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Commentary

DPP4 inhibitors for diabetes—What next?

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ABSTRACT

With vildagliptin and sitagliptin on the market for the treatment of type 2 diabetes, dipeptidyl peptidase 4 (DPP4, EC 3.4.14.5) research has entered a new era. Scientists aim to uncover the broader pharmacological profile of DPP4 inhibitors and search for therapeutic opportunities outside diabetes. During the pre-clinical and clinical evaluation of vildagliptin and sitagliptin, there has been a growing awareness of the presence of other DPP4-like peptidases in various cells and tissues. This fuelled the development of more inhibitors with defined selectivity for DPP2, 8 and 9 that were used to investigate the expression, distribution and regulation of these peptidases. In turn, these studies increased the insights in the role of DPP4 in the body's response to various insults.

1. DPP4 inhibitors for diabetes

Two dipeptidyl peptidase 4 (DPP4, EC 3.4.14.5) inhibitors are currently used for the treatment of type 2 diabetes (T2DM): vildagliptin (Galvus®, Novartis International AG, Basel, Switzerland) and sitagliptin (Januvia®, Merck & Co., Inc., Whitehouse Station, NJ, USA). Sitagliptin was approved by the FDA and EMEA for the treatment of T2DM patients who fail to achieve hyperglycemic control with diet and exercise, alone or in combination with another drug such as metformin or a glitazone. At the moment vildagliptin is only approved by the EMEA in Europe for combined treatment with other antidiabetic medications including metformin, sulfonylureas and thiazolidinediones. Several other DPP4 inhibitors are expected to become available in the near future; however, given that vildagliptin and sitagliptin were first to be developed, most reports of clinical trials relate to these two compounds. Updates and analyses of the performance of sitagliptin, vildagliptin and other awaited incretin-enhancing drugs are regularly published in field-specific literature (for recent reviews see [1–4]) and will not be repeated here. Instead, this paper will reflect on DPP4-related research that may have an impact on the future development of this class of drugs. The writing of this commentary coincided with the organization of the 3rd International Conference on Dipeptidyl Peptidases and Related Enzymes in Antwerp on 23–25 April 2008 [5].

Sitagliptin and vildagliptin, taken orally once or twice a day at indicated doses, lower post-prandial and fasting glucose levels. After prolonged use, T2DM patients have lower glycated hemoglobin levels indicative of an overall

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Abbreviations: BNP, brain-derived natriuretic peptide; DPP, dipeptidyl peptidase; G-CSF, granulocyte-colony stimulating factor; GIP, glucose dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; HIF-1, hypoxia inducible factor-1; HOMA-β, homeostasis model assessment β-cell function index; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating peptide; PYY, peptide YY; SDF-1, stromal cell derived factor-1; T2DM, type 2 diabetes.

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reduction of blood glucose over time. Clinical studies also show a significant increase in homeostasis model assessment beta-cell function index (HOMA-β) and fasting proinsulin/insulin ratio, which are markers of insulin secretion and beta-cell function [1,2]. Whether these results provide evidence for a pancreas-sparing effect of DPP4 inhibitors in the clinic remains an open question. In general, vildagliptin and sitagliptin are well tolerated. The incidence of hypoglycemia is low and few adverse effects were reported. DPP4 inhibitors are considered to be moderately effective, lowering HbA1c levels between 0.6 and 0.8% (compared to placebo) in patients with starting HbA1c levels between 6.5 and 10%. It is too soon to predict what their performance will be in a more diverse patient population. In a direct comparison with traditional antihyperglycemic drugs, the efficacy of these agents scored somewhat lower than metformin but similar to rosiglitazone and glizipide [6]. In combination with other glucose lowering drugs, such as metformin, sulfonylureas and thiazolidinediones, the effect of DPP4 inhibitors is additive [1,2,6].

The glucose-lowering action of DPP4 inhibitors derives from the prolongation of the active half-life of the incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). The incretins are peptide hormones secreted by intestinal endocrine cells upon food ingestion. Their immediate effect is to reduce the production of glucagon and to increase insulin secretion by pancreatic beta-cells. DPP4 proteolytically removes two amino acids from the N-terminal end of the incretins, thereby abolishing the interaction with their cognate receptors. Because DPP4 is present at the site of production, cleavage of the incretins starts almost immediately after their secretion, and this process has been shown to be an important determinant of the biological half-life of GLP-1 and GIP. Inhibition of DPP4 increases the life-time of the active forms by a few minutes before they are degraded by other proteases and cleared from the circulation [7].

