

Dipeptidyl peptidase iv inhibitors and diabetes therapy

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1. ABSTRACT

Current type 2 diabetes therapies are mainly targeted at stimulating pancreatic beta-cell secretion and reducing insulin resistance. A number of alternative therapies are currently being developed to take advantage of the actions of the incretin hormones Glucagon-Like Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP). These hormones are released from the small intestine in response to nutrient ingestion and stimulate insulin secretion in a glucose-dependent manner. One approach to potentiating their actions is based on inhibiting dipeptidyl peptidase IV (DPP IV), the major enzyme responsible for degrading the incretins *in vivo*. DPP IV exhibits characteristics that have allowed the development of specific orally administered inhibitors with proven efficacy in improving glucose tolerance in animal models of diabetes. A number of clinical trials have demonstrated that DPP IV inhibitors are effective in improving glucose disposal and reducing hemoglobin A1c levels in type 2 diabetic patients and one inhibitor, sitagliptin, is now in therapeutic use, with others likely to receive FDA approval in the near future. Studies aimed at elucidating the mode of action of the inhibitors are still ongoing. Both enhancement of insulin secretion and reduction in glucagon secretion, resulting from the blockade of incretin degradation, are believed to play important roles in DPP IV inhibitor action. Preclinical studies indicate that increased levels of incretins improve beta-cell secretory function and exert effects on beta-cell mitogenesis and survival that can preserve beta-cell mass. Roles for other hormones, neuropeptides and cytokines in DPP IV inhibitor-mediated responses are also possible.

2. INTRODUCTION

There is a worldwide epidemic of diabetes mellitus, with approximately 217 million people afflicted and WHO predictions of a minimum of 366 million diabetics by 2030 (1). Type 1 diabetes (T1DM) results from autoimmune destruction of beta-cells (2) and Type 2 (T2DM) from deficient β -cell function and insulin resistance (3). There is a genetic predisposition to both forms (2, 4). Currently, patients with T1DM are treated with insulin (5); a small percentage receiving whole pancreas (6, 7) or islet transplants (8-10). Treatment of T2DM (1, 11) involves lifestyle modification (diet, exercise) plus administration of antidiabetic drugs. The major drugs currently in use are members of the sulfonylurea or meglitinide family, targeted at stimulating insulin secretion (12, 13), biguanides (e.g. metformin) or thiazolidinediones to reduce insulin resistance (14) and α -glucosidase inhibitors for lowering starch and sucrose digestion (11). Combination therapy is also widely used (15) and insulin may be necessary when glucose management fails. Despite this armamentarium of pharmaceuticals, glucose is often poorly controlled in many type 2 diabetics (16) and monotherapy failure frequently occurs (17, 18). Alternative approaches, including new methods of stimulating insulin secretion, have therefore been sought and the most exciting recent development has been the design of drugs that take advantage of the incretin system (19) culminating in the recent introduction of exenatide (20-23) and the dipeptidyl peptidase IV (DPP IV) inhibitor sitagliptin (24-26) as therapeutics. This review will focus mainly on the preclinical studies underlying the development DPP IV inhibitors appropriate for diabetes therapy and their proposed mode of action, as well as a summary of the clinical experience to date.

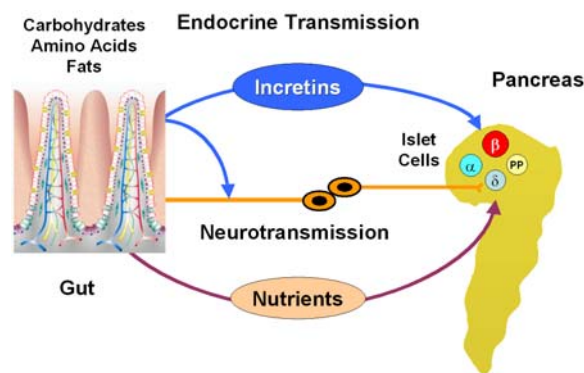


Figure 1. The Enteroinsular axis is a term used to describe the interaction between the intestine and the endocrine pancreas. Nutrients, autonomic nerves and hormones, the incretins, all contribute to the signaling.

3. THE ENTEROINSULAR AXIS AND INCRETINS

Following the discovery of the first hormone, secretin, Moore *et al.* proposed in 1906 that ‘the internal secretion of the pancreas might be stimulated and initiated (similar to the external secretion) by a substance of the nature of a hormone or secretin yielded by the duodenal mucous membrane’ (27). They reported a reduction in glucosuria in some of their type 1 diabetic patients receiving intestinal extracts orally but, not surprisingly, responses were not readily reproduced. A number of groups examined the effects of intravenous administration of various crude preparations of secretin on blood glucose levels, including La Barre, who introduced the term *incrétine* (28) (incretin) to describe a factor from the upper intestine capable of reducing circulating glucose levels, in the absence of any effect on the exocrine pancreas. The concept lay dormant, until the observation was made that humans handled an oral glucose load more efficiently than an equivalent amount of glucose administered intravenously (29, 30). A greater insulin response was also observed in those individuals receiving oral glucose, showing that the gut played an important regulatory role in insulin secretion and glucose disposal. Unger and Eisentraut (31) subsequently referred to the hormonal link between the gut and the endocrine pancreas as the entero-insular axis. Creutzfeldt (32) expanded the definition of the enteroinsular axis to include neural regulation (Figure 1) and both long autonomic reflexes, involving the central nervous system, and direct entero-pancreatic neural connections (33) are probably involved. He also emphasized the glucose-dependent nature of incretin action and it has been estimated that up to 60% of the total insulin secreted during a meal results from the incretin response (34). Identification of the first incretin came from an unexpected source. In studies aimed at isolating an enterogastrone, Brown discovered a 42 amino acid hormone that he termed Gastric Inhibitory Polypeptide (GIP) to reflect its ability to inhibit gastric acid secretion (35, 36). The name ‘Glucose-dependent Insulinotropic Polypeptide’ was later introduced as an alternative definition of the acronym GIP, to reflect its major role. As a result of bioassay studies on intestinal extracts it was proposed that additional incretins existed (37). Lund and

Habener established that the anglerfish proglucagon gene also coded for an additional glucagon-like peptide (38, 39) and, subsequently, a number of groups contributed to the identification of two mammalian glucagon-related peptides, glucagon-like peptide (GLP)-1 and GLP-2 (38), that are released from the large precursor proglucagon by tissue-selective processing. Two molecular forms, GLP-1₇₋₃₆ amide and GLP-1₇₋₃₇, produced by intestinal L-cells exhibit almost identical insulinotropic activity and they are now collectively referred to as GLP-1 (40). During a meal, the incretins are released from the gastrointestinal tract and stimulate insulin secretion in a glucose-dependent manner, i.e. they are incretins (40, 41). There is a reduced incretin response in many type 2 diabetic patients (41, 42) that is largely due to a reduction in circulating levels of GLP-1 (43, 44) and beta-cell resistance to the actions of GIP (45-47).

4. INCRETIN METABOLISM AND DIPEPTIDYL PEPTIDASE IV

Shortly following its isolation, measurements of renal arterio-venous differences in serum concentrations of canine GIP suggested that it was extracted by the kidneys (48). Subsequent animal studies on GLP-1 also indicated that renal metabolism occurred (49, 50). Since elevated serum levels of both GIP (48) and GLP-1 (51), were found in patients with compromised renal function it was concluded that the kidneys also played a major role in incretin clearance in humans. These early studies utilized radioimmunoassays that detected a number of different molecular forms of GIP and GLP-1 in serum or plasma. It was later shown, by Mentlein *et al.* and other groups, that incubation of GIP or GLP-1 in serum or plasma, or with purified forms of the enzyme dipeptidyl peptidase IV (DPP IV; CD26; E.C. 3.4.14.5), resulted in rapid N-terminal truncation and conversion of GIP₁₋₄₂ and GLP-1₇₋₃₆ to the non-insulinotropic peptides GIP₃₋₄₂ and GLP-1₉₋₃₆, respectively (52-54) (Figure 2). N-terminal degradation was also demonstrated with peptides administered to rodents (53, 55) or humans (56), as well as in studies on the endogenous peptides (56-58). By combining oral glucose tolerance tests with assays using site-directed antibodies, it was shown that responses of GIP₁₋₄₂ and GLP-1₇₋₃₆ did not differ between patients with chronic renal insufficiency and control subjects (59), whereas GIP₃₋₄₂ and GLP-1₉₋₃₆ concentrations were higher in the patients with renal failure. This implies that renal metabolism and/or uptake are important for the final elimination of metabolites (60). The liver is a major site of N-terminal degradation of GIP, with GLP-1 degradation performed by multiple body sites (57, 60).

Dipeptidyl Peptidase IV is a class II integral membrane glycoprotein a molecular weight of 110–150 kD per subunit (61, 62) that acts on oligopeptides by selectively removing N-terminal dipeptides. It exhibits a strong preference for peptides with proline or alanine as the penultimate (P1) amino acid (52, 61, 63-65). Aromatic amino acids are favored at the N-terminus, although the major criterion is a protonated amino group (65). Since the N-termini of GIP and GLP-1 consist of Tyr-Ala and His-

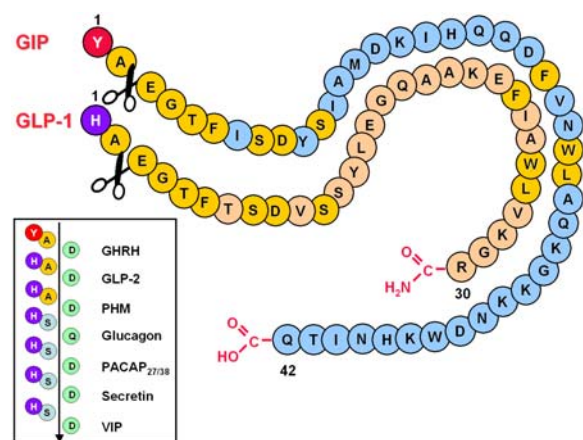


Figure 2. Amino acid sequences of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 and the cleavage sites for dipeptidyl peptidase IV. In the inset is shown the N-terminal sequences of major members of the glucagon-secretin superfamily and their cleavage sites.

Ala, respectively (Figure 2), they are excellent substrates of DPP IV. However DPP IV also acts on peptides with N-termini consisting of Xaa-Serine (Ser) (66), and other amino acids with less efficiency. Several other peptides belonging to the glucagon superfamily have been shown to be substrates *in vitro* (Figure 2), although the physiological significance of such degradation has not been established. The human DPP IV gene is localized to chromosome 2q24, adjacent to the proglucagon gene that codes for GLP-1 (67), and the enzyme is widely distributed throughout the body, with particularly high expression on the apical surfaces of endothelial and differentiated epithelial cells (68). The highest levels of DPP IV in humans are those in bone marrow and the brush border of the small intestine and kidney proximal tubules (61, 69, 70). However significant amounts of DPP IV are also present in the walls of blood vessels, including venules and capillary endothelial cells (61, 70), and DPP IV/CD26 is also expressed on specific subsets of CD4⁺ and CD8⁺ T-cells and natural killer cells (61, 70, 71). Soluble forms of DPP IV are found in blood plasma (72) and are believed to be derived from the endothelial cells, epithelial cells and leukocytes, with the endothelium probably making the largest contribution. Dipeptidyl peptidase IV is present in the plasma membrane as a homodimer (73), the monomers of which consist of a large globular extracellular domain, containing the catalytic site, and a single hydrophobic helix with a six amino acid intracellular region that anchors each subunit to the plasma membrane (74). The enzymatic mechanism responsible for detachment of the extracellular domain from the plasma membrane is obscure. Although activity measurements, following blood sampling, are generally used to determine the level of DPP IV inhibition with *in vivo* experiments or following clinical drug application, it is currently unclear as to the quantitative contribution of soluble and membrane bound DPP IV to the metabolism of GIP and GLP-1 *in vivo*. It has been estimated that as much as 75% of GLP-1 secreted by L-

cells of the gut is degraded to the non-insulinotropic GLP-1₉₋₃₆ by endothelial DPP IV (75, 76). There have also been reports that DPP IV is expressed by alpha-cells (55, 77) in the pancreatic islet. Although this remains to be confirmed, glucagon can be degraded by DPP IV (66, 78) and high local enzyme concentrations could be significant.

5. PRE-CLINICAL STUDIES ON DIPEPTIDYL PEPTIDASE IV INHIBITORS AND TYPE 2 DIABETES

Since the major actions of both GIP and GLP-1 were considered to be mediated via an endocrine route, and an intact N-terminus was critical for their insulinotropic activities, it was proposed that *in vivo* inhibition of DPP IV activity could lead to potentiation of endogenous incretin action during a meal (Figure 3), thus improving glucose homeostasis in diabetics (53, 55, 79-81). Studies on the effects of DPP IV inhibitors on glucose handling and incretin action in normal weight rats and pigs provided the first evidence for their potential. Isoleucine Thiazolidide (Ile-Thia; P32/98) administered to Wistar rats by gavage, in doses resulting in ~60-70% inhibition of plasma DPP IV activity, increased the integrated insulin response to an oral glucose load ~3-fold and improved tolerance (55, 82). Treatment of pigs with the DPP IV inhibitor valine pyrrolidide augmented glucose induced insulin secretion and improved glucose disposal in response to GLP-1 infusion (83). These studies therefore demonstrated that prolongation of incretin action by inhibiting DPP IV activity could form the basis of a viable therapy for T2DM. A number of animal models of diabetes were subsequently used to determine the effect of DPP IV inhibition in T2DM, including studies on a Vancouver strain of the Diabetic Fatty (VDF) Zucker rat (84-86). Homozygous obese *fa/fa* animals are hyperlipidemic and exhibit mild fasting hyperglycemia, but marked post-prandial hyperglycemia, accompanied by hyperinsulinemia and insulin resistance. Single oral doses of Ile-Thia potentiated insulin responses in both lean (*Fa/?*) and obese animals, and this was associated with 39% reductions in the integrated glucose profiles in *fa/fa* rats and 27% in their lean littermates (82, 87). Ile-Thia was administered chronically to VDF rats (10mg/kg bid orally) over 12 weeks (84, 85). Under this dosing regimen DPP IV was inhibited for approximately 9 hours per day. There were no significant effects of inhibitor on water or nutrient ingestion, but body weight gain (mainly fat deposition) was reduced by 12.5%. Fasting blood glucose in diabetic animals decreased from ~8 to 6 mM by 12 weeks of inhibitor treatment, accompanied by markedly improved glucose tolerance and early phase insulin secretion (85). A variety of different DPP IV inhibitors were shown to produce similar improvements in glucose tolerance in other diabetic animal models, including high fat-fed C57BL/6 mice (88-90) and ob/ob (91) and db/db (92) mice, Zucker Fatty (ZF) (93) and Zucker Diabetic Fatty (ZDF) (91, 93, 94) rats.

