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Pleiotropic Mechanisms for the Glucose-Lowering Action of DPP-4 Inhibitors

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Dipeptidyl peptidase (DPP)-4 inhibition is a glucose-lowering treatment for type 2 diabetes. The classical mechanism for DPP-4 inhibitors is that they inhibit DPP-4 activity in peripheral plasma, which prevents the inactivation of the incretin hormone glucagon-like peptide (GLP)-1 in the peripheral circulation. This in turn increases circulating intact GLP-1, which results in stimulated insulin secretion and inhibited glucagon secretion, in turn increasing glucose utilization and diminishing hepatic glucose production, which, through reduction in postprandial and fasting glucose, reduces HbA_{1c}. However, recent experimental studies in mainly rodents but also to a limited degree in humans have found additional mechanisms for DPP-4 inhibitors that may contribute to their glucose-lowering action. These nonclassical mechanisms include *1*) inhibition of gut DPP-4 activity, which prevents inactivation of newly released GLP-1, which in turn augments GLP-1-induced activations of autonomic nerves and results in high portal GLP-1 levels, resulting in inhibited glucose production through portal GLP-1 receptors; *2*) inhibition of islet DPP-4 activity, which prevents inactivation of locally produced intact GLP-1 in the islets, thereby augmenting insulin secretion and inhibiting glucagon secretion and possibly preventing islet inflammation; and *3*) prevention of the inactivation of other bioactive peptides apart from GLP-1, such as glucose-dependent insulinotropic polypeptide, stromal-derived factor-1α, and pituitary adenylate cyclase-activating polypeptide, which may improve islet function. These pleiotropic effects may contribute to the effects of DPP-4 inhibition. This Perspectives in Diabetes outlines and discusses these nonclassical mechanisms of DPP-4 inhibition.

Dipeptidyl peptidase (DPP)-4 inhibition is a strategy for glucose-lowering treatment for type 2 diabetes (1). It was developed on the basis that the gut-derived glucagon-like peptide (GLP)-1 is a potent antidiabetic hormone due to its ability to stimulate insulin secretion and inhibit glucagon secretion. DPP-4 inhibition prevents the inactivation of GLP-1 and, therefore, raises the circulating intact (active) GLP-1 levels above physiological levels that have antidiabetic actions.

After initial preclinical development, the first clinical proof-of-concept study for DPP-4 inhibition was reported in the early 2000s (2). DPP-4 inhibition was first approved for clinical use in 2006 with the DPP-4 inhibitor sitagliptin, and thereafter, several other DPP-4 inhibitors have been introduced into clinical practice (3). They are all oral agents taken once or twice daily and are also being developed for once-weekly administration. They reduce fasting and postprandial hyperglycemia, have a low risk for hypoglycemia, and are weight neutral (1). Currently, they are mainly used as an add-on to metformin, but they are also efficient in monotherapy in patients in whom metformin is unsuitable and in combination with other glucose-lowering agents.

THE CLASSICAL MECHANISM FOR THE GLUCOSE-LOWERING EFFECT OF DPP-4 INHIBITION

DPP-4 is an enzyme that is widely expressed throughout the body and abundantly expressed in endothelial cells. It is attached to the intravascular portion of vascular endothelial cells and also exists in a soluble circulating form (4). It is a serine protease that cleaves peptides between the amino acid 2 and 3 from the N-terminal end, particularly if the second amino acid is alanine or proline. GLP-1 has alanine as the second amino acid and is therefore a substrate for DPP-4, which cleaves the intact $GLP\text{-}1_{7–36}$ to $GLP\text{-}1_{9–36}$, which is largely inactive. The

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inactivation of GLP-1 by DPP-4 is rapid and extensive, and it has been estimated that the increase in GLP-1 concentration in peripheral venous plasma amounts to less than 10% of the increase in the portal concentration, with the consequence that after DPP-4 inhibition, much higher GLP-1 levels are seen in the portal vein than in peripheral plasma, as has been demonstrated for vildagliptin in pigs (5).