Many T2DM patients produce less than normal amounts of GLP-1 and appear to have an impaired response to GIP [8]. The efficacy of DPP4 inhibition in diabetes is attributed to an increased level of GLP-1. However, DPP4 inhibition differs from the pharmacological application of GLP-1 in several respects. Firstly, the levels of GLP-1 that may be achieved are restricted by secretion rate and intracellular pools of precursors and do not increase linearly with the degree of DPP4 inhibition. Because the secretion of incretins is glucose dependent, DPP4 inhibition is not expected to cause hypoglycemia in the fasting state. This may account for the lower incidence of hypoglycemia and the milder side effects found with DPP4 inhibition as compared to injection of a GLP-1 analog (which was recently introduced for T2DM management). Secondly, DPP4 cleaves many other peptides aside from GLP-1 and GIP [9,10]. Inhibition of DPP4 may also prolong the active half-life of these peptides and modulate their receptor interactions. Enhancement of the actions of additional substrates may be beneficial for glucose control and beta-cell function or other aspects of the disease. Lastly, every DPP4 inhibitor needs to be investigated individually because it may have a unique pharmacodynamic profile and compound-specific targets.

2. What next?

2.1. *Uncovering the full pharmacological spectrum of DPP4 inhibitors*

2.1.1. *Discovering new therapeutic niches*

In the coming years, the use of DPP4 inhibitors in the treatment of T2DM is expected to increase dramatically. Clinical investigators are keen to record effects on body weight, lipid levels, cardiovascular parameters and other clinically significant outcomes. Like GLP-1, several other DPP4 substrates or their analogs are currently being evaluated for therapeutic application in different disease settings, for example, brain-derived natriuretic peptide (BNP) for treatment of heart failure, pituitary adenylate cyclase activating peptide (PACAP) for treatment of neurodegenerative disease and stromal cell derived factor-1 (SDF-1) for use in tissue regeneration [11–13]. Whether the prolongation of the biological half-life of these and other peptides will be of therapeutic value depends on whether the peptides are involved in the pathophysiology of the disease and whether DPP4 is present at the sites where they are produced or exert their activity. Truncated forms of most of the reported substrates of DPP4 can be found in plasma, tissue extracts or other biological samples, such as cell culture supernatants; however, it is difficult to ascertain whether the truncation occurs in vivo or ex vivo. There are relatively few peptides for which there is solid experimental evidence that they are cleaved in vivo by DPP4. A selection is shown in Table 1 summarizing the relevant biological activities of the intact peptides and the DPP4-generated metabolites [9,10,14–16]. Most of the information of Table 1 comes from experiments with laboratory animals. Apart from studies of GLP-1, few data are available about changes in levels or ratios of truncated peptide forms in humans treated with sitagliptin or vildagliptin. To identify relevant in vivo substrates of DPP4, there is a need for tools to measure specific sets of truncated and intact peptides in large numbers of clinical samples. Recent technological developments in the field of mass spectrometry have allowed for the development of differential peptide profiling assays that have the potential to detect and identify endogenous DPP4 substrates in human plasma [17]. Currently, the reported methods have not been able to detect validated DPP4 substrates such as GLP-1, which are typically present in very low (picomolar) concentrations. However, the approach is promising for the identification of biomarkers for DPP4 activity in plasma, selectivity/efficacy profiles of specific DPP4 inhibitors and assays to determine several peptide/DPP4-metabolite ratios simultaneously in clinical samples. The full pharmacological spectrum of DPP4 inhibitors still remains to be discovered.

There are already indications that DPP4 inhibitors achieve more than just a lowering of glucose levels after meals. GLP-1 is a pleotropic peptide with many other activities [14]. Prolonged near-complete systemic inhibition of DPP4 results in an increased mass of pancreatic beta-cells in animals. This has been attributed to the growth promoting and anti-apoptotic action of GLP-1 and may be reflected in the positive outcome of DPP4 inhibitors in beta-cell performance and insulin sensitivity in patients [1–4,8].

Table 1 – Selected peptides for which there is experimental evidence for in vivo cleavage by DPP4

