Dipeptidyl Peptidase-4 Inhibition and the Treatment of Type 2 Diabetes

Preclinical biology and mechanisms of action

DANIEL J. DRUCKER, MD

Dipeptidyl peptidase (DPP)-4 is a complex enzyme that exists as a membrane-anchored cell surface peptidase that transmits intracellular signals via a short intracellular tail and as a second smaller soluble form present in the circulation. DPP-4 cleaves a large number of chemokines and peptide hormones in vitro, but comparatively fewer peptides have been identified as endogenous physiological substrates for DPP-4 in vivo. Both glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are endogenous physiological substrates for DPP-4, and chemical inhibition of DPP-4 activity, or genetic inactivation of DPP-4 in rodents, results in increased levels of intact bioactive GIP and GLP-1. Furthermore, mice and rats with genetic inactivation or inhibition of DPP-4 exhibit improved glucose tolerance, elevated levels of GLP-1 and GIP, and resistance to diet-induced obesity and hyperglycemia. Sustained DPP-4 inhibition lowers blood glucose via stimulation of insulin and inhibition of glucagon secretion and is associated with preservation of β-cell mass in preclinical studies. Although DPP-4 cleaves dozens of regulatory peptides and chemokines in vitro, studies of mice with genetic inactivation of incretin receptors demonstrate that GIP and GLP-1 receptor–dependent pathways represent the dominant mechanisms transducing the glucoregulatory actions of DPP-4 inhibitors in vivo. The available preclinical data suggests that highly selective DPP-4 inhibition represents an effective and safe strategy for the therapy of type 2 diabetes.

DPP-4 is a widely expressed cell surface peptidase that exhibits a complex biology encompassing cell membrane–associated activation of intracellular signal transduction pathways, cell-cell interaction, and enzymatic activity exhibited by both the membrane-anchored and soluble forms of the enzyme (1). DPP-4, also originally known as the lymphocyte cell surface marker CD26, or as the adenosine deaminase (ADA)-binding protein, is a 766–amino acid serine protease that preferentially cleaves peptide hormones containing a position two alanine or proline. The human gene encoding DPP has been localized to chromosome 2 locus 2q24.3 (2). The majority of the DPP-4 protein is extracellular, with a hydrophobic transmembrane sequence (amino acids 7–28) anchoring the protein in the cell membrane, followed by a very short six–amino acid intracellular sequence. DPP-4 is found on the cell surface as a glycosylated homodimer; however, glycosylation does not appear to be essential for enzymatic activity or binding of ADA. The catalytic region encompasses amino acids 511–766 and is also present in a soluble form of DPP-4 (sDPP-4), which is comprised of the majority of the extracellular DPP-4 protein (amino acids 39–766) (3). sDPP-4 is capable of exhibiting enzymatic activity and interacting with the mannose-6-phosphate/insulin-like growth factor II receptor (M6P-IGFIIR) on specific cell types (4). The wide tissue distribution of DPP-4 on numerous cell types and in different vascular beds and its presence as a soluble active enzyme in the circulation ensures that DPP-4–mediated proteolysis is a common event in most tissue compartments.

DPP-4 is a member of a complex gene family (Fig. 1), many members of which also cleave structurally related peptides (5,6). The DPP-4–related enzymes (Fig. 1) include seprase; fibroblast activation protein α; DPP-6, -8, and -9; attractin; *N*-acetylated-α–linked acidic dipeptidases I, II, and L; quiescent cell proline dipeptidase; thymus-specific serine protease; and DPP-4β (7). ADA immunoaffinity chromatography, which selectively binds and sequesters DPP-4, removed the majority (95%) of DPP-4–like enzymatic activity present in human plasma, thereby identifying DPP-4 as the predominant enzyme responsible for X-Pro or X-Ala cleavage in human serum (3). The multiple members of the DPP-4 family mandate a careful assessment of the selectivity and specificity of any agent used to inhibit DPP-4 activity (8).

DPP-4 AND THE INACTIVATION OF INCRETIN HORMONES

— Circulating levels of DPP-4 activity have been reported to be higher in some studies of subjects with chronic hyperglycemia and type 2 diabetes (9,10); however, whether circulating DPP-4 activity correlates with the levels of active plasma GLP-1 in individual human subjects is not known. The observation that DPP-4 was capable of cleaving the incretin peptides GIP and GLP-1 in human serum in vitro, together with the

From the Department of Medicine, Banting and Best Diabetes Centre, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Ontario, Canada.

Address correspondence and reprint requests to Dr. Daniel J. Drucker, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5. E-mail: d.drucker@utoronto.ca.

