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Dipeptidyl peptidase IV (DPP IV) and related molecules in type 2 diabetes

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1. ABSTRACT

Dipeptidyl peptidase IV (DPP IV) is a widely distributed physiological enzyme that can be found solubilized in blood, or membrane-anchored in tissues. DPP IV and related dipeptidase enzymes cleave a wide range of physiological peptides and have been associated with several disease processes including Crohn's disease, chronic liver disease, osteoporosis, multiple sclerosis, eating disorders, rheumatoid arthritis, cancer, and of direct relevance to this review, type 2 diabetes. Here, we place particular emphasis on two peptide substrates of DPP IV with insulin-releasing and antidiabetic actions namely, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The rationale for inhibiting DPP IV activity in type 2 diabetes is that it decreases peptide cleavage and thereby enhances endogenous incretin hormone activity. A multitude of novel DPP IV inhibitor compounds have now been developed and tested. Here we examine the information available on DPP IV and related enzymes, review recent preclinical and clinical data for DPP IV inhibitors, and assess their clinical significance.

2. INTRODUCTION

Two primary defects in the pathogenesis of type 2 diabetes are a relative loss of insulin secretion from pancreatic beta cells and a decreased sensitivity of liver and peripheral tissues to insulin (1). Drug treatments for type 2 diabetes have centred therefore on enhancing insulin secretion and action. In the case of sulphonylureas (e.g. glibenclamide) and meglitinides (e.g. nateglinide) insulin secretion is increased from the pancreas (2). The biguanides (e.g. metformin) and thiazolidinediones (e.g. pioglitazone) improve the body's sensitivity to insulin (3-5). Additionally, synthetic insulin and insulin analogues can be administered when oral drugs are no longer sufficient to provide adequate blood glucose control.

Insulin secretagogues and in particular the sulphonylureas suffer from a lack of glucose-dependency which can lead to episodes of hypoglycaemia (6). Also, as the disease progresses and beta-cell function declines, several years of use often lead to declining drug effectiveness. With the prevalence of type 2 diabetes reaching epidemic proportions (predicted to be about 350

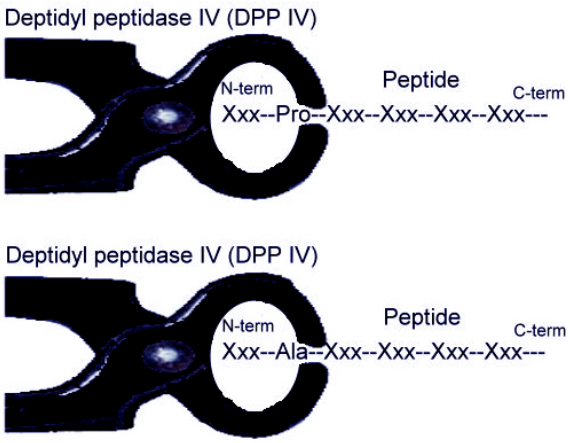

Figure 1. N-terminal cleavage activity and substrate specificity of dipeptidyl peptidase IV (DPP IV). DPP IV cleaves dipeptides from the N-terminus of peptides and polypeptides which have either a proline or an alanine residue in the penultimate position (examples of which can be found in Table 1). Xaa represents one of the 20 proteinogenic amino acids.

million by 2025, (7)) new antidiabetic drug treatments are urgently required. Incretin hormones are peptides secreted from endocrine cells in the small intestine which stimulate significant insulin secretion at physiological concentrations in a glucose-dependent manner (8-12). The two principal incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP). Expanding knowledge of the incretin hormones and their physiological inactivation by dipeptidyl peptidase IV (DPP IV) has lead to two new classes of antidiabetic drugs, incretin analogues/mimetics and DPP IV inhibitors/gliptins. In this article we focus on the enzyme DPP IV and the progress made towards the development of DPP IV inhibitors.

3. DPP IV AND RELATED ENZYMES

3.1. DPP IV (EC 3.4.14.5)

DPP IV is classified as a serine protease by virtue of the classical consensus motif Gly-Xxx-Ser-Xxx-Gly, which is Gly^{628}-Trp^{629}-Ser^{630}-Tyr^{631}-Gly^{632} in the case of human DPP IV (13). DPP IV was one of the earliest identified prolyl peptidases and over the years DPP IV has become one of the most intensively studied of its class (14). The proteolytic activity of DPP IV is relatively selective, cleaving only peptide bonds following proline or alanine amino acid residues located penultimate to the N-terminus (14; See Figure 1) (this also commonly occurs at sites following serine (See Table 1)). DPP IV is also the lymphocyte cell surface protein CD26 discussed in detail in other reviews (15). Structural studies with soluble human DPP IV reveal that the inhibitor diprotin A covalently bonds to Ser^{630} in the catalytic triad, irreversibly blocking the active site (13). Physiologically the role of DPP IV is wide ranging since it is capable of interacting with a number of diverse proteins such as collagen (16), fibronectin (17), adenosine deaminase (18), tyrosine phosphatase CD45 (19), and a plethora of regulatory peptides across a range of physiological systems (20-22). Table 1 lists many physiological peptide substrates (or potential substrates) of DPP IV showing N-terminal regions targeted by the enzyme.

DPP IV is expressed by endothelia and epithelia in most tissues, including bone marrow, kidney, intestine, pancreas, liver, lymphocytes, placenta, uterus, prostate and skin (14). Levels and expression and activity of DPP IV in certain tissues and blood plasma are known to vary significantly following the onset of disease, injury or inflammation (reviewed elsewhere 14, 22). Peptide substrates of DPP IV have such extensive physiological implications, that most body systems are likely to be affected including nervous, endocrine, neuroendocrine, immune, vascular, digestive, skeletal and reproductive systems (see substrates in Table 1). Among the most commonly recognized substrates of DPP IV are several chemokines that affect the immune system: (RANTES, eotaxin, IP-10, MCP-1, MCP-2, MCP-3, SDF-1α, SDF-1β, GCP-2 and MDC, see Table 1 (20); several neuropeptides: substance P, bradykinin, peptide YY (PYY), neuropeptide Y (NPY), and pituitary adenylate cyclase activating peptide (PACAP) (20); and glucagon, the counter-regulatory hormone of insulin (23). As we shall see later in this review, much attention has been focused towards the incretin hormones GLP-1 and GIP as substrates of DPP IV. Considered briefly below are other physiological proteases related to DPP IV which possess similar post-proline aminopeptidase activity.

3.2. Dipeptidyl peptidase II (DPP II, also known as DPP 7, or quiescent cell proline dipeptidase (QPP) (E.C. 3.4.14.2)

Evidence in recent years has suggested that three earlier identified proteases DPP II, DPP 7 and QPP are in fact the same enzyme (24-26). Discovered in 1968 by the extraction of Lys-Ala- hydrolytic activity from the anterior pituitary, DPP II appears to be widely distributed across a range of mammalian tissues (24, 27). The identification of physiological substrates of DPP II has been hindered by low purification yields from tissue and a lack of information regarding the molecular and catalytic properties. Although there are no totally selective DPP II inhibitors available, compounds such as Ala-ψ[CS-N]-Pyrr and Ala-ψ[CS-N]-Thia have more selectivity towards DPP II than DPP IV (28).

