REVIEW

GLP-1, the Gut-Brain, and Brain-Periphery Axes

Cendrine Cabou and Rémy Burcelin

INSERM (Institut National de la Santé et de la Recherche Médicale), U1048, Institute of Metabolic and Cardiovascular Diseases Rangueil, University of Toulouse III (Paul-Sabatier), (C.C, R.B), and the Faculty of Pharmacy, Toulouse, France.
Address correspondence to Remy Burcelin, e-mail: remy.burcelin@inserm.fr

■ Abstract

Glucagon-like peptide 1 (GLP-1) is a gut hormone which directly binds to the GLP-1 receptor located at the surface of the pancreatic β-cells to enhance glucose-induced insulin secretion. In addition to its pancreatic effects, GLP-1 can induce metabolic actions by interacting with its receptors expressed on nerve cells in the gut and the brain. GLP-1 can also be considered as a neuropeptide synthesized by neuronal cells in the brain stem that release the peptide directly into the hypothalamus. In this environment, GLP-1 is assumed to control numerous metabolic and cardiovascular functions such as insulin secretion, glucose production and utilization, and arterial blood flow. However, the exact roles of these two locations in the regulation of glucose homeostasis are not well understood. In this review, we highlight the latest experimental data supporting the role of the gut-brain and brain-periphery axes in the control of glucose homeostasis. We also focus our attention on the relevance of β-cell and brain cell targeting by gut GLP-1 for the regulation of glucose homeostasis. In addition to its action on β-cells, we find that understanding the physiological role of GLP-1 will help to develop GLP-1-based therapies to control glycemia in type 2 diabetes by triggering the gut-brain axis or the brain directly. This pleiotropic action of GLP-1 is an important concept that may help to explain the observation that, during their treatment, type 2 diabetic patients can be identified as 'responders' and 'non-responders'.

Keywords: type 2 diabetes · glycemic control · incretins · GLP-1 · DPP-4 · brain · exendin-4 · glucose homeostasis · area postrema

Introduction

Glucose-induced insulin secretion is regulated by several neural and hormonal stimuli. In particular, hormones secreted by intestinal endocrine cells potently stimulate glucose-induced insulin secretion after nutrient absorption. These hormones, called gluco-incretins or insulinotropic hormones, are major regulators of postprandial glucose homeostasis. The first incretin identified was called gastric inhibitory polypeptide (GIP) as it inhibits gastric acid secretion [1]. It was renamed glucose-dependent insulinotropic polypeptide to point to its incretin effect, thus better reflecting its physiological action. The second incretin to be discovered was called glucagon-like peptide 1 (GLP-1). Together, these incretins regulate the function of the endocrine pancreas and specific brain functions. Each incretin has its own specific action on the gastrointestinal tract, blood vessels, heart, adipose tissue, and liver. Altogether, incretins control body weight and glycemia. Consequently, the metabolic action of incretins has served as the basis for multiple strategies in the treatment of type 2 diabetes.

A considerable amount of data is now available regarding the insulin secretory effect of incretins and the efficacy of incretin-based therapies in type 2 diabetes. Most of the data focused on GLP-1. This is most likely because it is more suitable for

the treatment of diabetes since it is not lipogenic compared to GIP. Several studies have shown that type 2 diabetic patients are resistant to GIP treatment. Also, over the course of the last decade, GLP-1 was shown to play an important role in the control of cardiovascular functions and the brain Some findings point to an indirect role for the control of glucose metabolism, which has led to revisiting the metabolic action of the incretins. Firstly, 80-90% of the secreted GLP-1 is degraded by the dipeptidyl-peptidase 4 (DPP-4) before reaching insulin secreting β-cells. Secondly, a gut-to-brain GLP-1- and glucose-dependent axis has been described involving the vagus nerve for the transmission of nutritional signals towards the brain. Thirdly, GLP-1 and the GLP-1 receptor are present in the brain. Finally, multiple physiological effects are associated with the role of incretins in the brain. Consequently, the central action of the incretins in type 2 diabetic humans is now a matter of debate. As most studies have been performed on GLP-1, this review addresses the effects of GLP-1 on the brain in the regulation of energy homeostasis. It is noteworthy that recent literature points to a neuroprotective role for GLP-1, which, although important, is beyond the scope of this review.

The incretin concept

The incretin concept emerged from the observation that oral administration of an extract of pig duodenal mucosa could reduce hyperglycemia in diabetic patients [2]. The authors proposed that a stimulation of the internal pancreatic secretion would be mediated by this extract. Later, the development of an insulin immunoassay proved that this effect was due to an insulinotropic action [3]. At the same time, Elrick and Mc Intyre found that the insulin response to an oral glucose load was much greater than the response measured after an intravenous infusion of an equivalent amount of glucose [4, 5]. Their findings led to the conclusion that intestinal factors called incretins control postprandial insulin release. Subsequently, the two peptides secreted by the intestine were found to be involved in increased insulin secretion following an oral glucose load, GLP-1 and GIP.