The importance of removing the two N-terminal amino acids in GLP-1 by DPP-4 for the rapid inactivation of GLP-1 in vivo was initially demonstrated by Holst and Deacon, and they also showed that a DPP-4 inhibitor (valine pyrrolidide) prevented the inactivation of exogenously infused GLP-1, which augmented its insulinotropic effect in pigs (6). All of the DPP-4 inhibitors used in clinical practice have been shown to give robust and long-lasting inhibition of plasma DPP-4 activity (7). Several of the DPP-4 inhibitors have also been demonstrated to increase levels of (intact) GLP-1 after meal ingestion (8–11). For vildagliptin and sitagliptin, it has in addition been demonstrated that intact GLP-1 levels are increased not only after meal ingestion, but also throughout the entire 24-h period with elevated fasting levels (12,13).

Based on this knowledge, the classical mechanism for DPP-4 inhibition is that due to prevention of inactivation of GLP-1 in the peripheral circulation, the increased circulating intact GLP-1 results in stimulated insulin secretion and inhibited glucagon secretion, resulting in increased glucose utilization and diminished hepatic glucose production, which, through reduction in postprandial and fasting glucose, reduce HbA_{1c}. However, several recent findings, mainly in acute studies in nondiabetic rodents, have found that the classical mechanism of DPP-4 inhibition to reduce glucose by inhibiting the enzyme in peripheral plasma, thereby raising circulating levels of intact GLP-1, may not explain the full power of this approach and that tissue DPP-4 and/or neural effects may also contribute. This Perspectives in Diabetes summarizes these nonclassical effects to illustrate the mechanistic complexity of this strategy to lower glucose in type 2 diabetes.

DPP-4 INHIBITION IN THE GUT

As a challenge to the classical mechanism as the sole effect of DPP-4 inhibition to improve glycemia, recent studies have shown that DPP-4 inhibition can reduce glucose without also inhibiting plasma DPP-4 activity in the peripheral circulation; hence a nonsystemic plasma component seems to contribute. This idea was initially presented by Waget et al. in 2011 in acute studies in nondiabetic mice (14). They demonstrated that administration of low oral doses of sitagliptin (40–120 μg) improved glucose tolerance and increased circulating insulin without affecting DPP-4 activity in peripheral plasma. It should be emphasized that even though DPP-4 activity was not altered in peripheral plasma, the glucose-lowering effect of sitagliptin was still GLP-1 dependent since the effect was lost in mice with genetic deletion of GLP-1 receptors (14). While not inhibiting DPP-4 activity in peripheral plasma, these low doses of sitagliptin inhibited DPP-4 activity locally in the duodenum, jejunum, and ileum. In the gut, DPP-4 is localized to capillaries that are situated in close apposition to both the enteroendocrine cells and the nerve endings in the enteric autonomic nervous system (15). Inhibition of gut DPP-4 activity thus prevents the inactivation of intact GLP-1 immediately after its release from the L cells, which raises the tissue level of the active form of the hormone. This in turn may activate the autonomic nerves as well as result in increased portal GLP-1 levels to augment the activation of portal GLP-1 receptors. Therefore, the conclusion from the study by Waget et al. is that sitagliptin at low doses inhibits DPP-4 in the intestine compartment, which prevents the inactivation of GLP-1 immediately after its release from the L cells, rather than having an effect on inactivation of GLP-1 in the peripheral circulation by inhibiting DPP-4 activity in peripheral plasma.

