GLP-1-Based Strategies: A Physiological Analysis of Differential Mode of Action

DPP4 inhibitors and GLP-1 receptor agonists used in incretin-based strategies treat Type 2 diabetes with different modes of action. The pharmacological blood GLP-1R agonist concentration targets pancreatic and some extrapancreatic GLP-1R, whereas DPP4i favors the physiological activation of the gut-brain-periphery axis that could allow clinicians to adapt the management of Type 2 diabetes, according to the patient’s pathophysiological characteristics.

The new therapeutic avenues for the management of Type 2 diabetes developed over the last decade rely on the pivotal physiological role of incretin hormones, namely the glucagon-like peptide one (GLP-1) and the gastric inhibitory peptide (GIP), since they are produced by the gut in response to food intake and have been demonstrated to enhance glucose-induced insulin secretion (25, 98, 114). However, an endoprotease, dipeptidyl peptidase-4 (DPP4), degrades the peptides (31), and hence two distinct therapeutic classes have been developed. The first involves GLP-1 receptor agonists that are preserved from DPP4-induced degradation. Importantly, one should also distinguish between molecules derived from the native GLP-1 (named GLP-1 analogs), characterized by a near 95% homology to the native hormone, from exendin4, a GLP-1-like molecule discovered in the venom of a lizard, the Gila Monster (Heloderma suspectum), which only presents 50% homology to GLP-1 but is 100% homologous to Exenatide, a synthetic peptide used to treat Type 2 diabetic patients (39, 44). Eventually, chemical molecules binding to the GLP-1 receptor and orally available could also be used in the near future to control glycemia (57). Altogether, these types of molecules belong to the GLP-1 receptor agonist family. The second strategy implies the inhibition of the DPP4, resulting in a significant increase in the blood concentration of endogenous GLP-1 (FIGURE 1).

The respective clinical effects of GLP-1R agonists and DPP4 inhibitor support the conclusion that both therapeutic classes exert a sustained and durable hypoglycemic action in Type 2 diabetic patients (26, 28) (Table 1). However, the sparse, head-to-head studies indicate that the glucose-lowering effect induced by treatment with GLP-1 receptor agonists is somewhat higher than the result obtained with DPP4 inhibitors (32, 107, 134), whereas the GLP-1R agonists and DPP4 inhibitors clearly differ, regarding their influence on body weight. Altogether, clinical trials also confirmed their excellent profile in terms of global tolerance and safety. Finally, both therapeutic classes have been demonstrated to exert additional benefits, at least in animal models, including preservation of pancreatic β-cells (148, 176) and protective actions on the cardiovascular (15, 24) and nervous systems (1, 65, 142), although most of them remain to be definitively confirmed in long-term clinical trials.

Thus it is now obvious that the respective mechanisms involved in the glucose-lowering effect of these two incretin-based therapeutic classes are different and remain to be fully elucidated. One explanation is related to the plasma concentrations of active GLP-1 associated with the use of these agents since a 10-fold average increase is usually observed with GLP1-R agonists compared with only a 2- to 3-fold postprandial increase in systemic blood GLP-1 concentration with DPP4 inhibitors (32, 63, 97). Furthermore, the differences between DPP4 inhibitors and GLP-1 agonists could also result from their specific influence on the physiological incretin system and, for instance, the respect of the physiological gradient in incretin concentrations between the production site and the arterial circulation due to the progressive action of the DPP4 (43, 63). In the present review, we will first detail the physiological bases that support the differential influences of DPP4 inhibitors and GLP-1R agonists on the incretin system. Finally, we will discuss clinical perspectives resulting from these differential, but potentially complementary, properties for the management of Type 2 diabetic patients.

The Crucial Role of Incretins in the Gut-Brain Axis

The current dogma, which stipulates that upon food intake the incretins are secreted into the portal vein and then bind to the insulin-secreting pancreatic β-cells to induce insulin secretion, is still largely
debated for the following reasons (FIGURE 2). One major observation is that only 10–15% of newly secreted GLP-1, and most likely GIP, reach the β-cells as intact hormones via the arterial blood (31, 50). This is due to the continuous action of circulating DPP4 (50). Therefore, most of the active GLP-1 would most likely bind to its corresponding receptor in a paracrine rather than an endocrine manner. More precisely, endogenous GLP-1 directly binds to the GLP-1 receptor located on different neurons within the intestine and portal vein (16, 17, 109), thus acting as a key regulator of the “gut-brain axis,” such as in response to glucose intake. The presence of the GLP-1 receptor in the lumbar dorsal root ganglion was demonstrated by immunohistochemical analyses (16, 17, 109), and GLP-1 receptor agonists have been shown to accelerate the neurite outgrowth of DRG neurons from this lumbar dorsal root ganglion in culture (62) such that treatment with exendin-4 reduced the polyneuropathy of mice with streptozotocin-induced diabetes, as determined by quantifying the nerve fiber densities (62). Pioneering experiments have also shown that GLP-1 regulates the firing rate of the vagus nerve (123, 124). The nutritional signal is transformed into an enteric neural signal, which is sent to the brain stem (3, 4). Then, the hypothalamus relays the enteric information into a new signal, which targets peripheral organs such as the liver, the endocrine pancreas, the blood vessels, the muscle, or the intestine (18, 21, 60, 61, 75, 106, 119–121). In the mouse, administration of the GLP-1 receptor antagonist exendin-9 into the hepatoportal vein reduces glucose-induced insulin secretion by half (135). Sensory denervation of mice using capsaicin abolishes the insulinotropic effect of GLP-1 in response to hyperglycemia, demonstrating the indirect action of GLP-1 on sensory nerves (5). Furthermore, among incretin hormones, GLP-1 but not GIP triggers the enteric