Substrates	Relevant biological activities	
	Intact peptide	DPP4-generated metabolite
Glucagon-like peptide-1 (GLP-1)	Glucose dependent insulin secretion. Regulation of insulin biosynthesis. Inhibition of glucagon secretion. Beta-cell neogenesis, proliferation, apoptosis. Inhibition of gastric emptying and gastric acid secretion. Satiety, weight loss. Cardioprotection, vasodilation	Inactive. GLP-1 receptor antagonist (?). Insulin-independent glucose clearance. Cardioprotection, vasodilation
Glucose dependent insulinotropic peptide (GIP)	Glucose dependent insulin secretion. Regulation of insulin biosynthesis. Beta-cell neogenesis, proliferation, apoptosis. Inhibition of gastric acid secretion. Lipogenesis, lipid storage. Bone formation	Weak antagonist
Glucagon-like peptide-2 (GLP-2)	Intestinal growth factor. Increase of intestinal glucose transport. Inhibition of gastric emptying and gastric acid secretion. Inhibition of food intake	Inactive
Pituitary adenylate cyclase activating polypeptide (PACAP)	Glucose control (insulin secretion), islet function. Lipid metabolism. Adaptive thermogenesis. Smooth muscle cell relaxation, vasodilation	Inactive
Neuropeptide Y	In the central nervous system: anxiolytic and anti-epileptic action, inhibition of stress response, stimulation of food intake In the peripheral nervous system: stimulation of stress response, vasoconstriction, vascular smooth muscle cell proliferation, hyperlipidemia, glucose intolerance	Altered receptor subtype specificity
	Actions mainly mediated by Y_1 receptor (during stress, vascular injury or ischemia): atherosclerosis/restenosis	Actions mediated by the Y_2/Y_5 receptors (during stress, vascular injury or ischemia): angiogenesis
Peptide YY	Intestinal vasoconstriction (Y_1 receptor)	Altered receptor subtype specificity (Y_2)
	Inhibition of intestinal fluid secretion, gut motility and exocrine pancreas secretion (Y_2 receptor)	Inhibition of intestinal fluid secretion, gut motility and exocrine pancreas secretion. Appetite loss, reduction of food intake
SDF-1α/β (CXCL12)	Stem cell mobilization and engraftment (CXCR4 receptor) Hematopoiesis, lymphocyte homing, B-cell growth, angiogenesis	Inactive (CXCR4 receptor)
Brain derived natriuretic peptide (BNP)	Vasodilation. Natriuresis. Suppression of renin secretion. Relaxation of cardiac muscle and chambers. Inhibition of fibrosis. Actions of the cGMP-linked natriuretic type A receptor	Reduced diuretic, natriuretic and cGMP producing properties. Lacks vasodilating actions

The use of vildagliptin and sitagliptin does not cause an increase in body weight as is often seen with other glucose lowering therapies. A decrease in body weight could have been predicted because GLP-1 delays gastric emptying and increases gastric volume [14]. This creates a feeling of satiety but may also cause nausea and gastrointestinal problems, common side effects in clinical studies of GLP-1 analogs. That this type of side effect is less severe with DPP4 inhibition correlates well with the report of Vella et al., which states that vildagliptin does not alter gastric volume or satiety in T2DM patients [18]. The paper suggests that compensatory changes in enteroendocrine secretion of other peptides may account for this result. GIP is a possible candidate since it also affects satiety and appetite. Another DPP4 substrate that is often mentioned in this context is peptide YY (PYY). Vella et al. found that PYY secretion was decreased in patients using DPP4 inhibitors [18]. These observations may account for the fact that vildagliptin and sitagliptin are weight neutral. PYY shares receptors with neuropeptide Y (NPY), the most abundant neuropeptide in the central and peripheral nervous system and a substrate of DPP4.

Neuropeptide Y is also involved in energy homeostasis, but its main function is the regulation of stress [15]. It acts anxiolytically in the central nervous system and peripherally as a vasoconstrictor. Removal of the N-terminal Tyr-Pro dipeptide by DPP4 alters the receptor sub-type specificity from Y_1/Y_5 ($NPY_{1–36}$) to Y_2/Y_5 ($NPY_{3–36}$). In the rat, Y_1 signalling promotes atherosclerosis and restenosis after angioplasty. Y_2 receptor agonists stimulate angiogenesis and arteriogenesis. While histochemical observations confirm the presence of the NPY system in human atherosclerosis, it is not clear at present what role it plays in human pathology [15].

The intricate interactions between DPP4, NPY, PYY and their receptors have prompted several groups to investigate the effect of DPP4 inhibition on blood pressure regulation. Jackson and Mi reported that sitagliptin augments sympathetic enhancement of the renovascular effects of angiotensin II in genetic hypertension in rats. This process is also mediated