We recently confirmed that DPP-4 inhibition can reduce glycemia and increase insulin secretion by a mechanism independent of inhibition of DPP-4 activity in peripheral plasma. The results are shown in Fig. 1. In nondiabetic mice, the DPP-4 inhibitor vildagliptin was administered at four different doses, and plasma DPP-4 activity was measured. We found a dose-response relationship between dose of vildagliptin and plasma DPP-4 activity. When, at the same time, an oral glucose tolerance test with measurement of insulin secretion (insulinogenic index) was undertaken, we also found that vildagliptin dose-dependently improved insulin secretion and reduced glycemia. However, by comparing the glucose and insulin results with the inhibition of plasma DPP-4 activity, it is seen that the two lowest doses of vildagliptin did not significantly affect plasma DPP-4 activity yet clearly improved glycemia and increased insulin secretion. These results therefore suggest that DPP-4 inhibition may occur in the gut at lower dose levels of the DPP-4 inhibitors than inhibit DPP-4 activity in peripheral plasma and that this reduced gut DPP-4 activity prevents the inactivation of gut GLP-1, which would raise tissue level and portal level of intact GLP-1. This would be glucose lowering through two mechanisms: by activating enteric afferent autonomic nerves and by inhibiting hepatic glucose release through hepatoportal GLP-1 receptors.

Effects Through Neural Activation

The prevention of GLP-1 inactivation in the gut would allow a more powerful stimulation of GLP-1 on the local afferent gut autonomic nerves, which may in turn stimulate insulin secretion and inhibit glucagon secretion through a neural circuit since autonomic nerves are involved in the regulation of islet hormone secretion (16). The first suggestion that GLP-1 may stimulate insulin secretin through activation of the autonomic nervous system was presented in 2000 by Balkan and Li who demonstrated that the blockade of autonomic ganglia prevents

intraportally administered GLP-1 from stimulating insulin secretion in rats (17). Furthermore, we demonstrated in 2004 that a low dose of GLP-1 given intravenously did not stimulate insulin secretion in mice that had been rendered insensitive to sensory nerve activation by the neurotoxin capsaicin (18). These findings suggested that GLP-1 had the capacity to activate afferent autonomic nerves, in the gut and portal system, which, through central coupling mechanisms, activates efferent nerves that stimulate insulin secretion. In 2007, Vahl et al. demonstrated that the GLP-1 receptor is expressed in the nodose ganglia and in nerve terminals innervating the portal vein, which provided a further support for a neural component of GLP-1 effects (19). Vahl et al. also demonstrated that portal infusion of a low dose of a GLP-1 receptor antagonist (des-His[1],Glu[9] exendin-4) resulted in glucose intolerance in rats, suggesting a neural effect. Insulin secretion was the same after GLP-1 receptor antagonism as in controls, in spite of increased glucose levels, suggesting an impaired insulin secretion. Since DPP-4 inhibition prevents the local inactivation of GLP-1 in the gut, the afferent component of the neural effect may be targeted by the treatment. There is also a central component of the autonomic mechanisms, because the activation of afferent autonomic nerves results in central effects, which activates efferent nerves. It has also been reported that brain GLP-1 is involved in the central neural effects since central activation of GLP-1 receptors stimulate insulin secretion (20). DPP-4 inhibitors probably do not, however, target this central effect since DPP-4 is not expressed centrally. The efferent neural component of the neural circuit activated by gut/portal GLP-1 is most likely the vagus nerve, which is an important regulator of islet function (16). This conclusion is also supported by findings that portal GLP-1 administration stimulates the electrical activity in hepatic vagal afferents and pancreatic vagal efferents in rats (21). A recent study also presented direct evidence for a neural component of the glucose-lowering action of DPP-4 inhibition (22). Thus Fujiwara et al. found that the increase in insulin and reduction in glycemia achieved by portal infusion of a DPP-4 inhibitor (diprotin A) were significantly reduced by hepatic vagotomy in Sprague-Dawley rats.

Therefore it may be concluded that the prevention of local inactivation of GLP-1 in the gut by DPP-4 inhibitors may allow GLP-1 to activate these gut/portal afferent nerves. A neural effect may also be of importance for the effect of DPP-4 inhibition to inhibit glucagon secretion since glucagon secretion is dependent on neural effects (16), although this has not yet been studied and therefore remains to be established.