The majority of DPP IV inhibitors that have been developed can be grouped within three main classes (95-99); Figure 4):

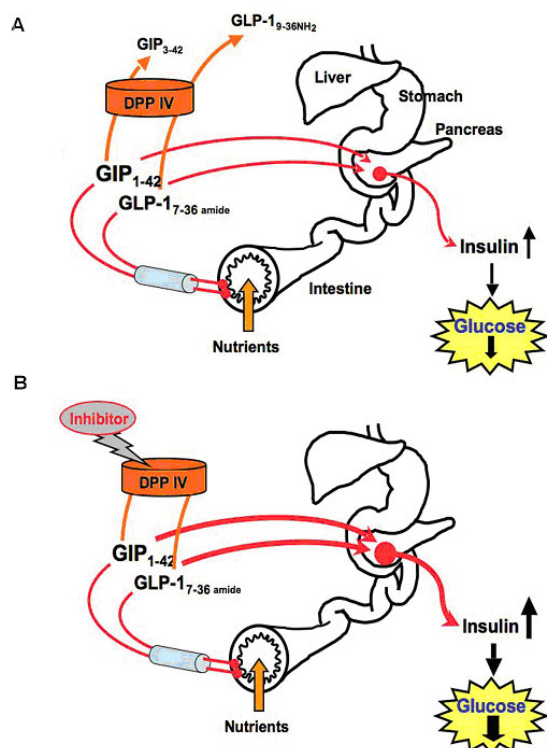


Figure 3. Diagrammatic representation of the roles of the incretins and dipeptidyl peptidase IV, and the effect of inhibitor on insulin responses to the incretins. A. During a meal, incretins (GIP and GLP-1) are released in response to nutrients and are transported through the vasculature to the endocrine pancreas, where they stimulate insulin secretion in a glucose-dependent manner. Dipeptidyl peptidase IV degrades the incretins at their N-termini, thus terminating their insulinotropic actions. B. DPP IV inhibition results in a potentiation of incretin signaling, one consequence of which is a greater insulin response and more efficient glucose handling.

1. Reversible product analogs (e.g. Pyrrolidines, Thiazolidines), including P32/98 and PSN9301 (Probiodrug, Prosidion)
2. Covalently modifying product analogs (e.g. Cyanopyrrolidines), including NVP DPP 728 and LAF 237 (Novartis) and BMS-477118 (Bristol-Myers Squibb).
3. Reversible non-peptide heterocyclic compounds, including Xanthine (NOVO), aminomethylpyridine (Roche) and MK-0431 (Merck).

In addition to the inhibitor studies outlined above, descriptions have been published of the chemical synthesis and biological testing of a number of inhibitors now undergoing clinical trials, including ABT-279 (100), ABT-341 (101), alogliptin (102), NVP DPP728 (94, 103, 104), saxagliptin (91), sitagliptin (26, 90, 105) and an analog (106) and vildagliptin (107-109). The level of inhibition of DPP IV achieved, as estimated from plasma/serum activity measurements, varied widely between the various compounds, and it is unclear

from studies to date as to the level and duration of inhibition that is most desirable. For example acute administration of the tight-binding inhibitor NVP-DPP728, produced almost complete DPP IV inhibition and achieved similar improvements in the ZDF rat (94) to those observed with the competitive inhibitor P32/98 in the VDF strain. A single dose of a second competitive inhibitor, Valine-Pyrrolidide (Val-Pyr; 100 μ mol/kg), resulted in a 4-6-fold potentiation of insulin responses and improved glucose tolerance in C57BL/6J mice fed either normal chow or a high fat diet (88). Chronic treatment of ZDF rats with the long-acting DPP IV inhibitor FE 999011 (10mg/kg oral bid) for 7 days delayed the onset of diabetes (93) and chronic (8-week) treatment of similar groups of animals with NVP DPP728 improved glucose tolerance substantially (89). Glucose and insulin responses to P32/98 in ZDF rats were similar to those obtained with rosiglitazone (110). Although numerous beneficial effects of DPP IV inhibitors treatment were reported, significant effects of inhibitor treatment on glucose tolerance and progression of diabetes were not observed in C57BLKS-db/db diabetic mice treated with Val-Pyr at 23 weeks, whereas improved glucose tolerance was observed when treated from 6 weeks of age (92). It was also difficult to reduce the time of onset of hyperglycemia in ZDF rats treated with the inhibitors P32/98 (110), FE 999011 (93), NN7201 or LAF237 (111) unless treatment commenced early in life.

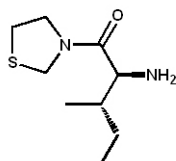
A number of preclinical studies have examined the benefits of dual therapy with DPP IV inhibitors plus drugs with proven efficacy in improving glucose tolerance in type 2 diabetics. Combined treatment with metformin and Val-Pyr was shown to be more effective than either drug alone in increasing circulating levels of intact GLP-1 in Fisher F344/Jcl rats (112), as well as reducing food intake, body weight and fasting glucose, and improving glucose tolerance in ZDF fa/fa rats (113). Metformin was proposed to act by stimulating GLP-1 secretion and not by inhibiting its degradation, in agreement with studies by Hinke *et al* (114). High-fat fed mice receiving a competitive DPP-IV inhibitor (E3024) in combination with either glibenclamide or nateglinide exhibited additive/synergistic improvements in glucose tolerance and increases in insulin levels during an OGTT (115) and rosiglitazone-treated ZDF rats administered a single dose of P32/98 showed further improvements in glucose tolerance (110).

6. DIPEPTIDYL PEPTIDASE IV INHIBITORS AND TREATMENT OF TYPE 2 DIABETES

Single dose oral administration of DPP IV inhibitors to type 2 diabetics showed that compounds such as P32/98 (116) were capable of reducing glucose excursions and increasing insulin responses during an OGTT. In a 4-week trial on patients with mild type 2 diabetes, NVP DPP728 (100 mg qd) produced ~1 mmol/l reductions in fasting glucose, prandial glucose excursions and mean 24-h glucose levels (117) as well as reductions in mean 24h insulin and hemoglobin (Hb)A1c. These studies therefore demonstrated therapeutic potential, and numerous

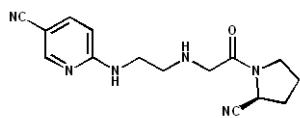
Dipeptidyl Peptidase IV Inhibitors Used in Preclinical or Clinical Studies

1. Reversible Product Analogs

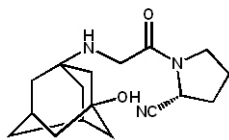


Isoleucine-Thiazolidide
P32/98 (Probiobdrug)
 $K_i = 80 \text{ nM}$

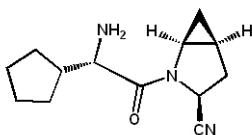
2. Covalently Modifying Product Analogs



NVP DPP728
(Novartis)
 $IC_{50} = 22 \text{ nM}$

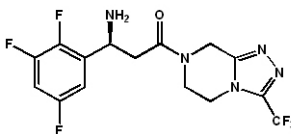


LAF237
Vildagliptin
Galvus (Novartis)
 $IC_{50} = 3.5 \text{ nM}$

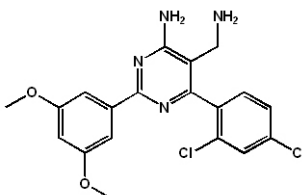


BMS-477118
Saxagliptin
(BMS/AstraZeneca)
 $K_i = 0.45 \text{ nM}$

3. Reversible Non-Peptide Heterocyclic Compounds



MK-0431
Sitagliptin
Januvia (Merck)
 $IC_{50} = 18 \text{ nM}$



R1438
Aminomethylpyridine
(Roche)
 $K_i = 0.1 \text{ nM}$

4. Compounds in Clinical Trials

ABT-279, ABT-341 (Abbott)
ALS 2-0426 (Alantox/Servier)
BI 1356 (Boehringer Ingelheim)
Denagliptin (GSK)
GRC8200 (Glenmark)
PSN-9301 (OSI)
PHX 1149 (Phenomix)
SSR-162369 (Sanofi-Aventis)
TS-021 (Taisho)
Alogliptin (Takeda)
TA-6666 (Tanabe)

Figure 4. Representative examples of dipeptidyl peptidase IV inhibitors applied in preclinical or clinical studies on type 2 diabetes therapy and additional compounds currently undergoing clinical trials.

DPP IV inhibitors are currently undergoing clinical trials (40, 95, 96, 99, 118-120). However, apart from those involving sitagliptin (MK0431; Merck) (25, 121, 122) and vildagliptin (LAF237; Novartis) (121-123), results from the majority of trials have only been published in abstract form (98, 124) and are therefore difficult to assess. Comprehensive reviews of the discovery (26, 98, 119, 125), pharmacokinetic and pharmacodynamic profiles (105, 121, 123) and clinical trials (19, 122, 126) of vildagliptin and sitagliptin have been published. Following administration at recommended clinical doses (100mg), both drugs demonstrate rapid absorption (105, 109, 123) and inhibition of DPP IV has been reported to reach >90% for 12-24 hours following single doses (123, 127-129). In type 2 diabetic patients monotherapy with either sitagliptin or vildagliptin (once or twice daily dosing for 4-24 weeks) decreased fasting and postprandial glucose and consistently reduced HbA1c levels by up to 1% (130-134) (Table 1). Insulin secretion was augmented and glucagon secretion inhibited effectively by vildagliptin during a meal tolerance test, resulting in effective suppression of endogenous glucose production (135). Long-term trials have shown sustained efficacy (123) with hypoglycemic episodes rarely occurring. There may also be a trend for improvements in

lipid profiles (136, 137). In combination with metformin, thiazolidinedione or insulin, sitagliptin or vildagliptin produced up to a further ~1% reduction in HbA1c when compared to placebo (121, 123, 138-140) (Table 1). To date there have been no major side effects reported in the larger clinical trials on DPP IV inhibitors (122, 123). Skin lesions in monkey studies with some inhibitors have been reported (141) and transient pruritis of the palms of the hands was speculated to be due to potentiation of the action of a peptide such as substance P (119).

7. Mode of Dipeptidyl Peptidase IV Inhibitor Action on Glucose Homeostasis

Inhibition of DPP IV should increase the circulating half-lives of GIP and GLP-1 resulting in potentiated insulin secretion, and considerable effort has been directed at testing this hypothesis. When assays capable of discriminating between intact and N-terminally truncated forms of the incretins were developed, studies in dogs showed that under fasting conditions only ~10% of total GLP-1 was in the active form. Following DPP IV administration this active fraction rose to ~90% (58).

Table 1. Summary of the major published clinical trials on sitagliptin and vildagliptin

Treatments	Dose	Trial	Reduced Fasting Glucose	Reduced Post-Prandial Glucose	Improved OGTT	HbA1c Reduction %	Ref
Monotherapy							
P32/98	60mg	Acute			✓		116
Sitagliptin	25/200mg	Acute			✓		148
NVP DPP728	100mg	4w	✓		✓	0.5%	117
Sitagliptin	5-50mg bid	12w	✓			0.38-0.78%*	132
	100/200mg qd	24w	✓	✓		0.8-0.94%*	197
Vildagliptin	100mg bid	4w	✓	✓		0.4%*	127
	25mg bid	12w	✓	✓		0.6%	131
	100mg qd	4w	✓	✓			143
	50/100mg	12w				0.40-0.46%	130
	50/100mg qd	24w				0.8-0.9%	133
	50/100mg qd	24w	✓			0.5-0.9%*	134
	100mg qd	24w	✓			1%	137
Add on to Metformin							
Sitagliptin	100mg qd	24w	✓	✓		0.65%*	198
Vildagliptin	50mg qd	12w	✓	✓		0.6%	146
	50mg qd or bid	24w	✓	✓		0.7-1.0%*	138
Add on to Pioglitazone							
Sitagliptin	100mg qd	24w	✓			0.7%	139
Add on to Insulin							
Vildagliptin	50mg bid	24w				0.5%	140
Non-diabetic Subjects							
Sitagliptin	200mg bid	4w			✓		128

w = weeks; bid = twice a day; qd = every day; * Placebo-controlled level.

Studies in rodents and pigs confirmed that both acute and chronic administration of DPP IV inhibitors increased levels of intact endogenous GLP-1 under basal conditions (58, 93, 94) and in response to a glucose load (88, 94). Additionally, in double GIP and GLP-1 receptor knockout mice, DPP IV inhibitor treatment failed to produce a major improvement in glucose handling, indicating that GIP and GLP-1 are largely responsible for inhibitor effects in mice (142). Similar increases in the intact forms of GIP and GLP-1 also occurred in type 2 diabetic patients during treatment with vildagliptin under basal conditions (127, 143) or in response to food (127, 143), and increases in active GLP-1 were demonstrated to be associated with a circadian rhythm throughout the day (121). However, factors in addition to GLP-1 and GIP may contribute to the marked improvements in glucose metabolism observed (145). Furthermore, although robust increases in insulin responses following DPP IV administration have been reported in animal studies (82, 87, 88, 94, 109), smaller changes in fasting and meal- or glucose-induced insulin secretion have been observed in the majority of human studies (117, 127, 146), supporting a role for non-insulin-mediated effects contributing significantly to responses to DPP IV inhibition. Three questions will be considered further: 1. Do incretins alone account for the effects of DPP IV inhibitors? 2. How may increases in incretins improve glucose homeostasis in type 2 diabetes? 3. What other enzymes and substrates could contribute to the effects of DPP IV inhibitors on glucose homeostasis?