First evidence on the existence of GLP-1 was found in patients with small-bowel resection who had a decreased incretin activity, despite plasma GIP remaining at normal level [6]. Later, GLP-1 was discovered following the cloning and sequencing of mammalian proglucagon genes [7, 8]. GIP is

secreted by enteroendocrine K-cells, found predominantly in the proximal gut (duodenum-jejunum) [9, 10]. GLP-1 secreting L-cells are mainly localized in the distal gut (ileum-colon) [11-12]. In healthy humans, the intravenous infusion of either of these two peptides contributes nearly equally to the incretin effect in the fasted state, or under an euglycemic clamp obtained with a glucose infusion [13]. However, during hyperglycemia GLP-1 is more insulinotropic than GIP. Importantly, in most cases, GLP-1 maintains its insulinotropic effect in diabetic patients, but not GIP [14-17].

Peripheral GLP-1: secretion, metabolism and insulinotropic action

In the intestine, GLP-1 is synthesized by the pre-proglucagon gene (Figure 1), which is mainly expressed in intestinal enteroendocrine L-cells,

Abbreviations:
3drV - dorsal third ventricle
3V - third ventricle
AMP - adenosine monophosphate
ANS - autonomic nervous system
AP - area postrema
ARC - arcuate
AgRP - agouti-related peptide
ATP - adenosine triphosphate
CART - cocaine- and amphetamine-regulated transcript
CC - central canal
cDNA - complementary deoxyribonucleic acid
DMN - dorsomedian
DMNX - dorsal motor nucleus of the vagus
DPP-4 - dipeptidyl peptidase-4
DVC - dorsal vagal complex
GIP - glucose-dependent insulinotropic polypeptide
GLP-1 - glucagon-like peptide-1
Glp1r - GLP-1 receptor
GLP-2 - glucagon-like peptide-2
GRPP - glycentin related polypeptide
HFD - high fat diet
icv - intracerebroventricular
IP-1 - intervening peptide 1
K_{ATP} - ATP-sensitive potassium
KO - knock-out
LV - lateral ventricle
MPF - major proglucagon fragment
mRNA - messenger ribonucleic acid
NEP - neutral endopeptidase
NPY - neuropeptide Y
NTS - solitary tract nucleus
PKA - protein kinases A
PKC - protein kinases C
POMC - propiomelanocortin
PVN - paraventricular

and to a lesser extent in pancreatic α-cells and neurons. Although the mRNA transcript sequence is identical in these organs, it leads to the generation of a peptide precursor, namely proglucagon, which undergoes different proteolytic cleavages, and yields different end products in these tissues [11, 18-20] (Figure 1). In L-cells, the end products are GLP-1, GLP-2, glicentin, and oxyntomodulin. GLP-1 is first synthesized as an immature form with 37 amino acids. It is then cleaved and amidated at the C-terminal end to compose the major bioactive form of GLP-1: a peptide with 30 amino acids, called GLP-1 (7-36)-amide, which is found in the brain and circulating blood of humans and rodents [21, 22].

Oral glucose absorption induces GLP-1 (7-36)-amide release from intestinal L-cells into intestinal capillaries. It reaches the portal vein, then liver and pancreas, where it binds to a specific receptor expressed on pancreatic β-cells, and enhances glucose-induced insulin secretion. This glucose-dependent physiological mechanism is the basis of an important therapeutic advantage that avoids iatrogenic hypoglycemia. Thus, oral glucose is a potent stimulus for the release of GLP-1, and this does not occur when glucose bypasses the absorptive processes in the gut. The molecular events leading to GLP-1 release by L-cells have been described in a recent review [23]. GLP-1 also decreases glucagon secretion by pancreatic α-cells [14], and increases the insulin to glucagon ratio, which improves insulin sensitivity. Furthermore, GLP-1 increases β-cell mass by stimulating proliferation, inducing islet neogenesis, and inhibiting apoptosis [24-25]. Because of these potent insulinotropic actions, GLP-1 has attracted considerable attention as the major candidate for the role of the putative 'incretin' hormone.
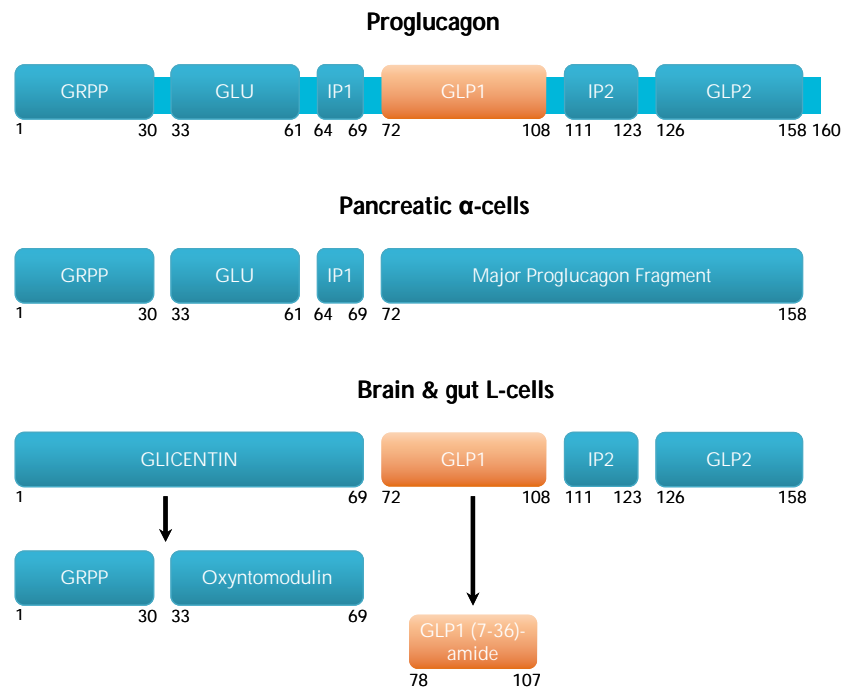