Effects Through Insulin-Independent Mechanisms of GLP-1

A major determinant of the reduction of fasting glucose by DPP-4 inhibition is a reduction in hepatic glucose output (11,12). This effect is mainly caused by reduction of circulating glucagon through the GLP-1–induced inhibition of glucagon secretion since prevention of changes in glucagon secretion also prevents GLP-1 from inhibiting hepatic glucose output in humans (23). However, recent studies suggest that there are indeed islet-independent glycemic effects of GLP-1 mediated by the hepatic portal system. Infusion of GLP-1 was found to reduce hepatic glucose production independent of changes in insulin or glucagon levels in dogs (24) and recently in humans (25). The local prevention of GLP-1 inactivation by DPP-4 inhibition in the gut and hepatic portal system may increase portal GLP-1 levels, allowing for such a direct effect of GLP-1 to suppress hepatic glucose output through activating portal GLP-1 receptors, which is also evident from several experimental studies (26,27). Indeed, there is

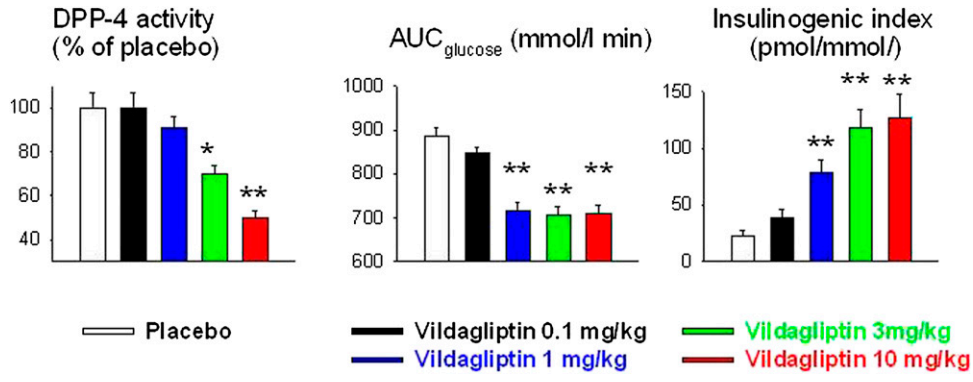

Figure 1—DPP-4 activity in peripheral plasma at 120 min after oral administration of the DPP-4 inhibitor vildagliptin at different doses or placebo (*left panel*), the area under the 120 min glucose curve after oral administration of glucose (35 mg) with or without vildagliptin at different doses (*middle panel*), and the 15 min insulinogenic index (15 min insulin level divided by the glucose level) after oral administration of glucose (35 mg) with or without vildagliptin at different doses (*right panel*). All experiments were performed in anesthetized C57BL/6J mice (n = 12 in each group), and samples were taken from the retro-orbital plexus in heparinized tubes and analyzed for DPP-4 (enzymatic measure), glucose (glucose oxidase method), and insulin (radioimmunoassay). Asterisks indicate the probability level of random difference when compared with placebo. *$P < 0.05$; **$P < 0.01$. AUC, area under the curve.

a higher increase in circulating levels of intact GLP-1 in the portal vein than in the peripheral circulation (5). We also recently confirmed that DPP-4 inhibition can lower glucose independent of changes in islet hormones in humans since in a study of acute administration of sitagliptin to healthy subjects, postprandial plasma glucose was significantly reduced without any differences in insulin or glucagon between the sitagliptin and placebo groups (28). Together these findings suggest that DPP-4 inhibition may increase portal GLP-1 levels, which reduces hepatic glucose output and increases peripheral glucose utilization by islet hormone–independent mechanisms.