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Abbreviations: ADA, adenosine deaminase; DFS, des-fluoro-sitagliptin; DPP, dipeptidyl peptidase; GHRH, growth hormone–releasing hormone; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IGF, insulin-like growth factor; M6P-IGFIIR, mannose-6-phosphate/insulin-like growth factor II receptor; NK, natural killer; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase activating peptide; PYY, peptide YY; QPP, quiescent cell proline dipeptidase; SDF, stromal cell–derived factor; sDPP-4, soluble form of DPP-4; VP, valine pyrrolidide.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

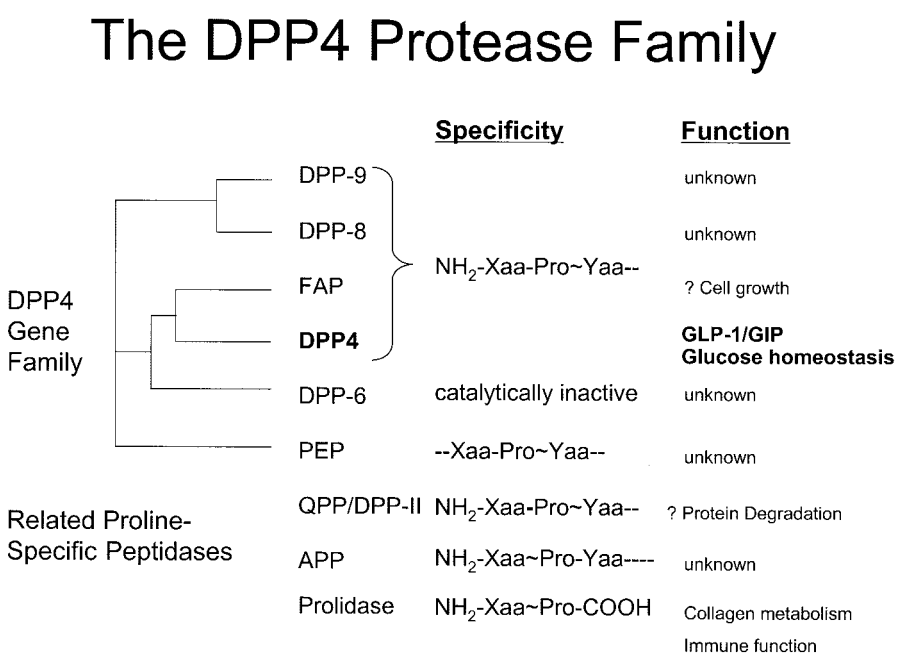

Figure 1—*Family of DPP-4–related proteases and their substrate specificities. For the majority of enzymes, the biological roles and identity of endogenous substrates remains poorly understood. APP, aminopeptidase P; FAP, fibroblast activation protein; PEP, prolyl endopeptidase.*

demonstration that chemical inhibitors of DPP-4 prevented the degradation of GIP and GLP-1, firmly established the importance of DPP-4 as a critical determinant of incretin inactivation (11). Subsequent studies demonstrated reduced cleavage of intact GLP-1(7-36)amide and GIP(1-42) in serum from DPP-4–deficient rats in vitro or following infusion of the peptides into DPP-4–deficient rats in vivo, providing complementary evidence for the importance of DPP-4 in the control of incretin inactivation (12). Moreover, both GLP-1(7-36)amide and the NH_2-terminal DPP-4–generated metabolite GLP-1(9-36)amide were identified in plasma from both fasted and fed humans, and inhibitors of DPP-4 prevented the conversion of GLP-1(7-36)amide to GLP-1(9-36)amide in human plasma in vitro (13). Similarly, the majority of circulating immunoreactive GIP in human plasma is the NH_2-terminally cleaved GIP(3-42) peptide, accounting for >70% of total plasma GIP immunoreactivity in the fasting state and 58% of total GIP after meal ingestion (14). Furthermore, exogenous administration of either GIP or GLP-1 via the subcutaneous or intravenous routes was associated with the rapid degradation of both peptides within minutes to the DPP-4 metabolites GIP(3-42) and GLP-1(9-36)amide, respectively. Hence, DPP-4 is a principal determinant of the circulating $t_{1/2}$ of intact bioactive GIP and GLP-1 (14,15).

DPP-4 INHIBITORS LOWER BLOOD GLUCOSE

— Related studies examined the effects of chemical inhibitors of DPP-4 enzymatic activity on the structure and activity of GLP-1 in normal animals and in experimental models of diabetes. The nonselective DPP-4 inhibitor valine pyrrolidide (VP) prevented the degradation of GLP-1 and GIP in anesthetized pigs and potentiated the incretin-mediated reduction of plasma glucose and stimulation of insulin secretion in response to an intravenous glucose challenge (16,17). Similarly, VP acutely improved oral glucose tolerance in high-fat–fed pigs, in association with increased levels of intact GLP-1 and increased levels of plasma insulin following oral glucose loading (18). A series of related studies then demonstrated that inhibition of DPP-4 activity preserved levels of intact GLP-1 and improved glucose tolerance in normal and diabetic rats and mice (19–26) in association with enhanced glucose-stimulated insulin secretion in islets isolated from DPP-4 inhibitor–treated mice (25).