Figure 1. Schematic representation of the structure of proglucagon and its post-translational processing in different tissues. In the pancreas, post-translational processing of proglucagon leads to the generation of glycentin related polypeptide (GRPP), glucagon, intervening peptide (IP-1), and a major proglucagon fragment (MPF). In the brain and the gut, post-translational processing of proglucagon liberates GLP-1, GLP-2, IP-2, glicentin, and oxyntomodulin.. Proconvertases and carboxypeptidases are responsible for the processing of the mature peptides. Numbers represent the position of amino acids following the signal peptide (not shown).

DPP-4, which is ubiquitously found in the capillary endothelium, rapidly inactivates GLP-1 (7-36)-amide in the intestinal capillaries, the hepatoportal vein, and the periphery [26]. DPP-4 mediates GLP-1 degradation by cleaving the di-peptide at the N-terminus and removing the histidine-alanine dipeptide, yielding GLP-1 (9-36)-amide [27]. DPP-4 is present in the brain, where it shows high activity when measured in the hypothalamus, hippocampus, circumventricular organs, choroid plexus, and leptomeninges [28-29]. GLP-1(7-36)-amide is also degraded by another membrane-bound peptidase, called neutral endopeptidase 24.11 (NEP24.11), which is expressed in the periphery and the brain [30]. This peptidase degrades GLP-1 (7-36)-amide into smaller peptides including GLP-1 (28-36)-amide and GLP-1 (32-36)-amide [31]. Thus, after release from intestinal L-cells, the highest GLP-1 concentration occurs in
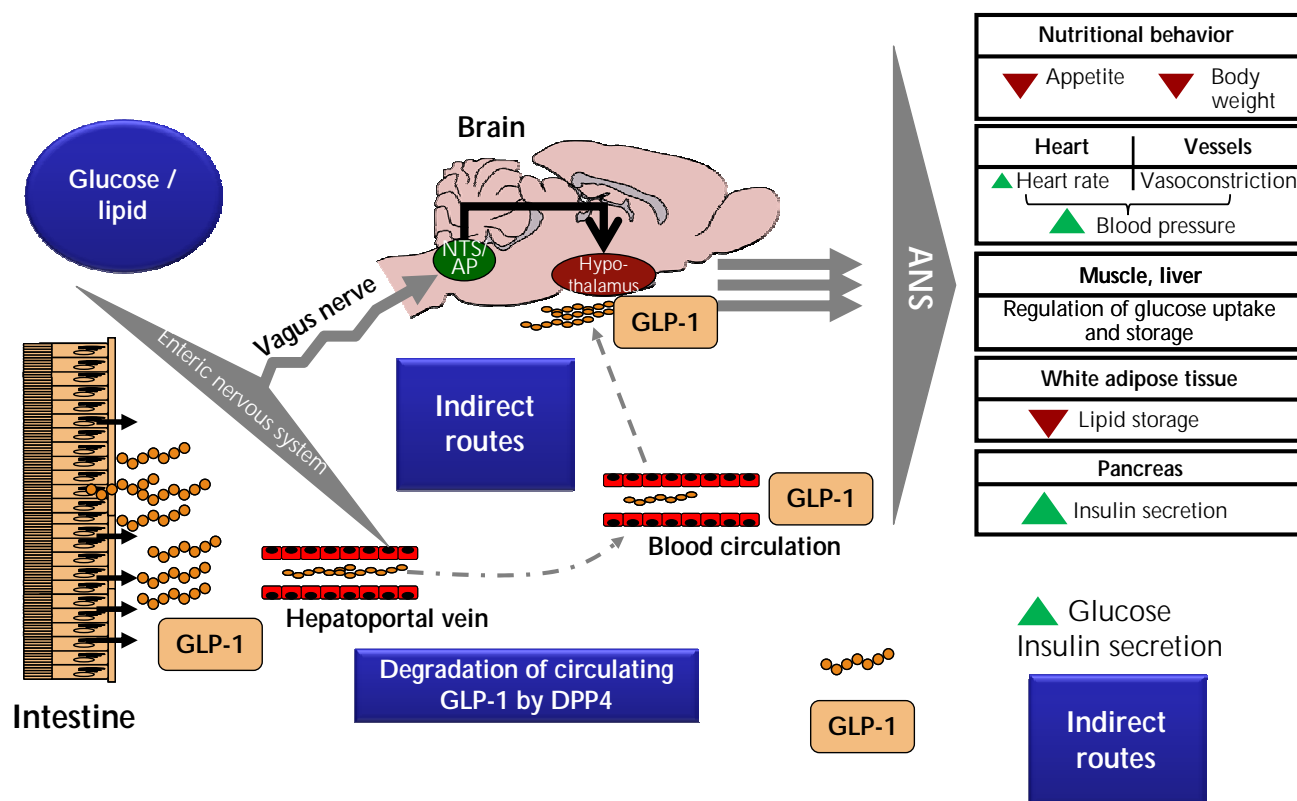
Figure 2. The gut-to-brain and the brain-to-periphery axes are part of GLP-1 metabolic and vascular functions. In response to glucose and lipid, GLP-1 is secreted by the intestine into the mesenteric capillaries and released into the hepatoportal vein. This activates termination ends from the vagus nerve to generate a neural signal towards the brain stem. The corresponding nuclei, such as the nucleus of the solitary tract nucleus (NTS) and the area postrema (AP), send axons to the hypothalamus, which release GLP-1 and activate the receptors. Then, a new signal is sent towards peripheral tissues through the autonomic nervous system (ANS) to regulate numerous functions. In blood and tissues, DPP-4 continuously degrades GLP-1. The remaining hormone could reach β-cells and enhance glucose-induced insulin secretion through the direct route, or by targeting the brain, through the indirect route.