3.3. Dipeptidyl peptidase 8 (DPP 8) and dipeptidyl peptidase 9 (DPP 9)

DPP 8 and DPP 9 are relative newcomers to the DPP IV enzyme family and their inadvertent inhibition appears to be responsible for at least some of the toxic side-effects of DPP IV inhibitors including alopecia, thrombocytopenia, anaemia, enlarged spleen, and multiple histological pathologies, including skin lesions and premature mortality in animals (29, 30). DPP 8 and 9 are monomeric soluble cytoplasmic enzymes sharing approximately 50% sequence similarity with human DPP IV (14). Like DPP IV they are widely distributed across human tissues and although they have not yet been

Table 1. Physiological regulatory peptides identified as substrates of DPP IV

Physiological System	Peptide	N-terminus
Nutrient metabolism and glucose homeostasis	GLP-1 (7-36)amide	His-Ala-Glu-
	GIP (1-42)	Tyr-Ala-Asp-
	GLP-1 (7-37)	His-Ala-Glu-
	Glucagon	His-Ser-Gln-
Digestive system	GLP-2 (1-33)	His-Ala-Asp-
	Trypsinogen	Phe-Pro-Thr-
	Trypsinogen pro-peptide	Phe-Pro-Thr-
	Gastrin releasing peptide (GRP)	Val-Pro-Leu-
	Pro-colipase	Val-Pro-Asp-
	Enterostatin	Val-Pro-Asp-
	β-Casomorphin	Tyr-Pro-Phe-
	Aprotinin	Arg-Pro-Asp-
Growth and development	Insulin-like growth factor-1 (IGF-1)	Gly-Pro-Glu-
	Growth hormone releasing factor (GHRF)	Tyr-Ala-Glu-
	Growth hormone-releasing hormone (GRH (1-29))	Tyr-Ala-Asp-
	GRH (1-44)	Tyr-Ala-Asp-
Neuroendocrine system	PACAP (1-27)	His-Ser-Asp-
	PACAP (1-38)	His-Ser-Asp-
Nervous system	Substance P	Arg-Pro-Lys-
	Neuropeptide Y	Tyr-Pro-Ser-
	Peptide YY (1-36)	Tyr-Pro-Ile
	Enkephalins	Tyr-Pro-Val-
	Corticotropin-like intermediate lobe peptide	Arg-Pro-Val-
	Endomorphin-2	Tyr-Pro-Phe-
Vascular system	Bradykinin	Arg-Pro-Pro-
Reproductive system	Human chorionic gonadotrophin α (hCGα)	Ala-Pro-Asp-
	Leutinising hormone α chain (LHα)	Phe-Pro-Asn-
	Prolactin	Thr-Pro-Val-
Immune system	Interleukin-2	Ala-Pro-Thr-
	Interleukin-1β	Ala-Pro-Val-
	α_1-Microglobulin	Gly-Pro-Val-
	RANTES	Ser-Pro-Tyr-
	Granulocyte chemtactic protein-2 (GCP-2)	Gly-Pro-Val-
	Stromal cell-derived factor-1α (SDF-1α)	Lys-Pro-Val-
	SDF-1β	Lys-Pro-Val-
	Macrophage-derived chemokine (MDC)	Gly-Pro-Tyr-
	Monocyte chemotactic protein-1 (MCP-1)	Glu-Pro-Asp-
	MCP-2	Glu-Pro-Asp-
	MCP-3	Glu-Pro-Val-
	Eotaxin	Gly-Pro-Ala-
	Interferon-γ-inducible protein-10 (IP-10)	Val-Pro-Leu-
Endocrine system	Thyrotropin α	Phe-Pro-Asp-
	Vasostatin-1	Leu-Pro-Val-
Other	Peptide histidine methionine	His-Ala-Asp-
	Tyr-Melanostatin	Tyr-Pro-Leu-

assigned any particular biological function, the undesirable consequences of unselective DPP IV inhibition may provide important clues. Their post-proline aminopeptidase activity has been evidenced by the hydrolysis of H-Ala-Pro- and H-Gly-Pro derived substrates (31). Selective inhibitors of DPP 8 and DPP 9 are already available and have been used to characterise DPP8/9 activity in human leukocytes (32).

3.4. Fibroblast Activation Protein (FAP)

The DPP IV-like activity of FAP has been confirmed by the rapid cleavage of an Ala-Pro-NH F_3 Mec substrate (33). FAP has 52% sequence similarity to DPP IV and has been linked with liver injury and chronic liver disease (31, 33). FAP is not as widely expressed as DPP IV and has been identified in serum and pancreatic alpha cells. The active site which carries out N-terminal dipeptide cleavage also possesses collagenolytic activity degrading gelatine and type 1 collagen (34). Selective fluorescent probes have been developed to detect and differentiate between the proteolytic activities of FAP and DPP IV (35).

4. THE INCRETIN HORMONES, DPP IV AND DIABETES

4.1. The incretins

Although the concept of targeting DPP IV in type 2 diabetes is relatively recent, the origin can be traced back to early work establishing the importance of the gut in regulating post-prandial glucose homeostasis (36, 37). The gut contributes neural and endocrine signals that account for the enhanced physiological insulin response after a meal, a signalling pathway known as the enteroinsular axis (36, 37). GLP-1 and GIP secreted from intestinal L- and K-cells, respectively, account for most of the enteroinsular (or "incretin") effect (38-42). A list of well characterised

actions of GLP-1 and GIP relevant to type 2 diabetes can be found in Table 2. The glucose-dependent nature of their insulinotropic activity provides a clear advantage to enhance postprandial insulin secretion and reduce the risk of interprandial hypoglycaemia (8-10). Insulin biosynthesis is also increased. Furthermore, GLP-1 and GIP possess extrapancreatic mechanisms which contribute to limit hyperglycaemia, e.g. reducing hepatic insulin extraction (43, 44), reported 'insulin-like' effects on skeletal muscle, liver and adipose tissue (45-49), and a reduction of gastric acid secretion or gastric emptying (50-52). Incretin hormones have additional potential benefits over other insulin-releasing drugs through improved islet morphology and protective and proliferative effects on the pancreatic beta-cell (53-59). Such properties might help to counter the characteristic age-related decline of beta-cell mass in diabetes. Although these properties are evident in animal models, substantiating effects of incretin hormones on beta-cell morphology in humans remains problematic. Finally, an important yet occasionally overlooked effect is the suppression of glucagon secretion by GLP-1 (60).

4.2. Inactivation of the incretins by DPP IV

Mentlein and co-workers were amongst the first to demonstrate degradation of GLP-1 and GIP by DPP IV *in vitro* (61). Using DPP IV purified from human placenta they observed the enzymatic removal of N-terminal dipeptides His^{7}-Ala^{8} and Tyr^{1}-Ala^{2} from GLP-1 and GIP, respectively. More significantly, they observed that this degradation also took place when GLP-1 and GIP were incubated in human serum (61). It was subsequently confirmed that DPP IV-mediated metabolism of GLP-1 and GIP did indeed occur *in vivo* (40, 62). The action of DPP IV *in vivo* reduces the half-life of GLP-1 and GIP to <2 min (40, 62, 63). Since the predominant and active forms of incretin hormones are GLP-1(7-36)amide and GIP(1-42), degradation by DPP IV leads to major degradation fragments GLP-1(9-36)amide and GIP(3-42), respectively.

The activities and binding characteristics of these fragments have been elucidated. Since GLP-1(9-36)amide and GIP(3-42) are both non-insulinotropic peptides, it was initially suggested that these were relatively inert and inactive metabolites (64, 65). Although the affinity of GLP-1(9-36)amide for the GLP-1 receptor is 100-fold lower than the parent molecule it appears to act as a weak receptor antagonist (64, 66, 67). While GLP-1(9-36)amide does not antagonise the insulinotropic activity of GLP-1(7-36)amide *in vivo*, there is evidence that this metabolite possesses weak antihyperglycaemic activity through a mechanism not involving insulin secretion (68). This concept currently remains controversial due to conflicting findings in mice, pigs and humans (68-70).

The receptor affinity of GIP fairs comparatively better following truncation by DPP IV. The binding affinity of GIP(3-42) is approximately 4-fold lower than that of GIP(1-42) (71). *In vitro* studies have demonstrated that GIP(3-42) antagonises the GIP receptor (72, 73). However, *in vivo* studies have been conflicting (70-73). A recent study confirmed that GIP(3-42) antagonises GIP-stimulated cAMP production and insulin secretion, but found that *in vivo* it does not behave as an antagonist at physiological concentrations (71). However, pharmacological doses of GIP(3-42) (25 nmol/kg) administered to obese diabetic *(ob/ob)* mice once daily for 14 days enhanced insulin sensitivity and improved glycaemic control (70). This appears to involve extrapancreatic mechanisms which lead to improved insulin sensitivity. During this study neither GIP(3-42) or GLP-1(9-36)amide affected body weight, food intake, pancreatic insulin content or islet morphology (70).