Verification that DPP-4 was the dominant molecular target for the glucose lowering properties of NVP-DPP728 was illustrated in studies demonstrating that this compound acutely lowered blood glucose following oral glucose challenge in wild-type Wistar rats but not in Fischer 344 rats with an inactivating mutation in the DPP-4 gene (27). Nevertheless, DPP-4 inhibition is not capable of exerting significant antidiabetic actions in all preclinical models, as acute VP administration increased plasma levels of intact GLP-1 in older *db/db* mice but VP did not lower blood glucose in 24-week-old severely hyperglycemic (fasting blood glucose 29 mmol/l) *db/db* mice (28).

DPP-4 INHIBITORS AND β-CELL MASS AND SURVIVAL

— DPP-4 inhibitors exhibit favorable actions on islet and β-cell mass, morphology, and survival. Wistar rats treated with streptozotocin and twice-daily P32/98 for 7 weeks exhibited increased body weight, lowered fed blood glucose, and increased levels of plasma insulin (29). Furthermore, P32/98 improved glucose tolerance, enhanced glucose-stimulated insulin release in perfused pancreas experiments, and increased pancreatic insulin content. Histological analyses demonstrated an increased number of small islets and a greater proportion of β-cells within islets in rats treated with P32/98 (29).

The DPP-4 inhibitor des-fluorositagliptin (DFS) significantly reduced ambient and fed blood glucose and A1C levels in diabetic ICR mice, in association with decreased liver weight and reduced levels of hepatic and plasma triglycerides and plasma free fatty acids (30). Furthermore DFS-treated animals exhibited increased β-cell mass and a reduction in the α-cell–to–β-cell ratio. A head-to-head comparison of glipizide and DFS for 10 weeks in diabetic mice demonstrated comparatively greater improvement of glycemia and A1C in DFS-treated mice, and improvements in pancreatic insulin content and relative β-cell area were observed in mice treated with DFS but not in glipizide-treated animals (30). Furthermore, islets isolated from DFS-treated mice exhibited improved insulin secretion in response to KCl or glucose and increased islet insulin content (30).

A comparative study of the DPP-4 inhibitor vildagliptin versus the GLP-1R agonist liraglutide was carried out in candy-fed rats for 12 weeks (31). Liraglutide reduced food intake and attenuated weight gain; however, there were no major differences in glucose or A1C in the two treatment arms, whereas plasma insulin levels were significantly higher in rats treated with vildagliptin (31). Both vildagliptin- and liraglutide-treated rats exhibited a relative normalization of

β-cell mass compared with vehicle-treated candy-fed rats.

ROLE OF ENDOGENOUS DPP-4

Studies in rats and mice with inactivating DPP-4 mutations

The biological importance of DPP-4 has been examined in rats with a naturally occurring loss of function mutation in the DPP-4 gene and in mice with targeted genetic inactivation of DPP-4. A strain of Fischer 344 (F344) rats originally identified in Japan harbor a Gly633-Arg mutation in the DPP-4 gene within the active site of the enzyme. The mutant DPP-4 protein is synthesized appropriately, yet it is not exported out of the endoplasmic reticulum and is rapidly degraded without being processed to the mature active enzyme (32–34). Subsequent studies identified heterogeneity in baseline levels of DPP-4 activity in different inbred rat strains, emphasizing the importance of careful characterization of enzymatic activity in different rodent models (35). F344 rats exhibit improved glucose tolerance and increased levels of plasma GLP-1 and insulin following oral glucose challenge. Furthermore, high-fat feeding of F344 rats for 7 weeks was associated with reduced weight gain, increased levels of intact GLP-1, improved glucose tolerance, and enhanced insulin sensitivity as assessed by homeostatic model assessment (36). Hence, loss of DPP-4 activity in rats is associated with potentiation of endogenous GLP-1 action and improvement of glucose tolerance.

Targeted inactivation of the DPP-4 gene in mice also leads to increased plasma levels of GIP, GLP-1, and insulin and reduced glycemic excursion following oral glucose challenge (37). Consistent with findings in DPP-4–deficient rats, DPP-$4^{-/-}$ mice exhibit resistance to diet-induced obesity, reduced fat accumulation, decreased plasma levels of leptin, and reduced food intake but increased energy expenditure on a high-fat diet (38). DPP-$4^{-/-}$ mice appear to be more insulin sensitive and fail to develop hyperinsulinemia, hepatic steatosis, or islet hyperplasia after high-fat feeding. Moreover, DPP-$4^{-/-}$ mice were resistant to the development of streptozotocin-induced diabetes following a single injection of streptozotocin (38).