the intestinal submucosal extracellular space, while intermediate levels can be found in the hepatoportal vein. Comparatively low concentrations are found in the systemic circulation [32]. It is estimated that less than 10% of intestinal GLP-1 is active after the first hepatic pass, and reaches the blood circulation to target peripheral organs such as the brain [32].

Because of its rapid inactivation by enzymes, GLP-1 (7-36)-amide has a very short half-life in the blood circulation, approximately 2 minutes in human and most mammalian species. It has been proposed that GLP-1 secretion in response to meal ingestion is reduced in type 2 diabetes [16, 33], although its relative insulinotropic activity is preserved [15]. However, a recent meta-analysis concluded that GLP-1 secretion in patients could be increased, normal, or reduced [34]. Therefore, debate is still ongoing, although long-lasting active analogues of GLP-1 and inhibitors of DPP4 have already been developed as drugs. These drugs are designed to increase the stability of the physiologically released hormone in type 2 diabetes [35]. At present, there is debate whether both drugs belong to the same therapeutic class.

Brain GLP-1 signaling and metabolic actions

Experimental studies carried out in rodent brains show that GLP-1 and its receptor are synthesized in selective brain areas, mainly in brain-

stem and hypothalamus. The localization of GLP-1 receptors in the brain may help us to understand the physiological role of brain GLP-1 signaling.

Brain GLP-1 synthesis

In the brain, preproglucagon expression is found in the solitary tract nucleus (NTS) and in the brainstem [20, 22]. Post-translational processing of proglucagon is similar to L-cell processing, and leads to GLP-1 with roughly equimolecular amounts of glicentin and oxyntomodulin [20, 22] (Figure 2). GLP-1 (7-36)-amide is synthesized by neuronal cell bodies in the caudal region of the NTS in the brainstem. There is a nucleus that forms the border of the caudal part of the fourth ventricle (Figure 3). The NTS is located in the dorsal vagal complex (DVC), which includes the area postrema (AP) and the dorsal motor nucleus of the vagus (DMNX). This complex receives visceral sensory inputs, generated by the vagal nerves that innervate the gastro-duodenal tract [36]. The distribution of cells expressing pre-proglucagon is not limited to a cluster of neurons in the caudal part of the NTS. It is also found in a small number of neurons that extend laterally from the NTS through the dorsal reticular area into the A1 area located in the medulla [20, 37-39].

In retrograde tracing studies, it has been shown that GLP-1 immunoreactive fibers in the hypothalamus originate from NTS cell bodies [37]. These efferent projections from the NTS densely innervate the hypothalamus and mainly the paraventricular and the dorsomedial nuclei [20, 37, 39]. Moreover, moderate innervations are located in the arcuate nuclei and the subfornical organ [20]. Numerous GLP-1 immunoreactive fibers
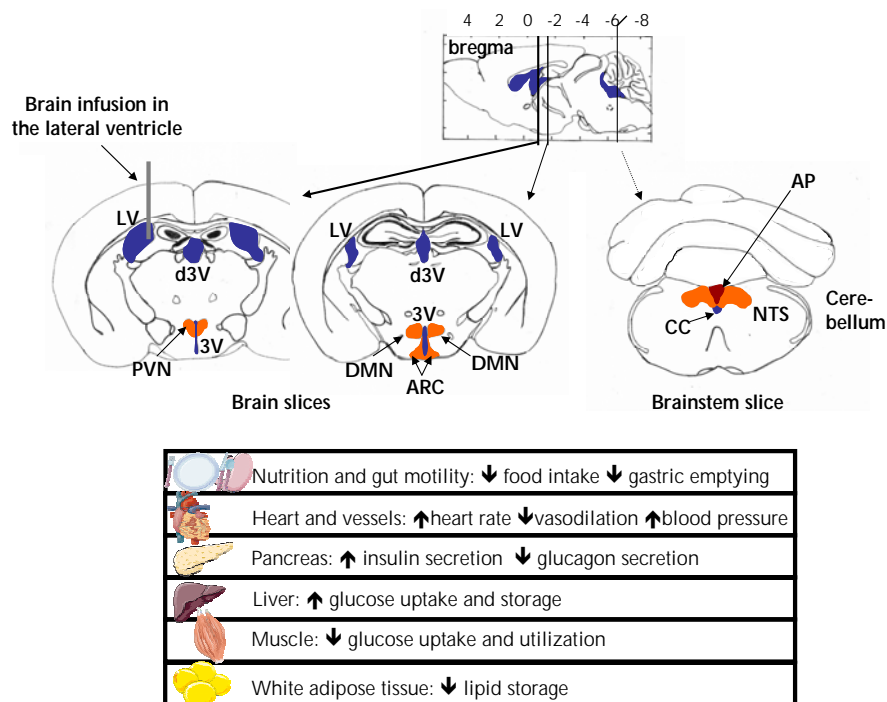