7.1. Do Incretins Alone Mediate the Effects of Dipeptidyl Peptidase IV inhibitors?

It has been unambiguously demonstrated that levels of intact endogenous GLP-1 and GIP increase following treatment with DPP IV inhibitors (58, 88, 93, 94, 127, 143, 147-149). At present, it is unclear as to the contribution of GIP to DPP IV inhibitor responses in

humans due to the beta-cell resistance to GIP in T2DM (45-47). It is also not known whether such resistance applies to other cell types bearing GIP receptors or if it is reversible. Similar beta-cell resistance to GIP in *fa/fa* VDF (150, 151) or 90% pancreatectomized (Px) hyperglycemic (152) rats, involves hyperglycemia-associated down-regulation of GIP receptor expression at both the mRNA and protein levels (150, 151), although the origin of resistance in humans is still undetermined (153). Normalization of circulating glucose level in ZDF (Piteau, McIntosh et al; Unpublished) or Px (152) rats results in a restoration of GIP receptor expression and, with optimized control of glucose in type 2 diabetics, it is possible that GIP responsiveness may return. Additionally, the 'GIP-resistant' *fa/fa* VDF rat is capable of responding to high pharmacological levels of GIP or DPP IV-resistant analogs (154) and DPP IV inhibitor treatment could result in sufficient bioactive GIP to contribute to the improved glucose homeostasis. However, if we consider that GLP-1 is the major incretin involved, there is a discrepancy between the 2-3-fold increase in meal-associated active GLP-1 levels (144) and the marked improvements in metabolic status (145). In a well reasoned discussion of this apparent enigma, Holst and Deacon (76) have pointed out that systemic venous blood levels of GLP-1 would be significantly lower than those in blood in the portal venous system, due to dilution. An additional complication is the dual mode of action demonstrated by GLP-1. A direct islet action, via an endocrine route, is supported by the presence of GLP-1 receptors in islet beta-cells (155, 156), the responsiveness of islets to GLP-1 *in vitro* (157) and the ability of GLP-1 to stimulate insulin secretion following orthotopic pancreas transplantation (158). However, GLP-1 also appears to activate afferent autonomic nerves (Figure 5). The proximity of nerve terminals in the lamina propria to the GLP-1-releasing L-cells of the intestine suggests that

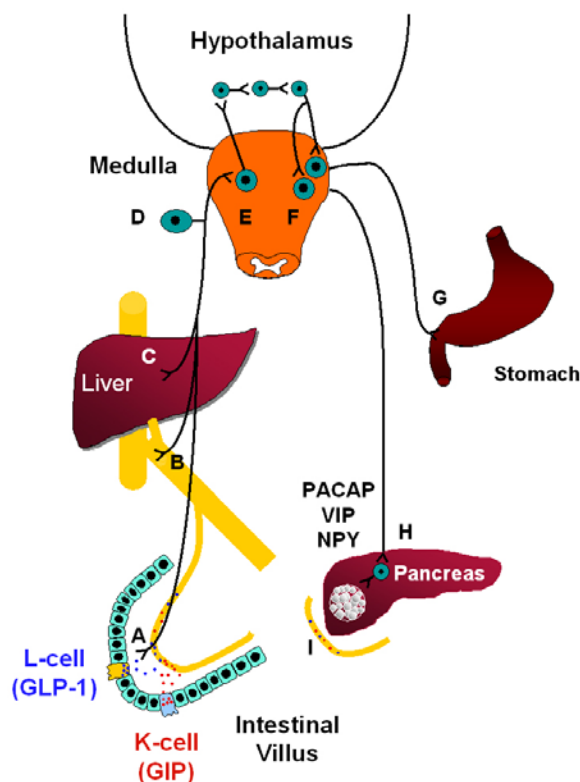


Figure 5. Diagram of possible pathways by which GLP-1 and GIP exert their actions on the stomach and pancreas. In response to nutrients, GIP and GLP-1 are released from K- and L-cells in the intestinal mucosa, respectively. During passage to the capillary GLP-1 may activate afferent sensory neurons (A) with cell bodies in the nodose ganglion (D). Through activation of neuronal pathways involving the nucleus tractus solitarius (E) and the hypothalamus (F) efferent vagal nerves are believed to be activated and are, at least partially, responsible for inhibition of gastric emptying (G) and stimulation of insulin secretion (H). The transmitters released from intrinsic pancreatic nerves have not been identified with certainty, but may include NPY, PACAP and VIP. GLP-1 may also activate sensory nerves in the hepatoportal area (B) and the liver itself (C). Both GIP and GLP-1 can stimulate insulin secretion via the bloodstream (I). (Based on Figure 2 in Holst, JJ, Deacon CF. Glucagon-like peptide-mediates the therapeutic actions of DPP IV inhibitors. Reproduced with permission from 76.

locally released peptide could activate afferent autonomic nerve fibres that arise from the nodose ganglion (76). GLP-1 receptors have recently been detected in nodose ganglion cells, an appropriate site for their synthesis prior to axonal transport to the periphery (159). Since endothelial cell DPP IV was detected in close proximity to L-cells, inhibitor treatment could therefore prolong the action of GLP-1 at this site (75). A further target of GLP-1 action was suggested by the demonstration that intra-portal infusion of GLP-1, but not GIP (160), increased the rate of afferent nerve spike discharge in the hepatic branch of the vagus nerves (161, 162) as well as the firing rate of the efferent

pancreatic branch, in a reflex fashion (161). Support for important multiple roles for intrahepatic portal neural activation by GLP-1 was also provided by studies showing that ganglionic blockade (163) or sensory autonomic nerve ablation with capsaicin reduced GLP-1-induced insulin secretion (164) and a vagal-dependence of GLP-1-induced inhibition of gastric emptying (165, 166). An additional role for GLP-1 in the maintenance of the glucose competence of hepatoportal glucose sensors has also been proposed (167). Clearly it is important to establish whether DPP IV inhibition is capable of raising GLP-1 levels sufficiently to support the functioning of these various neurally mediated responses.

7.2. How may increases in incretins improve glucose homeostasis in type 2 diabetes?

Inhibition of plasma DPP IV activity has been demonstrated to result in increased insulin responses to an oral glucose load in preclinical studies (55, 82), but in many human studies responses have not been dramatic. Mari and co-workers recently used mathematical modeling to determine insulin secretory rates as a function of the prevailing glucose level and showed that 28-days treatment with vildagliptin significantly increased the pattern of insulin secretion (143). In addition to beta-cell secretion, a number of potential alternative targets of incretin action have also been studied, including improvements in beta-cell glucose responsiveness, growth promoting and anti-apoptotic actions on the pancreatic islet (168-178), suppression of glucagon secretion (179-182), decreasing gastric emptying and reducing food intake (120). The evidence for or against such actions will be considered.

7.2.1. Improving Islet Glucose Responsiveness and Stimulating Insulin Biosynthesis

In addition to acutely stimulating insulin secretion in a glucose-dependent manner, GLP-1 has been shown to confer glucose-sensitivity (competence) on beta-cells (183). *In vitro* studies have also provided limited evidence for improved beta-cell responsiveness to glucose following chronic DPP IV inhibitor treatment. After 12 weeks of Ile-Thia treatment, perfusion of isolated pancreata from VDF rats showed a marked increase in glucose-responsiveness, although islet size, morphology and beta-cell mass were not altered (85). Treatment of C57BL/6J mice with the DPP IV inhibitors NVP DPP728 (89) or vildagliptin (184) for 8-9 weeks resulted in augmented glucose-induced insulin secretion in isolated islets, associated with increased islet GLUT-2 expression (89). The improved responsiveness was ablated by targeted beta-cell expression of a dominant-negative mutant form of hepatocyte nuclear factor-1 alpha (184). Although both GLP-1 (185, 186) and GIP (187, 188) have been shown to stimulate insulin biosynthesis (44, 120, 178, 189, 190), there is currently no evidence for similar responses to DPP IV inhibitor treatment *in vivo*.

7.2.2. Increasing Beta Cell Mitogenesis and Survival

During life, beta-cell mass is regulated through replication, neogenesis, growth and apoptosis (191). *In vivo* and *in vitro* studies have shown that GIP (168, 169, 171), GLP-1 (175, 176, 192) and analogs such as exendin-4 (173,

176) can promote beta-cell growth and replication. The only evidence that DPP IV inhibitors can exert similar effects *in vivo* is the report, in abstract form, that neonatal rats treated with vildagliptin for 21 days showed increased numbers of replicating islets (193). Apoptosis plays a central role in both the normal development of islet mass and the pathology of T2DM. During progression to T2DM, both lean and obese patients initially show increases in beta-cell mass (194), followed by loss of beta-cells via apoptosis at a rate that outstrips the low levels of replication and neogenesis (192). Contributing factors underlying increased apoptosis include chronic hyperglycemia and hyperlipidemia, endoplasmic reticulum (ER)- and oxidative-stress and cytokine actions (191). Both GLP-1 (174, 175, 177, 178, 195) and GIP (170, 172) exert profound prosurvival effects on the beta-cell. In view of this, it is not surprising that there is a growing interest in studying the possibility that DPP IV inhibition could protect beta-cells against apoptosis and promote islet growth. Preclinical studies have provided some support for this proposal. High fat fed mice receiving a low-dose of the beta-cell toxin streptozotocin (STZ) develop diabetes resembling T2DM. Treatment with a des-fluoro-sitagliptin analog was shown to result in increased numbers of insulin-positive beta-cells in islets and a normalization of beta-cell mass. Insulin content and glucose-induced insulin secretion were also greatly improved (196). (Studies on a type 1 model of diabetes induced by STZ in rats will be discussed in Section 8). The vildagliptin-treated rats, mentioned above, also showed a reduced number of apoptotic cells at the end of the treatment period (193). There are currently no direct data available to support similar preservation of islet mass in humans during DPP IV inhibitor treatment, but indirect estimates have suggested that treatment can sustain insulin responses, possibly through beta-cell protection (146). For example, homeostasis model assessment (HOMA) of beta-cell function and the proinsulin-to-insulin ratio were shown to be improved with sitagliptin and sitagliptin add-on-to metformin treatments (197, 198).

7.2.3. Reducing Glucagon Secretion

Inappropriately elevated plasma glucagon in T2DM is considered to be a major contributor to both fasting and postprandial hyperglycemia. GLP-1 (179, 180, 182, 199) and analogs (200) strongly suppress glucagon levels in type 2 diabetics (182, 199). Four-weeks treatment of type 2 diabetic patients with vildagliptin (127, 136) reduced meal-stimulated glucagon levels and the importance of the reduction was indicated by the high correlation between post-meal glucagon decreases and postprandial glucose levels (127). In a recent study, suppression of glucagon during a meal-tolerance test was found to be five-fold greater in T2DM patients treated with a single dose of vildagliptin (120, 135). It is unclear as to the underlying mechanisms that result in suppressing glucagon (201, 202). GLP-1 has been shown to inhibit glucagon secretion in the isolated perfused pancreas (203), however this may not be through a direct action since there is now doubt as to whether GLP-1 receptors are expressed in alpha-cells (204), and the majority of recent data suggest that beta-cell derived factors, including insulin (204), are

mediators of GLP-1 actions on the alpha-cell (202).

7.2.4. Reducing Insulin Resistance

Chronic DPP IV inhibitor (Ile-Thia) treatment led to a reduction in insulin resistance in VDF rats, as assessed using a 3-stage conscious euglycemic-hyperinsulinemic clamp, that resulted from a reduction in hepatic glucose output and increased responsiveness of peripheral glucose uptake to insulin (85). *In vitro* studies on tissues from DPP IV-treated animals showed improvements in insulin-induced inhibition of isoproterenol-stimulated lipolysis in abdominal adipocytes and insulin-stimulated skeletal muscle glucose uptake. The mechanisms underlying such improvement are unclear, but long-term GLP-1 infusion (6-week) also resulted in a reduction in insulin resistance in type 2 diabetic subjects (206) and it will be interesting to see whether there are similar responses following extended DPP IV inhibitor treatment.

7.2.5. Inhibiting Gastric Emptying

Administration of GLP-1 (207-211) or analogs such as exendin-4 (Exenatide) (212) to humans strongly inhibits gastric emptying. However, no evidence for a role for altered gastric emptying was found in Zucker rats treated with NVP-DPP728 (94) and none reported in the majority of studies in humans (145, 213), apart from one recent report in abstract form (214). Although the inhibition produced by exogenous GLP-1 is thought to be mediated via both vagal afferent activation (165, 215) and direct effects within the brain (216), it is likely that the negative responses following DPP IV inhibitor treatment are mainly due to insufficient levels of intact peptide accessing the portal receptors (76).

7.2.6. Reducing Food Intake

GLP-1 injected into the brain reduces food intake in rodents (217), mainly over the short-term (218), and repeated injections result in reduced body weight (219). Intravenous infusion of GLP-1 over 4-8 hours was also shown to reduce nutrient intake and extend periods of postprandial satiety in obese and diabetic humans (220, 221). Extensive studies have been performed on the effect of long-acting GLP-1 analogs in humans (120, 178, 217), with sustained weight loss obtained in type 2 diabetic patients treated with exenatide (23). In contrast, no major effects of DPP IV inhibitor treatment on body weight in clinical trials have been reported. In a recent comparative study in candy-fed rats, the long-acting GLP-1 analog liraglutide was shown to reduce food intake and body weight, whereas vildagliptin did not. It therefore seems likely that levels of circulating active GLP-1 or analog have to reach high pharmacological concentrations in order to impact on feeding (222).

7.3. Do Other Enzymes and Substrates Contribute to the Effects of Dipeptidyl Peptidase IV Inhibitors on Glucose Homeostasis?

There are a number of enzymes structurally related to dipeptidyl peptidase IV that exhibit similar substrate specificity (96, 223, 224), including fibroblast activation protein alpha (FAP-alpha; Seprase; DP 5) (225), DPP-8 (226) and DPP-9 (227), and the catalytically

inactive DPP-6 (DPL1;DPX) (228) and DPP-10 (DPL2) (229). DPP II (DPP2; quiescent cell proline dipeptidase (QPP); DPP7) (230, 231) is a structurally unrelated enzyme also exhibiting similar specificity (232). DPP IV is believed to be the major enzyme involved in the normal metabolism of incretin hormones, although neutral endopeptidase 24.11 may contribute to GLP-1 degradation (233). Using an adenosine deaminase binding assay, authentic DPP IV was estimated by Durinx and co-workers to account for approximately 95% of DPP IV-like enzyme activity in serum/plasma (234). However, when using DPP IV inhibitors clinically it is important to know whether additional enzymes that act on DPP IV substrates are present in the circulation and if an inhibitor can gain access to intracellular enzymes with DPP IV activity. Since FAP (seprase) and attractin are integral plasma membrane proteins, they could be cleaved from their cells of origin in a similar fashion to DPP IV. Seprase has been identified in serum (235), as have a number of partially characterized molecular species, such as DPPIV-beta (236, 237), a 110 kDa form similar to kidney DPP IV (238) and a 250 kDa dimer (239). The current status of attractin (240), the human ortholog of mouse mahogany (241), is unclear since recent studies indicated that purified recombinant enzyme did not exhibit DPP IV activity (242) and it has been proposed that attractin may act as an extracellular receptor or adhesion protein (242) and in the regulation of intracellular membrane biogenesis and vesicle trafficking (243). The intracellular enzymes, DPP II, DPP-8 and DPP-9 should presumably only be released from damaged or dying cells and plasma levels would be expected to be very low, although appreciable levels of DPPII have been found in plasma (244). The potential consequences of altering the activity of the DPP IV-like enzymes was vividly portrayed by Lankas *et al* (245) in studies on a range of inhibitors with varying specificities. In 2-week rat toxicity studies, treatment with DPP IV inhibitors showing off-target activity with DPP8/9, or a selective DPP-8/9 inhibitor, was associated with alopecia, thrombocytopenia, reticulocytopenia, enlarged spleen and multi-organ histopathological changes, with mortality in some cases. In acute dog tolerability studies a DPP8/9 inhibitor produced gastrointestinal toxicity, whereas a QPP inhibitor produced reticulocytopenia only in rats. The selective DPP8/9 inhibitor also attenuated human T-cell activation with *in vitro* models. Sitagliptin, with a selectivity of >2500 fold greater for DPP IV than for DPP-8 and DPP-9 (26) showed no toxic effects.