The importance of DPP-4 for control of immune function and behavior has also been examined in F344 rats and DPP-$4^{-/-}$ mice. DPP-4–deficient rats exhibited increased pain sensitivity, reduced stress-like responses, and decreased susceptibility to the sedative effects of ethanol (39). Furthermore, splenocytes isolated from DPP-4–deficient rats exhibited decreased natural killer (NK) cell–mediated tumor lysis using syngeneic MADB106 tumor cells as antigen (35). Modest yet detectable abnormalities in immune responses and behavior have also been described in DPP-$4^{-/-}$ mice, including changes in stress-associated mobility, curiosity, and exploratory behaviors (40). In separate studies, the relative number of $CD4^+$ T-cells was lower and NK cells higher in spleen cells, and the numbers of circulating $CD4^+$ NK T-cells were reduced in DPP-$4^{-/-}$ mice (41). Furthermore, interleukin-4 production was significantly reduced, and levels of interleukin-10 and interferon-γ were increased following stimulation with pokeweed mitogen in splenic DPP-$4^{-/-}$ lymphocytes. Following immunization with pokeweed mitogen, serum levels of total IgG, IgG1, IgG2, and IgE were significantly lower, accompanied by lower levels of plasma cytokines, in serum from DPP-$4^{-/-}$ mice (41). Analysis of nociceptive responses revealed reduced latencies to stimuli such as the hot plate or tail pinch test, in association with increased plasma levels of substance P. The abnormal latencies were abolished following treatment of DPP-$4^{-/-}$ mice with a substance P (neurokinin 1) receptor antagonist, and administration of two different DPP-4 inhibitors reduced latencies in wild-type mice (41). These findings implicate a role for DPP-4 as a critical regulator of substance P–mediated inflammatory responses in vivo.

A role for DPP-4 in the modulation of the inflammatory response is suggested by differences in the severity of experimental arthritis in wild-type vs. DPP-$4^{-/-}$ mice. Antigen-induced arthritis and plasma levels of the proinflammatory chemokine stromal cell–derived factor (SDF)-1 were significantly increased in DPP-$4^{-/-}$ mice, in association with increased numbers of SDF-1 receptor (CXCR4)-positive cells infiltrating arthritic joints. Furthermore, plasma DPP-4 activity was reduced in wild-type mice with antigen-induced arthritis and in human subjects with rheumatoid arthritis, and the levels of circulating DPP-4 and DPP-4 activity were inversely correlated with the severity of rheumatoid arthritis in affected subjects (42). Taken together, the data clearly implicate a role for DPP-4 in the control of immune function, inflammatory responses, and behavior. However, whether these phenotypes can be selectively ascribed to loss of the catalytic activity of the enzyme or more generalized loss of DPP-4 function independent of the catalytic activity cannot yet be determined.

IMPORTANCE OF DPP-4–SELECTIVE INHIBITION

— Although experimental results obtained using nonselective DPP-4 inhibitors implicated a role for DPP-4 in the control of immune regulation, transplantation biology, cancer cell growth, and metastasis (43,44), there is limited data for similar studies using highly selective DPP-4 inhibitors that have been generated for the treatment of type 2 diabetes. More recent experiments comparing the actions of DPP-4–selective versus –nonselective inhibitors suggest that preferential inhibition of DPP-8/9 and quiescent cell proline dipeptidase (QPP) in vivo was associated with a species- and tissue-specific profile of different toxicities. Inhibition of DPP-8/9 produced alopecia, thrombocytopenia, splenomegaly, thrombocytopenia, and multiorgan pathology, leading to death in rats and gastrointestinal toxicity in dogs. Moreover, similar toxicities were observed in wild-type and DPP-$4^{-/-}$ mice treated with DPP-8/9 inhibitors (8). In contrast, inhibition of the related enzyme QPP produced reticulocytopenia in rats, whereas selective inhibition of DPP-4 was not associated with detectable toxicity in rats or dogs (8). Similarly, inhibition of DPP-8/9, but not DPP-4, was associated with reduction of mitogen-stimulated proliferation of human mononuclear cells in vitro (8). Curiously, some but not all DPP-4 inhibitors have been reported to produce skin lesions in monkey studies. The extent to which these findings reflect differential selectivity of specific agents for the monkey enzymes and whether the lesions are completely attributable to non–DPP-4–dependent mechanisms remains poorly understood. Collectively, these findings illustrate that data obtained using nonselective DPP inhibitors needs to be interpreted with caution in regard to the putative role of DPP-4 in the development of specific organ pathologies.

Table 1—*DPP-4 peptide substrates*

	Pharmacological	Physiological
Aprotinin	IP-10	GLP-1
Bradykinin	MDC	GLP-2
β-Casomorphin-2	MCP-1	GIP
CG	MCP-2	SDF-1α/β
CLIP	MCP-3	Substance P
Endomorphin-2	Tyr-melanostatin	
Enterostatin	α1-microglobulin	
Eotaxin	NPY	
GCP-2	PHM	
GHRH	Prolactin	
GRP	PYY	
IGF-1	RANTES	
IL-2	Trypsinogen	
Il-1β	Tryspinogen pro-peptide Colipase	

Peptides cleaved by DPP4 may be pharmacological or physiological substrates. Physiological peptide substrates are defined as those peptides whose endogenous levels of intact to cleaved forms are significantly different following genetic inactivation or chemical inhibition of DPP4 activity in vivo. CG, chromogranin; CLIP, corticotropin-like intermediate lobe peptide; GCP-2, granulocyte chemotactic protein-2; GRP, gastrin-releasing peptide; IL-1β, interleukin-1β; IL-2, interleukin-2; IP-10, interferon-γ–inducible protein 10, also known as CXCL10 or chemokine (C-X-C motif) ligand 10; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; PHM, peptide histidine methionine; RANTES, regulated on activation normal T-cell expressed and secreted.