**FIGURE 1. GLP-1-based therapeutic molecules and strategies**

Two classes of molecules: incretin receptor agonists and DPP4 inhibitors. In the first, among incretin receptor agonists, we should discriminate between GLP-1 receptor and GIP receptor agonists, the latter being under development. Among the GLP-1 receptor agonists, we should discriminate between the native molecules (GLP-1 7-37/36 and exendin-4) and analogs of native GLP-1 such as Liraglutide, Taspoglutide, Albiglutide, Dulaglutide, or of native exendin-4 such as Lixisenatide. The analogs have the specific feature of binding albumin and hiding from the DPP4. In the second class among the DPP4 inhibitors, we should discriminate the Vildagliptin, Sitagliptin, Saxagliptin, and Linagliptin. Altogether, such strategies aim at increasing the concentration of GLP-1 agonist receptor in the blood.
We and others have always supported this concept and demonstrated that the regulation of the hepatoportal glucose sensor by GLP-1 controls glucose homeostasis and the gut-brain axis (17, 27, 69, 74, 86, 122). This set of arguments strongly suggests that increasing the efficiency of the gut-brain-periphery axis by increasing the enteric concentration of active GLP-1 might be of crucial importance for the control of glycemia in response to food intake.

**DPP4 Therapy Stimulates the Gut-Brain Axis Induced by Endogenous Incretins**

The major characteristic of DPP4 inhibitors is that they increase the concentration of GLP-1 at its physiological release site and therefore stimulate the gut-brain axis for the control of glycemia (FIGURE 2) (167). Recent data from our group demonstrate in the mouse that the glucoregulatory effect of DPP4 inhibitors depends on the inhibition of the enteroluminal DPP4 activity, i.e., in the vicinity of the GLP-1 secretion site (167). The inhibitors then enhance the neural activity of the vagus nerve, which leads to the control of insulin secretion (FIGURE 2). In the rat, direct administration of DPP4 inhibitor into the portal vein enhanced the vagus nerve firing rate activity (43). Accordingly, vagotomy reduced the satiety effect of GLP-1 (2). Although this concept has not yet been fully validated in humans, it is supported by the fact that vagotomy is known to block GLP-1-mediated gastric acid secretion (169).

This mode of action of DPP4 inhibitors must be considered as a major difference with GLP-1 receptor agonists since the latter molecules are administered systemically and most likely do not stimulate the gut-brain axis. Hence, neuropathy, which can occur rapidly during the development of diabetes (141), could alter the gut-brain axis, especially the enteric GLP-1 signal, and consequently hamper the therapeutic efficacy of DPP4 inhibitors. Therefore, the question remains as to whether DPP4i would efficiently control glycemia in Type 2 diabetic patients affected by autonomic diabetic neuropathy, which is able to induce an impaired gut-to-brain transmission signal, as previously described in animal models of Type 2 diabetes (85, 86).

### GLP-1R Agonists Recruit the Cerebral Effects of GLP-1 in Contrast to DPP4 Inhibitors

A meaningful difference between the mode of action of GLP-1R agonists and DPP4 inhibitors could...
also be due to the direct targeting of brain GLP-1 receptors (9, 45, 161) by the high pharmacological concentrations of circulating GLP-1-like molecules observed during the corresponding therapeutic strategy (Table 1). It is less conceivable, although we cannot rule out this hypothesis, that the increased circulating concentration of endogenous GLP-1 induced by DPP4 inhibitors targets brain GLP-1 receptors.