The incretins are considered to be the most important DPP IV substrates impacted on by inhibitor treatment in T2DM, but other members of the glucagon superfamily (Figure 2) have also been shown to be *in vitro* substrates, as have a range of other hormones, neuropeptides (peptide YY (PYY), neuropeptide Y (NPY), substance P and endomorphin) and a large family of chemotactic cytokines (chemokines) involved in the development and function of the immune system (e.g. RANTES (Regulated upon Activation, Normally T-cell Expressed and Secreted), GCP-2 (Granulocyte Chemotactic Protein-2), SDF-1 (Stromal-Derived Factor), eotaxin, MDC (Monocyte-Derived Chemokine) and ITAC (Interferon

gamma-Inducible T-Cell Chemoattractant) (61, 62, 67, 97, 124, 246)). Although it has not been established which peptides, apart from GIP, GLP-1 and, possibly, FDF-1 and substance P are physiological substrates of DPP IV (141), PACAP (Pituitary Adenylate Cyclase Activating Peptide) and VIP are located in intrinsic neurons within the pancreas (247) and changes in their local concentrations could have important effects on insulin secretion. Additionally, N-terminal truncation can produce altered receptor binding specificity in some peptides (e.g. NPY and PYY), rather than loss of activity, and inhibition could therefore result in altered physiological responses. However, there may also be redundant systems that circumvent the effects of DPP IV inhibitor treatment. Metabolism of PYY₁₋₃₆ by DPP IV results in the production of a ligand (PYY₃₋₃₆) (248) with high selectivity for the Y2 sub-type of receptor that is involved in the suppression of appetite (249). When the effect of PYY₁₋₃₆ in DPP IV-deficient rats was examined, it was found to be ineffective in reducing food intake, as expected. However, inhibition of DPP IV in control Fisher 344 rats did not alter the acute anorexic effect of exogenous PYY₁₋₃₆ (250), suggesting that additional enzyme(s) are capable of cleaving PYY₁₋₃₆ in a DPP IV-like manner. It is not known whether this also occurs in humans.

8. PRE-CLINICAL STUDIES ON DIPEPTIDYL PEPTIDASE IV INHIBITORS AND TYPE 1 DIABETES

A potential application for DPP IV inhibitors in type 1 diabetic patients arose from studies on patients receiving a GLP-1 infusion who demonstrated lowered fasting hyperglycemia (251), reduced glycemic excursions (179, 252, 253) and requirement for less insulin (179). These effects have been attributed to reductions in glucagon levels and delayed gastric emptying (254). Type 1 diabetes is an autoimmune disorder (2) and DPP IV has been shown to degrade several important chemokines *in vitro*, as well as having specific roles in the regulation of T-cell proliferation and chemotaxis (246, 255). Inhibition of surface DPP IV has a number of effects on immune functions, including the suppression of T-lymphocyte (T-cell) proliferation, T-cell stimulation of B-lymphocyte immunoglobulin release, Th1 cytokine production and trans-endothelial migration of T-cells (Reviewed in: (61, 62, 70, 246)). An association between autoimmune disease and elevated CD26⁺ cells occurs in multiple sclerosis (256) and Graves' disease (257), suggesting that DPP IV inhibition modulate the disease process. Therefore, DPP IV inhibition could exert positive effects on type-1 diabetes by promoting β -cell growth and survival, as well as reducing the autoimmune response.

To date there are only limited preclinical studies on the potential use of DPP IV inhibitors in T1DM. The effect of long-term DPP IV-inhibitor treatment on the development of T1DM was studied in rats treated with a single dose of STZ (50 mg/kg). Under these conditions, a rapid phase of cell death is initiated that involves both apoptotic and necrotic mechanisms without a strong autoimmune component (86, 205). Rats were treated with DPP IV inhibitor (Ile-Thia; 20 mg/kg daily po) for one

week before or after STZ administration treatment over a seven-week period. The Ile-thia treated groups demonstrated increased weight gain (230%) and nutrient intake, ~5 mM reductions in fed blood glucose levels and increased insulin. Glucose tolerance, fasting glucose and glucose-stimulated insulin secretion were all improved, as were *in vitro* perfused rat pancreas responses. Of particular importance was an 8-fold increase in total pancreatic insulin content, compared to untreated STZ-control animals and increases in the number of very small islets that exhibited a normal β -cell complement. DPP IV inhibitor treatment in the STZ model of type 1 diabetes model therefore has a number of beneficial effects, including stimulation of insulin biosynthesis and increasing the pancreatic content of a specific islet fraction, either via neogenesis or protection, thus improving endocrine pancreatic function (86). In the biobreeding (BB) rat, a model of type 1 diabetes that spontaneously develops extensive insulinitis, Ile-Thia treatment from 3 weeks of age delayed the onset of diabetes by about 10 days and prevented the onset of diabetes in ~20% of the animals (Pospisilik *et al.*, Unpublished observations). Glucose tolerance in both pre-diabetic and diabetic rats was improved and the severity of diabetes slightly reduced. Surviving animals showed long-term improvements in islet function and blood glucose levels, although there was no evidence for marked changes in the insulinitis. Further studies on type-1 diabetes models are underway.

9. THE FUTURE OF DIPEPTIDYL PEPTIDASE IV INHIBITORS IN THE TREATMENT OF DIABETES

Results from clinical trials on a range of different DPP IV inhibitors have been extremely positive and sitagliptin was approved by the FDA for type 2 diabetes therapy in October 2006, with vildagliptin receiving approvable status. It is likely that several of the inhibitors listed in Figure 4 will also be in clinical use in 2008/09. Nevertheless, there are still questions that require further investigation regarding the physiological roles and regulation of dipeptidyl peptidase IV, the implications of long-term inhibition and the possibility of achieving even greater inhibitor selectivity.

The wide range of DPP IV substrates identified with *in vitro* studies was a concern from the onset of inhibitor development for clinical use, since there appears to be a high potential for non-target effects. In addition, DPP IV/CD26 has non-catalytic functions mediated through interaction with a number of proteins that play important structural and signaling roles, including adenosine deaminase (258), a renal Na^+/H^+ exchanger (259), fibronectin (260) and tyrosine phosphatase CD45 (261). These various DPP IV/CD26-protein interactions are involved in immune function, ion transport, regulation of extracellular matrix binding and cell-cell signaling (61, 259, 262, 263), but it has not been demonstrated that they are influenced by inhibitor binding. Additionally, it appears that rodents, at least, can compensate for loss of DPP IV activity. Studies on DPP IV (CD-26) deficient strains of Fischer 344 rats revealed a fully functional enteroinsular axis (264), minor behavioral changes (265) and no major

defects in immune function (266) apart from small reductions in CD4^+ T lymphocyte responses to immunization (267). Dipeptidyl peptidase IV-knockout mice are healthy and exhibit increased active incretin and insulin levels, as well as resistance to the development of obesity (268, 269), and glucose tolerance is improved in both knockout mice and DPP IV-deficient rats (270). Altered nociception, reported in knockout mice (271) and DPP IV-negative rats (265), is of interest since it appears to be associated with altered substance P metabolism (271).

As discussed earlier, plasma/serum enzyme activity levels are the only read-out of the effectiveness of DPP IV inhibition that we have. This may provide an accurate reflection of the inhibition obtained at the tissue (e.g. endothelial, hepatic) level, although we have no direct supporting evidence, and we also have no information on whether changes in plasma enzyme levels occur during prolonged inhibitor therapy. It is important to gain a deeper understanding of the mode of DPP IV gene regulation, the mechanisms by which DPP IV is cleaved from the plasma membrane and the clearance of the soluble enzyme. Expression of the human DPP IV gene appears to be under tissue specific regulation (272). Insulin-dependent phosphorylation of hepatic DPP IV in rodents has been suggested to result in altered enzyme turnover and increased surface shedding (273), thus potentially altering circulating levels. There is also evidence that plasma DPP IV levels change with metabolic status, although no clear pattern has emerged. Plasma levels of DPP IV activity increased during fasting in type 2 diabetics (274) and elevated levels were found in anorexia-nervosa and bulimia-nervosa patients (275). It is unclear whether increases result from altered gene expression, changes in membrane shedding or some other process. Could prolonged DPP IV treatment result in altered enzyme levels as glucose homeostasis improves? A clearer understanding of plasma DPP IV turnover is clearly needed and could assist in optimizing drug administration regimens.

Finally, can the selectivity and efficacy of DPP IV inhibitors used in diabetes therapy be improved upon? A wide range of inhibitors, from multiple chemical classes, have shown tremendous potential as therapeutics. Physicochemical studies, X-ray crystallography and cryo-transmission electron microscopy have provided extensive information on the structure and mode of action of DPP IV, thus facilitating the rational design of new inhibitors. The catalytic triad is situated in a large cavity with the substrate binding region for peptide N-termini consisting of a short helix containing a Glu205-Glu206 sequence motif (276, 277). However, a secondary binding site has also been identified, located in close proximity to the catalytic triad, that exhibits differential binding to individual peptides of the glucagon superfamily (278), potentially allowing development of more selective inhibitors. Additionally, although combination therapy of DPP IV inhibitors and biguanide or thiazolidinedione is proving very effective, such therapy may prove even more effective if incretin secretion from the intestinal enteroendocrine cells can also be selectively increased, and research with this objective is currently underway.

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11. REFERENCES

1. Smyth, S. & A. Heron: Diabetes and obesity: the twin epidemics. *Nat Med* 12, 75-80 (2005)
2. Atkinson, M.: Thirty years of investigating the autoimmune basis for type I diabetes. Why can't we prevent or reverse this disease? *Diabetes* 54, 1253-1263 (2005)
3. Chiasson, J.-L. & R. Rabassa-Lhoret: Prevention of diabetes. Insulin resistance and β -cell function. *Diabetes* 53 (Suppl. 3), S34-S38 (2004)
4. O'Rahilly, S., I. Barroso & N. Wareham: Genetic factors in type 2 diabetes. The end of the beginning? *Science* 307, 370-373 (2005)
5. Gómez-Pérez, F. & J. Riull: Insulin therapy: Current alternatives. *Arch Med Res* 36, 258-272 (2005)
6. Stock, P. & J. Bluestone: Beta-cell replacement for type I diabetes. *Annu Rev Med* 55, 133-156 (2004)
7. Thivolet, C.: New therapeutic approaches to type 1 diabetes: from prevention to cellular or gene therapies *Clin Endocrinol* 55, 565-574 (2001)
8. Ryan, E., B. Paty, P. Senior, D. Bigam, E. Alfadhli, N. Kneteman, J. Lakey & A. Shapiro: Five-year follow-up after clinical islet transplantation. *Diabetes* 54, 2060-2069 (2005)
9. Shapiro, A., J. Lakey, E. Ryan, G. Korbitt, E. Toth, G. Warnock, N. Kneteman & R. Rajotte: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *New Engl J Med* 343, 230-238 (2000)
10. Warnock, G., N. Kneteman, E. Ryan, R. Seelis, A. Rabinovitch & R. Rajotte: Normoglycemia after transplantation of freshly isolated and cryopreserved pancreatic islets in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 34, 55-58 (1991)
11. Stumvoll, M., B. Goldstein & T. van Haeften: Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365, 1333-1346 (2005)
12. Doyle, M. & J. Egan: Pharmacological agents that directly modulate insulin secretion. *Pharmacol Rev* 55, 105-131 (2003)
13. Perfetti, R. & A. Ahmad: Novel sulfonylurea and non-sulfonylurea drugs to promote the secretion of insulin *Trends Endocrinol Metab* 11, 218-223 (2000)
14. Matthaei, S., M. Stumvoll, M. Kellerer & H.-U. Häring: Pathophysiology and pharmacological treatment of insulin resistance. *Endocrine Rev* 21, 585-618 (2000)
15. Padwal, R., S. Majumda, J. Johnson, J. Varney & F. McAlister: A systematic review of drug therapy to delay or prevent type 2 diabetes. *Diabetes Care*, 28, 736-744 (2005)
16. American Diabetes Association: Implications of the diabetes control and complications trial. *Diabetes Care* 26, S25-S27 (2003)
17. Eurich, D., S. Simpson, S. Majumdar & J. Johnson: Secondary failure rates associated with metformin and sulfonylurea therapy for type 2 diabetes mellitus. *Pharmacother* 25, 810-816 (2005)
18. Kahn, S. E., S. M. Haffner, M. A. Heise, W. H. Herman, R. R. Holman, N. P. Jones, B. G. Kravitz, J. M. Lachin, M. C. O'Neill, B. Zinman, G. Viberti & A. S. Group: Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *New Engl J Med* 355, 2427-2443 (2006)
19. Roges, O., M. A. Baron & Philis-Tsimikas: The incretin effect and its potentiation by glucagon-like peptide 1-based therapies: a revolution in diabetes management. *Expert Opin Invest Drugs* 14, 705-727 (2005)
20. McIntyre, J. A. & M. Bayes: Exenatide. Treatment of type 2 diabetes GLP-1 receptor agonist. *Drug Future* 29, 23-31 (2004)
21. Nielsen, L. L., A. A. Young & D. G. Parkes: Pharmacology of exenatide (Synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Peptides* 117, 77-88 (2004)
22. Davidson, M. B., G. Bate & P. Kirkpatrick: Exenatide. *Nat Rev Drug Disc*, 4, 713-714 (2005)
23. DeFronzo, R. A., R. E. Ratner, J. Han, D. D. Kim, M. S. Fineman & A. D. Baron: Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 28, 1092-1100 (2005)
24. Miller, S. & E. L. St Onge: Sitagliptin: a dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Annal Pharmacother* 40, 1336-43 (2006)
25. Deacon, C. F.: Dipeptidyl peptidase 4 inhibition with sitagliptin: a new therapy for type 2 diabetes. *Expert Opin Invest Drugs* 16, 533-545 (2007)
26. Thornberry, N. A. & A. E. Weber: Discovery of JANUVIA (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Curr Topics Med Chem* 7, 557-568 (2007)
27. Moore, B., E. Edie & J. Abram: On the treatment of diabetes mellitus by acid extract of duodenal mucous membrane *Biochem J* 1, 28-38 (1906)
28. La Barre, J. Sur les possibilités d'un traitement du diabète par l'incrétine. *Bull. Acad. R. Med. Belg.* 12 620-634 (1932)
29. Elrick, H., L. Stimmler, C. Hlad, Jr & Y. Arai: Plasma insulin response to oral and intravenous glucose administration. *J Clin Endocrinol Metab* 24, 1076-1082 (1964)
30. McIntyre, N., C. Holdsworth & D. Turner: New interpretation of oral glucose. *Lancet* II, 20-21 (1964)
31. Unger, R. & A. Eisentraut: Entero-insular axis. *Arch Intern Med* 123, 261-265 (1969)
32. Creutzfeldt, W.: The incretin concept today. *Diabetologia* 16, 75-85 (1979)
33. Kirchgessner, A. & M.D.Gershon: Innervation of the pancreas by neurons in the gut. *J Neurosci* 10, 1626-1642 (1990)