DPP-4 SUBSTRATES AND REDUCED DPP-4 ENZYME ACTIVITY

Physiology versus pharmacology

Numerous endocrine peptides, chemokines, and neuropeptides contain an alanine or proline at position 2 and are putative DPP-4 substrates (Table 1). An endogenous physiological DPP-4 substrate is defined as a peptide whose endogenous circulating levels of intact versus NH_2-terminally cleaved forms are altered following reduction or elimination of DPP-4 activity in vivo. For the majority of peptides listed in Table 2, it is reasonable to assume that they may be pharmacological substrates, as DPP-4 produces NH_2-terminal cleavage of the peptide(s) in vitro. In contrast, there is limited evidence that the majority of these peptides are physiological substrates. Moreover, even small changes in the ratios of intact to cleaved peptide for physiological DPP-4 substrates may not always be sufficient to produce predicted biological changes in specific target tissues, as discussed below.

Both GIP and GLP-1 are physiological substrates for DPP-4, as DPP-4 inhibition is associated with increased circulating levels of intact GIP and GLP-1 in vivo (16,17), and levels of intact GIP and GLP-1 are increased, relative to their NH_2-terminally cleaved forms in rats and mice with inactivating DPP-4 gene mutations (37). Similarly, the chemokines SDF-1α and SDF-β are cleaved by DPP-4 at the NH_2-terminus, and plasma levels of intact SDF-1α (1–67) are increased in DPP-$4^{-/-}$ mice (42). Hence, endogenous levels of intact SDF-1 are clearly dependent on DPP-4 activity.

Substance P may also be a physiological substrate for DPP-4. Levels of tissue DPP-4 are reduced in nasal tissue of human subjects with chronic rhinosinusitis, and the vasodilatory effects of substance P are attenuated by DPP-4 in vivo (45). Conversely, DPP-4 inhibition potentiates the vasodilatory effects of exogenous substance P, findings consistent with reports of nasopharyngitis in human subjects treated with DPP-4 inhibitors (46,47). Moreover, plasma levels of substance P were more than twofold higher in DPP-$4^{-/-}$ versus DPP-$4^{+/+}$ mice (48). Hence, substance P fulfills the criteria for an endogenous substrate of DPP-4. Whether clinically meaningful increases in the levels of SDF-1 or substance P occur in humans following partial reduction of DPP-4 activity with selective DPP-4 inhibitors remains uncertain.

Although the majority of peptide hormones listed in Table 1 may be cleaved by DPP-4 in vitro, the endogenous levels of intact-to-cleaved peptide may not be significantly different in DPP-$4^{-/-}$ versus DPP-$4^{+/+}$ mice or rats or following administration of DPP-4 inhibitors in vivo. For example, glucagon is cleaved by DPP-4 in vitro to yield glucagon (3–29), and this cleavage is inhibited by the DPP-4 inhibitor isoleucine thiazolidide (49); however, increased plasma levels of intact versus cleaved glucagon have not been reported following administration of DPP-4 inhibitors in vivo or in rats or mice with inactivating mutations of the DPP-4 gene.

GLP-2(1-33) is cleaved by DPP-4 at the position 2 alanine both in vitro and following exogenous administration in vivo, leading to the generation of GLP-2(3-33) (50,51). Moreover, DPP-4–resistant GLP-2 analogs exhibit much greater potency than native GLP-2 in vivo (50). Nevertheless, although DPP-4 inhibition increased the plasma levels of intact nutrient-stimulated GLP-2 (1–33) in rats, chronic administration of the DPP-4 inhibitor VP alone had no effect on intestinal growth, a key biological readout of enhanced GLP-2 activity in vivo (52).

Growth hormone–releasing hormone (GHRH) was one of the first peptides demonstrated to be a substrate for DPP-4 (53). Circulating levels of GHRH are low and difficult to measure in plasma. Nevertheless, increased levels of intact bioactive GHRH in the hypothalamic-pituitary axis would be predicted to stimulate growth hormone secretion, leading to increased circulating levels of insulin-like growth factor (IGF)-1 and somatic growth. However, DPP-$4^{-/-}$ mice and F344 mutant rats do not exhibit increased body size or organ growth. Furthermore, treatment of young pigs for 72 h with a sitagliptin analog that produced 90% inhibition of plasma DPP-4 activity was not associated with alterations in the circulating concentrations of IGF-1 (54). Similarly, 10 days of sitagliptin administration to healthy nondiabetic male subjects did not produce significant elevations in IGF-1 or IGF binding protein-3 relative to placebo-treated control subjects (55). Hence, DPP-4 inhibition may not always produce predictable changes in downstream biological pathways, despite altering the relative levels of intact-to-cleaved peptide substrates.