4.3. Overcoming DPP IV mediated incretin inactivation

As knowledge of incretin hormone inactivation expanded greater emphasis was placed on ways to overcome this problem. The pharmacological strategies adopted have led to the development of two fundamentally new ways to treat type 2 diabetes. The first approach has involved generating GLP-1 and GIP agonists resistant to the action of DPP IV (reviewed elsewhere (74)). Production and testing of numerous modified forms of the incretin hormones have generated effective DPP IV-resistant analogues of GLP-1 and GIP (74). In human subjects GLP-1 analogues have demonstrated sustained improvements in glycaemic control in type 2 diabetes. To date one GLP-1 agonist, exendin (exenatide/Byetta), has been clinically approved, and another, liraglutide (NN2211) is in phase III clinical trials (75). A second and more recently adopted approach, which is the focus of this review, has been the development of DPP IV inhibitors (22, 76, 77). As illustrated in Figure 2 the concept of DPP IV inhibitors is to enhance endogenous incretin activity by preventing the rapid inactivation of incretin hormones. The preclinical and clinical data for DPP IV inhibitors (or 'gliptins' as they are termed) is reviewed later in this article.

4.4. Rodent models lacking DPP IV activity

The generation of rodent models lacking functional DPP IV has brought major advances in our understanding of this enzyme's role in metabolism, and has strengthened the rationale for developing specific inhibitors of DPP IV (78-81). Of particular note is the fact that mice lacking DPP IV activity have significantly reduced glycaemic excursions, greater levels of glucose-stimulated insulin, while the degradation of both GLP-1 and GIP is reduced (78). Similarly, DPP IV-deficient rats have improved glucose tolerance, enhanced insulin release and higher levels of active GLP-1 (79). Evidence gathered from these rodent models underpins the role of DPP IV in regulating incretin activity and consequently glucose homeostasis.

It is especially interesting that DPP IV 'knockout' mice are relatively resistant to the development of glucose intolerance and diabetes following 20 weeks on a high fat diet (80). These mice exhibited reduced food intake and enhanced metabolic energy expenditure and did not develop obesity (80). This has been substantiated by similar observations in DPP IV-deficient Fischer rats (81). Furthermore, DPP IV 'knockout' appears to confer protection from the diabetogenic effects of modest amounts of the beta-cell toxin streptozotocin (80).

Table 2. Characterised actions of GLP-1 and GIP relevant to type 2 diabetes

	GLP-1		GIP	
	Effect	Reference	Effect	Ref
Released in response to a mixed meal	√	111, 112	√	111, 112
Lower blood glucose	√	113	√	114, 115
Glucose-dependent stimulation of insulin secretion	√	8	√	9, 10
Suppress glucagon secretion	√	60	-	-
Extrapancreatic glucose-lowering actions	√	45-47	√	48, 49
Extend beta cell mass and survival	√	53-56	√	57-59
Suppress gastric acid secretion	-	-	√	52
Inhibition of gastric emptying	√	50, 51	-	-
Inhibition of hepatic insulin extraction	√	43	√	44
Enhance satiety	√	116	-	-
Reduce body weight	√	117	-	-

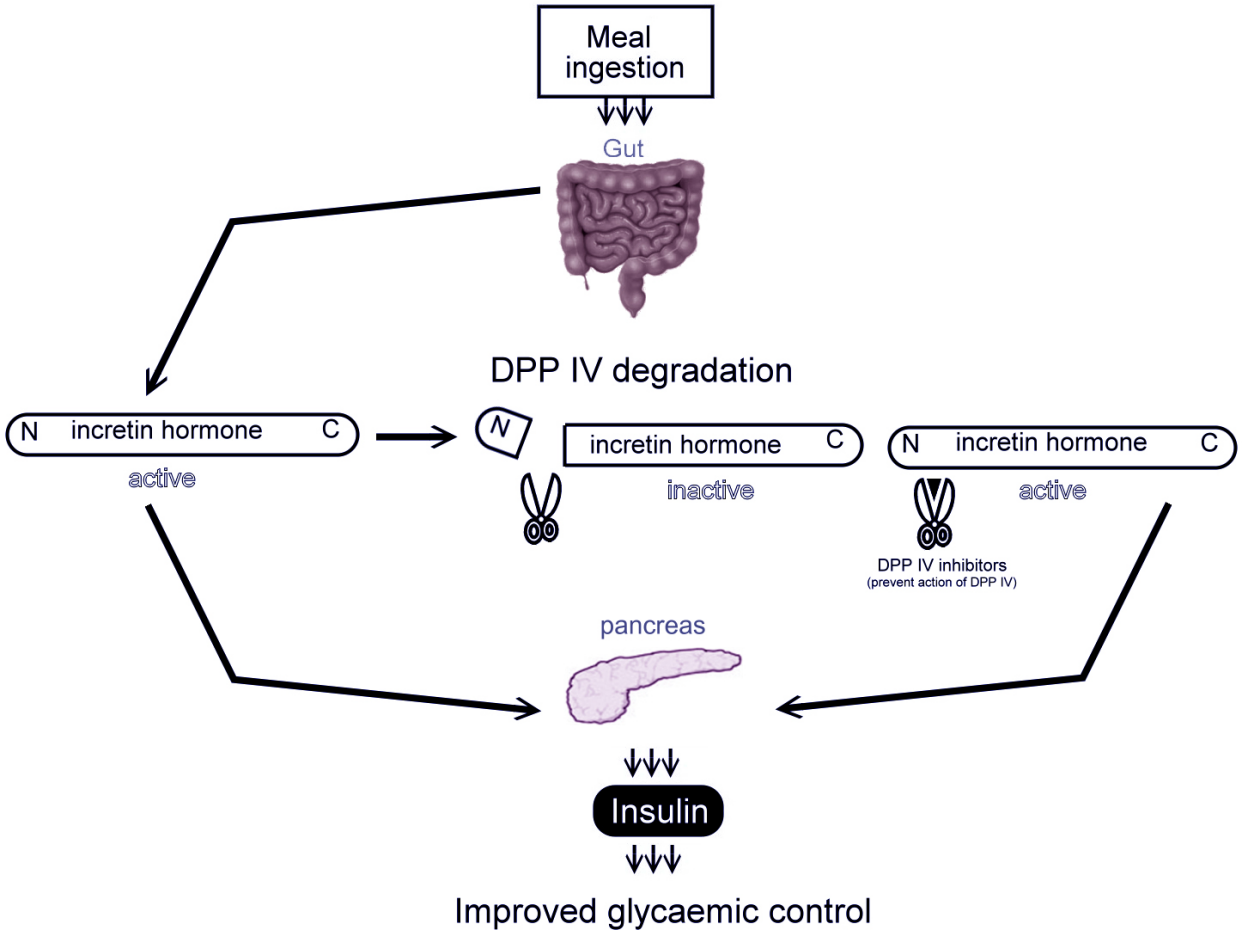

Figure 2. Incretin hormone inactivation and DPP IV inhibitor mode of action. The incretin hormones (GLP-1 and GIP) are released from the intestine following meal ingestion. Incretins circulate to the pancreas where they stimulate insulin-release leading to a lowering of plasma glucose concentrations. However, enzymatic cleavage by ubiquitous DPP IV renders them non-insulinotropic. DPP IV inhibitors prevent processing by DPP IV and therefore enhance endogenous incretin hormone activity.

Although, animal models lacking DPP IV activity are viable and appear relatively normal, recent evidence is emerging of some neurological, immunological and inflammatory alterations (82-87). Mice lacking DPP IV have shortened latencies to nociceptive stimuli, perhaps due to observed higher plasma levels of substance P (82). In the context of experimental asthma, rats lacking DPP IV demonstrate decreased T-cell recruitment associated with significantly reduced ovalbumin-specific IgE-titres (83). Furthermore, marked changes in the cytokine responses of interleukins and tumour necrosis factor have been observed (84; 85). DPP IV deficient rats are more susceptible to angiooedema caused by ACE inhibitor administration (86) and it has been reported that DPP IV inhibition in some species (e.g. dogs and monkeys) cause gastrointestinal disturbances and skin lesions, although these remain to be confirmed (31). Finally, the severity of antigen-induced arthritis is increased in DPP IV-deficient mice which may be due to increased levels of circulating active stromal cell-derived factor-1 (87).