34. Perley, M. & D. Kipnis: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* 46, 1954-1962 (1967)
35. Brown, J. C.: A gastric inhibitory polypeptide. I. The amino acid composition and the tryptic peptides. *Can J Biochem* 49, 255-261 (1971)
36. Brown, J. C. & J. R. Dryburgh: A gastric inhibitory polypeptide. II. The complete amino acid sequence. *Can J Biochem* 49, 867-872 (1971)
37. Ebert, R., H. Unger & W. Creutzfeldt: Preservation of incretin activity after removal of gastric inhibitory polypeptide (GIP) from rat gut extracts by immunoadsorption. *Diabetologia* 24, 449-454 (1983)
38. Lund, P. K.: The discovery of glucagon-like peptide 1. *Regul Pept* 128, 93-96 (2005)
39. Lund, P. K., R. H. Goodman, P. C. Dee & J. F. Habener: Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc Natl Acad Sci USA* 79, 345-349 (1982)
40. Drucker, D.: The role of gut hormones in glucose homeostasis. *J Clin Invest* 117, 24-32 (2007)
41. Vahl, T. & D. D'Alessio: Enteroinsular signaling: perspectives on the role of the gastrointestinal hormones glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide in normal and abnormal glucose metabolism. *Curr Opin Clin Nutr Metab Care* 6, 461-468 (2003)
42. Nauck, M., F. Stockmann, R. Ebert & W. Creutzfeldt: Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 29, 46-52 (1986)
43. Vilsbøll, T., T. Krarup, C. F. Deacon, S. Madsbad & J. J. Holst: Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 50, 609-613 (2001)
44. Holst, J. J. & J. Gromada: Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol - Endocrinol Metab* 287, E199-206 (2004)
45. Nauck, M. A., M. M. Heimesaat, C. Ørskov, J. J. Holst, R. Ebert & W. Creutzfeldt: Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91, 301-307 (1993)
46. Meier, J. J., M. A. Nauck, W. E. Schmidt & B. Gallwitz: Gastric inhibitory polypeptide: the neglected incretin revisited. *Regul Pept* 107, 1-13 (2002)
47. Krarup, T., N. Saurbrey, A. J. Moody, C. Kuhl & S. Madsbad: Effect of porcine gastric inhibitory polypeptide on beta-cell function in type I and type II diabetes mellitus. *Metabolism* 36, 677-682 (1987)
48. O'Dorisio, T. M., K. R. Sirinek, E. L. Mazzaferri & S. Cataland: Renal effects on serum gastric inhibitory polypeptide (GIP). *Metabolism* 26, 651-656 (1977)
49. Ruiz-Grande, C., J. Pintado, C. Alarcon, C. Castilla, I. Valverde & J. M. Lopez-Novoa: Renal catabolism of human glucagon-like peptides 1 and 2. *Can J Physiol Pharmacol* 68, 1568-1573 (1990)
50. Deacon, C. F., L. Pridal, L. Klarskov, M. Olesen & J. J. Holst: Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol* 271, E458-464 (1996)
51. Ørskov, C., J. Andreasen & J. Holst: All products of proglucagon are elevated in plasma from uremic patients. *J Clin Endocrinol Metab* 74, 379-384 (1992)
52. Mentlein, R., B. Gallwitz & W. E. Schmidt: Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214, 829-835 (1993)
53. Kieffer, T. J., C. H. S. McIntosh & R. A. Pederson: Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 136, 3585-3596 (1995)
54. Deacon, C. F., A. H. Johnsen & J. J. Holst: Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 80, 952-957 (1995)
55. Pauly, R. P., H.-U. Demuth, F. Rosche, J. Schmidt, H. A. White, C. H. S. McIntosh & R. A. Pederson: Inhibition of dipeptidyl peptidase IV (DP IV) in rat results in improved glucose tolerance. *Regul Pept* 64, 148 (1996)
56. Deacon, C. F., M. A. Nauck, J. Meier, K. Hucking & J. J. Holst: Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab* 85, 3575-3581 (2000)
57. Deacon, C. F.: What do we know about the secretion and degradation of incretin hormones. *Regul Pept* 128, 117-124 (2005)
58. Deacon, C. F., S. Wamberg, P. Bie, T. E. Hughes & J. J. Holst: Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. *J Endocrinol* 172, 355-62 (2002)
59. Meier, J. J., M. A. Nauck, D. Kranz, J. J. Holst, C. F. Deacon, D. Gaeckler, W. E. Schmidt & B. Gallwitz: Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* 53, 654-662 (2004)
60. Deacon, C. F.: Circulation and degradation of GIP and GLP-1. *Horm Metab Res* 36, 761-765 (2004)
61. Lambeir, A. M., C. Durinx, S. Scharpe & I. De Meester: Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit Rev Clin Lab Sc*, 40, 209-294 (2003)
62. de Meester, I., A.-M. Lambeir, P. Proost & S. Scharpe: Dipeptidyl peptidase IV substrates. An update on in vitro peptide hydrolysis by human DPP-IV. *Adv Exp Med Biol* 524, 3-17 (2003)
63. Mentlein, R.: Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul Pept* 85, 9-24 (1999)
64. Augustyns, K., G. Bal, G. Thonus, A. Belyaev, X. M. Zhang, W. Bollaert, A. M. Lambeir, C. Durinx, F. Goossens & A. Haemers: The unique properties of dipeptidyl-peptidase IV (DPP IV / CD26) and the therapeutic potential of DPP IV inhibitors. *Curr Med Chem* 6, 311-327 (1999)

65. de Meester, I., A. M. Lambeir, P. Proost & S. Scharpe: Dipeptidyl peptidase IV substrates. An update on in vitro peptide hydrolysis by human DPP-IV. *Adv Exp Med Biol* 524, 3-17 (2003)
66. Hinke, S. A., J. A. Pospisilik, H. U. Demuth, S. Mannhart, K. Kuhn-Wache, T. Hoffmann, E. Nishimura, R. A. Pederson & C. H. S. McIntosh: Dipeptidyl peptidase IV (DPIV/CD26) degradation of glucagon. Characterization of glucagon degradation products and DPIV-resistant analogs. *J Biol Chem* 275, 3827-3934 (2000)
67. Drucker, D. J.: Enhancing incretin action for the treatment of type 2 diabetes. *Diabetes Care* 26, 2929-2940 (2003)
68. Low, S. H., S. H. Wong, B. L. Tang, V. N. Subramaniam & W. J. Hong: Apical cell surface expression of rat dipeptidyl peptidase IV in transfected Madin-Darby canine kidney cells. *J Biol Chem* 266, 13391-13396 (1991)
69. Vanhoof, G., I. De Meester, M. van Sande, S. Scharpe & A. Yaron: Distribution of proline-specific aminopeptidases in human tissues and body fluids. *Eur J Clin Chem Clin Biochem* 30, 333-338 (1992)
70. Gorrell, M. D., V. Gysbers & G. W. McCaughan: CD26: A multifunctional integral membrane and secreted protein of activated lymphocytes. *Scand J Immunol* 54, 249-264 (2001)
71. De Meester, I., S. Korom, J. Van Damme & S. Scharpe: CD26, let it cut or cut it down. *Immunol Today* 20, 367-375 (1999)
72. Maes, M., S. Scharpe, I. De Meester, P. Goossens, A. Wauters, H. Neels, R. Verkerk, F. De Meyer, P. D'Hondt, D. Peeters & et al.: Components of biological variation in prolyl endopeptidase and dipeptidyl-peptidase IV activity in plasma of healthy subjects. *Clin Chem* 40, 1686-1691 (1994)
73. Chien, C.-H., L.-H. Huang, C.-Y. Chou, Y.-S. Chen, Y.-S. Han, G.-G. Chang, P.-H. Liang & X. Chen: One site mutation disrupts dimer formation in human DPP-IV proteins. *J Biol Chem* 279, 52338-52345 (2004)
74. Engel, M., T. Hoffmann, L. Wagner, M. Wermann, U. Heiser, R. Kiefersauer, R. Huber, W. Bode, H.-U. Demuth & H. Brandstetter: The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. *Proc Natl Acad Sci USA* 100, 5063-5068 (2003)
75. Hansen, L., C. F. Deacon, C. Orskov & J. J. Holst: Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 140, 5356-5363 (1999)
76. Holst, J. J. & C. F. Deacon: Glucagon-like peptide-1 mediates the therapeutic actions of DPP-IV inhibitors. *Diabetologia* 48, 612-615 (2005)
77. Poulsen, M. D., G. H. Hansen, E. Dabelsteen, P. E. Hoyer, O. Noren & H. Sjostrom: Dipeptidyl peptidase IV is sorted to the secretory granules in pancreatic islet A-cells. *J Histochem Cytochem* 41, 81-88 (1993)
78. Pospisilik, J. A., S. A. Hinke, R. A. Pederson, T. Hoffmann, F. Rosche, D. Schlénzig, K. Glund, U. Heiser, C. H. S. McIntosh & H.-U. Demuth: Metabolism of glucagon by dipeptidyl peptidase IV (CD26). *Regul Pept* 96, 133-141 (2001)
79. Holst, J. J. & C. F. Deacon: Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. *Diabetes*, 47, 1663-1670 (1998)
80. McIntosh, C. H. S., H.-U. Demuth, J. A. Pospisilik & R. A. Pederson: Dipeptidyl Peptidase IV (DP-IV) Inhibitors: How do they work as new antidiabetic agents? *Regul Pept* 128, 159-165 (2005)
81. Deacon, C. F., M. A. Nauck, M. Toft-Nielsen, L. Pridal, B. Willms & J. J. Holst: Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH₂-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44, 1126-31 (1995)
82. Pauly, R. P., H.-U. Demuth, F. Rosche, J. Schmidt, H. A. White, F. Lynn, C. H. McIntosh & R. A. Pederson: Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor Ile-thiazolidide. *Metabolism* 48, 385-389 (1999)
83. Deacon, C. F., T. E. Hughes & J. J. Holst: Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. *Diabetes* 47, 764-769 (1998)
84. Pospisilik, J. A., S. G. Stafford, H. U. Demuth, C. H. McIntosh & R. A. Pederson: Long-term treatment with dipeptidyl peptidase IV inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat: a euglycemic-hyperinsulinemic clamp study. *Diabetes* 51, 2677-2683 (2002)
85. Pospisilik, J. A., S. G. Stafford, H.-U. Demuth, R. Brownsey, W. Parkhouse, D. T. Finegood, C. H. S. McIntosh & R. A. Pederson: Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats. *Diabetes* 51, 943-950 (2002)
86. Pospisilik, J. A., J. A. Ehses, T. Doty, C. H. S. McIntosh, H.-U. Demuth & R. A. Pederson: Dipeptidyl peptidase IV inhibition in animal models of diabetes. *Adv Exp Med Biol* 524, 281-291 (2003)
87. Pederson, R. A., H. A. White, D. Schlénzig, R. P. Pauly, C. H. S. McIntosh & H.-U. Demuth: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* 47, 1253-1258 (1998)
88. Åhrén, B., J. J. Holst, H. Martensson & B. Balkan: Improved glucose tolerance and insulin secretion by inhibition of dipeptidyl peptidase IV in mice. *Eur J Pharmacol* 404, 239-245 (2000)
89. Reimer, M. K., J. J. Holst & B. Åhrén: Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. *Eur J Endocrinol* 146, 717-727 (2002)
90. Kim, D., L. Wang, M. Beconi, G. J. Eiermann, M. H. Fisher, H. He, G. J. Hickey, J. E. Kowalchick, B. Leitang, K. Lyons, F. Marsilio, M. E. McCann, R. A. Patel, A. Petrov, G. Scapin, S. B. Patel, R. S. Roy, J. K. Wu, M. J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry & A. E. Weber: (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 48, 141-151 (2005)