Neuropeptide Y (NPY) and peptide YY (PYY) exert opposing actions on control of food intake, and both peptides are cleaved by DPP-4 in vitro, resulting in the generation of NH_2-terminally truncated peptides with different receptor affinities. Inhibition of DPP-4 activity prevents the generation of the anorectic PYY(3-36) from PYY(1-36), and reduced levels of PYY(3-36) have been detected following

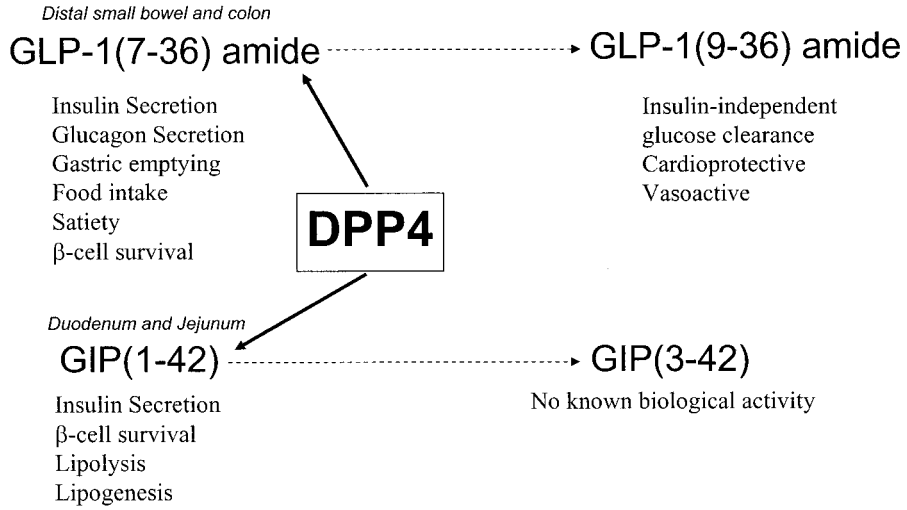

Figure 2—*The principal biological actions of the active incretin hormones GLP-1(7-36)amide and GIP(1-42) and the actions of the peptides GLP-1(9-36)amide and GIP(3-42) generated following cleavage by DPP-4.*

infusion of PYY(1-36) into rats treated with a DPP-4 inhibitor (56). Although DPP-4 is clearly important for cleavage of exogenous PYY(1-36), biologically significant alterations in the levels of endogenous PYY(1-36)-to-PYY(3-36) have not yet been described in rodents or humans with reductions in DPP-4 activity. DPP-4 cleavage of NPY(1-36) in vitro leads to the generation of NPY(3-36), which exhibits a markedly reduced affinity for the orexigenic Y1 receptor but interacts with the Y2/Y5 receptor. Exogenous administration of NPY also exert effects on vasomotor activity, angiogenesis, and vascular remodeling; however, the importance of endogenous basal levels of NPY(1-36) and NPY(3-36) for control of these activities remains uncertain. Although administration of NPY produces potent anxiolytic and sedative-like effects in DPP-4–deficient F344 rats (57), there is little evidence that endogenous circulating or tissue levels of NPY(1-36) versus NPY(3-36) are significantly altered following reduction of DPP-4 activity in vivo.

BIOLOGICAL ACTIVITIES OF DPP-4 NOT RELATED TO CONTROL OF GLUCOSE HOMEOSTASIS

— DPP-4 has been implicated in the control of lymphocyte and immune function, cell migration, viral entry, cancer metastasis, and inflammation (rev. in 1,58). DPP-4 expression often varies with the state of cellular differentiation, and loss of DPP-4 expression has been associated with changes in tumor growth and enhanced metastatic or invasive behavior (59–61). The importance of DPP-4 for retention of chemotherapy sensitivity and topoisomerase-2 expression has been mapped, using site-directed mutagenesis, to a region of the protein essential for its enzymatic activity (62,63). DPP-4/CD26 is expressed at low levels on resting T-cells; however, DPP-4 expression increases following T-cell activation. DPP-4 functions as a T-cell costimulatory molecule that enhances antigen-specific T-cell proliferation (64), and sDPP-4 enhances T-cell transendothelial migration in vitro, actions that require the catalytic activity of the DPP-4 enzyme and a functional M6P-IGFIIR (4).

DPP-4 also regulates migration of human cord blood $CD34^+$ progenitor cells and the homing and engraftment of hematopoetic stem cells. Inhibition of DPP-4 enzymatic activity promotes human hematopoetic stem cell migration and bone marrow engraftment via potentiation of the levels of intact CXCL12/SDF-1α, a physiological substrate for DPP-4 activity (Table 1) (65,66). Furthermore, inhibition of DPP-4 activity enhanced homing and engraftment of bone marrow cells or enriched hematopoetic stem cells in the liver of allogeneic fetal mice following in utero hematopoetic cell transplantation (67), likely due to potentiation of SDF-1α interaction with the CXCR4 receptor.