GLP-1 is most likely produced in the brain stem since proglucagon mRNA-expressing entities were detected in several nuclei, such as the solitary tract (NST) and the dorsal and ventral medulla, as well as in the olfactory bulb (38, 90, 91, 101, 139, 140, 178), where the corresponding neurons project into the hypothalamus (7–9, 14, 125, 138). However, a formal demonstration of the processing of the proglucagon peptide into GLP-1 by the brain is still needed. Interestingly, the postprandial raise in plasma GLP-1 concentration correlates with an increased regional cerebral blood flow in the left dorsolateral prefrontal cortex, as shown by PET studies exploring human satiation and the hypothalamus (126). However, in physiological situations, almost none of the endogenous GLP-1 released by the gut following a meal can reach brain nuclei, unlike in pharmacological states where large amounts of exogenous GLP-1 are administered and dramatically increase the global plasma GLP-1 concentration. This conclusion strongly supports the role of brain-released GLP-1 (7, 138) in the control of the above central effects in physiological situations that could be mimicked during pharmacological treatments using GLP-1R agonists. Over the past two decades, numerous studies have pointed out that brain GLP-1, as a neuropeptide, not only regulates insulin secretion but also controls food intake and body weight (2, 40, 82, 158, 171). It has been demonstrated that the suppressive effect of intraperitoneal administration of Exendin-4 on food intake was attenuated by the concomitant administration of the GLP-1 receptor antagonist Exendin-9 (76). Furthermore, Exendin-4 also required sensory afferents to reduce food intake, which triggered noncerebral GLP-1 receptor at low doses, whereas higher doses were required to target the brain (53, 55, 76).

Incretin based therapies: pharmacological routes

![Incretin-based therapies: pharmacological routes](http://physiologyonline.physiology.org/)

**FIGURE 2. Incretin-based therapies: pharmacological routes**

Nutrient absorption of glucose/lipids increases secretion of gut-released GLP-1, which, released into the hepatoporial vein, is mostly degraded by DPP4 before reaching the systemic blood. The remaining GLP-1 then triggers glucose-induced insulin secretion. At the same time, enteric GLP-1 activates the gut-brain axis. A new signal is sent to peripheral tissues, which control glycemia and cardiovascular functions. The GLP-1 receptor agonists directly target the β-cells and could trigger brain cells as well. We cannot rule out that these molecules also recruit the gut-brain axis, although no gradient is generated by systemic administration. Conversely, DPP4 inhibitors enhance the hepatoporal concentration of GLP-1, which favors the activation of the gut-brain axis. We cannot rule out the influence of DPP4 inhibitors on a significant increase in plasma GLP-1 concentration that would still trigger glucose-induced insulin secretion.
Other arguments for the central role of GLP-1 are related to adverse effects, such as nausea and vomiting observed when GLP-1 receptor agonists are used. This could be due to the excessive inhibition of gastric emptying (67), when the circulating GLP-1 analog crosses the blood-brain barrier or when it reaches regions of the hypothalamus, such as the arcuate nucleus, which are not fully protected by the blood-brain barrier. These central actions of GLP-1R agonists also give the main explanation for the taste aversion and food intake reduction observed with such therapies (146, 147). This conclusion is also supported by recent data from the literature, since the central role of GLP-1 has now gained a wide range of effects, including neuroprotection from neurotoxins and cognitive functions such as learning (38). The GLP-1 receptor was also shown to play an important role in the synaptic plasticity, which has been linked to some forms of memory formation (1). Such an effect could be involved in the treatment of Alzheimer’s disease (94, 129, 131) by increasing cell proliferation (49), reducing beta amyloid peptide production (94), and protecting neurons from oxidative stress (130).

**Regulation of Insulin Secretion by GLP-1: Endocrine Action vs. Indirect Mechanisms**

Glucose-induced GLP-1 secretion that targets the pancreatic β-cells directly to enhance glucose-induced insulin secretion is the established dogma to explain the glucose-lowering effects of this incretin. However, the respective contributions of neural and endocrine actions of GLP-1 on insulin secretion have been recently addressed using a transgenic animal model in which the GLP-1 receptor is specifically expressed in pancreatic β-cells (FIGURE 2). The authors demonstrate that, in response to a glucose challenge, insulin secretion was increased similarly to what is observed in wild-type mice, suggesting that GLP-1 signaling in the β-cells is sufficient to ensure normal insulin secretion (92). This important set of data illustrates that a low concentration of GLP-1, resulting from the secretory activity of gut endocrine cells, is capable of resuming its overall glucostatic activity via pancreatic GLP-1 receptors, thus independently from communication with neural pathways. However, this conclusion only implies that the GLP-1 receptors on the β-cells are sufficient for the regulation of glucose-induced insulin secretion in this specific animal model. It does not imply that gut GLP-1 can actually reach the β-cells to trigger insulin secretion. Recently, another hypothesis proposed that GLP-1 could be produced locally by differential processing of proglucagon into GLP-1 by pancreatic α-cells in vivo as shown in a mouse model of β-cell regeneration (81, 118). The pancreatic α-cells could process some GLP-1 by activation of prohormone convertase 1/3, which is normally not the case in these cells (81). This hypothesis could explain why GLP-1 was also found in isolated human islets and why in rat islets cultured for 7 days the ectopic overexpression of a proconvertase was observed (170). Furthermore, the ectopic expression of GLP-1 was even observed in min6 cells that maintain insulin secretion and ensure cell survival (112). Similarly, it could also be speculated that the proGLP-1 could be expressed in α-cells and undergo similar processing by PC1/3 to generate and release GIP and help trigger insulin secretion.