91. Augeri, D. J., J. A. Robl, D. A. Betebenner, D. R. Magnin, A. Khanna, J. G. Robertson, A. Wang, L. M. Simpkins, P. Taunk, Q. Huang, S.-P. Han, B. Abboa-Offei, M. Cap, L. Xin, L. Tao, E. Tozzo, G. E. Welzel, D. M. Egan, J. Marcinkeviciene, S. Y. Chang, S. A. Biller, M. S. Kirby, R. A. Parker & L. G. Hamann: Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 48, 5025-5037 (2005)
92. Nagakura, T., N. Yasuda, K. Yamazaki, H. Ikuta & I. Tanaka: Enteroinular axis of db/db mice and efficacy of dipeptidyl peptidase IV inhibition. *Metabolism* 52, 81-86 (2003)
93. Sudre, B., P. Broqua, R. B. White, D. Ashworth, D. M. Evans, R. Haigh, J. L. Junien & M. L. Aubert: Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male Zucker diabetic fatty rats. *Diabetes* 51, 1461-1469 (2002)
94. Balkan, B., L. Kwasnik, R. Miserendino, J. J. Holst & X. Li: Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7-36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* 42, 1324-1331 (1999)
95. Augustyns, K., P. Van der Veken, K. Senten & A. Haemers: The therapeutic potential of inhibitors of dipeptidyl peptidase IV (DPP IV) and related proline-specific dipeptidyl aminopeptidases. *Curr Med Chem* 12, 971-998 (2005)
96. Demuth, H.-U., C. H. S. McIntosh & R. A. Pederson: Type 2 diabetes--therapy with dipeptidyl peptidase IV inhibitors. *Biochim Biophys Acta* 1751, 33-44 (2005)
97. McIntosh, C. H. S., H.-U. Demuth, S.-J. Kim, J. A. Pospisilik & R. A. Pederson: Applications of dipeptidyl peptidase IV inhibitors in diabetes mellitus. *Int J Biochem Cell Biol* 38, 860-872 (2006)
98. Chyan, Y.-J. & L.-M. Chuang: Dipeptidyl peptidase-IV inhibitors: An evolving treatment for type 2 diabetes from the incretin concept. *Rec Pat Endo Metab Imm Drug Disc* 1, 15-24 (2007)
99. Wiedeman, P. E. & J. M. Trevillyan: Dipeptidyl peptidase IV inhibitors for the treatment of impaired glucose tolerance and type 2 diabetes. *Curr Opin Invest Drugs* 4, 412-420 (2003)
100. Madar, D. J., H. Kopecka, D. Pireh, H. Yong, Z. Pei, X. Li, P. E. Wiedeman, S. W. Djuric, T. W. Von Geldern, M. G. Fickes, L. Bhagavatula, T. McDermott, S. Wittenberger, S. J. Richards, K. L. Longenecker, K. D. Stewart, T. H. Lubben, S. J. Ballaron, M. A. Stashko, M. A. Long, H. Wells, B. A. Zinker, A. K. Mika, D. W. A. Beno, A. J. Kempf-Grote, J. Polakowski, J. Segreti, G. A. Reinhart, R. M. Fryer, H. L. Sham & J. M. Trevillyan: Discovery of 2-[4-{2-(2S,5R)-2-cyano-5-ethynyl-1-pyrrolidinyl]-2-oxoethyl]amino]-4-methyl-1-piperidinyl]-4-pyridinecarboxylic acid (ABT-279): a very potent, selective, effective, and well-tolerated inhibitor of dipeptidyl peptidase-IV, useful for the treatment of diabetes. *J Med Chem* 49, 6416-6420 (2006)
101. Pei, Z., X. Li, T. W. von Geldern, D. J. Madar, K. Longenecker, H. Yong, T. H. Lubben, K. D. Stewart, B. A. Zinker, B. J. Backes, A. S. Judd, M. Mulhern, S. J. Ballaron, M. A. Stashko, A. K. Mika, D. W. A. Beno, G. A. Reinhart, R. M. Fryer, L. C. Preusser, A. J. Kempf-Grote, H. L. Sham & J. M. Trevillyan: Discovery of ((4R,5S)-5-amino-4-(2,4,5-trifluorophenyl)cyclohex-1-enyl)-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)methanone (ABT-341), a highly potent, selective, orally efficacious, and safe dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 49, 6439-6442 (2006)
102. Feng, J., Z. Zhang, M. B. Wallace, J. A. Stafford, S. W. Kaldor, D. B. Kassel, M. Navre, L. Shi, R. J. Skene, T. Asakawa, K. Takeuchi, R. Xu, D. R. Webb & S. L. Gwaltney, 2nd: Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. *J Med Chem* 50, 2297-22300 (2007)
103. Villhauer, E. B., J. A. Brinkman, G. B. Naderi, B. E. Dunning, B. L. Mangold, M. D. Mone, M. E. Russell, S. C. Weldon & T. E. Hughes: 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(S)-pyrrolidinecarbonitrile: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* 45, 2362-2365 (2002)
104. Hughes, T. E., M. D. Mone, M. E. Russell, S. C. Weldon & E. B. Villhauer: NVP-DPP728 (1-[[[2-[(5-cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(S)-pyrrolidine], a slow-binding inhibitor of dipeptidyl peptidase IV. *Biochem* 38, 11597-11603 (1999)
105. Deacon, C. F.: MK-431 (Merck). *Curr Opin Invest Drugs* 6, 419-426 (2005)
106. Biftu, T., D. Feng, X. Qian, G.-B. Liang, G. Kieczykowski, G. Eiermann, H. He, B. Leiting, K. Lyons, A. Petrov, R. Sinha-Roy, B. Zhang, G. Scapin, S. Patel, Y.-D. Gao, S. Singh, J. Wu, X. Zhang, N. A. Thornberry & A. E. Weber: (3R)-4-[(3R)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(2,2,2-trifluoroethyl)-1,4-diazepan-2-one, a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Bioorg Med Chem Letts* 17, 49-52 (2007)
107. Burkey, B. F., X. Li, L. Bolognese, B. Balkan, M. Mone, M. Russell, T. E. Hughes & P. R. Wang: Acute and chronic effects of the incretin enhancer vildagliptin in insulin-resistant rats. *J Pharmacol Exp Therapeut* 315, 688-695 (2005)
108. Brandt, I., J. Joossens, X. Chen, M.-B. Maes, S. Scharpe, I. De Meester & A.-M. Lambeir: Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by Vildagliptin ((2S)-{[(3-hydroxyadamantan-1-yl)amino]acetyl}-pyrrolidine-2-carbonitrile). *Biochem Pharmacol* 70, 134-143 (2005)
109. Villhauer, E. B., J. A. Brinkman, G. B. Naderi, B. F. Burkey, B. E. Dunning, K. Prasad, B. L. Mangold, M. E. Russell & T. E. Hughes: 1-[[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(S)-pyrrolidine]: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* 46, 2774-2789 (2003)
110. Wargent, E., C. Stocker, P. Augstein, P. Heinke, A. Meyer, T. Hoffmann, A. Subramanian, M. V. Sennitt, H. U. Demuth, J. R. S. Arch & M. A. Cawthorne: Improvement of glucose tolerance in Zucker diabetic fatty rats by long-term treatment with the dipeptidyl peptidase inhibitor P32/98: comparison with and combination with rosiglitazone. *Diabetes Obes Metab* 7, 170-181 (2005)

111. Rolin, N., H. Nygaard, M. Wilken, A. B. Kanstrup & R. D. Carr: The novel, xanthine-based, DPP-IV inhibitor NN7201 and LAF237 improve glucose tolerance, but do not prevent progression of diabetes in diabetic fatty rats. *Diabetes* 53 (Suppl. 2), A340 (2004)
112. Yasuda, N., T. Inoue, T. Nagakura, K. Yamazaki, K. Kira, T. Saeki & I. Tanaka: Enhanced secretion of glucagon-like peptide 1 by biguanide compounds. *Biochem Biophys Res Commun* 298, 779-784 (2002)
113. Yasuda, N., T. Inoue, T. Nagakura, K. Yamazaki, K. Kira, T. Saeki & I. Tanaka: Metformin causes reduction of food intake and body weight gain and improvement of glucose intolerance in combination with dipeptidyl peptidase IV inhibitor in Zucker fa/fa rats. *J Pharmacol Exp Ther* 310, 614-619 (2004)
114. Hinke, S. A., K. Kühn-Wache, T. Hoffmann, R. A. Pederson, C. H. McIntosh & H. U. Demuth: Metformin effects on dipeptidylpeptidase IV degradation of glucagon-like peptide-1. *Biochem Biophys Res Commun* 291, 1302-1308 (2002)
115. Yamazaki, K., N. Yasuda, T. Inoue, E. Yamamoto, Y. Sugaya, T. Nagakura, M. Shinoda, R. Clark, T. Saeki & I. Tanaka: Effects of the combination of a dipeptidyl peptidase IV inhibitor and an insulin secretagogue on glucose and insulin levels in mice and rats. *J Pharmacol Exp Ther* 320, 738-746 (2007)
116. Hoffmann, T., K. Glund, C. H. S. McIntosh, R. A. Pederson, M. Hanefeld, B. Rosenkranz & H. U. Demuth: DPP-IV-inhibition as treatment of type II diabetes. In: *Cell-Surface Aminopeptidases: Basic and Clinical Aspects*. Ed. S. Mizutani. Kluwer, New York 381-387 (2001)
117. Åhrén, B., E. Simonsson, H. Larsson, M. Landin-Olsson, H. Torgeirsson, P. A. Jansson, M. Sandqvist, P. Bavenholm, S. Efendic, J. W. Eriksson, S. Dickinson & D. Holmes: Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. *Diabetes Care* 25, 869-875 (2002)
118. Evans, D. M.: Dipeptidyl peptidase IV inhibitors. *Drugs* 5, 577-585 (2002)
119. Weber, A. E.: Dipeptidyl peptidase IV inhibitors for the treatment of diabetes. *J Med Chem* 47, 4135-4141 (2004)
120. Drucker, D. J. & M. A. Nauck: The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 368, 1696-1705 (2006)
121. Åhrén, B.: Dipeptidyl peptidase-4 inhibitors: clinical data and clinical implications. *Diabetes Care* 30, 1344-1350 (2007)
122. Barnett, A.: DPP-4 inhibitors and their potential role in the management of type 2 diabetes. *Int J Clin Pract* 60, 1454-1470 (2006)
123. Henness, S. & S. J. Keam: Vildagliptin. *Drugs*, 66 1989-2001 (2006)
124. Green, B. D., P. R. Flatt & C. J. Bailey: Inhibition of dipeptidylpeptidase IV activity as a therapy of type 2 diabetes. *Exp Opin Emerg Drugs* 11, 525-539 (2006)
125. Peters, J.-U.: 11 Years of cyanopyrrolidines as DPP-IV inhibitors. *Curr Topics Med Chem* 7, 579-595 (2007)
126. Pratley, R. E. & A. Salsali: Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. *Curr Med Res Opin* 23, 919-931 (2007)
127. Åhrén, B., M. Landin-Olsson, P.-A. Jansson, M. Svensson, D. Holmes & A. Schweizer: Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 89, 2078-2084 (2004)
128. Herman, G. A., A. Bergman, F. Liu, C. Stevens, A. Q. Wang, W. Zeng, L. Chen, K. Snyder, D. Hilliard, M. Tanen, W. Tanaka, A. G. Meehan, K. Lasseter, S. Dilzer, R. Blum & J. A. Wagner: Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects. *J Clin Pharmacol* 46, 876-886 (2006)
129. Bergman, A. J., C. Stevens, Y. Zhou, B. Yi, M. Laethem, M. De Smet, K. Snyder, D. Hilliard, W. Tanaka, W. Zeng, M. Tanen, A. Q. Wang, L. Chen, G. Winchell, M. J. Davies, S. Ramael, J. A. Wagner & G. A. Herman: Pharmacokinetic and pharmacodynamic properties of multiple oral doses of sitagliptin, a dipeptidyl peptidase-IV inhibitor: a double-blind, randomized, placebo-controlled study in healthy male volunteers. *Clin Ther* 28, 55-72 (2006)
130. Ristic, S., S. Byiers, J. Foley & D. Holmes: Improved glycaemic control with dipeptidyl peptidase-4 inhibition in patients with type 2 diabetes: vildagliptin (LAF237) dose response. *Diabetes Obes Metab* 7, 692-698 (2005)
131. Pratley, R. E., S. Jauffret-Kamel, E. Galbreath & D. Holmes: Twelve-week monotherapy with the DPP-4 inhibitor vildagliptin improves glycemic control in subjects with type 2 diabetes. *Horm Metab Res* 38, 423-428 (2006)
132. Scott, R., M. Wu, M. Sanchez & P. Stein: Efficacy and tolerability of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy over 12 weeks in patients with type 2 diabetes. *Int J Clin Pract* 61, 171-180 (2007)
133. Dejager, S., S. Razac, J. E. Foley & A. Schweizer: Vildagliptin in drug-naïve patients with type 2 diabetes: a 24-week, double-blind, randomized, placebo-controlled, multiple-dose study. *Horm Metab Res* 39, 218-223 (2007)
134. Pi-Sunyer, F. X., A. Schweizer, D. Mills & S. Dejager: Efficacy and tolerability of vildagliptin monotherapy in drug-naïve patients with type 2 diabetes. *Diabetes Res Clin Pract* 76, 132-138 (2007)
135. Balas, B., M. R. Baig, C. Watson, B. E. Dunning, M. Ligueros-Saylan, Y. Wang, Y. L. He, C. Darland, J. J. Holst, C. F. Deacon, K. Cusi, A. Mari, J. E. Foley & R. A. DeFronzo: The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single dose administration in type 2 diabetic patients. *J Clin Endocrinol Metab* 92, 1249-1256 (2007)
136. Matikainen, N., S. Manttari, A. Schweizer, A. Ulvestad, D. Mills, B. E. Dunning, J. E. Foley & M. R. Taskinen: Vildagliptin therapy reduces postprandial intestinal triglyceride-rich lipoprotein particles in patients with type 2 diabetes. *Diabetologia* 49, 2049-2057 (2006)
137. Rosenstock, J., M. A. Baron, S. Dejager, D. Mills & A. Schweizer: Comparison of vildagliptin and rosiglitazone monotherapy in patients with type 2 diabetes: a 24-week, double-blind, randomized trial. *Diabetes Care* 30, 217-223 (2007)
138. Bosi, E., R. P. Camisasca, C. Collober, E. Rochotte & A. J. Garber: Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. *Diabetes Care* 30, 890-895 (2007)

139. Rosenstock, J., R. Brazg, P. J. Andryuk, K. Lu, P. Stein & G. Sitagliptin Study: Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Therapeut* 28, 1556-68 (2006)
140. Fonseca, V., A. Schweizer, D. Albrecht, M. A. Baron, I. Chang & S. Dejager: Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes. *Diabetologia* 50, 1148-1155 (2007)
141. Drucker, D. J.: Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. *Diabetes Care* 30, 1335-1343 (2007)
142. Hansotia, T. & D. J. Drucker: GIP and GLP-1 as incretin hormones: lessons from single and double incretin receptor knockout mice. *Regul Pept* 128, 125-134 (2005)
143. Mari, A., W. M. Sallas, Y. L. He, C. Watson, M. Ligueros-Saylan, B. E. Dunning, C. F. Deacon, J. J. Holst & J. E. Foley: Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed beta-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metabol* 90, 4888-4894 (2005)
144. Herman, G. A., C. Stevens, K. Van Dyck, A. Bergman, B. Yi, M. De Smet, K. Snyder, D. Hilliard, M. Tanen, W. Tanaka, A. Q. Wang, W. Zeng, D. Musson, G. Winchell, M. J. Davies, S. Ramael, K. M. Gottesdiener & J. A. Wagner: Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin Pharmacol Ther* 78, 675-688 (2005)
145. Nauck, M. A. & A. El-Ouaghlidi: The therapeutic actions of DPP-IV inhibition are not mediated by glucagon-like peptide-1. *Diabetologia* 48, 608-611 (2005)
146. Ahrén, B., R. Gomis, E. Standl, D. Mills & A. Schweizer: Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. *Diabetes Care* 27, 2874-2880 (2004)
147. Hoffmann, T., B. Wolf, F. Rosche, S. Fischer, M. Hanefeld, R. A. Pederson, M. C.H.S., K. Glund & H.-U. Demuth: Kinetics of active and inactive plasma GIP in type 2 diabetic patients during treatment with the DPIV-inhibitor P32/98: Direct determination by immunoprecipitation LC-MS. *Diabetes* 51(Suppl. 2), A340 (2002)
148. Herman, G. A., A. Bergman, C. Stevens, P. Kotey, B. Yi, P. Zhao, B. Dietrich, G. Golor, A. Schroder, B. Keymeulen, K. C. Lasseter, M. S. Kipnes, K. Snyder, D. Hilliard, M. Tanen, C. Cilissen, M. De Smet, I. de Lepeleire, K. Van Dyck, A. Q. Wang, W. Zeng, M. J. Davies, W. Tanaka, J. J. Holst, C. F. Deacon, K. M. Gottesdiener & J. A. Wagner: Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metabol* 91, 4612-4619 (2006)
149. Larsen, M. O., B. Rolin, U. Ribel, M. Wilken, C. F. Deacon, O. Svendsen, C. F. Gotfredsen & R. D. Carr: Valine pyrrolidide preserves intact glucose-dependent insulinotropic peptide and improves abnormal glucose tolerance in minipigs with reduced beta-cell mass. *Exp Diabes Res* 4, 93-105 (2003)
150. Lynn, F. C., N. Pamir, E. H. Ng, C. H. S. McIntosh, T. J. Kieffer & R. A. Pederson: Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 50, 1004-1011 (2001)
151. Lynn, F. C., S. A. Thompson, J. A. Pospisilik, J. A. Ehse, S. A. Hinke, N. Pamir, C. H. S. McIntosh & R. A. Pederson: A novel pathway for regulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in beta cells. *FASEB J* 17, 91-93 (2003)
152. Xu, G., H. Kaneto, D. R. Laybutt, V. F. Duvivier-Kali, N. Trivedi, K. Suzuma, G. L. King, G. C. Weir & S. Bonner-Weir: Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. *Diabetes* 56, 1551-1558 (2007)
153. Vilsboll, T. & J. J. Holst: Incretins, insulin secretion and type 2 diabetes mellitus. *Diabetologia* 47, 357-366 (2004)
154. Hinke, S. A., R. W. Gelling, R. A. Pederson, S. Manhart, C. Nian, H.-U. Demuth & C. H. S. McIntosh: Dipeptidyl peptidase IV-resistant [D-Ala(2)]glucose-dependent insulinotropic polypeptide (GIP) improves glucose tolerance in normal and obese diabetic rats. *Diabetes* 51, 652-661 (2002)
155. Thorens, B., A. Porret, L. Buhler, S. P. Deng, P. Morel & C. Widmann: Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes* 42, 1678-1682 (1993)
156. Moens, K., D. Flamez, C. Van Schravendijk, Z. Ling, D. Pipeleers & F. Schuit: Dual glucagon recognition by pancreatic beta-cells via glucagon and glucagon-like peptide 1 receptors. *Diabetes* 47, 66-72 (1998)
157. Gromada, J., K. Bokvist, W. G. Ding, J. J. Holst, J. H. Nielsen & P. Rorsman: Glucagon-like peptide 1 (7-36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. *Diabetes*, 47 57-65 (1998)
158. Kissler, H. J., H. Gepp, A. Schmiedl & P. O. Schwill: Preservation of the incretin effect after orthotopic pancreas transplantation in inbred rats. *Metabolism* 48, 645-650 (1999)
159. Nakagawa, A., H. Satake, H. Nakabayashi, M. Nishizawa, K. Furuya, S. Nakano, T. Kigoshi, K. Nakayama & K. Uchida: Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton Neurosci* 110, 36-43 (2004)
160. Nishizawa, M., H. Nakabayashi, K. Uchida, A. Nakagawa & A. Nijima: The hepatic vagal nerve is receptive to incretin hormone glucagon-like peptide-1, but not to glucose-dependent insulinotropic polypeptide, in the portal vein. *J Auton Nerv Syst* 61, 149-154 (1996)
161. Nakabayashi, H., M. Nishizawa, A. Nakagawa, R. Takeda & A. Nijima: Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. *Am J Physiol* 271, E808-E813 (1996)
162. Nishizawa, M., H. Nakabayashi, K. Kawai, T. Ito, S. Kawakami, A. Nakagawa, A. Nijima & K. Uchida: The hepatic vagal reception of intraportal GLP-1 is via receptor