Figure 3. Schematic brain GLP-1 receptors localization in the mouse brain. GLP-1 receptors have been primarily identified in the paraventricular (PVN), dorsomedian (DMN), and arcuate (ARC) hypothalamic nuclei. In the brainstem, they were also localized in the nucleus of the tractus solitarius (NTS) and the area postrema (AP). These two last areas in the brainstem also contain the cell bodies of neurons synthesizing the GLP-1 which projects into the hypothalamus. All these localizations contribute to the metabolic effects described in the figure. Ventricles are represented in blue. CC: central canal. 3V: third ventricle. LV: lateral ventricle. 3drV: dorsal third ventricle.

can also be found in extrahypothalamic areas such as thalamic and cortical areas. In the brainstem, fibers project towards reticular formation and spinal cord [40, 41].

Brain GLP-1 receptors

GLP-1 receptor cDNA from human [42] and rat [43] brains has been cloned and sequenced. The deduced amino acid sequences are the same as those found in pancreatic islets [44]. More precisely, both receptors are 463 amino acids in length, and show 90% amino acid sequence homology [44-46]. The receptor belongs to the class B family of 7 transmembrane-spanning G-protein-coupled receptors [47]. This receptor is linked to the adenylate cyclase system and protein kinase A (PKA) activation [48]. However, it has been shown that the receptor can also activate the phospholi-

pase C pathway on pancreatic β-cells, which leads to protein kinases C (PKC) stimulation [46, 49, 50].

In situ hybridization studies with GLP-1 receptor mRNA and binding studies using radiolabeled GLP-1 have found labeled cells distributed throughout the entire brain in rodents [41, 51, 52] and humans [53]. These studies have also shown a high level of GLP-1 receptor-expressing neurons in the hypothalamus and the brainstem. These are two brain areas which are involved in the central control of energy homeostasis and autonomous functions. In the hypothalamus, the receptor is mainly located in the following nuclei: the arcuate nucleus, the paraventricular nucleus, the dorsomedial nucleus, and the supraotic nucleus (Figure 3). It can also be found in the DVC, especially in the NTS and the area postrema (Figure 3). Moreover, GLP-1 binding sites are present in the circumventricular organs such as the subfornical organ and the area postrema [41, 51, 54]. These last two locations could be the target for both peripheral GLP-1 of intestinal origin and GLP-1 synthesized in the nervous system.

The gut-to-brain GLP-1-dependent axis

The relatively low plasma level, and rapid metabolism of GLP-1 in the blood, raise questions about an alternative neural pathway that account for part of its endocrine effects on target organs such as pancreatic β-cells (especially on its effects on glucose tolerance). Thus, it has been suggested that GLP-1 secreted from L-cells could potentially influence brain neuronal activities via an alternative neural pathway initiated by sensors in the hepatic portal region [55] (Figure 2).

Indeed, experimental studies on rodents in our laboratory, and from others, have shown that glucose detection is associated with GLP-1 secretion and action on peripheral receptors localized on vagal nerve fibers in the enteric area [56], which includes the hepatoportal veins [55, 57-58]. Thereby, the vagus nerve transmits the metabolic information to the NTS in the brainstem [59-63], which relays the glucose signal to hypothalamic nuclei [59, 64]. The brain centralizes the metabolic information and generates new signals to guide the energetic flux towards tissues, either for energy use or storage. This process is called the gut-to-brain-to-periphery axis. Initially, we showed that a low rate infusion of glucose into the portal vein caused a paradoxical hypoglycemia [65-66]. This implied the activation of glucose sensors located in the portal vein, and the mechanism required the

glucose transporter GLUT2 to detect glucose [67]. Hence, we suggested that mechanisms similar to those observed in insulin secreting β-cells were responsible for the activation of the gut-to-brain axis. Therefore, GLP-1 and somatostatin are hormones positively and negatively involved in the transmission of the nutritional signals towards the brain, respectively [65].

Another hypothesis is that circulating GLP-1 can access the brain to exert its metabolic effects, but this is still a matter of debate. Experimental studies in rodents have shown that circulating GLP-1, or its agonist exendin-4 (see below), can reach the brain as they can bind to blood-brain barrier-free circumventricular organs such as the subfornical organ close to the hypothalamus [68], and the area postrema in the brainstem [68-69]. Another mechanism determining central GLP-1 action could be GLP-1 neuronal secretion in the brain which appears in response to glucose detection by intestinal cells. In line with this observation, our laboratory found that a brain infusion of exendin-9, an antagonist of the GLP-1 receptor, can reduce muscle glucose utilization and its storage as glycogen in mice receiving glucose from an intragastric catheter [70]. However, the molecular mechanisms controlling this secretion remain unclear [71].