Table 3. DPP IV inhibitors in clinical development

Inhibitor (reference number)	Generic name *(Proprietary name)*	Company	Clinical Phase	Specificity data
MK-0431	Sitagliptin[a] (***Januvia***)	Merck	4	IC_{50} = 18 nM K_i = 9 nM
LAF237	Vildagliptin[b] (***Galvus***)	Novartis	3	IC_{50} = 3.5 nM K_i = 17 nM
BMS-477118	Saxagliptin	Bristol-Myers Squib and AstraZeneca	3	IC_{50} = 26nM K_i = <1 nM
SYR-322	Alogliptin	Takeda	3	-
R1438	Aminomethylpyridine deivative	Roche	2	K_i = 0.1 nM
P32/98	Isoleucine thiazolidide	Probiodrug	2	K_i = 80 nM
PSN9301	-	Prosidion	2	-
GRC-8200	Carbazole compound	Glenmark	2	IC_{50} = 1.6 nM
PHX-1149	-	Phenomix	2	-
SSR-162369	Pyrrolidinoxanthine derivative	Sanofi-Aventis	1	-
ALS 2-0426	-	Alantos/Amgen	1	-
NN-7201	-	NovoNordisk	?	-

[a] Sitagliptin was launched in Mexico in 2006, and in USA, UK and several other European countries in 2007, [b] A New Drug Application (NDA) for vildagliptin was submitted to FDA (American Food and Drug Administration) and EMEA (European Medicines Evaluation Agency) in 2006, '-' = information not available.

5. INHIBITORS OF DPP IV ACTIVITY

5.1. Preclinical studies

In the late 1990s the first DPP IV inhibitors were developed and tested in animal models of type 2 diabetes. One of the initial studies demonstrated that oral administration of P32/98 (isoleucine thiazolidide), improved glucose tolerance in Zucker fatty rats (88). The effectiveness of another inhibitor vildagliptin (LAF-237) to control glycaemia was similarly demonstrated (89). These studies indicated that effects were dose-dependent and caused no tachyphylaxis over the 3 weeks treatment period (89). NVP-DPP728 similarly improved glucose tolerance and the mechanism was verified by the presence of higher plasma levels of GLP-1(7-36)amide (90). In high fat fed mice, valine-pyrrolidide potentiated the plasma insulin response to intra-gastric glucose and improved glucose tolerance (91). Interestingly, valine-pyrrolidide did not affect glucose-stimulated insulin secretion from isolated islets, suggesting the mechanism was indirect, although enhancement of GLP-1 (91) and GIP (92) activity was confirmed. Investigation of DPP IV inhibitor in isolated perfused porcine ileum revealed that active levels of secreted GLP-1 could be increased from ~50% to over 85% (93).

Chronic treatment (12 weeks) of Zucker rats with P32/98 improved glucose tolerance and increased plasma insulin levels, but did not lead to any measurable changes in beta cell mass or islet morphology (94). P32/98 reduced body weight gain, an effect which was associated with GLP-1 activity, but there was no change in food intake. An almost identical P32/98 study indicated that hepatic and peripheral insulin sensitivity was significantly improved (95).

Preclinical studies have also demonstrated that DPP IV inhibitors are most effective in mild to moderate models of diabetes but fail to control glycaemia in severely hyperglycaemic mice (79, 96). The effects of DPP IV inhibitors in combination with other antidiabetic drugs have been examined in diabetic mouse models. Combining vildagliptin with rosiglitazone did not provide any additional efficacy to rosiglitazone alone, but it did appear to reduce common side-effects such as weight gain and haemodilution (96). Studies combining a DPP IV inhibitor with an alpha-glucosidase inhibitor have demonstrated additive improvements of glucose tolerance and increased active GLP-1 levels (97).

5.2. Clinical studies

Following the encouraging results of studies in animal models, there is now a substantial list of DPP IV inhibitors in clinical development with at least 8 others at earlier stages (Table 3). These have received the class name of 'gliptins' or 'incretin enhancers' (21, 30, 77, 98). The first gliptin to be approved for clinical use and marketed as a treatment for type 2 diabetes is sitagliptin (*Januvia*), launched in Mexico in 2006, and in the USA and several European countries in 2007. Vildagliptin (*Galvus*) was submitted for evaluation by the regulatory agencies in 2006. Two further gliptins, saxagliptin and alogliptin, are advanced in phase III clinical development.

As discussed above, the antidiabetic effect of DPP IV inhibitors relates mainly to increased nutrient-stimulated (prandial) insulin secretion. This is particularly effective in lowering postprandial hyperglycaemia, but there is a substantial carry-over effect to benefit the control of interprandial glycaemia (21, 77, 98). However, DPP IV inhibitors do not increase basal insulin secretion, and only suppress glucagon secretion in the hyperglycaemic state, so there is low risk of interprandial 'over-shoot' into hypoglycaemia. To assess overall long-term glycaemic control in diabetic patients it is customary to measure the percentage of glycated haemoglobin (HbA1c): the normal range is <6.0%, and the target for treatment of diabetic patients is usually to achieve a sustained HbA1c < 6.5-7.5% depending on country and regional guidelines.

5.2.1. Sitagliptin

Sitagliptin is a piperazine derivative with a high (~87%) bioavailability, rapid absorption in man (Tmax 1-4h) and low (~38%) plasma protein binding. Near complete inhibition of DPP IV activity for about 12h is achieved in man with a single daily dose of 100 mg. Clinical trials to assess the efficacy, tolerability and safety of sitagliptin in type 2 diabetes patients have recently been reported (98, 99). The long-term effects on HbA1c when sitagliptin is administered as monotherapy or add-on therapy to certain other types of antidiabetic agents are listed in Table 4 (100-104). Sitagliptin typically reduced

Table 4. Long-term (≥24 weeks) clinical trials with the DPP IV inhibitor sitagliptin (Januvia) in patients with type 2 diabetes

Study design	Duration (weeks)	n	Dose (mg/day)	Other antidiabetic treatment	Mean Baseline HbA1c	Placebo subtracted decrease in HbA1c	Ref
RDBPC	24	741	100	Diet only	8.0 %	↓ 0.79 %	100
			200	Diet only	8.0 %	↓ 0.94 %	
RDBPC	24	701	100	Diet + metformin	7.96 %	↓ 0.65 %	101
RDBPC	24	353	100	Diet + pioglitazone	8.05 %	↓ 0.70 %	102
RDBPC	24	340[a]	100	Diet only	8.8 %	↓ 0.83 %	103
RDBAC	52	1,172	100	Diet + metformin	7.5%	↓ 0.67 %	104

Adapted with permission from 98, [a]Number is the diet+placebo and the diet+sitagliptin arms only, [b]Same decrease in HbA1c (0.67%) for diet+metformin+sitagliptin as for diet+metformin+glipizide. RDBPC = randomised double blind placebo control. RDBAC = randomised double blind active comparator.

Table 5. Long-term (≥24 weeks) clinical trials with the DPP IV inhibitor vildagliptin (Galvus) in patients with type 2 diabetes

Study design	Duration (weeks)	n	Dose (mg/day)	Other antidiabetic treatment	Mean Baseline HbA1c	Placebo subtracted decrease in HbA1c	Ref
RDBPC	52	107	100	Diet + metformin	7.7 %	↓ 1.1 %	105
RDBPC	24	463	50	Diet + pioglitazone	8.7 %[a]	↓ 0.8 %	106
			100	Diet + pioglitazone	8.7 %[a]	↓ 1.0 %	
RDBPC	24	607[b]	100	Diet only	8.7 %	↓ 1.1 %	107,108
RDBAC	52	780	100	Diet + metformin	8.7 %	↓ 1.0 %[c]	109
RDBPC	24	544	50	Diet + metformin	≥7.4 %	↓ 0.7 %	110
			100	Diet + metformin		↓ 1.1 %	

Adapted with permission from 98, [a]Approximate HbA1c value estimated from illustration, [b]Number is the diet+placebo and the diet+vildagliptin arms only, [c]Decrease in HbA1c was 1.0% diet+vildagliptin versus 1.4% for diet+metformin. RDBPC = randomised double blind placebo control. RDBAC = randomised double blind active comparator.