DPP-4 INHIBITION IN PANCREATIC ISLETS

There is accumulating evidence that GLP-1 is expressed in islets, which is not surprising considering its coding in the proglucagon sequence in α-cells. It has, however, been thought that proglucagon is processed to glucagon in α-cells due to cell-specific expression of the processing enzymes prohormone convertase 2. However, as recently demonstrated by Marchetti et al. in human islets, GLP-1 may also be expressed in α-cells (29). It has also been shown that under certain conditions, such as high glucose, GLP-1 production is increased in α-cells due to a specific overexpression of prohormone convertase 1/3 (30). Therefore, in subjects with diabetes, there is the possibility that GLP-1 is produced in islets to such a degree that it will be of relevance after DPP-4 inhibition. This assumption has become even more likely due to accumulating evidence that DPP-4 is also expressed in islets. This was initially demonstrated 20 years ago in studies in pig islets where it was found to be localized to secretory granules of the α-cells (31). We have also demonstrated that DPP-4 is expressed in mouse and human islets and is exclusively expressed in the α-cells in human islets (32). In line with these observations, Shah et al. showed recently that long-term incubation of isolated human islets with high glucose, palmitate, and cytokines increases apoptosis and impairs insulin secretion and that the DPP-4 inhibitor linagliptin prevents this by stabilizing and increasing GLP-1 secretion along with inhibited islet DPP-4 activity (33). We have also confirmed stimulation of insulin secretion in isolated mouse islets using the DPP-4 inhibitor NVP DPP728 (32). These findings therefore suggest that a local islet mechanism may contribute to the increased insulin secretion during DPP-4 inhibition.

A local islet effect may also have anti-inflammatory effects, which may be of importance since local islet inflammation may contribute to the development of β-cell deterioration in type 2 diabetes. This may be caused by cytokines released from infiltrating immune cells. An additional mechanism may be that hyperglycemia may cause a secretion of interleukin-1β from β-cells (34). A recent study by Dobrian et al. reported that sitagliptin reduces expression of inflammatory cytokines in islets from dietary-induced obesity in mice (35). We also found recently that chronic treatment of high-fat–fed mice with vildagliptin prevented fat-induced pancreatic inflammation and peri-insulitis (36). This may be mediated by the two incretin hormones since it has been shown that treatment of splenic T cells with both GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) diminishes pancreatic infiltration of T cells after splenic T cells are injected into NOD mice (37). Hence, in addition to stimulating insulin secretion and inhibiting glucagon secretion through preventing GLP-1 inactivation in islets, DPP-4 inhibition may also prevent islet inflammation as a mechanism for improving islet function.

PREVENTION OF INACTIVATION OF OTHER BIOLOGICALLY ACTIVE PEPTIDES THAN GLP-1

DPP-4 inhibition may exert pleiotropic effects through biologically active peptides that are substrates for DPP-4. Indeed, several biological peptides apart from the GLP-1 are potentially substrates for DPP-4 although most of these findings are not relevant in vivo (4). Indirect evidence that other peptides than GLP-1 may contribute to the effects of DPP-4 inhibition was recently presented by Aulinger et al. (38). They showed that the GLP-1 receptor antagonist exendin-9 could inhibit the effect of sitagliptin on glucose and insulin secretion by only ~50% after 4 weeks of treatment with the DPP-4 inhibitor in type 2 diabetes. An obvious candidate for the GLP-1 independent mechanism is the incretin hormone GIP since GIP is inactivated by DPP-4 and stimulates insulin secretion, although this effect is reduced in type 2 diabetes. This would also be supported by animal studies showing that the glucose-lowering action of DPP-4 inhibition is only partially inhibited in mice with genetic deletion of GLP-1 receptors (or GIP receptors) but totally suppressed in mice with genetic deletion of both GIP and GLP-1 receptors (39). GIP may also be of relevance for the low risk of hypoglycemia during treatment with DPP-4 inhibition since the peptide stimulates glucagon secretion during hypoglycemia (40), which is an important counterregulatory mechanism allowing a preserved or even augmented glucagon secretion during hypoglycemia in patients treated with DPP-4 inhibition, as has been demonstrated for vildagliptin (41).