Furthermore, this does not rule out the hypothesis that, in wild-type animals, the regulation of the gut-brain-periphery axis by GLP-1 is also involved in the control of insulin secretion. The pharmacological blockage of brain GLP-1 receptor by exendin9 prevents glucose-induced insulin secretion (84). Altogether, both the direct endocrine and the neural paracrine actions of GLP-1 can probably exert simultaneous regulatory actions on β-cell function. However, their respective contribution is yet to be determined, which poses new questions regarding the contribution of the DPP4 inhibitor strategy of the control of glucose-induced insulin secretion compared with the direct effect of GLP-1 receptor agonists. Furthermore, enhancement of the transient physiological concentration of native GLP-1 and GIP, or the establishment of a continuous pharmacological circulating concentration of GLP-1 receptor agonist are most likely to trigger the insulin secretory machinery and β-cell mass in the long term in different ways.

**The Role of GIP: A Major Difference Between DPP4 Inhibitors and GLP-1R Agonists**

Inhibiting DPP4 activity prevents the degradation of numerous other peptides, primarily GIP, which regulates insulin secretion (100). Furthermore, since the GIP receptor expression has been identified in extrapancreatic tissues such as the adipose tissue, the gut, the bones, and the brain, increasing the circulating levels of GIP by DPP4 inhibitors could enhance the specific actions of this incretin on extrapancreatic tissues (71, 128). Accordingly, it has been shown that obesity-associated metabolic disturbances could be reduced by the chemical and genetic ablation of GIP receptor signaling in mice (102, 128). Therefore, this point is important since a DPP4 strategy will lead to an increase of GIP (93, 135, 167), a feature not observed with GLP-1 receptor agonists. The extrapancreatic effects of
GIP, whether good or bad for health, could be challenged in Type 2 diabetic patients, since the action of GIP has been proposed to be reduced (113, 157). Similarly, DPP4 is involved in the transformation of PYY(1–36) into PYY(3–36) (11, 83). Since the latter peptide inhibits food intake, the DPP4 inhibitors would prevent PYY from being synthesized and, hence, from the satiety effect of PYY(3–36) (83, 87). Therefore, the overall action of DPP4 inhibitors is most likely not restricted to GLP-1, and this could be part of the reason why DPP4 inhibitors are neutral on body weight gain. GLP-1R agonist would have a more straightforward action, restricted to GLP-1 receptors on different locations of the body.

Incretin Secretion: A Specific Concern for DPP4 Inhibitor and GLP-1 Receptor Agonist Efficacy

The obvious importance of incretins on the regulation of glycemia suggests that impaired GLP-1 secretion (145) could be considered as an early step among the mechanisms leading to glucose intolerance (51, 103). Considering this important question, it is conceivable that, in patients with no or a very low GLP-1 secretory capacity, the DPP4 inhibitors would have a lower therapeutic efficacy. Hence, the mechanisms regulating glucose-induced GLP-1 secretion should be considered as important regulators of DPP4 inhibition efficacy. They are most likely related to genes regulating glucose-sensing mechanisms, previously identified for glucose-induced insulin secretion (151, 152), glucokinase (163), glucose transporter (SGLT-1, GLUT2) (143, 144), or the genes involved in glucose-sensing mechanisms, previously identified on mRNA and protein levels (149, 150). Differences in GLP-1 receptor phosphorylation, and intracellular Ca2+ accumulation, extracellular signal-regulated kinase 1/2 (ERK1/2) activation, and PKA signaling across the receptor and lead to physiologically relevant differences in signaling pathways (cAMP accumulation, extracellular signal-regulated kinase 1/2 phosphorylation, and intracellular Ca2+ mobilization) (13, 89). However, no clear data demonstrate the direct relevance of the polymorphisms in the regulation of glucose-induced insulin secretion. Knowing these polymorphisms could also help to identify the timing of reduced therapeutic efficacy available from human studies to determine the hepatoportal concentration of incretins following a meal in healthy or Type 2 diabetic subjects. However, recent experiments have been conducted in the pig to assess the active and total concentrations of GLP-1 in different vascular beds such as the carotid artery and the femoral, hepatic, and portal veins (63). The data show that, in response to a meal, the GLP-1 secreting molecule, the concentrations of total GLP-1 increased six- to eightfold, both before and after DPP4 inhibitor administration in the portal vein, and decreased progressively as the distance from the site of release increased, with lowest concentrations being found in the peripheral venous plasma (63). This observation is of major importance since it further demonstrates the importance of the temporal hepatoportal to peripheral GLP-1 gradient due to the progressive degradation of GLP-1 by the DPP4. No incretin effect was observed when a postprandial rise in systemic GLP-1 and glucose concentrations was modeled in the dog (70). This set of data confirms the notion of gut-brain axis for the control of insulin secretion and glucose homeostasis (20). Unfortunately, no such data are yet available in Type 2 diabetic humans, and, therefore, the efficacy of incretins to be secreted in response to a meal is purely hypothetical since they are all based on peripheral venous plasma. A recent meta-analysis did not support the contention of a generalized defect in nutrient-related GLP-1 secretory responses in Type 2 diabetes patients, opening the route, in all patients, to a DPP4 inhibitor-based therapy (117).