The importance of the interaction of human DPP-4 with ADA remains incompletely understood. Adenosine exerts acute anti-inflammatory effects during tissue hypoxia; however, chronically elevated levels of adenosine may be deleterious in experimental models of inflammation. Experimental hypoxia induces the cell surface expression of both ADA and DPP-4 on vascular endothelial cells, and ADA activity is also increased in plasma from hypoxic human subjects (68). However, there does not seem to be a correlation between the ability of ADA to bind DPP-4 and the development of immunodeficiency in human subjects with ADA mutations (69). Hence, the functional importance, if any, of selective DPP-4 inhibition on ADA binding and activity remains obscure.

BIOLOGICAL IMPORTANCE OF GLP-1(9-36)AMIDE AND GIP(3-42)

— Intact GLP-1 and GIP are rapidly cleaved by DPP-4 to yield GLP-1(9-36)amide and GIP(3-42) (Fig. 2), and the levels of NH_2-terminally truncated incretins are greater than the levels of intact GIP and GLP-1 in both the fasting and postprandial states (13,14). Following sustained inhibition of DPP-4 activity, plasma levels of GLP-1(7-36)amide and GIP(1-42) are increased (70), whereas levels of GLP-1(9-36)amide and GIP(3-42) are substantially decreased. Hence, it seems reasonable to consider whether differences in the ratios of intact to cleaved incretin peptides have biological implications. Although GIP(3-42) may be a weak GIP receptor antagonist in vitro, it does not exert glucoregulatory actions in vivo (71,72).

There is considerable evidence that GLP-1(9-36)amide has biological actions in vivo (Fig. 2). GLP-1(9-36)amide modestly enhances glucose clearance independent of changes in insulin secretion in pigs (73), whereas studies in mice show no effect of GLP-1(9-36)amide on insulin secretion or glucose clearance (74). GLP-1(9-36)amide had no effect on glucose clearance or insulin secretion in healthy human volunteers following intravenous glucose infusion (75). In contrast, acute infusion of GLP-1(9-36)amide lowered postprandial glucose following meal ingestion independent of changes in levels of insulin or glucagon or gastric emptying in human subjects (76). Although substantial amounts of GLP-1(9-36) are generated following meal ingestion, GLP-1(9-36)amide does not appear to antagonize the glucose-lowering properties of GLP-1(7-36)amide in diabetic human subjects (77). Remarkably, GLP-1(9-

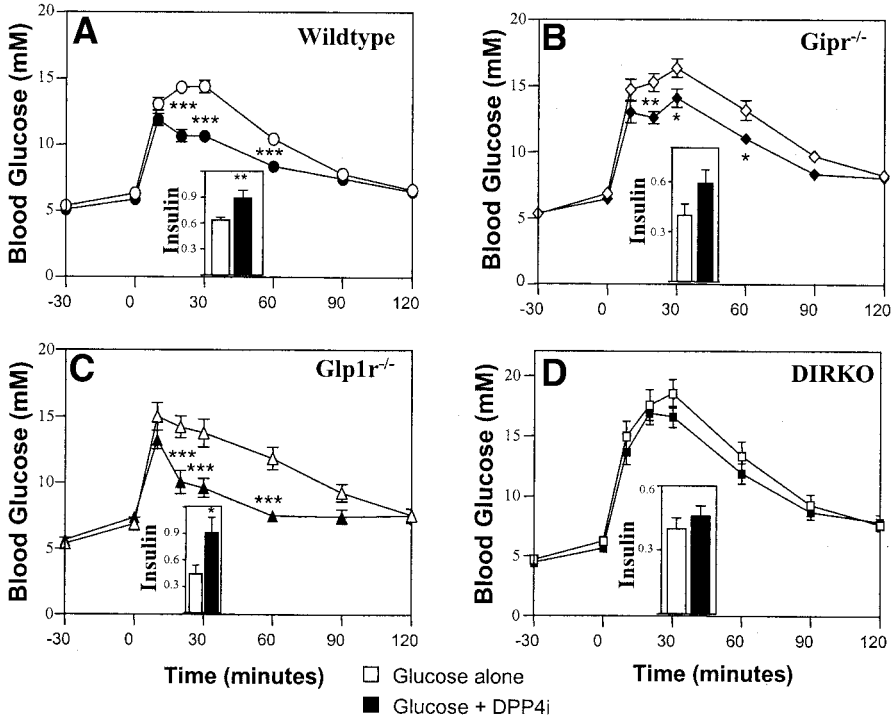

Figure 3—*Four different DPP-4 inhibitors (LAF237/vildagliptin, VP, Syrrx106124, and TP8211) acutely lower blood glucose and enhance glucose-stimulated insulin secretion in wild-type mice and in mice with targeted disruption of single incretin receptors (Glp1r$^{-/-}$ and Gipr$^{-/-}$). In contrast, none of the DPP-4 inhibitors lowers blood glucose or stimulates insulin secretion in mice with genetic disruption of both the GIP and GLP-1 receptors (dual incretin receptor knockout [DIRKO]). Data shown is from experiments using VP; however, identical results were obtained with the three other DPP-4 inhibitors, as described in ref. 82.*

36)amide increased myocardial glucose uptake and improved left ventricular function in dogs with pacing-induced dilated cardiomyopathy (78). The mechanisms through which GLP-1(9-36)amide mediates its emerging biological actions are currently poorly understood and the subject of active investigation.