There is also, however, potential for involvement of nonincretin bioactive peptides in the effect of DPP-4 inhibitors. One such potential peptide is stromal-derived factor (SDF)-1α. It is a small peptide chemokine that is a substrate for DPP-4 since its active form (SDF-1α [1–68]) is rapidly degraded to an inactive form (SDF-1α [3–68]) by the enzyme (42). A recent study showed that SDF-1α is also expressed in rat islets, that its expression is increased by cellular injury, and that SDF-1α increases the expression of prohormone convertase 1/3 in α-cells, which increases the islet production of GLP-1 (43). In cellular injury in islets, such as oxidative stress or glucolipotoxicity, it is therefore possible that SDF-1α expression is increased with increased production of GLP-1 as a consequence. This would potentially be of relevance during

DPP-4 inhibition when inactivation of both SDF-1α and GLP-1 are inhibited, thereby facilitating further the intraislet GLP-1 production, with effects on insulin and glucagon secretion.

Another potential mediator of the insulinotropic effect of DPP-4 inhibition is pituitary adenylate cyclase-activating polypeptide (PACAP). It is a widespread neuropeptide that is secreted by intraislet nerves and stimulates insulin secretion (44). It is a substrate for DPP-4 (45), and therefore its rapid inactivation might be inhibited during treatment with DPP-4 inhibition. We have found a functional correlate to this in that the insulinotropic action of intravenously injected PACAP in mice is augmented by DPP-4 inhibition (46) and that glucose tolerance after oral glucose administration is impaired in mice with genetic deletion of PAC1 receptors compared with wild-type mice (unpublished observations). The potential contribution of prevention of PACAP inactivation by DPP-4 inhibition for the glucose-lowering action of treatment remains to be studied in more detail since PACAP, besides stimulating insulin secretion, also stimulates, and does not inhibit, glucagon secretion (43). Nevertheless, long-term administration of PACAP in animal models of diabetes has been shown to improve glycemia (47).

CONCLUSIONS

This Perspectives in Diabetes has outlined pleiotropic mechanisms that may contribute to the insulinotropic, glucagonostatic, and glucose-lowering effect seen during treatment with DPP-4 inhibitors. These effects, which have been demonstrated in several experimental conditions in rodents and to a limited degree also in humans, are illustrated together with the classical mechanism in Fig. 2. The main conclusion is that changes in DPP-4 activity in peripheral plasma may not fully explain the glucose-lowering ability of DPP-4 inhibition but that effects on tissue DPP-4 activity may contribute, either through effects of tissue-bound DPP-4 or DPP-4 in tissue plasma. These pleiotropic actions of DPP-4 inhibition may explain that DPP-4 inhibition reduces glucose almost as much as GLP-1 receptor agonists, which have a stronger direct GLP-1 receptor activation (48). The pleiotropic effects will also allow differentiation in mechanisms from other glucose-lowering strategies, including GLP-1 receptor agonists, and may also suggest that differentiation between the different DPP-4 inhibitions may not entirely depend on different degrees of inhibition of DPP-4 activity in peripheral plasma but may also be dependent on tissue penetration and local effects of DPP-4. In fact, even nonabsorbed DPP-4 inhibitors may have glucose-lowering actions through the inhibition of DPP-4 activity in the gut.

It should be emphasized that the evidence presented here is based mainly on acute studies in nondiabetic rodents. Therefore it is important to also explore the potential of the nonclassical effects of DPP-4 inhibitors in other models, including in subjects with type 2 diabetes. A few studies already exist in humans, however, that support the notion of a nonclassical mechanism for DPP-4