Resistance to the Action of Incretin as a Modulator of Therapeutic Responses

Incretin receptors enhance glucose-induced insulin secretion through mechanisms requiring increased cAMP production, leading to the activation of PKA and numerous other intracellular signaling pathways (149, 150). Differences in GLP-1 receptor signal transduction could contribute to impairing the incretin signal and, hence, the therapeutic action of the corresponding receptor agonists. This could be illustrated by the presence of several single nucleotide polymorphisms (SNPs) that are distributed across the receptor and lead to physiologically relevant differences in signaling pathways (cAMP accumulation, extracellular signal-regulated kinase 1/2 phosphorylation, and intracellular Ca2+ mobilization) (13, 89). However, no clear data demonstrate the direct relevance of the polymorphisms in the regulation of glucose-induced insulin secretion. Knowing these polymorphisms could also help to identify the timing of reduced therapeutic efficacy...
and therefore to switch rapidly to another therapeutic strategy. Overcoming putative GLP-1 resistance would most likely be difficult by means of DPP4 inhibitors, which moderately increase the concentration of active GLP-1. So far, no markers are available to define such subgroups of patients, but arguments related to the detailed mode of action of each therapeutic strategy could provide answers.

**DPP4 Biological Characteristics Could be Discriminating Arguments for the Preferential Use of DPP4 Inhibitors or Analogs in Therapeutic Strategies**

*Dpp4−/−* mice exhibit reduced glycemic excursion following a glucose challenge in association with increased levels of glucose-stimulated insulin and of the intact insulinitropic forms of GLP-1 and GIP (96), illustrating the importance of endogenous DPP4 for the control of the incretin axis. The different DPP4 inhibitors that have been approved to date (sitagliptin, vildagliptin, saxagliptin, and linagliptin) block the activity of the enzyme very specifically in a test tube. However, some differences remain between these inhibitors regarding their features of absorption, distribution, metabolism, and elimination, which, combined with differences in their potency and duration of action, suggest that their mode of action is not similar.

From a clinical point of view, the glucostatic efficacy of the different inhibitors is similar. The quantitative determination of the circulating amount of DPP4 is estimated to be a very small fraction of the overall enzyme, which is most likely not responsible for the overall degradation of the GLP-1 produced by the gut (50). A key point is that the inhibition of the blood DPP4 activity does not seem to be mandatory for the glucostatic action of DPP4 inhibitors (167). Conversely, most of the glycemia control during an oral glucose challenge could be attributed to the inhibition of the enteric luminal and mucosal DPP4 activity (167). It is noteworthy that a side effect directly linked to the inhibition of DPP4 is the reduced production of dipeptides. Although His-Ala is specifically released during GLP-1 processing by the DPP4, it is suspected that many other dipeptides could be released by the endoluminal endo-protease essentially during and following a protein-enriched meal. The role of the lack of dipeptide release following DPP4 inhibition was recently shown to involve the simultaneous and opposite control of insulin and glucagon secretion from mouse and human islets (167). Altogether, the DPP4 inhibitors that have different pharmacokinetic characteristics could target the DPP4 in various locations and hence impact glucose metabolism differently. No data are yet available in humans to identify whether this hypothesis is true, and, therefore, room is available for the identification of putative differential effects between inhibitors. Eventually, clinicians will have to pay attention to unexpected or previously reported symptoms mediated by the regulation of other less well understood peptides than the incretins.