HbA1c (from a baseline of ~ 8%) by about 0.7 – 0.8% after 24-52 weeks. Efficacy was similar whether the sitagliptin was taken as monotherapy or add-on therapy to metformin or a thiazolidinedione. In these trials fasting plasma glucose concentrations were reduced by about 1.0 – 1.5 mmol/L, and postprandial glucose levels measured 2 hours after a standard mixed meal were usually reduced by about 3 mmol/L.

Consistent with the increase in nutrient-stimulated (but not basal) insulin secretion, sitagliptin therapy did not cause a clinically significant increase in the incidence of reported hypoglycaemia in any of the trials. Indeed, a combination of sitagliptin + metformin for 52 weeks was associated with only 4.9% of patients reporting hypoglycaemia events compared with 32% of patients receiving glipizide + metformin (104). This was achieved with similar overall levels of glycaemic control in the two groups as indicated by HbA1c. It is also noteworthy that [delete space] sitagliptin did not increase body weight compared to placebo in any of the trials.

Parameters of tolerability and the adverse events profile for sitagliptin were generally similar to the placebo or comparator groups in these trials, providing no signals for concern in these patient groups over these time periods. Despite the potential to slow gastric emptying, this does not seem to be a clinical issue as there was little reporting of abdominal discomfort or nausea. Patients receiving sitagliptin are required to have good renal function since the drug is mostly eliminated unchanged in the urine. However, the drug does not appear to induce or inhibit P450 isoforms, so it should have little effect on the metabolism of other drugs and can be used in patients with mild liver disease.

5.2.2. Vildagliptin and other gliptins

Vildagliptin is a cyanopyrrolidine: absorption is rapid, bioavailability is >90%, and there is extensive hepatic metabolism of the drug to metabolites that are mostly eliminated in the urine. Inhibition of DPP IV by >90% persists for more than 12h after a single daily dose of 100 mg, which is likely to be the preferred therapeutic dose. Vildagliptin has received a similar battery of clinical trials to sitagliptin and shown similar results when used as monotherapy or add-on to metformin or a thiazolidinedione (105-110; Table 5). Slightly greater lowering of HbA1c in some studies with vildagliptin may relate in part to a higher baseline (starting) HbA1c. As with sitagliptin there was low risk of hypoglycaemia and no effect on body weight compared to placebo.

5.3. Developmental issues

Initial preclinical development of DPP IV inhibitors as antidiabetic agents focused on their selectivity: studies in rodents, dogs and monkeys have suggested that possible interference with DPP 8/9 or other dipeptidyl peptidases could cause blood dyscrasias, gastrointestinal disturbances and various histopathological changes including skin lesions (29, 30). Inhibitors of DPP 8/9 have also reduced T-cell activation in human *in vitro* models. However the DPP IV inhibitors presently advanced in clinical development have shown highly selective and potent inhibition of DPP IV. They have exhibited sufficient potency and duration of action that therapeutic doses achieved nearly complete inhibition of DPP IV activity for >12h with a diminishing effect thereafter.

Although DPP IV inhibition could potentially affect a wide range of biological peptides, no apparent serious untoward effects have emerged during the

clinical trials with sitagliptin and vildagliptin. Nevertheless, the urinary metabolites of vildagliptin and possible interference with DPP 8/9 are receiving further investigation. Available information regarding other gliptins such as saxagliptin and alogliptin remain preliminary as these agents are proceeding in phase III clinical trials. As with all new drugs, phase IV pharmacovigilance will watch for any evidence of possible long-term side effects during large population clinical use. In the case of DPP IV inhibitors, particular attention will be given to the side effects seen in some animal studies (described above), and on effects that could relate to other peptide substrates (Table 1) as well as the immunological role of CD26 and its implications for infection and inflammatory disease. Since launch there have been occasional reports of hypersensitivity reactions to sitagliptin, including some necrolytic lesions of skin and mucous membranes similar to Stevens-Johnson syndrome.

5.4. Future outlook for DPP IV inhibitors

As clinical experience with sitagliptin (and other DPP IV inhibitors) increases, attention will undoubtedly focus on the durability of the antihyperglycaemic effect. Since incretin hormones have been associated with morphological integrity, proliferation and increased mass of islet beta-cells in experimental models (53-59), there is an expectation that DPP IV inhibitors might offer some recourse to prevent the progressive deterioration of beta-cell function and mass in human type 2 diabetes. Definitive answers are likely to require several years of thorough phase IV trials, and will probably rely heavily on assessments of overall glycaemic control and insulin responses to stimulatory tests. Currently available non-invasive measures of beta-cell mass are probably not accurate enough to provide the required information.

The present indication for DPP IV inhibitors as oral antidiabetic agents sees their use as second-line agents, mainly in combination with metformin or a thiazolidinedione. In this respect the ability of DPP IV inhibitors to increase nutrient-induced insulin secretion is complementary to the actions of metformin and thiazolidinediones to counter insulin resistance. There is also low risk of precipitating hypoglycaemia with these agents, favouring their use in patients with glucose values close to the normal range. Lack of weight gain is a further advantage, and the tolerability of DPP IV inhibitors seen in trials to date suggests that these agents will be acceptable to most patients. Additionally, DPP IV inhibitors do not appear to require dose titration or regular home blood glucose monitoring, which may be considered a potential cost saving, and their availability as once daily tablets could assist compliance.

6. SUMMARY AND PERSPECTIVE

In conclusion, information and awareness of the regulatory roles of DPP IV have increased dramatically in the last 20 years. A previously obscure yet ubiquitous enzyme is now the basis of a therapeutic approach against type 2 diabetes. Much of this can be accredited to initial observations relating to the therapeutic potential of GLP-1 and GIP and their rapid inactivation by DPP IV. Preclinical work involving the generation of animal models lacking DPP IV activity and DPP IV inhibitors compounds have demonstrated the potential of DPP IV as a target to alter glycaemic status. Furthermore, clinical studies involving DPP IV inhibitors so far indicate that these are efficacious and generally well tolerated compounds exhibiting apparently few adverse effects and low risk of hypoglycaemia. Although issues over enzyme selectivity remain, DPP IV inhibitors and incretin analogues appear to offer two fundamentally new classes of agents to improve glycaemic control in type 2 diabetes.

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8. REFERENCES

1. C.J. Bailey, P.R. Flatt, New Antidiabetic drugs, London: *Smith-Gordon,* (1990)
2. A. Dornhorst: Insulinotropic meglitinide analogues. *Lancet.* 358 (9294), 1709-1716 (2001)
3. K. Cusi, R.A. Defronzo, Metformin: A review of its metabolic effects. *Diabetes Rev* 6 (2), 89-131 (1998)
4. C.J. Bailey, R.C. Turner: Metformin. *N Engl J Med.* 334 (9):574-579 (1996)
5. Yki-Jarvinen H. Thiazolidinediones. N Engl J Med. 2004 Sep 9;351 (11):1106-18.
6. A.J. Krentz, C.J. Bailey: Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs. 65* (3):385-411 (2005)
7. Diabetes Atlas, 3rd edn. *International Diabetes Federation, Brussels,* 322pp, (2006)
8. D.M. Hargrove, N.A. Nardone, L.M. Persson, J.C. Parker, R.W. Stevenson: Glucose-dependent action of glucagon-like peptide-1 (7-37) *in vivo* during short- or long-term administration. *Metabolism.* 44 (9), 1231-1237 (1995)
9. D.K. Andersen, D. Elahi, J.C. Brown, J.D. Tobin, R. Andres: Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. *J Clin Invest.* 62 (1), 152-61 (1978)
10. J. Dupre, S.A. Ross, D. Watson, J.C. Brown: Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab.* 37 (5), 826-828 (1973)
11. D.J. Drucker. The role of gut hormones in glucose homeostasis. *J Clin Invest.* 117 (1):24-32 (2007)
12. J.J. Holst: Glucagon-like peptide-1: from extract to agent. *Diabetologia* 49, 253-260 (2006)
13. R. Thoma, B. Loffler, M. Stihle, W. Huber, A. Ruf, M. Hennig: Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. *Structure.* 11 (8), 947-959 (2003)
14. C.A. Abbott, M.D. Gorrell: The family of CD26/DPIV and related ectopeptidases. In