The physiologic role of GLP-1 receptors in the control of metabolic functions (Figure 2) has often been studied in rodents, following intracerebroventricular (icv) infusions of GLP-1 (7-36)-amide, or its analogues such as exendin-4 that are resistant to enzyme degradation (by NEP and DPP-4) [31]. Exendin-4, or exendin (4-39), is a natural peptide, purified from the salivary secretions of the Gila monster lizard (*Heloderma suspectum*). This peptide shows 53% sequence homology with the mammalian GLP-1 (7-36)-amide [72-73]. Binding studies using radiolabeled iodinated GLP-1 or exendin-4 have shown that both peptides specifically interact with the same receptor expressed on pancreatic β-cells isolated from rodents [73-74] and humans [44]. In the brain, these two peptides show an identical distribution pattern of binding sites [51]. Furthermore, these peptides increase the production of intracellular cyclic AMP and stimulate glucose-induced insulin secretion [44, 73-74]. All the abovementioned effects are inhibited by the GLP-1 receptor antagonist, exendin-9 or exendin (9-39) that is a truncated form of exendin-4 [44, 74]. The properties of exendin-4 as an agonist, and of exendin-9 as an antagonist, open the possibility of using these peptides as tools to study the physiology of the GLP-1 receptor.

Nutritional behavior and body weight

The anorectic properties of centrally administrated GLP-1 were described for the first time during the 1990's. These studies showed that a GLP-1 infusion into the brain ventricles of rodents inhibits short-term food and water intake, and decreases body weight in the long term [75-80]. These properties were also demonstrated in clinical studies: an intravenous GLP-1 infusion both decreased food intake and increased satiety in healthy [81], diabetic [82], and obese [83] subjects. Moreover, weight loss is reported in diabetic subjects treated for 6 weeks with GLP-1 administrated subcutaneously [84].

The mechanism by which GLP-1 inhibits food intake is not fully understood. It has been suggested that it could result from a central action of GLP-1. It has also been suggested that part of the anorectic effects of GLP-1 could result from its side effects such as nausea and inhibition of gastric emptying [85-87]. Another mechanism could be related to activation of peripheral GLP-1 receptors expressed by the vagus nerve [88-89], which relays metabolic signals to the brain. Ruttinmann *et al.* have shown that a peripheral injection of GLP-1, irrespective of its site of infusion (vena cava, intraperitoneal cavity, or portal vein), reduced food intake without affecting the subsequent inter-meal interval, the size of subsequent meals, or the cumulative food intake [88]. These effects were only attenuated when GLP-1 was intraperitoneally administered to rats with subdiaphragmatic vagal deafferentations. Furthermore, intraperitoneal injection of exenatide dose-dependently increases c-fos expression both in myenteric and submucosal neurons of the rat duodenum, but not in other locations like jejunum and ileum. Exenatide is a synthetic version of exendin-4 recently approved for clinical use in type 2 diabetic patients [90-92]. These peripheral effects are also associated with brain neuronal activations in the brainstem (area postrema, NTS, and dorsal motor nucleus of the vagus), and can be prevented by an intraperitoneal injection of exendin-9 [63]. Thus, the satiating effect of peripherally administered GLP-1 at least requires an activation of peripheral GLP-1 receptors, which metabolically transmits the signal of satiety to the brain using the vagus nerve. It is well demonstrated that GLP-1 can act on brain neuronal circuits in the hypothalamus involved in appetite control [77].

GLP-1 receptor mRNA is highly expressed in the hypothalamic arcuate nucleus, and precisely overlaps the area occupied by neurons co-expressing propiomelanocortin (POMC) [93]. Furthermore, icv infusion of GLP-1 in fasted rodents increases the synthesis of anorexigenic peptides (POMC and cocaine- and amphetamine-regulated transcript (CART)) both in arcuate and paraventricular nuclei [94]. In these brain locations, the peptide decreases the synthesis of orexigenic peptides (neuropeptide Y (NPY) and agouti-related peptide (AgRP)). We should note that in studies with GLP-1 injected *in situ* into rat brain hypothalamic nuclei the paraventricular nucleus [79], and not the arcuate nucleus [93], is involved in the anorectic response to GLP-1.

Cardiovascular effects

The first studies in the 1990's described insulin-induced vasodilatation of the femoral artery in humans, and its impact on the control of muscle glucose utilization and glucose tolerance [95-98]. Today, it is well known that the cardiovascular system plays a key role in regulating glucose homeostasis. In 1999, Barragan *et al.* demonstrated for the first time that brain GLP-1 infusion into the lateral ventricle of conscious rats increased blood pressure and heart rate in these rodents [99]. Later, these effects were replicated with a brain infusion of exendin-4, the agonist of the GLP-1 receptor [69, 100]. It is noteworthy that GLP-1 and exendin-4 both increase blood pressure and heart rate when they are infused into the blood in rats [54, 101], and that these effects can be prevented by a cerebral injection of exendin-9 [99]. This last observation suggests that circulating GLP-1 can regulate cardiovascular function by acting on the brain. We believe that the effect on blood pressure could be due to an indirect action on blood vessels. This hypothesis has been further demonstrated by our laboratory in healthy, conscious mice receiving an exendin-4 brain infusion in the lateral ventricle [102]. Our experimental work has shown that exendin-4 decreases insulin-induced vasodilatation of the femoral artery, and that this mechanism correlates with a decrease in insulin sensitivity.