**Glucagon Secretion and Gastric Emptying: An Additional Argument to Discriminate Between the Use of DPP4 Inhibitors and GLP-1R Agonists**

In Type 2 diabetic patients, glucagon-induced excessive hepatic glucose production is a major feature of hyperglycemia in the fasting and fed states (22, 159, 160). Both GLP-1R agonists and DPP4 inhibitors regulate glucagon secretion and, hence, hepatic glucose production (26). GLP-1 may exert its glucagonostatic effect via direct interaction with GLP-1 receptors on pancreatic α-cells, but this is highly controversial since the presence of these receptors on glucagon-producing cells has been detected in some studies (35, 59) but not in others (155). If GLP-1 receptors are indeed expressed, then, one could suggest that the glucagonostatic effect of GLP-1R agonists could reflect direct targeting of these cells (34, 58, 78). If not expressed, an indirect route should be responsible for the physiological regulation of glucagon secretion, such as the gut-brain-periphery axis recruited by DPP4 inhibitors (16, 19). An observation has been proposed to reconcile the two hypotheses related to the regulation of glucagon secretion by GLP-1R agonists. This hypothesis implies that only a subset of α-cells expresses GLP-1 receptors, which could explain the discrepancies. In addition, pancreatic α-cells could also express GIP to promote insulin secretion (42), which could be processed by PC1/3 proconvertases and then inhibit glucagon secretion. However, the low concentration of circulating native GLP-1 during DPP4 inhibitor therapies still cannot distinguish between the simple existence of the direct route or whether it is predominant. Conversely, the use of GLP-1R agonists certainly recruit the GLP-1 receptors expressed at the surface of the subset of glucagon-producing cells to control glucagon secretion. Furthermore, the presence of the GLP-1 receptor at the surface of pancreatic α-cells, if any, seems to be a feature of cell lines since GLP-1R mRNA was only detectable in the β-cells in normal pancreatic tissue from mice and rats (155). GLP-1R has been shown to be exclusively colocalized with insulin-expressing cells since the use of two different GLP-1R antibodies simultaneously
with others directed against glucagon, somatostatin, or the pancreatic polypeptide failed to show co-staining with noninsulin containing cells, and, hence, another indirect mechanism needs to be suggested. It was proposed that glucose-induced insulin secretion is responsible for the glucagonostatic effect of GLP-1. However, this hypothesis is only partly supported by data from the literature. To demonstrate this feature, C-peptide negative type 1 diabetic patients were treated acutely by an infusion of GLP-1 during a concomitant hyperinsulenic and hyperglucyemic glucose clamp study (80). In such conditions, GLP-1 decreases glucagon secretion as well as the arginine-induced glucagon response during hyperglycemia (80), which is, in addition to the insulinotropic action of GLP-1, considered to equally contribute to the glucose-lowering action of GLP-1 (52). From a therapeutic point of view, GLP-1R agonists showed strong glucagonostatic effects. A short-term, 2-wk treatment with exenatide reduced postprandial glucagon in Type 2 diabetic patients (32). Conversely, in nondiabetic subjects submitted to intense physical exercise to induce hypoglycemia, a single administration of exenatide did not reduce plasma glucagon concentration (79), suggesting that the glucagonostatic feature was mainly observed in physiopathological situations, such as Type 2 diabetes.

DPP4 inhibitors have also been described to have a glucagonostatic activity leading to the suppression of hepatic glucose production following a meal in humans (10). Vildagliptin preserves proper glycemic control by enhancing the sensitivity of the α-cell to reduce glucagon secretion during hyperglycemia and conversely stimulating its secretion in hypoglycemia (10). These effects likely contribute to the efficacy of vildagliptin to improve glycemic control as well as to its low hypoglycemic potential (6). Similarly, sitagliptin reduces postprandial glucagon secretion (32) and endogenous glucagon production (37). These important clinical features were linked in animal models with the role of both GLP-1 and GIP (41).

Another important feature that could discriminate between the mode of action of DPP4i and GLP-1 receptor agonist is gastric emptying. By triggering the gut brain axis, GLP-1 is thought to control gastric emptying, thus slowing both hyperglycemia and lipid absorption (16, 20, 54, 64, 162). This effect is reported for GLP-1 receptor agonist and mostly for short acting, also named prandial, molecules (12, 88, 116) and almost absent for DPP4i (164). Hence, in response to the lowering of blood glucose, plasma insulin secretion is only mildly increased in response to oral glucose challenge. This strategy could further reduce the impact of hyperinsulinemia on body weight gain and insulin resistance (23, 95). However, it could be expected that the reduction of gastric emptying could be accompanied by nausea and vomiting (33, 77, 132, 133), although this does not seem to hold for all molecules (52). The reason for the different mode of action between DPP4i and GLP-1 receptor agonist could be the direct activation of brain GLP-1 receptor by the pharmacological concentrations of circulating GLP-1 obtained with GLP-1 receptor agonists. This has been described following the direct administration of GLP-1 into the brain stem (56, 108, 110) and of the co-secreted peptide GLP-2, which triggers proopiomelanocortin-expressing neurons (48). GLP-2 is a peptide distinct from GLP-1 with its own receptor and ~50% homology to both glucagon and GLP-1. Importantly, the effect of long-acting GLP-1 receptor agonists such as liraglutide on gastric emptying vanishes overtime through a desensitization mechanism called tachyphylaxis (72, 115). This important mechanism was also described following intravenous infusion of the endogenous peptide, which decelerated gastric emptying more rapidly after the first meal than after the second, suggesting a reduction of the effect of GLP-1 (115, 172).