through the Y_1 receptor [19]; the enhancement of the renovascular effects of angiotensin II is independent of the structure of the DPP4 inhibitor used. These authors advise caution because DPP4 inhibitors will often be prescribed to patients already taking anti-hypertensive medication. Suppressing DPP4 activity may also increase the risk of angiotensin converting enzyme-I inhibitor associated angioedema in susceptible patients [20]. This process is mediated by yet another DPP4 substrate, the neuropeptide substance P. The increased incidence of nasopharyngitis in patients taking sitagliptin and vildagliptin [1,2] may be linked to the prolonged action of substance P. In addition to NPY and PYY, GLP-1 and therapeutically used GLP-1 analogs have an effect on blood pressure in rodents [8,14,21]. GLP-1 has vasodilatory actions in isolated mouse hearts and is cardioprotective after ischemia–reperfusion injury. Unlike glucose homeostasis, this effect does not depend solely on the known GLP-1 receptor. Moreover, the DPP4-generated GLP-1 metabolite also contributes to the GLP-1 receptor-independent process [22]. These findings underscore the interest to investigate cardiovascular actions of DPP4 inhibitors in T2DM patients. A recent study revealed a small blood pressure lowering effect of sitagliptin in non-diabetic mildly or moderately hypertensive patients [23]. Although this result is reassuring, it is important to identify genetic or environmental factors that increase the risk of blood pressure deregulation in certain patient groups.

Brain-derived natriuretic peptide is involved in cardiorenal homeostasis. The active form $BNP_{1–33}$ has vasodilator and natriuretic activity. Natriuretic peptides antagonize the rennin–angiotensin system and the sympathetic nervous system. Once released $BNP_{1–33}$ is rapidly converted by DPP4 into $BNP_{3–33}$ [16,24]. The short form has reduced natriuretic and diuretic properties and lacks vasodilatory activity, but it has not lost the ability to activate cGMP response in cardiac fibroblasts and cardiomyocytes [16,25]. High levels of BNP are produced by cardiomyocytes during heart failure in response to increased heart volume and pressure [11]. BNP levels are used as a diagnostic marker of heart failure and as a marker of treatment response, while the BNP peptide is used as a therapeutic agent in heart failure. It is perhaps too soon to speculate about the effects of DPP4 inhibition in heart failure, but, at the very least, DPP4 inhibitors may be useful as tools to stabilize the various biological BNP forms ex vivo and to fine-tune their diagnostic and prognostic value.

A large body of work on DPP4 in the immune system predates the development of inhibitors as incretin-enhancing drugs (for a recent review see [26]). DPP4 is involved in immune response and inflammation by cleaving several more peptides (including a number of chemokines) and by its participation in cell–cell and cell–extracellular matrix interactions.

Several compounds originally developed as DPP4 inhibitors have shown efficacy in T-cell mediated disease models (acute lung injury, experimental encephalomyelitis, colitis, arthritis) and fibrotic skin diseases. The beneficial effect of these compounds is enhanced by combining them with aminopeptidase N inhibitors. These studies were reviewed quite recently and will not be discussed further, except to illustrate some points raised about inhibitor selectivity [27–30].

During the pre-clinical and clinical evaluation of vildagliptin and sitagliptin, there has been a growing awareness of the presence of other DPP4-like peptidases in various cells and tissues. This fuelled the development of more selective inhibitors (by research teams in pharmaceutical companies as well as academia, reviewed in [31]). Several fairly recently discovered proteins have functional or structural similarity to DPP4 and in vitro cleave the same substrates [32–35]. Studies with DPP4 negative animals confirmed that DPP4 is the molecule that needs to be targeted to improve glucose tolerance [36]. However, this may not be the case for immunosuppression. Although the DPP4 protein undoubtedly plays a role in T-cell mediated immune responses, DPP8 and 9 are present in leukocytes [37] and there is evidence that they are involved in T-cell activation and processes of cell adhesion, cell migration and apoptosis, perhaps even through non-catalytic interactions [38,39]. Inhibition of DPP8 and 9 suppresses mitogen stimulated T-cell responses whereas selective inhibition of DPP4 and DPP2 does not [39]. The compounds used in the immune disease models of [27–30] have a broad selectivity (DPP4, 8 and 9), and some of the effects that were formerly attributed to DPP4 inhibition are now assigned to the inhibition of DPP8 and 9. Sitagliptin is a very selective DPP4 inhibitor, whereas vildagliptin has some affinity for DPP8 and 9 and inhibits them at pharmacological concentrations in vitro. At the moment, it is not certain whether this has any physiological significance in vivo [40].

The consequences of chronic DPP4 inhibition in healthy individuals can be extrapolated from observations in genetically DPP4-deficient animals [39,41]. DPP4-deficient rats are lean, with an improved glucose tolerance associated with increased GLP-1 and bound leptin levels and decreased triglycerides. Moreover, they cope better with stress and show anxiolytic behavior, due to potentiation of the NPY-Y_1 receptor interactions in the CNS. A hyperactive and anti-depressive behavior is observed in $DPP4^{-/-}$ knock-out mice as well [42]. Several alterations in the immune system of DPP4 deficient animals are also reported, including differences in leukocyte subset compositions (eosinophils, NK cells, B cells), reduced NK and T-cell function and altered cytokine profiles [43,44]. These effects can in part be reproduced by administration of DPP4 inhibitors to normal rats. Some aspects of DPP4 deficiency may be the result of non-enzymatic functions of the protein that are generally preserved in case of pharmacological DPP4 inhibition. It is uncertain whether such changes also occur upon DPP4 inhibition in humans and whether they modify the severity of inflammatory diseases such as allergy, asthma or arthritis.