It is well demonstrated that GLP-1 recruits the autonomic nervous system to modulate the cardiovascular tone [39]. Indeed, studies have reported on a role for the parasympathetic and sympathetic branch in this control mechanism. However, it is not clear which branch plays the more important role. Initially, experiments on healthy rodents

demonstrated a potential role of the parasympathetic branch in the control of heart rate and blood pressure in response to peripheral or central GLP-1 action. This observation was demonstrated by vagotomy experiments [99] and pharmacological experiments using an icv injection of cholinergic tone inhibitors [100] in rodents receiving an exendin-4 brain infusion into the lateral ventricle. Other studies have assumed a role for the sympathetic tone. This has been suggested by an experiment showing that an intravenous or cerebral infusion of exendin-4 in rodents can stimulate c-fos expression (a marker of neuronal activation) in catecholaminergic neurons of the brainstem and in particular in the area postrema [54, 69]. Consequently, the area postrema has been thought to regulate these hemodynamic effects. In humans, a clinical study on healthy subjects assessed the effects of GLP-1 infusion on blood pressure and heart rate, and the possible activation of the sympathetic nervous system. The authors did not find any clear variation in systolic or diastolic blood pressure, but there was increased sympathetic nerve activity in skeletal muscles [103].

Cardiac effects have not been described in clinical studies with type 2 diabetic subjects. Although a 48 hour continuous subcutaneous GLP-1 infusion has no chronotropic effect, it can decrease both systolic and diastolic blood pressure in patients [104]. Interestingly, the same observations are reported in long-term studies (12 weeks to 6 months) with exenatide [105-106]. However, in non-diabetic patients with congestive heart failure, a minor increase in heart rate and diastolic blood pressure was observed during a 48 hour continuous subcutaneous GLP-1 infusion [107]. In conclusion, these observations suggest that the effects of GLP-1 on cardiovascular function seem to differ in clinical studies, and still remain to be unraveled. It is important to note that a recent review based on clinical trials has reported a positive beneficial impact of treatments with GLP-1, or its agonists (exenatide or liraglutide), on cardiac function in diabetic subjects. In part, these therapies show cardioprotective effects by reducing cardiovascular risk factors such as glycemia, lipidemia, blood pressure, and body weight [108].

Action on peripheral glucose homeostasis and fuel partitioning

Data from healthy rodents

Data from our laboratory, and from others, have shown that brain GLP-1 receptor signaling has metabolic effects on peripheral glucose tolerance. We were able to show in mice that intragastric low-rate glucose infusion (insufficient to affect systemic glucose levels) triggers skeletal muscle glucose uptake and glycogen synthesis, which highlights the significance of the gut-to-brain-to-periphery axis [70]. This effect is controlled by brain GLP-1 receptor activation, as it is abolished by prior icv administration of the GLP-1 receptor antagonist, exendin (9-39), and it is absent in GLP-1 receptor knock-out (KO) mice. We concluded that prandial perception of food facilitates a central GLP-1 receptor-dependent mechanism to improve glucose disposal. The quantification of the c-fos expression pattern in brainstem and hypothalamus showed that the enteric glucose sensor system sent signals to these brain areas. Neuronal cells in the NTS were activated by a low-rate intragastric glucose infusion, whereas cells from the arcuate nucleus of the hypothalamus were switched to rest [59]. These effects were attenuated in GLP-1 receptor KO mice, suggesting a role for brain GLP-1 signaling in influencing neural circuits to regulate glucose metabolism.

We have also demonstrated that brain GLP-1 signaling monitors the development of energy reserves in preparation for the fasting phase following a meal. We used hyperglycemic and hyperinsulinemic glucose clamps to raise systemic glucose and insulin concentrations to levels similar to those in prandial states. After exendin-4 activation of central GLP-1 receptors, we found reduced insulin-induced glucose uptake in skeletal muscles which favored hepatic glycogen stores [70]. In these experimental conditions, our results also showed that icv exendin-4 infusion increases insulin secretion by pancreatic β-cells, but prevents the vasodilatatory action of this hormone on the femoral artery [102]. We have further demonstrated that exendin-4 impairs muscle blood flow, an effect which contributes in part to the decline in skeletal muscle glucose utilization and muscle glucose uptake. During these experiments, we also found that icv brain infusion of exendin-4 activates neural pathways such as the vagus nerve [102] and muscle innervation [70], which mediates peripheral effects on muscle blood flow and insulin resistance. We assume that these physiological mechanisms prevent an overutilization of glucose by muscles during feeding to save glucose for the liver, and to prepare efficiently for the next fasting state [70]. However, the brain molecular and cellular mechanisms controlling the metabolic effects of brain GLP-1 remain unknown, and need to be investigated.

Sandoval *et al.* showed that the hypothalamic arcuate nucleus, and not the paraventricular nucleus, could be involved in this regulation. Indeed, the group showed that a GLP-1 infusion directly into the arcuate, and not the paraventricular nucleus, decreased glucose production and glucose uptake by peripheral tissues during clamp studies [93]. Also, co-infusion of a K_{ATP} channel blocker with GLP-1 into the arcuate nucleus inhibited the effect of GLP-1 on peripheral glucose metabolism [93]. These data confirm that central glucose-sensing neurons in the hypothalamic brain area are involved in brain GLP-1 signaling and control glucose tolerance. Finally, Nogueiras *et al.* demonstrated that a continuous icv infusion of GLP-1 in mice, directly and potently decreased lipid storage in white adipose tissue. This mechanism is independent of food intake and appears to be, in part, mediated by the sympathetic nervous system and the β-2 adrenoreceptors expressed in white adipose tissue [109].