different from the pancreatic GLP-1 receptor. *J Auton Nerv Syst* 80, 14-21 (2000)

163. Balkan, B. & X. Li: Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am J Physiol Regul Integr Comp Physiol* 279, R1449-R1454 (2000)

164. Ahrén, B.: Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 286, R269-272 (2004)

165. Imeryuz, N., B. C. Yegen, A. Bozkurt, T. Coskun, M. L. Villanueva-Penacarrillo & N. B. Ulusoy: Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol*, 273, G920-G927 (1997)

166. Wettergren, A., M. Wojdemann & J. J. Holst: Glucagon-like peptide-1 inhibits gastropancreatic function by inhibiting central parasympathetic outflow. *Am J Physiol* 275, G984-G992 (1998)

167. Burcelin, R., A. Da Costa, D. Drucker & B. Thorens: Glucose competence of the hepatoporal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. *Diabetes* 50, 1720-1728 (2001)

168. Trümper, A., K. Trümper & D. Hörsch: Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells. *J Endocrinol* 174, 233-246 (2002)

169. Trümper, A., K. Trümper, H. Trusheim, R. Arnold, B. Göke & D. Hörsch: Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. *Mol Endocrinol* 15, 1559-1570 (2001)

170. Ehses, J. A., V. R. Casilla, T. Doty, J. A. Pospisilik, K. D. Winter, H. U. Demuth, R. A. Pederson & C. H. S. McIntosh: Glucose-dependent insulinotropic polypeptide promotes beta-(INS-1) cell survival via cyclic adenosine monophosphate-mediated caspase-3 inhibition and regulation of p38 mitogen-activated protein kinase. *Endocrinology* 144, 4433-4445 (2003)

171. Ehses, J. A., S. L. Pelech, R. A. Pederson & C. H. S. McIntosh: Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J Biol Chem* 277, 37088-37097 (2002)

172. Kim, S.-J., K. Winter, C. Nian, M. Tsuneoka, Y. Koda & C. H. S. McIntosh: Glucose-dependent insulinotropic polypeptide (GIP) stimulation of pancreatic beta-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1, and down-regulation of bax expression. *J Biol Chem* 280, 22297-22307 (2005)

173. Xu, G., D. Stoffers, J. Habener & S. Bonner-Weir: Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48, 2270-2276 (1999)

174. Brubaker, P. L. & D. J. Drucker: Minireview: Glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. *Endocrinology* 145, 2653-2659 (2004)

175. Perfetti, R. & H. Hui: The role of GLP-1 in the life and death of pancreatic beta cells. *Horm Metab Res* 36, 804-810 (2004)

176. Turrel, C., D. Bailbe, M. J. Meile, M. Kergoat & B. Portha: Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 50, 1562-1570 (2001)

177. Drucker, D. J.: Glucagon-like peptide-1 and the islet beta-cell: augmentation of cell proliferation and inhibition of apoptosis. *Endocrinology* 144, 5145-5148 (2003)

178. Drucker, D. J.: The biology of incretin hormones. *Cell Metab* 3, 153-165 (2006)

179. Gutniak, M., C. Ørskov, J. J. Holst, B. Ahrén & S. Efendic: Antidiabetogenic effect of glucagon-like peptide-1 (7-36) amide in normal subjects and patients with diabetes mellitus *New Engl J Med* 326, 1316-1322 (1992)

180. Ritzel, R., C. Orskov, J. J. Holst & M. A. Nauck: Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. *Diabetologia* 38, 720-725 (1995)

181. Ahrén, B., H. Larsson & J. J. Holst: Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82, 473-478 (1997)

182. Dunning, B. E., J. E. Foley & B. Ahren: Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* 48, 1700-1713 (2005)

183. Holz, G. G. T., W. M. Kuhtreiber & J. F. Habener: Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 361, 362-365 (1993)

184. Ahrén, B., M. Sorhede Winzell, B. Burkey & T. E. Hughes: Beta-cell expression of a dominant-negative HNF-1alpha compromises the ability of inhibition of dipeptidyl peptidase-4 to elicit a long-term augmentation of insulin secretion in mice. *Eur J Pharmacol* 521, 164-168 (2005)

185. Wang, Y., J. M. Egan, M. Raygada, O. Nadiv, J. Roth & C. Montrose-Rafizadeh: Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. *Endocrinology* 136, 4910-4917 (1995)

186. Wang, X., C. M. Cahill, M. A. Pineyro, J. Zhou, M. E. Doyle & J. M. Egan: Glucagon-like peptide-1 regulates the beta cell transcription factor, PDX-1, in insulinoma cells. *Endocrinology* 140, 4904-4907 (1999)

187. Schafer, R. & H. Schatz: Stimulation of (Pro-)insulin biosynthesis and release by gastric inhibitory polypeptide in isolated islets of rat pancreas. *Acta Endocrinol* 91, 493-500 (1979)

188. Fehmann, H. C. & B. Göke: Characterization of GIP(1-30) and GIP(1-42) as stimulators of proinsulin gene transcription. *Peptides* 16, 1149-1152 (1995)

189. Meier, J. J. & M. A. Nauck: Glucagon-like peptide 1(GLP-1) in biology and pathology. *Diabetes Metab Res Rev* 21, 91-117 (2005)

190. Baggio, L. L. & D. J. Drucker: Biology of incretins: GLP-1 and GIP. *Gastroenterol* 132, 2131-2157 (2007)

191. Rhodes, C.: Type 2 diabetes-a matter of beta-cell life and death? *Science* 307, 380-384 (2005)

192. Wajchenberg, B. L.: Beta-cell failure in diabetes and preservation by clinical treatment. *Endocrine Rev* 28, 187-218 (2007)

193. Duttaroy, A., F. Voelker, L. Q. Merriam, X. Zhang, X. Ren & B. Burley: The DPP-4 inhibitor Vildagliptin increases pancreatic beta-cell neogenesis and decreases apoptosis. *Diabetes* 54 (Suppl 1), A141 (2005)
194. Butler, A., J. Janson, S. Bonner-Weir, R. Ritzel, R. Rizza & P. Butler: beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52 102-110 (2003)
195. Urusova, I. A., L. Farilla, H. Hui, E. D'Amico & R. Perfetti: GLP-1 inhibition of pancreatic islet cell apoptosis. *Trends Endocrinol Metab* 15, 27-33 (2004)
196. Mu, J., J. Woods, Y.-P. Zhou, R. S. Roy, Z. Li, E. Zychband, Y. Feng, L. Zhu, C. Li, A. D. Howard, D. E. Moller, N. A. Thornberry & B. Zhang: Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic β -cell mass and function in a rodent model of type 2 diabetes. *Diabetes* 55, 1695-1704 (2006)
197. Aschner, P., M. S. Kipnes, J. K. Luncford, M. Sanchez, C. Mickel, D. E. Williams-Herman & S. S. Group: Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care* 29, 2632-2637 (2006)
198. Charbonnel, B., A. Karasik, J. Liu, M. Wu, G. Meininger & S. S. Group: Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. *Diabetes Care* 29, 2638-2643 (2006)
199. Dunning, B. E. & J. E. Gerich: The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. *Endocrine Rev* 28, 253-283 (2007)
200. Degen, K. B., B. Brock, C. B. Juhl, C. B. Djurhuus, J. Grubert, D. Kim, J. Han, K. Taylor, M. Fineman & O. Schmitz: Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes* 53, 2397-2403 (2004)
201. Gromada, J. & P. Rorsman: New insights into the regulation of glucagon secretion by glucagon-like peptide-1. *Horm Metab Res* 36, 822-829 (2004)
202. Gromada, J., I. Franklin & C. Wollheim: alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocrine Rev* 28, 84-116 (2007)
203. Suzuki, S., K. Kawai, S. Ohashi, H. Mukai & K. Yamashita: Comparison of the effects of various C-terminal and N-terminal fragment peptides of glucagon-like peptide-1 on insulin and glucagon release from the isolated perfused rat pancreas. *Endocrinology* 125, 3109-3114 (1989)
204. Franklin, I., J. Gromada, A. Gjinoi, S. Theander & C. B. Wollheim: Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release. *Diabetes* 54, 1808-1815 (2005)
205. Pospisilik, J. A., J. Martin, T. Doty, J. A. Ehses, N. Pami, F. C. Lynn, S. Piteau, H.-U. Demuth, C. H. S. McIntosh & R. A. Pederson: Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* 52, 741-750 (2003)
206. Zander, M., S. Madsbad, J. L. Madsen & J. J. Holst: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359, 824-830 (2002)
207. Wettergren, A., B. Schjoldager, P. E. Mortensen, J. Myhre, J. Christiansen & J. J. Holst: Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 38, 665-673 (1993)
208. Nauck, M. A., D. Wollschlaeger, J. Werner, J. J. Holst, C. Orskov, W. Creutzfeldt & B. Willms: Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia* 39, 1546-1553 (1996)
209. Schirra, J., M. Katschinski, C. Weidmann, T. Schafer, U. Wank, R. Arnold & B. Göke: Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest* 97, 92-103 (1996)
210. Nauck, M. A., U. Niedereichholz, R. Ettl, J. J. Holst, C. Orskov, R. Ritzel & W. H. Schmiegell: Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 273, E981-988 (1997)
211. Meier, J. J., B. Gallwitz, S. Salmen, O. Goetze, J. J. Holst, W. E. Schmidt & M. A. Nauck: Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 88, 2719-2725 (2003)
212. Kolterman, O. G., J. B. Buse, M. S. Fineman, E. Gaines, S. Heintz, T. A. Bicsak, K. Taylor, D. Kim, M. Aisporna, Y. Wang & A. D. Baron: Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 88, 3082-3089 (2003)
213. Vella, A., G. Bock, P. D. Giesler, D. B. Burton, D. B. Serra, M. L. Saylan, B. E. Dunning, J. E. Foley, R. A. Rizza & M. Camilleri: Effects of dipeptidyl peptidase-4 inhibition on gastrointestinal function, meal appearance, and glucose metabolism in type 2 diabetes. *Diabetes* 56, 1475-1480 (2007)
214. Woerle, H., T. Lindenberg, R. Linke, J. Foley, M. Ligueros-Saylan, Y. Zhang, Y.-L. He, C. Beglinger, B. Goeke & J. Schirra: A single dose of vildagliptin (VILDA) decelerates gastric emptying (GE) in patients with type 2 diabetes (T2DM). *Diabetes* 56 (Suppl 1), A133 (2007)
215. Abbott, C. R., M. Monteiro, C. J. Small, A. Sajedi, K. L. Smith, J. R. C. Parkinson, M. A. Ghatei & S. R. Bloom: The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res*, 1044, 127-131 (2005)
216. Nagell, C. F., A. Wettergren, C. Orskov & J. J. Holst: Inhibitory effect of GLP-1 on gastric motility persists after vagal deafferentation in pigs. *Scand J Gastroenterol* 41, 667-672 (2006)
217. Turton, M. D., D. O'Shea, I. Gunn, S. A. Beak, C. M. Edwards, K. Meeran, S. J. Choi, G. M. Taylor, M. M. Heath, P. D. Lambert, J. P. Wilding, D. M. Smith, M. A. Ghatei, J. Herbert & S. R. Bloom: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379, 69-72 (1996)
218. Donahay, J. C., G. van Dijk, S. C. Woods & R. J. Seeley: Intraventricular GLP-1 reduces short- but not long-