Ectopeptidases:CD13/Aminopeptidase N and CD26/Dipeptidyl peptidase IV in Medicine and Biology New York: Kluwer/Plenum, 2002 (Langer J and Ansorge S Eds) pp171-191.
15. D. Darmoul, M. Lacasa, I. Chantret, D.M. Swallow, G. Trugnan: Isolation of a cDNA probe for the human intestinal dipeptidylpeptidase IV and assignment of the gene locus DPP4 to chromosome 2. *Ann Hum Genet.* 54 (3), 191-197 (1990)
16. C. Hanski, T. Huhle, R. Gossrau, W. Reutter: Direct evidence for the binding of rat liver DPP IV to collagen *in vitro*. *Exp Cell Res.* 178 (1), 64-72 (1988)
17. H.C. Cheng, 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 (37), 24207-24215 (1998)
18. T. Hashikawa, 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 (1), 131-133 (2004)
19. T. Ishii, 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 U S A.* 98 (21), 12138-12143 (2001)
20. R. Mentlein: Dipeptidyl-peptidase IV (CD26)--role in the inactivation of regulatory peptides. *Regul Pept.* 85 (1), 9-24 (1999)
21. B.D. Green, P.R. Flatt, C.J. Bailey: Inhibition of dipeptidyl peptidase IV activity as a therapy of type 2 diabetes. *Expert Opin Emerging Drugs.* 11 (3): 525-539 (2006)
22. D.J. Drucker: Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes. *Expert Opin Investig Drugs.* 12 (1), 87-100 (2003)
23. S.A. Hinke, J.A. Pospisilik, H.U. Demuth, S. Mannhart, K. Kuhn-Wache, T. Hoffmann, E. Nishimura, R.A. Pederson, C.H. McIntosh: Dipeptidyl peptidase IV (DPIV/CD26) degradation of glucagon. Characterization of glucagon degradation products and DPIV-resistant analogs *J Biol Chem.* 275 (6), 3827-3834 (2000)
24. M.B. Maes, 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 (2), 315-324 (2005)
25. M.B. Maes, S. Scharpe, I. De Meester. Dipeptidyl peptidase II (DPPII), a review. *Clin Chim Acta.* 380 (1-2), 31-49 (2007)
26. B. Leiting, K.D. Pryor, J.K. Wu, F. Marsilio, R.A. Patel, C.S. Craik, J.A. Ellman, R.T. Cummings, N.A. Thornberry: Catalytic properties and inhibition of proline-specific dipeptidyl peptidases II, IV and VII. *Biochem. J.* 371, 525–532 (2003)
27. J.K. McDonald, F.H. Leibach, R.E. Grindeland, S. Ellis: Purification of dipeptidyl aminopeptidase II (dipeptidyl arylamidase II) of the anterior pituitary gland. Peptidase and dipeptide esterase activities. *J Biol Chem.* 243 (15), 4143-4150 (1968)
28. A. Stockel-Maschek, C. Mrestani-Klaus, B. Stiebitz, H. Demuth, K. Neubert: Thioxo amino acid pyrrolidides and thiazolidides: new inhibitors of proline specific peptidases. *Biochim Biophys Acta.* 1479 (1-2), 15-31 (2000)
29. G.R. Lankas, 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 (10), 2988-2994 (2005)
30. Y.J. Chyan, L.M. Chuang. Dipeptidyl peptidase IV inhibitors: an evolving treatment for type 2 diabetes from the incretin concept. *Recent Patents on Endocrine, Metabolic and Immune Drug Discovery* 1 (1): 15-24 (2007)
31. M.D. Gorrell: Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clin Sci (Lond)* 108 (4), 277-292 (2005)
32. M.B. Maes, V. Dubois, I. Brandt, A.M. Lambeir, P. Van der Veken, K. Augustyns, J.D. Cheng, X. Chen, S. Scharpe, I. De Meester: Dipeptidyl peptidase 8/9-like activity in human leukocytes. *J. Leukoc. Biol.* 81 (5):1252-1257, (2007)
33. J. Niedermeyer, B. Enenkel, J.E. Park, M. Lenter, W.J. Rettig, K. Damm, A. Schnapp: Mouse fibroblast-activation protein--conserved Fap gene organization and biochemical function as a serine protease. *Eur J Biochem.* 254 (3), 650-654 (1998)
34. K. Aertgeerts, I Levin, L. Shi, G.P. Snell, A. Jennings, G.S. Prasad, Y. Zhang, M.L. Kraus, S. Salakian, V. Sridhar, R. Wijnands, M.G: Tennant. Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein alpha. *J Biol Chem.* 280 (20), 19441-19444 (2005)
35. K.S. Lai, N.H. Ho, J.D. Cheng, C.H. Tung: Selective Fluorescence Probes for Dipeptidyl Peptidase Activity-Fibroblast Activation Protein and Dipeptidyl Peptidase IV. *Bioconjug Chem.* [Epub ahead of print] (2007)
36. M.J. Perley, D.M. Kipnis: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J Clin Invest.* 46 (12), 1954-1962 (1967)
37. R.H. Unger, A.M. Eisentraut: Entero-insular axis. *Arch Intern Med.* 123 (3), 261-266 (1969)
38. T. Vilsboll, T. Krarup, S. Madsbad, J.J. Holst: Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. *Regul Pept.* 114 (2-3), 115-121 (2003)
39. J.J. Holst, C. Orskov, O.V. Nielsen, T.W. Schwartz: Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett.* 211 (2), 169-174 (1987)
40. T.J. Kieffer, C.H. 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 (8), 3585-3596 (1995)
41. C. Orskov, L. Rabenhoj, A. Wettergren, H. Kofod, J.J. Holst: Tissue and plasma concentrations of amidated and