**From Switch to Combination Strategies: Clinical Perspectives for the Management of DPP4 Inhibitor and GLP-1 Agonist Therapies**

Although both therapeutic classes are directly derived from the incretin concept, pharmacological as well as experimental and clinical data reviewed here fully support the conclusion that DPP4 inhibitors and GLP-1R agonists must be considered to involve two distinct approaches. Initially, the major question was whether these two therapeutic strategies, aiming at increasing the concentration of circulating active GLP-1, exerted a similar or different impact on the control of glycemia and energy homeostasis. Although most clinical studies indicate that GLP-1 receptor agonists induce weight loss and exert greater glycemic control than DPP4 inhibitors, it is also clear from clinical trials that these latter molecules have a more favorable profile in terms of tolerance and safety. As reported in the recent ADA-EASD position statement for the management of hyperglycemia in Type 2 diabetes, both therapeutic classes can be prescribed as soon as oral monotherapy with metformin fails to maintain optimal glycemic control (68).

However, numerous questions remain to be resolved to optimize the use of these therapies for the management of Type 2 diabetes. First, retrospective analysis of clinical studies has, until now, failed to identify specific clinical or pathophysiological features able to unambiguously predict the response to either DPP4 inhibitors or GLP-1R
agonists (104, 153). It is thus not easy to establish clinical recommendations to optimize the use of these treatments. For instance, in the recent NICE guidelines, DPP4 inhibitors are only proposed on the basis of their safety profile, instead of sulfonylureas in subjects at risk of hypoglycemia, and instead of thiazolidinediones when weight gain is feared. Similarly, GLP-1 receptor agonists are only recommended in obese patients with BMI values above 35 kg/m$^2$ due to their ability to promote weight loss. Importantly, these guidelines emphasize the need to systematically evaluate the beneficial effects obtained in the 6 mo following treatment introduction, underlining the impossibility to predict a priori the effects of these molecules at the individual level. Besides some safety and socio-economic issues, some hypotheses could be inferred from the preclinical and some rare clinical data to use DPP4i or GLP-1 receptor agonists. First, DPP4i could be preferred at the onset of Type 2 diabetes, i.e., when the enteric nervous system is most likely not impaired to fully ensure the action of the endogenous GLP-1 on the gut-brain axis. Conversely, the pharmacological doses of circulating GLP-1 reached when using GLP-1 receptor agonists indicate the use of the corresponding molecules in patients with a longer history of diabetes and most likely in association with basal insulin therapy. However, these speculations must be validated by appropriate clinical trials, and it is thus crucial to improve our knowledge of the respective mechanisms involved in the actions of DPP4 inhibitors or GLP-1 receptor agonists to characterize clinical or biological criteria, which will help to identify responder and nonresponder subjects to either therapy. For instance, as suggested by experimental data, the integrity of the gut-brain-periphery axis seems to be a pivotal notion for the efficacy of DPP4 inhibitor-based therapy. It is thus tempting to suggest that, although not so easy in clinical practice, systemic screening and gradation of autonomic neuropathy would be helpful to adapt the therapeutic strategy.

Second, it has been unclear until now whether subjects considered as primary or secondary nonresponders to DPP4 inhibitors could be successfully switched to GLP-1 receptor agonist treatment and vice versa. Clinical data are sparse to date, restricted to information from the DURATION-2 study, which evaluated the safety and efficacy of switching from sitagliptin to exenatide once weekly (174). At the end of the 26-wk, double-blind treatment period, subjects initially randomized in the sitagliptin arm were indeed proposed to enter an additional 26-wk, open-label, uncontrolled observational period, stopping this oral medication and receiving a 2-mg exenatide injection weekly. These patients demonstrated significant incremental improvements in HbA1c ($-0.3 \pm 0.1\%$), fasting plasma glucose ($-0.7 \pm 0.2 \text{mM}$), and weight ($-1.1 \pm 0.3 \text{kg}$), and, as expected, nausea was the most frequent adverse event in this assessment period (174). Inversely, to the best of our knowledge, it has not been evaluated whether switching from GLP-1R agonists to DPP4 inhibitors is a valid strategy when the first therapy did not provide the expected metabolic effect or when it induced side-effects. According to differences in their mode of action, and mainly the capacity of DPP4 inhibitors to restore an adequate hepatoportal-to-periphery gradient in postprandial GLP-1 concentrations, it is probably important to encourage clinical studies testing this strategy in the near future.