Post-proline cleaving dipeptidyl peptidase (DPP) activity is measured using dipeptide-derived chromogenic or fluorigenic substrates (e.g. Ala-Pro-*p*-nitroanilide). Such activity in cell lysates and biological fluids derives from DPP4 itself and several ''DPP4-like'' peptidases that cleave this synthetic substrate. Post-proline DPP activity is present in all major organs. The highest specific activity is found in kidney and liver, and the lowest is found in plasma and thymus. Data on the expression and distribution of the different DPP4-like enzymes in the body have been communicated by several research groups over the last few years, using DPP4 knock-out mice, RT-PCR, selective inhibition, activity staining and immunohistochemistry. During the Antwerp conference, S. Ansorge showed that the contribution of DPP4 to the total

post-proline DPP activity ranges between 70 and 95% in most tissues, except in the pancreas, colon and brain (10–30%) [45]. These results are very valuable for scientists hoping to purify DPP8 or 9 from tissues [46], to study colocalization with substrates, receptors or ligands, or to investigate cell type specific expression within organs. Studies of the expression and distribution of the various DPP4-like enzymes open perspectives for new therapeutic applications of DPP4 inhibitors because they reveal that various ''stressors'' induce the expression of DPP4 in tissues, like the brain, that have very little DPP4 in normal conditions [47]. DPP2 and DPP8/9 are constitutively expressed in healthy cerebral tissue. Expression of DPP4 is induced in rat brain after focal cerebral ischemia. A similar phenomenon was observed after allergic reactions in rat lungs [48]. This offers prospects for DPP4 inhibitors in neuroprotection after ischemic stroke or in the management of asthma.

There are several other factors that suggest that DPP4 plays a role in the tissues' reaction to hypoxia and in the process of ischemia/reperfusion damage. Inhibition of DPP4 and DPP4-like peptidases using AB192 abrogates acute organ rejection in several lung and heart transplantation models [49]. Ex vivo perfusion of the lungs with this inhibitor limits ischemia/reperfusion injury and preserves early graft function [50]. Dang et al. describe that DPP4 expression is highly induced by hypoxia inducible factor-1 (HIF-1), a transcription factor that increases the expression of genes in response to oxygen deprivation [51]. Also among the genes triggered by HIF-1 are a DPP4 substrate, stromal cell-derived factor-1α (SDF-1α, CXCL12), and presumably adenosine deaminase, a cell surface ligand of DPP4 [52,53]. SDF-1α is the chemokine responsible for stem cell homing and engraftment. Stabilization of the full length SDF-1α by inhibition of DPP4 is considered beneficial during transplantation of hematopoietic stem cells, especially since the agents (e.g., G-CSF) used in the clinic for stem cell mobilization increase expression of DPP4 [54,55]. In this context, it is interesting to note that inhibition of angiotensin converting enzyme or administration of cyclosporine has a positive influence on the mobilization of endothelial progenitor cells after ischemia via modulation of the DPP4 system [56,57].

Cellular reactions to hypoxia, cell migration and extracellular matrix interactions have all been addressed in the literature concerning a role of DPP4 in cancer. At present, one hesitates to predict a role of DPP4 inhibitors in cancer treatment. However, therapeutic opportunities for DPP4 inhibitors appear realistic in the clinical context of stem cell mobilization, transplantation and engraftment [58] and the prevention of ischemia/reperfusion damage. Applications in the field of tissue repair/regeneration seem quite remote and still need solid experimental support.

2.1.2. *A new generation of DPP4 inhibitors*

Research teams in pharmaceutical companies are already developing second generation DPP4 inhibitors. Extrapolating from vildagliptin and sitagliptin, these molecules will be very potent inhibitors with high selectivity for DPP4 over other perceived targets. They will sustain high levels of DPP4 inhibition for prolonged time periods at a relatively low dose taken once a day. Feed-back from post-marketing surveillance will incite discussions between medical practitioners on the type of patient eligible to receive DPP4 inhibitors as monotherapy or in specific combinations. Some investigators advocate that short-term inhibition of DPP4 at meal times may benefit specific patient groups and that DPP4 inhibitors have a potential role in the prevention of T2DM [59].

Research on DPP4-like enzymes is coming of age, and it is hard to predict what direction it will take. Inhibitors will be developed with high selectivity between the closely related DPP8 and 9 to be used as instruments in the determination of their tissue distribution, regulation and role in cellular processes.