DPP-4 INHIBITION AND REDUCTION OF BLOOD GLUCOSE

Mechanisms of action

A considerable number of glucoregulatory peptides, in addition to GLP-1 and GIP, have been identified as exogenous substrates susceptible to DPP-4 cleavage (Table 1). For example, DPP-4 cleaves vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide (PACAP), oxyntomodulin, and gastrin-releasing peptide (GRP) (79,80), and differential metabolism of exogenously infused PACAP38 was observed in wild-type versus DPP-4$^{-/-}$ mice (80). Furthermore, DPP-4 inhibition potentiates the insulinotropic response to exogenous PACAP and GRP in mice in vivo (81). Hence, it is reasonable to postulate that one or more of these peptides, together with GLP-1 and GIP, contribute to the reduction in glycemia observed following acute or chronic DPP-4 inhibition.

In contrast, studies in mice with disruption of single incretin receptors, or analysis of mice with combined genetic disruption of both the GIP and GLP-1 receptors (double incretin receptor knockout or DIRKO mice), strongly suggest that GIP and GLP-1 are the principal peptide substrates responsible for transducing the glucose-lowering actions of DPP-4 inhibitors (Fig. 3). Although DPP-4 inhibitors lower blood glucose and stimulate insulin secretion in Gipr$^{-/-}$ or Glp1r$^{-/-}$ mice (37,82), four different DPP-4 inhibitors failed to reduce blood glucose following acute oral glucose challenge in normoglycemic DIRKO mice (82) (Fig. 3). To determine the importance of GIP and GLP-1 receptor signaling for the chronic glucoregulatory actions of DPP-4 inhibitors, high-fat–fed DIRKO mice were treated with vildagliptin continuously in the drinking water for 8 weeks. Although vildagliptin improved insulin secretion and lowered blood glucose in wild-type mice, no effect of vildagliptin on glucose control or insulin secretion was observed in DIRKO mice (83). Hence, the available preclinical data strongly support the essential importance of the GIP and GLP-1 receptors as dominant mediators for the antidiabetic actions of DPP-4 inhibitors.

DPP-4 INHIBITORS

Current concepts and major unanswered questions

A large number of actions ascribed to inhibition of DPP-4 activity were originally delineated in experiments using nonselective DPP-4 inhibitors. However, many of these inhibitors were subsequently shown to exhibit inhibitory "off target" actions on related proteases in the absence of DPP-4 activity (8,84). Hence, the available literature on the pleiotropic effects of DPP-4 inhibition using first-generation nonselective inhibitors must be interpreted with caution, pending analysis of data from confirmatory experiments carried out using highly selective inhibitors of the DPP-4 enzyme. Similarly, although intriguing metabolic, behavioral, and immunologic phenotypes have been described in rodents with inactivating mutations in the DPP-4 gene, F344 rats and DPP-4$^{-/-}$ mice exhibit a complete loss of DPP-4 activity. In contrast, there is little data on these parameters following administration of highly selective DPP-4 inhibitors that produce a 50–80% reduction in enzymatic activity. Thus, whether biological results obtained with selective DPP-4 enzyme inhibitors will be identical to data obtained in studies of rodents with complete absence, during both development and adult life, of a multifunctional DPP-4 protein requires more careful investigation. Furthermore, it will be important to monitor DPP-4–treated human subjects carefully for the development of inflammatory conditions, angioedema, rhinitis, and urticaria, given the potential importance of SDF-1 and/or substance P as DPP-4 substrates.

Equally compelling questions arise from attempts to understand how DPP-4 inhibitors lower blood glucose in diabetic subjects. The major actions of DPP-4 inhibitors in vivo include suppression of glucagon secretion and enhancement of insulin secretion, consistent with the known actions of GLP-1 and GIP. Preclinical data in rodents with loss of incretin

receptor signaling support a critical role for the GLP-1 and GIP receptors for transduction of the antidiabetic actions of DPP-4 inhibitors (82,83). Nevertheless, prolonged DPP-4 inhibition in diabetic human subjects may recruit additional as yet unidentified mechanisms that promote glucose lowering. Moreover, the long-term consequences of DPP-4 inhibition on β-cell function and the durability of glucose lowering achieved with sustained DPP-4 inhibition require careful clinical assessment. Taken together, it seems prudent to pursue additional detailed studies of the biological role(s) of DPP-4 and the consequences and safety of highly selective DPP-4 inhibition in experimental and clinical models of diabetes.

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