Third, since both incretin-based therapeutic classes exert their beneficial effects by recruiting different mechanisms, it is also tempting to hypothesize that these molecules could act additively. However, whereas DPP4 inhibitors have often been used to potentiate the actions of GLP-1 receptor agonists in experimental conditions, the effectiveness and the tolerance of this therapeutic combination had not been addressed in diabetic patients until this year. Indeed, in a recently published clinical trial, 208 Type 2 diabetic patients experiencing inadequate glycemic control under sitagliptin plus metformin therapy Type 2 diabetic patients were randomized to receive either twice daily exenatide injections plus metformin and sitagliptin (combination strategy) or twice daily exenatide injections plus metformin and placebo (switch strategy) (166). Interestingly, the data failed to demonstrate the non-inferiority of the switch strategy compared with the combination of both incretin-based therapies. Change in HbA1c from baseline to week 20 was significantly greater in the combination therapy group (exenatide + sitagliptin, $-0.68 \pm 0.08\%$) than in subjects who switched to exenatide (exenatide + placebo, $-0.38 \pm 0.09\%$; $P = 0.12$), with a greater proportion of subjects achieving HbA1c of $<7\%$ (41.7 vs. 26.6%; $P = 0.027$). No significant differences were observed between the two groups in terms of weight loss ($-2.20 \pm 0.24$ vs. $-2.58 \pm 0.25$ kg) and side-effects, especially the incidence of hypoglycemia. This study thus provides the first evidence that the combination of one DPP4 inhibitor and one GLP-1 agonist should represent an interesting strategy for the management of Type 2 diabetes.

Finally, the hypothesis that, other than their glucose-lowering action, GLP-1R agonists and DPP4 inhibitors could exert cardiovascular protective effects has emerged in recent years. Evidence was first provided by animal studies since GLP-1 and exendin-4 were demonstrated to exert beneficial effects in ischemia/reperfusion and cardiac failure models (15). Similar experimental observations...
have been reported with GLP-1R agonists and DPP4 inhibitors (66). Furthermore, these treatments were demonstrated to favorably influence cardiovascular risk factors in Type 2 diabetic subjects enrolled in phase 3 clinical trials. For instance, GLP-1R agonists exert a slight but significant lowering effect on systolic blood pressure without a significant change in diastolic blood pressure (reviewed in Refs. 165, 173). Few clinical studies have reported on the influence of DPP4 inhibitors on blood pressure, but almost none found a significant effect. In addition, corroborating data indicate that GLP-1R agonists can induce a small increase in heart rate (+2–3 pulses/min). This undesirable effect is thought to result from the high pharmacological GLP-1 circulating levels induced by these therapies, since no change in heart rate was reported with DPP4 inhibitors (165). Altogether, data from clinical trials also indicate that both GLP-1R agonists and DPP4 inhibitors only exert a weak effect on fasting plasma lipid but are able to reduce postprandial hyperlipidemia, especially the rise in triglyceride-rich particles (99, 156). Finally, although these data must be interpreted with caution, meta-analyses of phase 3 clinical trials have been reported recently, demonstrating a lower rate of cardiovascular events in Type 2 diabetic subjects that received DPP4 inhibitors (73, 104). Large prospective studies are already ongoing, each including more than 15,000 Type 2 diabetic subjects with the aim of determining the influence of some DPP4 inhibitors and GLP-1 receptor agonists on cardiovascular outcomes (73).

In conclusion, GLP-1 receptor agonists and DPP4 inhibitors have so far been considered to belong to the same class of incretin-based therapy molecules. Although this statement is surely true, recent data from the literature underlining their respective modes of action demonstrate that their effects on the control of glycemia and other physiological functions present major differences in their modes of action. Although both strategies lead to an increase in the circulating concentration of active GLP-1, significant divergence in their glucostatic mode of action has been identified. The factors involved are 1) their circulating concentrations, which are much higher when using GLP-1 receptor agonists; 2) the blood gradient of the incretins, which is respected when using DPP4 inhibitor; 3) the restricted activation of the GLP-1 receptor by the GLP-1-like molecules, whereas DPP4 inhibitors extend their action to other molecules like GIP and NPY; 4) the pharmacokinetics and release of the pharmacological molecules, which could be very different from each other and therefore reach different active sites for a different efficacy or even function. Better knowledge of the specific mechanisms recruited by each therapeutic class should provide new perspectives to improve their use in the global management of Type 2 diabetes.


Author contributions: R.B., P.G., and S.D. drafted manuscript.

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