DPP inhibitors with narrow or broad specificity may find applications beyond diabetes in the treatment of fibrotic skin disease and immune disorders, in leukapheresis and stem cell transplantation and in the prevention of ischemia/reperfusion damage.

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REFERENCES

[1] Garber AJ, Sharma MD. Update: vildagliptin for the treatment of Type 2 diabetes. Expert Opin Investig Drugs 2008;17:105–13.
[2] Karasik A, Aschner P, Katzeff H, Davies MJ, Stein PP. Sitagliptin, a DPP-4 inhibitor for the treatment of patients with type 2 diabetes: a review of recent clinical trials. Curr Med Res Opin 2008;24:489–96.
[3] Deacon CF. Alogliptin, a potent and selective dipeptidyl peptidase-IV inhibitor for the treatment of type 2 diabetes. Curr Opin Investig Drugs 2008;9:402–13.
[4] Amori RE, Lau J, Pittas AG. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. JAMA 2007;298:194–206.
[5] Abstracts. 3rd International Conference on Dipeptidyl Peptidases and Related Proteins. Clin Chem Lab Med 2008; 46:A1–A43.
[6] Inzucchi SE, McGuire DK. New drugs for the treatment of diabetes. Part II. Incretin-based therapy and beyond. Circulation 2008;117:574–84.
[7] Deacon CF. What do we know about the secretion and degradation of incretin hormones? Regul Pept 2005;128:117–24.
[8] Deacon CF, Carr RD, Holst JJ. DPP-4 inhibitor therapy: new directions in the treatment of type 2 diabetes. Front Biosci 2008;13:1780–94.
[9] Brandt I, Lambeir AM, Maes MB, Scharpé S, De Meester I. Peptide substrates of dipeptidyl peptidases. Adv Exp Med Biol 2006;575:3–18.
[10] Lambeir AM, Durinx C, Scharpé S, De Meester I. 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 Sci 2003;40:209–94.
[11] Rubattu S, Sciarretta S, Valenti V, Stanzione R, Volpe M. Natriuretic peptides: an update on bioactivity, potential