glycine-extended glucagon-like peptide I in humans. *Diabetes.* 43 (4), 535-539 (1994)
42. D. Elahi, D.K. Andersen, J.C. Brown, H.T. Debas, R.J. Hershcopf, G.S. Raizes, J.D. Tobin, R. Andres: Pancreatic alpha- and beta-cell responses to GIP infusion in normal man. *Am J Physiol.* 237 (2), E185-191 (1979)
43. H. Larsson, J.J. Holst, B. Ahren: Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. *Acta Physiol Scand.* 160 (4), 413-422 (1997)
44. N.N. Rudovich, H.J. Rochlitz, A.F. Pfeiffer: Reduced hepatic insulin extraction in response to gastric inhibitory polypeptide compensates for reduced insulin secretion in normal-weight and normal glucose tolerant first-degree relatives of type 2 diabetic patients. *Diabetes.* 53 (9), 2359-2365 (2004)
45. I. Valverde, M. Morales, F. Clemente, M.I. Lopez-Delgado, E. Delgado, A. Perea, M.L. Villanueva-Penacarrillo: Glucagon-like peptide 1: a potent glycogenic hormone. *FEBS Lett.* 349 (2), 313-316 (1994)
46. M.L. Villanueva-Penacarrillo, A.I. Alcantara, F. Clemente, E. Delgado, I. Valverde. Potent glycogenic effect of GLP-1 (7-36)amide in rat skeletal muscle. *Diabetologia.* 37 (11),1163-1166 (1994)
47. F.P.M. O'Harte, A.M. Gray, Y.H. Abdel-Wahab, P.R. Flatt: Effects of non-glycated and glycated glucagon-like peptide-1 (7-36) amide on glucose metabolism in isolated mouse abdominal muscle. *Peptides.*18 (9), 1327-1333 (1997)
48. D. Elahi, G.S. Meneilly, K.L. Minaker, J.W. Rowe, D.K. Andersen: *Can. J. Physiol. Pharmacol.* 65, 18 (1986)
49. F.P.M. O'Harte, Y.H. Abdel-Wahab, J.M. Conlon, P.R. Flatt: Amino terminal glycation of gastric inhibitory polypeptide enhances its insulinotropic action on clonal pancreatic B-cells. *Biochim Biophys Acta.* 1425 (2), 319-327 (1998)
50. P.K. Chelikani, A.C. Haver, R.D. Reidelberger: Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol.* 288 (6), R1695-1706 (2005)
51. T.J. Little, A.N. Pilichiewicz, A. Russo, L. Phillips, K.L. Jones, M.A. Nauck, J. Wishart, M. Horowitz, C. Feinle-Bisset: Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. *J Clin Endocrinol Metab.* 91 (5), 1916-1923 (2006)
52. V. Maxwell, A. Shulkes, J.C. Brown, T.E. Solomon, J.H. Walsh, M.I. Grossman MI.Effect of gastric inhibitory polypeptide on pentagastrin-stimulated acid secretion in man. *Dig Dis Sci.* 25 (2):113-116 (1980)
53. D.J. Drucker: Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. *Mol Endocrinol.* 17 (2), 161-171 (2003)
54. J. Buteau, M.L. Spatz, D. Accili: Transcription factor FoxO1 mediates glucagon-like peptide-1 effects on pancreatic beta-cell mass. *Diabetes.* 55 (5), 1190-1196 (2006)
55. B.D. Green, K.S. Lavery, N. Irwin, F.P.M. O'Harte, P. Harriott, B. Greer, C.J. Bailey, P.R. Flatt: Novel glucagon-like peptide-1 (GLP-1) analog (Val^8)GLP-1 results in significant improvements of glucose tolerance and pancreatic beta-cell function after 3-week daily administration in obese diabetic (ob/ob) mice. *J Pharmacol Exp Ther.* 318 (2), 914-921 (2006)
56. D.A. Stoffers: The development of beta-cell mass: recent progress and potential role of GLP-1. *Horm Metab Res.* 36 (11-12), 811-821 (2004)
57. S.J. Kim, K. Winter, C. Nian, M. Tsuneoka, Y. Koda, C.H. 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 (23), 22297-222307 (2005)
58. J.A. Ehses, V.R. Casilla, T. Doty, J.A. Pospisilik, K.D. Winter, H.U. Demuth, R.A. Pederson, C.H. 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 (10):4433-4445 (2003)
59. A. Trumper, K. Trumper, D. Horsch: Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta (INS-1)-cells. *J Endocrinol.* 174 (2), 233-246.
60. W.O. Creutzfeldt, N. Kleine, B. Willms, C. Orskov, 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 (6), 580-586 (1996)
61. R. Mentlein, 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 (3), 829-835 (1993)
62. C.F. Deacon, A.H. Johnsen, J.J. Holst: Degradation of glucagon-like peptide-1 by the 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)
63. L. Pridal, C.F. Deacon, O. Kirk, J.V. Christensen, R.D. Carr, J.J. Holst: Glucagon-like peptide-1 (7-37) has a larger volume of distribution than glucagon-like peptide-1 (7-36)amide in dogs and is degraded more quickly *in vitro* by dog plasma. *Eur J Drug Metab Pharmacokinet.* 21 (1), 51-59 (1996)
64. L.B. Knudsen, L. Pridal: Glucagon-like peptide-1- (9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after *in vivo* administration to dogs, and it acts as an antagonist on the pancreatic receptor. *Eur J Pharmacol.* 318 (2-3), 429-435 (1996)
65. W.E. Schmidt, E.G. Siegel, H. Kummel, B. Gallwitz, W. Creutzfeldt: Commercially available preparations of porcine glucose-dependent insulinotropic polypeptide (GIP) contain a biologically inactive GIP-fragment and cholecystokinin-33/-39. *Endocrinology.* 120 (2), 835-837 (1987)
66. B.D. Green, M.H. Mooney, V.A. Gault, N. Irwin, C.J. Bailey, P. Harriott, B. Greer, P.R. Flatt, F.P.M O'Harte: Lys9 for Glu9 substitution in glucagon-like peptide-1 (7-36)amide confers dipeptidylpeptidase IV resistance with

cellular and metabolic actions similar to those of established antagonists glucagon-like peptide-1 (9-36)amide and exendin (9-39) *Metabolism.* 53 (2), 252-259 (2004)
67. A. Wettergren, M. Wojdemann, J.J. Holst: The inhibitory effect of glucagon-like peptide-1 (7-36)amide on antral motility is antagonized by its N-terminally truncated primary metabolite GLP-1 (9-36)amide. *Peptides.*19 (5), 877-882 (1998)
68. C.F. Deacon, A. Plamboeck, S. Moller, J.J. Holst: GLP-1- (9-36) amide reduces blood glucose in anesthetized pigs by a mechanism that does not involve insulin secretion. *Am J Physiol Endocrinol Metab.* 282 (4), E873-879 (2002)
69. B. Rolin, C.F. Deacon, R.D. Carr, B. Ahren: The major glucagon-like peptide-1 metabolite, GLP-1- (9-36)-amide, does not affect glucose or insulin levels in mice. *Eur J Pharmacol.* 494 (2-3), 283-288 (2004)
70. J.C. Parker, K.S. Lavery, N. Irwin, B.D. Green, B. Greer B, P. Harriott, F.P.M. F.P.M. O'Harte, V.A. Gault, P.R. Flatt: Effects of sub-chronic exposure to naturally occurring N-terminally truncated metabolites of glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), GIP (3-42) and GLP-1 (9-36)amide, on insulin secretion and glucose homeostasis in ob/ob mice. *J Endocrinol.* 191 (1), 93-100 (2006)
71. C.F. Deacon, A. Plamboeck, M.M. Rosenkilde, J. de Heer, J.J. Holst: GIP- (3-42) does not antagonize insulinotropic effects of GIP at physiological concentrations. *Am J Physiol Endocrinol Metab.* 291 (3), E468-475 (2006)
72. V.A. Gault, J.C. Parker, P. Harriott, P.R. Flatt, F.P.M. O'Harte: Evidence that the major degradation product of glucose-dependent insulinotropic polypeptide, GIP (3-42), is a GIP receptor antagonist *in vivo*. *J Endocrinol.* 175 (2):525-533 (2002)
73. S.A. Hinke, R.W. Gelling, R.A. Pederson, S. Manhart, C. Nian, H.U. Demuth, C.H. McIntosh: Dipeptidyl peptidase IV-resistant [D-Ala (2)]glucose-dependent insulinotropic polypeptide (GIP) improves glucose tolerance in normal and obese diabetic rats. *Diabetes.* 51 (3), 652-661 (2002)
74. B.D. Green, V.A. Gault, F.P.M. O'Harte, P.R. Flatt: Structurally modified analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) as future antidiabetic agents. *Curr Pharm Des.*10 (29), 3651-3662 (2004)
75. C. Gonzalez, V. Beruto, G. Keller, S. Santoro, G. Di Girolamo: Investigational treatments for Type 2 diabetes mellitus: exenatide and liraglutide. *Expert Opin Investig Drugs.* 15 (8), 887-895 (2006)
76. B.D. Green, P.R. Flatt, C.J. Bailey: Inhibition of dipeptidylpeptidase IV activity as a therapy of type 2 diabetes. *Expert Opin Emerg Drugs.* 11 (3), 525-539 (2006)
77. B.D. Green, P.R. Flatt, C.J. Bailey: Dipeptidyl peptidase IV (DPP IV) inhibitors: a newly emerging drug class for the treatment of type 2 diabetes. *Diabetes Vasc Dis Res.* 3 (3): 159-65 (2006)
78. D. Marguet, L. Baggio, T. Kobayashi, A.M. Bernard, M. Pierres, P.F. Nielsen, U. Ribel, T. Watanabe, D.J. Drucker, N. Wagtmann: Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci U S A.* 97 (12):6874-6879 (2000)
79. T. Nagakura, N. Yasuda, K. Yamazaki, H. Ikuta, I. Tanaka: Enteroinsular axis of db/db mice and efficacy of dipeptidyl peptidase IV inhibition. *Metabolism.* 52 (1), 81-86 (2003)
80. S.L. Conarello, Z. Li, J. Ronan, R.S. Roy, L. Zhu, G. Jiang, F. Liu, J. Woods, E. Zycband, 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 U S A.* 100 (11), 6825-6730 (2003)
81. N. Yasuda, T. Nagakura, K. Yamazaki, T. Inoue, I. Tanaka: Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats. *Life Sci.* 71 (2), 227-238 (2002)
82. R. Guieu, 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 (2), 230-235 (2006)
83. C. Kruschinski, 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 (1), 17-24 (2005)
84. V. Preller, A. Gerber, S. Wrenger, M. Togni, D. Marguet, J. Tadje, U. Lendeckel, C. Rocken, J. Faust, K. Neubert, B. Schraven, R. Martin, S. Ansorge, S. Brocke, D. Reinhold: TGF-beta1-mediated control of central nervous system inflammation and autoimmunity through the inhibitory receptor CD26. *J Immunol.* 178 (7), 4632-4640 (2007)
85. S. Yan, D. Marguet, J. Dobers, W. Reutter, H. Fan: Deficiency of CD26 results in a change of cytokine and immunoglobulin secretion after stimulation by pokeweed mitogen. *Eur J Immunol.* 33 (6), 1519-1527 (2003)
86. J.B. Byrd JB, A. Shreevatsa A, P. Putlur, D. Foretia, L. McAlexander, T. Sinha, M.D. Does, N.J. Brown: Dipeptidyl peptidase IV deficiency increases susceptibility to angiotensin-converting enzyme inhibitor-induced peritracheal edema. *J Allergy Clin Immunol.* 2007 [Epub ahead of print].
87. N. Busso, N. Wagtmann, C. Herling, V. Chobaz-Peclat, A. Bischof-Delaloye, A. So, E. Grouzmann: Circulating CD26 is negatively associated with inflammation in human and experimental arthritis. *Am J Pathol.* 166 (2), 433-442 (2005)
88. R.A. Pederson, H.A. White, D. Schlenzig, R.P. Pauly, C.H. McIntosh, H.U. Demuth: Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes.* 47 (8), 1253-1258 (1998)
89. B.F. Burkey, 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 Ther.* 315 (2), 688-695 (2005)
90. B. Balkan, 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 (11), 1324-1331 (1999)

