund, K.; Hoffmann, T.; Wolf, R.; Hoffman, A.; Metzner, J.; Demuth, H.U. *Diabetes* **2004**, *53*, A128.
[117] Herman, G.A.; Zhao, P.L.; Dietrich, B.; Golor, G.; Schrodter, A.; Keymeulen, B.; Lasseter, K.C.; Kipnes, M.S.; Hilliard, D.; Tanen, M.; De Lepeleire, I.; Cilissen, C.; Stevens, C.; Tanaka, W.; Gottesdiener, K.M.; Wagner, J.A. *Diabetes* **2004**, *53*, A82.

Received: August 15, 2004 **Accepted: September 19, 2004**