therapeutic use, and implication in cardiovascular diseases. Am J Hypertens 2008. doi: 10.1038/ajh.2008.174.
[12] Bourgault S, Vaudry D, Botia B, Couvineau A, Laburthe M, Vaudry H, et al. Novel stable PACAP analogs with potent activity towards the PAC1 receptor. Peptides 2008;29: 919–32.
[13] Segers VF, Tokunou T, Higgins LJ, MacGillivray C, Gannon J, Lee RT. Local delivery of protease-resistant stromal cell derived factor-1 for stem cell recruitment after myocardial infarction. Circulation 2007;116:1683–92.
[14] Drucker DJ. The role of gut hormones in glucose homeostasis. J Clin Invest 2007;117:24–32.
[15] Kuo LE, Abe K, Zukowska Z. Stress NPY and vascular remodeling: implications for stress-related diseases. Peptides 2007;28:435–40.
[16] Boerrigter G, Costello-Boerrigter LC, Harty GJ, Lapp H, Burnett Jr JC. Des-serine-proline brain natriuretic peptide 3-32 in cardiorenal regulation. Am J Physiol Regul Integr Comp Physiol 2007;292:RR897–901.
[17] Yates NA, Deyanova EG, Geissler W, Wiener MC, Sachs JR, Wong KK, et al. Identification of peptidase substrates in human plasma by FTMS based differential mass spectrometry. Int J Mass Spectrom 2007;259:174–83.
[18] Vella A, Bock G, Giesler PD, Burton DB, Serra DB, Saylan ML, et al. The effect of dipeptidyl peptidase 4 inhibition on gastric volume, satiation and enteroendocrine secretion in Type 2 diabetes: a double blind, placebo-controlled crossover study. Clin Endocrinol (Oxf) 2008;69: 737–44.
[19] Jackson EK, Mi Z. Sitagliptin augments sympathetic enhancement of the renovascular effects of angiotensin II in genetic hypertension. Hypertension 2008;5:1637–42.
[20] Byrd JB, Touzin K, Sile S, Gainer JV, Yu C, Nadeau J, et al. Dipeptidyl peptidase IV in angiotensin-converting enzyme inhibitor associated angioedema. Hypertension 2008;51:141–7.
[21] Gardiner SM, March JE, Kemp PA, Bennett T. Mesenteric vasoconstriction and hindquarters vasodilatation accompany the pressor actions of exendin-4 in conscious rats. J Pharmacol Exp Ther 2006;316:852–9.
[22] Ban K, Noyan-Ashraf MH, Hoefer J, Boltz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of the glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 2008;117:2340–50.
[23] Mistry GC, Maes AL, Lasseter KC, Davies MJ, Gottesdiener KM, Wagner JA, et al. Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on blood pressure in nondiabetic patients with mild to moderate hypertension. J Clin Pharmacol 2008;48:592–8.
[24] Brandt I, Lambeir AM, Ketelslegers JM, Vanderheyden M, Scharpé S, De Meester I. Dipeptidyl-peptidase IV converts intact B-type natriuretic peptide into its des-SerPro form. Clin Chem 2006;52:82–7.
[25] Heublein DM, Huntley BK, Boerrigter G, Cataliotti A, Sandberg SM, Redfield MM, et al. Immunoreactivity and guanosine 3′,5′-cyclic monophosphate activating actions of various molecular forms of human B-type natriuretic peptide. Hypertension 2007;49:1114–9.
[26] Ohnuma K, Dang NH, Morimoto C. Revisiting an old acquaintance: CD26 and its molecular mechanisms in T cell function. Trends Immunol 2008;29:295–301.
[27] Thielitz A, Ansorge S, Bank U, Tager M, Wrenger S, Gollnick H, et al. The ectopeptidases dipeptidyl peptidase IV (DP IV) and aminopeptidase N (APN) and their related enzymes as possible targets in the treatment of skin diseases. Front Biosci 2008;13:2364–75.
[28] Reinhold D, Bank U, Täger M, Ansorge S, Wrenger S, Thielitz A, et al. DP IV/CD26 APN/CD13 and related enzymes as regulators of T cell immunity: implications for experimental encephalomyelitis and multiple sclerosis. Front Biosci 2008;13:2356–63.
[29] Thielitz A, Vetter RW, Schultze B, Wrenger S, Simeoni L, Ansorge S, et al. Inhibitors of dipeptidyl peptidase IV-like activity mediate antifibrotic effects in normal and keloid-derived skin fibroblasts. J Invest Dermatol 2008;128:855–66.
[30] Bank U, Heimburg A, Helmuth M, Stefin S, Lendeckel U, Reinhold D, et al. Triggering endogenous immunosuppressive mechanisms by combined targeting of Dipeptidyl peptidase IV (DPIV/CD26) and Aminopeptidase N (APN/CD13)—a novel approach for the treatment of inflammatory bowel disease. Int Immunopharmacol 2006;6:1925–34.
[31] Van der Veken P, Haemers A, Augustyns K. Prolyl peptidases related to dipeptidyl peptidase IV: potential of specific inhibitors in drug discovery. Curr Top Med Chem 2007;7:621–35.
[32] Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond) 2005;108:277–92.
[33] Bjelke JR, Christensen J, Nielsen PF, Branner S, Kanstrup AB, Wagtmann N, et al. Dipeptidyl peptidases 8 and 9: specificity and molecular characterization compared with dipeptidyl peptidase IV. Biochem J 2006;396:391–9.
[34] Ajami K, Pitman MR, Wilson CH, Park J, Menz RI, Starr AE, et al. Stromal cell-derived factors 1alpha and 1beta, inflammatory protein-10 and interferon-inducible T cell chemo-attractant are novel substrates of dipeptidyl peptidase 8. FEBS Lett 2008;582(5):819–25.
[35] Frerker N, Wagner L, Wolf R, Heiser U, Hoffmann T, Rahfeld JU, et al. Neuropeptide Y (NPY) cleaving enzymes: structural and functional homologues of dipeptidyl peptidase 4. Peptides 2007;28:257–68.
[36] Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, et al. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. Proc Natl Acad Sci USA 2000;97:6874–9.
[37] Maes MB, Dubois V, Brandt I, Lambeir AM, Van der Veken P, Augustyns K, et al. Dipeptidyl peptidase 8/9-like activity in human leukocytes. J Leukoc Biol 2007;81:1252–7.
[38] Yu DM, Wang XM, McCaughan GW, Gorrell MD. Extraenzymatic functions of the dipeptidyl peptidase IV-related proteins DP8 and DP9 in cell adhesion, migration and apoptosis. FEBS J 2006;273:2447–60.
[39] Reinhold D, Goihl A, Kühlmann U, Bank U, Lendeckel U, Faust J, et al. On the role of dipeptidyl peptidase IV-like enzymes in T lymphocyte activation. Clin Chem Lab Med 2008;46:A10.
[40] Burkey BF, Hoffmann PK, Hassiepen U, Trappe J, Juedes M, Foley JE. Adverse effects of dipeptidyl peptidases 8 and 9 inhibition in rodents revisited. Diabetes Obes Metab 2008;10:1057–61.
[41] Von Hörsten S, et al. Role of DP4 in neuro-endocrine-immune-interactions. Clin Chem Lab Med 2008;46:A13.
[42] El Yacoubi M, Vaugeois JM, Marguet D, Sauze N, Guieu R, Costentin J, et al. Behavioral characterization of CD26 deficient mice in animal tests of anxiety and antidepressant-like activity. Behav Brain Res 2006;171:279–85.
[43] Kruschinski C, Skripuletz T, Bedoui S, Tschernig T, Pabst R, Nassenstein C, et al. CD26 (dipeptidyl-peptidase IV)-dependent recruitment of T cells in a rat asthma model. Clin Exp Immunol 2005;139:17–24.
[44] Preller V, Gerber A, Wrenger S, Togni M, Marguet D, Tadje J, et al. TGF-beta1-mediated control of central nervous system inflammation and autoimmunity through the inhibitory receptor CD26. J Immunol 2007;178:4632–40.
[45] Ansorge S, Bank U, Heimburg A, Helmuth M, Koch G, Tadje J, et al. Recent insights on the DPIV and APN