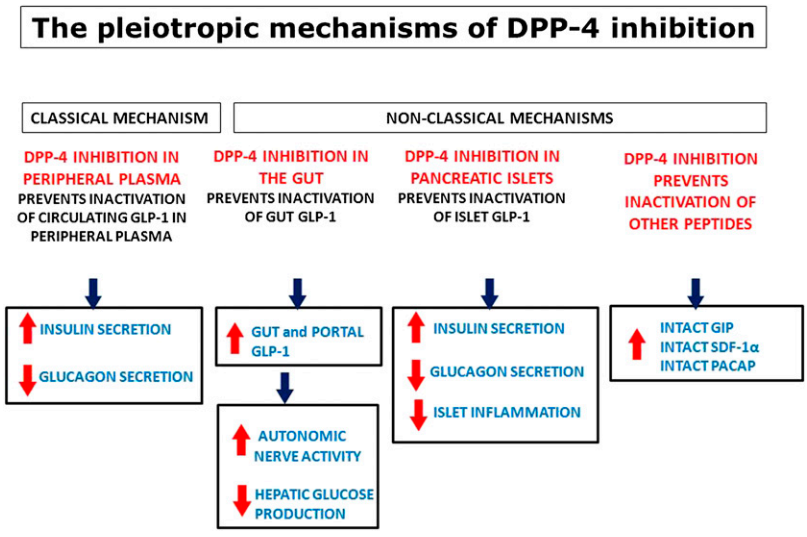

Figure 2—The classical and nonclassical mechanisms for the glucose-lowering effect of DPP-4 inhibition. GLP-$1_{7–36}$ is released from the gut into the circulation and is rapidly degraded to the largely inactive GLP-$1_{9–36}$ by DPP-4. In the classical mechanism, DPP-4 inhibitors inhibit DPP-4 in peripheral plasma, which prevents the inactivation of circulating intact GLP-1, which raises the circulating concentration of intact GLP-$1_{7–36}$. This stimulates insulin secretion and inhibits glucagon secretion, which reduces hepatic glucose production and increases fat and muscle glucose utilization, which lowers fasting and postprandial glucose. The nonclassical mechanisms of DPP-4 inhibition involve inhibition of gut DPP-4 activity, which raises tissue level of intact GLP-1 in the gut immediately after its release, in turn activating gut autonomic nerves in addition to raising the portal concentration of intact GLP-1, thereby activating portal GLP-1 receptors. Another nonclassical mechanism of DPP-4 inhibitors is inhibition of islet DPP-4, which prevents inactivation of islet GLP-1, thereby improving islet function. Finally, DPP-4 inhibitors may also prevent the inactivation of other biologically active peptides than GLP-1, such as GIP, SDF-1α, and PACAP, which may result in improved islet function.

inhibition. Thus it has been demonstrated in subjects with type 2 diabetes both for sitagliptin (14) and vildagliptin (49) that insulin secretion also after intravenous glucose administration is stimulated during DPP-4 inhibition with no or only minimal increase in circulating GLP-1 levels, which would suggest contribution by autonomic nerves and/or other bioactive peptides. A finding by Salehi et al. that GLP-1 receptor antagonism reduces the insulin response to intravenous glucose in humans may support the neural hypothesis (50). Furthermore, studies in healthy subjects (28) and in subjects with type 2 diabetes (11) have shown that sitagliptin can reduce glucose without increasing circulating insulin, which also suggests nonclassical mechanisms. Further studies are, however, important, and these should also include exploration of the relative impact of these different mechanisms. Studies in subjects with autonomic neuropathy also need to be undertaken to explore whether diabetes neuropathy would affect the neural signaling involved in the glucose-lowering action of DPP-4 inhibition. It should also be emphasized that even though effects of DPP-4 inhibitors that are different from changes in DPP-4 activity in peripheral plasma seem to contribute to their glucose-lowering action, it is not possible to distinguish whether the effect is mediated through local cell-associated or tissue DPP-4 or whether it is actually plasma DPP-4 activity present in virtually all tissue compartments. Future studies have to develop novel techniques to distinguish between these different tissue components of DPP-4 to establish the mechanisms and plasma versus nonplasma site of action of DPP-4 inhibitors. The conclusion that can be firmly drawn now is that changes in peripheral plasma DPP-4 activity to prevent the inactivation of circulating GLP-1 cannot fully account for the mechanism of DPP-4 inhibitors to lower glucose and, therefore, that nonclassical pleiotropic mechanisms may exist for the glucose-lowering action of DPP-4 inhibition.

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Author Contributions. The manuscript was written by B.O. and B.A. B.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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