- term food intake or body weight in lean and obese rats. *Brain Res* 779, 75-83 (1998)
219. Meeran, K., D. O'Shea, C. M. Edwards, M. D. Turton, M. M. Heath, I. Gunn, S. Abusnana, M. Rossi, C. J. Small, A. P. Goldstone, G. M. Taylor, D. Sunter, J. Steere, S. J. Choi, M. A. Ghatei & S. R. Bloom: Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* 140, 244-250 (1999)
220. Naslund, E., M. Gutniak, S. Skogar, S. Rossner & P. M. Hellstrom: Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. *Am J Clin Nutr* 68, 525-530 (1998)
221. Gutzwiller, J. P., J. Drewe, B. Goke, H. Schmidt, B. Rohrer, J. Lareida & C. Beglinger: Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 276, R1541-R1544 (1999)
222. Raun, K., P. von Voss, C. F. Gotfredsen, V. Golozoubova, B. Rolin & L. B. Knudsen: Liraglutide, a long-acting glucagon-like peptide-1 analog, reduces body weight and food intake in obese candy-fed rats, whereas a dipeptidyl peptidase-IV inhibitor, vildagliptin, does not. *Diabetes* 56, 8-15 (2007)
223. Chen, T., K. Ajami, G. W. McCaughan, M. D. Gorrell & C. A. Abbott: Dipeptidyl peptidase IV gene family. The DPIV family. *Adv Exp Med Biol* 524, 79-86 (2003)
224. Venalainen, J. I., R. O. Juvonen & P. T. Mannisto: Evolutionary relationships of the prolyl oligopeptidase family enzymes. *Eur J Biochem*, 271, 2705-2715 (2004)
225. Chen, W.-T. & T. Kelly: Seprase complexes in cellular invasiveness. *Cancer Metast Rev* 22, 259-269 (2003)
226. Abbott, C. A., D. M. Yu, E. Woollatt, G. R. Sutherland, G. W. McCaughan & M. D. Gorrell: Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8. *Eur J Biochem* 267, 6140-6150 (2000)
227. Olsen, C. & N. Wagtmann: Identification and characterization of human DPP9, a novel homologue of dipeptidyl peptidase IV. *Gene* 299, 185-193 (2002)
228. Radicke, S., D. Cotella, E. M. Graf, U. Ravens & E. Wettwer: Expression and function of dipeptidyl-aminopeptidase-like protein 6 as a putative beta-subunit of human cardiac transient outward current encoded by Kv4.3. *J Physiol* 565, 751-756 (2005)
229. Qi, S. Y., P. J. Riviere, J. Trojnar, J.-L. Junien & K. O. Akinsanya: Cloning and characterization of dipeptidyl peptidase 10, a new member of an emerging subgroup of serine proteases. *Biochem J* 373, 179-189 (2003)
230. Underwood, R., M. Chiravuri, H. Lee, T. Schmitz, A. K. Kabacnel, K. Yardley & B. T. Huber: Sequence, purification, and cloning of an intracellular serine protease, quiescent cell proline dipeptidase. *J Biol Chem* 274, 34053-34058 (1999)
231. Maes, M.-B., A.-M. Lambeir, K. Gilany, K. Senten, P. Van der Veken, B. Leiting, K. Augustyns, S. Scharpe & I. De Meester: Kinetic investigation of human dipeptidyl peptidase II (DPPII)-mediated hydrolysis of dipeptide derivatives and its identification as quiescent cell proline dipeptidase (QPP)/dipeptidyl peptidase 7 (DPP7). *Biochem J* 386, 315-324 (2005)
232. Sedo, A., J. S. Duke-Cohan, E. Balaziová & L. R. Sedova: Dipeptidyl peptidase IV activity and/or structure homologs: contributing factors in the pathogenesis of rheumatoid arthritis? *Arthritis Res Ther* 7, 253-269 (2005)
233. Plamboeck, A., J. J. Holst, R. D. Carr & C. F. Deacon: Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. *Diabetologia* 48, 1882-1890 (2005)
234. Durinx, C., A. M. Lambeir, E. Bosmans, J. B. Falmagne, R. Berghmans, A. Haemers, S. Scharpe & I. De Meester: Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur J Biochem* 267, 5608-5613 (2000)
235. Collins, P. J., G. McMahon, P. O'Brien & B. O'Connor: Purification, identification and characterisation of seprase from bovine serum. *Int J Biochem Cell Biol* 36, 2320-2333 (2004)
236. Jacotot, E., C. Callebaut, J. Blanco, B. Krust, K. Neubert, A. Barth & A. G. Hovanesian: Dipeptidyl-peptidase IV-beta, a novel form of cell-surface-expressed protein with dipeptidyl-peptidase IV activity. *Eur J Biochem* 239, 248-258 (1996)
237. Blanco, J., C. Nguyen, C. Callebaut, E. Jacotot, B. Krust, J. P. Mazaleyrat, M. Wakselman & A. G. Hovanesian: Dipeptidyl-peptidase IV-beta--further characterization and comparison to dipeptidyl-peptidase IV activity of CD26. *Eur J Biochem* 256, 369-378 (1998)
238. Iwaki-Egawa, S., Y. Watanabe, Y. Kikuya & Y. Fujimoto: Dipeptidyl peptidase IV from human serum: purification, characterization, and N-terminal amino acid sequence. *J Biochem* 124, 428-433 (1998)
239. Shibuya-Saruta, H., Y. Kasahara & Y. Hashimoto: Human serum dipeptidyl peptidase IV (DPPIV) and its unique properties. *J Clin Lab Anal* 10, 435-440 (1996)
240. Duke-Cohan, J. S., W. Tang & S. F. Schlossman: Attractin: a cub-family protease involved in T cell-monocyte/macrophage interactions. *Adv Exp Med Biol* 477, 173-185 (2000)
241. Tang, W., T. M. Gunn, D. F. McLaughlin, G. S. Barsh, S. F. Schlossman & J. S. Duke-Cohan: Secreted and membrane attractin result from alternative splicing of the human ATRN gene. *Proc Natl Acad Sci USA* 97, 6025-6030 (2000)
242. Friedrich, D., T. Hoffmann, J. Bar, M. Wermann, S. Manhart, U. Heiser & H.-U. Demuth: Does human attractin have DP4 activity? *Biol Chem* 388, 155-162 (2007)
243. Duke-Cohan, J. S., J. H. Kim & A. Azouz: Attractin: cautionary tales for therapeutic intervention in molecules with pleiotropic functionality. *J Environ Pathol Toxicol Oncol* 23, 1-11 (2004)
244. Kojima, K., R. Mihama, T. Said, A. Togari, T. Matsui, K. Shinpo, K. Fujita, K. Fukasawa, M. Harada & Y. Nagatsu: Serum activities of dipeptidyl- aminopeptidase II and dipeptidyl-aminopeptidase IV in tumor-bearing animals and in cancer patients. *Biochem Med Metabol Biol* 37, 35-41 (1987)
245. Lankas, G. R., B. Leiting, R. S. Roy, G. J. Eiermann, M. G. Beconi, T. Biftu, C.-C. Chan, S. Edmondson, W. P. Feeney, H. He, D. E. Ippolito, D. Kim, K. A. Lyons, H. O.

- Ok, R. A. Patel, A. N. Petrov, K. A. Pryor, X. Qian, L. Reigle, A. Woods, J. K. Wu, D. Zaller, X. Zhang, L. Zhu, A. E. Weber & N. A. Thornberry: Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9. *Diabetes* 54, 2988-2994 (2005)
246. Aytac, U. & N. H. Dang: CD26/dipeptidyl peptidase IV: a regulator of immune function and a potential molecular target for therapy. *Curr Drug Targets Immune Endocr Metabol Disord* 4, 11-18 (2004)
247. Ahrén, B.: What mediates the benefits associated with dipeptidyl peptidase-IV inhibition? *Diabetologia*, 48, 605-607 (2005)
248. Medeiros, M. D. & A. J. Turner: Processing and metabolism of peptide-YY: pivotal roles of dipeptidylpeptidase-IV, aminopeptidase-P, and endopeptidase-24.11. *Endocrinology* 134, 2088-2094 (1994)
249. Ballantyne, G. H.: Peptide YY(1-36) and peptide YY(3-36): Part I. Distribution, release and actions. *Obesity Surg* 16, 651-658 (2006)
250. Unniappan, S., C. H. S. McIntosh, H. U. Demuth, U. Heiser, R. Wolf & T. J. Kieffer: Effects of dipeptidyl peptidase IV on the satiety actions of peptide YY. *Diabetologia* 49, 1915-1923 (2006)
251. Creutzfeldt, W. O., N. Kleine, B. Willms, C. Ørskov, J. J. Holst & M. A. Nauck: Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type I diabetic patients. *Diabetes Care* 19, 580-586 (1996)
252. Dupre, J., M. T. Behme, I. M. Hramiak, P. Mcfarlane, M. P. Williamson, P. Zabel & e. al: Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* 44, 626-630 (1995)
253. Dupre, J., M. T. Behme, I. M. Hramiak & T. J. McDonald: Subcutaneous glucagon-like peptide I combined with insulin normalizes postcibal glycemic excursions in IDDM. *Diabetes Care* 20, 381-384 (1997)
254. Dupre, J.: Glycaemic effects of incretins in Type 1 diabetes mellitus: a concise review, with emphasis on studies in humans. *Regul Pept* 128, 149-157 (2005)
255. McCaughan, G. W., M. D. Gorrell, G. A. Bishop, C. A. Abbott, N. A. Shackel, P. H. McGuinness, M. T. Levy, A. F. Sharland, D. G. Bowen, D. Yu, L. Slaitini, W. B. Church & J. Napoli: Molecular pathogenesis of liver disease: an approach to hepatic inflammation, cirrhosis and liver transplant tolerance. *Immunol Rev* 174, 172-191 (2000)
256. Hafler, D. A., D. A. Fox, M. E. Manning, S. F. Schlossman, E. L. Reinherz & H. L. Weiner: In vivo activated T lymphocytes in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *New Engl J Med* 312, 1405-1411 (1985)
257. Eguchi, K., Y. Ueki, C. Shimomura, T. Otsubo, H. Nakao, K. Migita & e. al.: Increment in the Tα1+ cells in the peripheral blood and thyroid tissue of patients with Graves' disease. *J Immunol* 142, 4233-4240 (1989)
258. Hashikawa, T., S. W. Hooker, J. G. Maj, C. J. Knott-Craig, M. Takedachi, S. Murakami & L. F. Thompson: Regulation of adenosine receptor engagement by ecto-adenosine deaminase. *FASEB J* 18, 131-133 (2004)
259. Girardi, A. C. C., F. Knauf, H.-U. Demuth & P. S. Aronson: Role of dipeptidyl peptidase IV in regulating activity of Na⁺/H⁺ exchanger isoform NHE3 in proximal tubule cells. *Am J Physiol Cell Physiol* 287, C1238-C1245 (2004)
260. Cheng, H. C., M. Abdel-Ghany, R. C. Elble & B. U. Pauli: Lung endothelial dipeptidyl peptidase IV promotes adhesion and metastasis of rat breast cancer cells via tumor cell surface-associated fibronectin. *J Biol Chem* 273, 24207-24215 (1998)
261. Ishii, T., K. Ohnuma, A. Murakami, N. Takasawa, S. Kobayashi, N. H. Dang, S. F. Schlossman & C. Morimoto: CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO. *Proc Natl Acad Sci USA* 98, 12138-12143 (2001)
262. Morimoto, C. & S. F. Schlossman: The structure and function of CD26 in the T-cell immune response. *Immunol Rev* 161, 55-70 (1998)
263. Wang, X. M., D. M. T. Yu, G. W. McCaughan & M. D. Gorrell: Extra-enzymatic roles of DPIP and FAP in cell adhesion and migration on collagen and fibronectin. *Adv Exp Med Biol* 575, 213-222 (2006)
264. Pederson, R. A., T. J. Kieffer, R. Pauly, H. Kofod, J. Kwong & C. H. McIntosh: The enteroinsular axis in dipeptidyl peptidase IV-negative rats. *Metabolism* 45, 1335-1341 (1996)
265. Karl, T., T. Hoffmann, R. Pabst & S. von Horsten: Extreme reduction of dipeptidyl peptidase IV activity in F344 rat substrains is associated with various behavioral differences. *Physiol Behav* 80, 123-134 (2003)
266. Coburn, M., D. Hixson & J. Reichner: In vitro immune responsiveness of rats lacking dipeptidylpeptidase IV. *Cell Immunol* 158, 269-280 (1994)
267. Kruschinski, C., T. Skripuletz, S. Bedoui, T. Tschernig, R. Pabst, C. Nassenstein, A. Braun & S. von Horsten: CD26 (dipeptidyl-peptidase IV)-dependent recruitment of T cells in a rat asthma model. *Clin Exp Immunol* 139, 17-24 (2005)
268. Marguet, D., L. Baggio, T. Kobayashi, A. M. Bernard, M. Pierres, P. F. Nielsen, U. Ribet, T. Watanabe, D. J. Drucker & N. Wagtmann: Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci USA* 97, 6874-6879 (2000)
269. Conarello, S. L., Z. Li, J. Ronan, R. S. Roy, L. Zhu, G. Jiang, F. Liu, J. Woods, E. Zychband, D. E. Moller, N. A. Thornberry & B. B. Zhang: Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci USA* 100, 6825-6830 (2003)
270. Nagakura, T., N. Yasuda, K. Yamazaki, H. Ikuta, S. Yoshikawa, O. Asano & I. Tanaka: Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats. *Biochem Biophys Res Commun* 284, 501-516 (2001)
271. Guieu, R., E. Fenouillet, C. Devaux, Z. Fajloun, L. Carrega, J.-M. Sabatier, N. Sauze & D. Marguet: CD26 modulates nociception in mice via its dipeptidyl-peptidase IV activity. *Behav Brain Res* 166, 230-235 (2006)
272. Bohm, S. K., J. R. Gum, Jr., R. H. Erickson, J. W. Hicks & Y. S. Kim: Human dipeptidyl peptidase IV gene promoter: tissue-specific regulation from a TATA-less GC-rich sequence characteristic of a housekeeping gene promoter. *Biochem J* 311, 835-843 (1995)

273. Bilodeau, N., A. Fiset, G. G. Poirier, S. Fortier, M.-C. Gingras, J. N. Lavoie & R. L. Faure: Insulin-dependent phosphorylation of DPP IV in liver. Evidence for a role of compartmentalized c-Src. *FEBS J* 273, 992-1003 (2006)
274. Ryskjaer, J., C. F. Deacon, R. D. Carr, T. Krarup, S. Madsbad, J. Holst & T. Vilsboll: Plasma dipeptidyl peptidase-IV activity in patients with type-2 diabetes mellitus correlates positively with HbA1c levels, but is not acutely affected by food intake. *Eur J Endocrinol* 155, 485-493 (2006)
275. Hildebrandt, M., M. Rose, H. Monnikes, W. Reutter, W. Keller & B. F. Klapp: Eating disorders: a role for dipeptidyl peptidase IV in nutritional control. *Nutrition* 17, 451-454 (2001)
276. Thoma, R., B. Löffler, M. Stihle, W. Huber, A. Ruf & M. Hennig: Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. *Structure* 11, 947-959 (2003)
277. Kuhn, B., M. Hennig & P. Mattei: Molecular recognition of ligands in dipeptidyl peptidase IV. *Curr Topics Med Chem* 7, 609-619 (2007)
278. Kühn-Wache, K., T. Hoffmann, S. Manhart, W. Brandt & H. U. Demuth: The specificity of DP IV for natural substrates is peptide structure determined. *Adv Exp Med Biol* 524, 57-63 (2003)

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