family in immune functions. Clin Chem Lab Med 2008;46: A15.
[46] Dubois V, Lambeir AM, Veken PV, Augustyns K, Creemers J, Chen X, et al. Purification and characterization of a DPP9-like enzyme from bovine testes. Front Biosci 2008;13: 3558–68.
[47] Röhnert P, Emmerlich D, Reinhold D, Schmidt W, Striggow F. Activity and immunohistochemical profile of DASH's in the brain and regulation in ischemia and stroke. Clin Chem Lab Med 2008;46:A12.
[48] Schade J, Stephan M, Schmiedl A, Wagner L, Niestroj AJ, Demuth HU, et al. Regulation of expression and function of dipeptidyl peptidase 4 (DP4), DP8/9, and DP10 in allergic responses of the lung in rats. J Histochem Cytochem 2008;56:147–55.
[49] Jung FJ, Yang L, De Meester I, Augustyns K, Cardell M, Hillinger S, et al. CD26/dipeptidylpeptidase IV-targeted therapy of acute lung rejection in rats. J Heart Lung Transplant 2006;25:1109–16.
[50] Zhai W, Cardell M, De Meester I, Augustyns K, Hillinger S, Inci I, et al. Intragraft DPP IV inhibition attenuates post-transplant pulmonary ischemia/reperfusion injury after extended ischemia. J Heart Lung Transplant 2007;26: 174–80.
[51] Dang DT, Chun SY, Burkitt K, Abe M, Chen S, Havre P, et al. Hypoxia-inducible factor-1 target genes as indicators of tumor vessel response to vascular endothelial growth factor inhibition. Cancer Res 2008;68:1872–80.
[52] Kast RE. How lithium treatment generates neutrophilia by enhancing phosphorylation of GSK-3, increasing HIF-1 levels and how this path is important during engraftment. Bone Marrow Transplant 2008;41:23–6.
[53] Eltzschig HK, Faigle M, Knapp S, Karhausen J, Ibla J, Rosenberger P, et al. Endothelial catabolism of extracellular adenosine during hypoxia: the role of surface adenosine deaminase and CD26. Blood 2006;108:1602–10.
[54] Broxmeyer HE. Chemokines in hematopoiesis. Curr Opin Hematol 2008;15:49–58.
[55] Christopherson 2nd KW, Uralil SE, Porecha NK, Zabriskie RC, Kidd SM, Ramin SM. G-CSF- and GM-CSF-induced upregulation of CD26 peptidase downregulates the functional chemotactic response of CD34+ CD38-human cord blood hematopoietic cells. Exp Hematol 2006;34: 1060–8.
[56] Wang CH, Verma S, Hsieh IC, Chen YJ, Kuo LT, Yang NI, et al. Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. Mol Cell Cardiol 2006;41:34–43.
[57] Wang CH, Cherng WJ, Yang NI, Hsu CM, Yeh CH, Lan YJ, et al. Cyclosporine increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. Am J Physiol Regul Integr Comp Physiol 2008;294:R811–8.
[58] Focosi D, Kast RE, Metelli MR, Benedetti E, Galimberti S, Papineschi F, et al. Enhancement of hematopoietic stem cell engraftment by inhibition of CXCL12 proteolysis with sitagliptin, an oral dipeptidyl-peptidase IV inhibitor: a report in a case of delayed graft failure. Leuk Res 2009;33:178–81.
[59] Augstein P, Berg S, Heinke P, Altmann S, Salzsieder E, Demuth HU, et al. Efficacy of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide (P32/98) in fatty Zucker rats with incipient and manifest impaired glucose tolerance. Diabetes Obes Metab 2007;10:850–61.