In a recently published work, we have investigated the molecular mechanisms activated by GLP-1 in the brain that coordinate both vascular function and peripheral muscle glucose metabolism [110]. *In vitro* studies using rat pancreatic β-cells had previously shown that GLP-1 can activate PKC to modulate insulin secretion [50]. According to this observation, we support the hypothesis that these enzymes could be part of the molecular mechanisms of brain GLP-1 signaling. PKC are serine/threonine kinases that belong to intracellular signaling pathways. These enzymes can contribute to the regulation of synthesis and release of neurotransmitters, and to electrical activation of neuronal cells [111]. Therefore, we have repeated our experimental hyperglycemic and hyperinsulinemic clamp studies described above [102]. We observed that the brain exendin-4 infusion acutely triggers the translocation of cytoplasmic PKC-δ to the plasma membrane in cells from the hypothalamus. These functional and molecular effects were blocked by an icv brain exendin-9 infusion, or the genetic invalidation of the GLP-1 receptor (Glp1r$^{-/-}$ mice). Thus, our recent data have shown for the first time that brain GLP-1 can activate hypothalamic PKC-δ to decrease insulin-stimulated vasodilatation and whole body glucose utilization.

Data from diabetic and obese rodents

What is known about brain GLP-1 signaling during a state of overnutrition such as obesity or type 2 diabetes? Experimental studies on diabetic rodents have shown that brain GLP-1 signaling is stimulated during diabetes, and may derange glycemic control. This observation is supported by previous data from our group, and by others. It has been shown that expression of the proglucagon gene encoding GLP-1 is increased in the brainstem of diabetic high fat diet (HFD)-fed mice [112], and obese Zucker rats [38], which both contain cell bodies of GLP-1 neurons oriented towards the hypothalamus. Following this observation, and based on the hypothesis that GLP-1 can activate PKC, as demonstrated in pancreatic β-cells [50] or during an exendin-4 brain infusion, we have demonstrated that overall PKC activity is increased in the hypothalamus of diabetic HFD-fed mice in the fed state and during hyperinsulinemic euglycemic clamps [110]. This increased activity is associated with a translocation of both PKC-δ and -α to the plasma membrane of hypothalamic cells. However, exendin-9 brain infusion only inhibits PKC-δ translocation and improves the otherwise impaired insulin sensitivity and vasoconstriction observed in the control group.

Furthermore, the disruption of the GLP-1 receptor in Glp1r$^{-/-}$ mice prevents both overall PKC activation and PKC-δ translocation to the membrane fraction in healthy and diabetic Glp1r$^{-/-}$ mice. In this latter group, the genetic deletion seems to prevent both the impairment of the vascular blood flow and the insulin sensitivity during clamp studies. Thus, all these observations suggest that brain GLP-1 activity is increased during metabolic diseases. We believe that this overactivity might be due to an insidious increase in glycemia, which chronically stimulates the basal activity of brain neuronal cells. Therefore, this central mechanism could alter the metabolic effects of hormones released during the postprandial state, such as insulin.

Conclusions

Brain GLP-1 action has been studied over the last decade, but its physiological impact is still not completely understood. Numerous studies reported in the literature indicate a common effect of both peripheral and central GLP-1 receptor signaling on glucose homeostasis to promote glucose tolerance. These observations point to a cross-talk between circulating GLP-1 and the brain, but the relative importance of peripheral versus central GLP-1 for the control of glucose homeostasis is not known.

We think that the peripheral action of GLP-1 may play a critical role in glycemic regulation,

perhaps even more important than its central action, because it initiates the metabolic information which is then redirected towards peripheral organs by the brain. It is also possible that the peripheral secretion indirectly regulates the level of activation of brain neuronal circuits expressing the GLP-1 receptor. We raise this hypothesis from observations obtained in mice invalidated for the GLP-1 receptor. Our recent data show that these mice do not develop the metabolic phenotype which was very similar to that observed in the group of diabetic mice brain-infused with the GLP-1 receptor antagonist, exendin-9 [110]. This observation suggests that the peripheral receptor may play a critical role in diabetes development.

It is also important to find out whether long-acting GLP-1 analogs could improve glucose tolerance in type 2 diabetes by acting on the brain. A similar conceptual question could be applied to DPP-4 inhibitors. As opposed to GLP-1 analogues, DPP-4 inhibitor treatment increases portal vein GLP-1 concentrations, and activates the gut-to-brain vagus nerve activity [113].

Hence, the identification of the gut-to-brain-to-periphery axis opens new avenues for the treatment of type 2 diabetes. The high concentration of circulating GLP-1 analogue obtained during the treatment of type 2 diabetes could reach the brain to favor neuroprotection and control of food intake. It is noteworthy that the effect of brain GLP-1 on insulin action and vascular blood flow is clearly analyzed in animals, but have never been mentioned in relation to humans. We consider that there is a strong case for such studies in humans.

Beside GLP-1 analogues, the use of DPP4 inhibitors could offer different therapeutic strategies. We can be sure that they recruit the gut-to-brain vagus nerve-dependent axis, which is unlikely the case for GLP-1 analogs. Different patient conditions could be treated, and therapies could be combined where appropriate. Such new incretin-based therapies, utilizing newly discovered modes of action, would need clinical trials to validate them, but have a great potential for a new era of diabetes treatment.

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