91. B. Ahren, 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 (1-2), 239-245 (2000)
92. C.F. Deacon, P. Danielsen, L. Klarskov, M. Olesen, J.J. Holst: Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes.* 50 (7), 1588-1597 (2001)
93. L. Hansen, 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 (11), 5356-5363 (1999)
94. J.A. Pospisilik, S.G. Stafford, H.U. Demuth, R. Brownsey, W. Parkhouse, D.T. Finegood, C.H. 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 (4), 943-950 (2002)
95. J.A. Pospisilik, 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 (9), 2677-2683 (2002)
96. S. Roy, V. Khanna, S. Mittra, A. Dhar, S. Singh, D.C. Mahajan, P. Priyadarsiny, J.A. Davis, J. Sattigeri, K.S. Saini, V.S. Bansal: Combination of dipeptidylpeptidase IV inhibitor and low dose thiazolidinedione: Preclinical efficacy and safety in db/db mice. *Life Sci.* [Epub ahead of print] (2007)
97. K. Yamazaki, T. Inoue, N. Yasuda, Y. Sato, T. Nagakura, O. Takenaka, R. Clark, T. Saeki, I. Tanaka: Comparison of efficacies of a dipeptidyl peptidase IV inhibitor and alpha-glucosidase inhibitors in oral carbohydrate and meal tolerance tests and the effects of their combination in mice. *J Pharmacol Sci.* 104 (1), 29-38 (2007)
98. B.D. Green, P.R. Flatt, C.J. Bailey: Gliptins: dipeptidyl peptidase-4 (DPP-4) inhibitors to treat type 2 diabetes. *Future Prescriber* in press (2007)
99. I.W. Campbell, C. Day: Sitagliptin – enhancing incretin action. *Br J Diabetes Vasc Dis.* 7 (3), 134-139 (2007)
100. P. Aschner, M.S. Kipnes, J.K. Lunceford, M. Sanchez, C. Mickel, D.E. Williams-Herman: Sitagliptin Study 021 Group. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes.*Diabetes Care.* 29 (12):2632-2637 (2006)
101. B. Charbonnel, A. Karasik, J. Liu, M. Wu, G. Meininger : Sitagliptin Study 020 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 (12):2638-2643 (2006)
102. J. Rosenstock, R. Brazg, P.J. Andryuk, K. Lu, P. Stein: Sitagliptin Study 019 Group. 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 Ther.* 28 (10):1556-1568 (2006)
103. B.J. Goldstein, M.N. Feinglos, J.K. Lunceford, J. Johnson, D.E. Williams-Herman. Effect of Initial Combination Therapy with Sitagliptin, a Dipeptidyl Peptidase-4 Inhibitor, and Metformin on Glycemic Control in Patients with Type 2 Diabetes. *Diabetes Care.* [Epub ahead of print] (2007)
104. M.A. Nauck, G. Meininger, D. Sheng, L. Terranella, P.P. Stein: Sitagliptin Study 024 Group. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, non-inferiority trial. *Diabetes Obes Metab.* 9 (2):194-205 (2007)
105. B. Ahren, 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 (12): 2874-2880 (2004)
106. A.J. Garber, A. Schweizer, M.A. Baron, E. Rochotte, S. Dejager: Vildagliptin in combination with pioglitazone improves glycaemic control in patients with type 2 diabetes failing thiazolidinedione monotherapy: a randomized, placebo-controlled study. *Diabetes Obes Metab.* 9 (2):166-174 (2007)
107. J. Rosenstock, M.A. Baron, R.P. Camisasca, F. Cressier, A. Couturier, S. Dejager: Efficacy and tolerability of initial combination therapy with vildagliptin and pioglitazone compared with component monotherapy in patients with type 2 diabetes. *Diabetes Obes Metab.* 9 (2):175-185 (2007)
108. J. Rosenstock, 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 (2):217-223 (2007)
109. A. Schweizer, A. Couturier, J. Foley, S. Dejagers: Comparison between vildagliptin and metformin to sustain reductions in HbA1c over 1 year in drug-naïve patients with type 2 diabetes. *Diabetic Med.* Epub ahead of print PMID17509069 to be completed at proof (2007)
110. E. Bosi, 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 (4):890-895 (2007)
111. R.M. Elliott, L.M. Morgan, J.A. Tredger, S. Deacon, J. Wright, V. Marks. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol.* 138 (1), 159-166 (1993)
112. C. Herrmann, R. Goke, G. Richter, H.C. Fehmann, R. Arnold, B. Goke: Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion.*56 (2), 117-126 (1995)
113. M.B. Toft-Nielsen, S. Madsbad, J.J. Holst: Continuous subcutaneous infusion of glucagon-like peptide 1 lowers

plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care.* 1999 22 (7), 1137-1143 (2004)
114. F.P.M. O'Harte, M.H. Mooney, P.R. Flatt: NH_2-terminally modified gastric inhibitory polypeptide exhibits amino-peptidase resistance and enhanced antihyperglycemic activity. *Diabetes.* 48 (4), 758-765 (1999)
115. S.A. Hinke, R.W. Gelling, R.A. Pederson, S. Manhart, C. Nian, H.U. Demuth, C.H. McIntosh: Dipeptidyl peptidase IV-resistant [D-Ala (2)]glucose-dependent insulinotropic polypeptide (GIP) improves glucose tolerance in normal and obese diabetic rats. *Diabetes.* 51 (3):652-661 (2002)
116. E. Naslund, 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 (3), 525-530 (1998)
117. M.D. Turton, 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 (6560):69-72, (1996)

Abbreviations: DPP: dipeptidyl peptidase, FAP: fibroblast activation protein, GIP: glucose-dependent insulinotropic polypeptide, GLP-1: glucagon-like peptide-1, QPP: quiescent cell proline dipeptidase

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