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MINI REVIEW

Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) is derived from both the enteroendocrine L cells and preproglucagon-expressing neurons in the nucleus tractus solitarius (NTS) of the brain stem. As GLP-1 is cleaved by dipeptidyl peptidase-4 yielding a half-life of less than 2 min, it is plausible that the gut-derived GLP-1, released postprandially, exerts its effects on the brain mainly by interacting with vagal afferent neurons located at the intestinal or hepatic portal area. GLP-1 neurons in the NTS widely project in the central nervous system and act as a neurotransmitter. One of the physiological roles of brain-derived GLP-1 is restriction of feeding. GLP-1 receptor agonists have recently been used to treat type 2 diabetic patients, and have been shown to exhibit pleiotropic effects beyond incretin action, which involve brain functions. GLP-1 receptor agonist administered in the periphery is stable because of its resistance to dipeptidyl peptidase-4, and is highly likely to act on the brain by passing through the blood–brain barrier (BBB), as well as interacting with vagal afferent nerves. Central actions of GLP-1 have various roles including regulation of feeding, weight, glucose and lipid metabolism, cardiovascular functions, cognitive functions, and stress and emotional responses. In the present review, we focus on the source of GLP-1 and the pathway by which peripheral GLP-1 informs the brain, and then discuss recent findings on the central effects of GLP-1 and GLP-1 receptor agonists.

INTRODUCTION

The anti-obesity effect of incretin-based medicines is mainly mediated by the central anorectic effect of glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonist. They also provoke various effects that involve the central and peripheral nervous systems, which include regulation of glucose and lipid metabolism, cardiovascular functions, cognitive functions, and stress and emotional responses. In the present review, we focus on the properties and functions of gut-derived vs brain-derived GLP-1, distinct roles of humoral vs neural pathways linking peripheral GLP-1 to the brain, and different modes of action between endogenous GLP-1 vs GLP-1 receptor agonists.

GUT-DERIVED VS BRAIN-DERIVED GLP-1

GLP-1 is released from the enteroendocrine L cells in response to meals, and enhances glucose-induced insulin secretion from pancreatic islets[1], being recognized as an incretin hormone. GLP-1 is also produced by preproglucagon-expressing neurons in the nucleus tractus solitarius (NTS) of the brain stem[2], and acts as a neurotransmitter. It is important to distinguish the central action of gut-derived GLP-1 from that of brain-derived GLP-1.

Generally, the peripheral factors send their information to the brain through two distinct pathways, the humoral and neural pathways (Figure 1). In detail, the humoral pathway is composed of the blood–brain barrier (BBB) and the circumventricular organ (CVO) that has a leaky BBB. It is reported that GLP-1 can enter the brain through the BBB[3], and radiolabeled GLP-1 is bound to its receptors expressed in the CVOs[4,5]. However, endogenous GLP-1 derived from the gut is rapidly cleaved by dipeptidyl peptidase-4 (DPP-4), with its half-life being less than 2 min[6]. Hence, it is reasonable that gut-derived GLP-1 influences the brain mainly through the neural pathway, which is composed of the vagal afferent fibers at the intestinal or hepatic portal area. We previously reported that GLP-1 evokes action potentials and increases cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in the neurons of nodose ganglion, where cell bodies of the vagal afferent fibers are located

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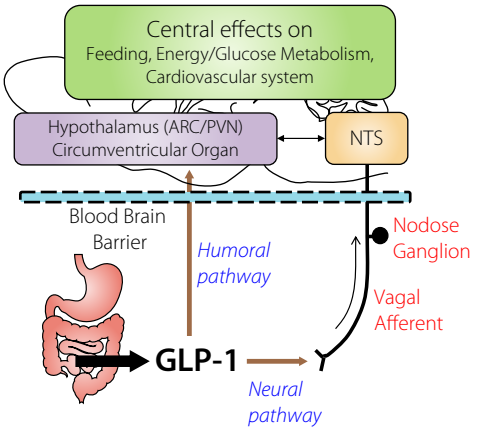

Figure 1 | Glucagon-like peptide-1 (GLP-1), released from the intestine, informs the brain by passing through blood–brain barrier (humoral pathway) and by interacting with vagal afferent nerves (neural pathway). Consequent activation of the hypothalamus, circumventricular organ, and brain stem including nucleus tractus solitarius (NTS), regulates feeding, energy/glucose metabolism and the cardiovascular system. ARC, arcuate nucleus; PVN, paraventricular nucleus.

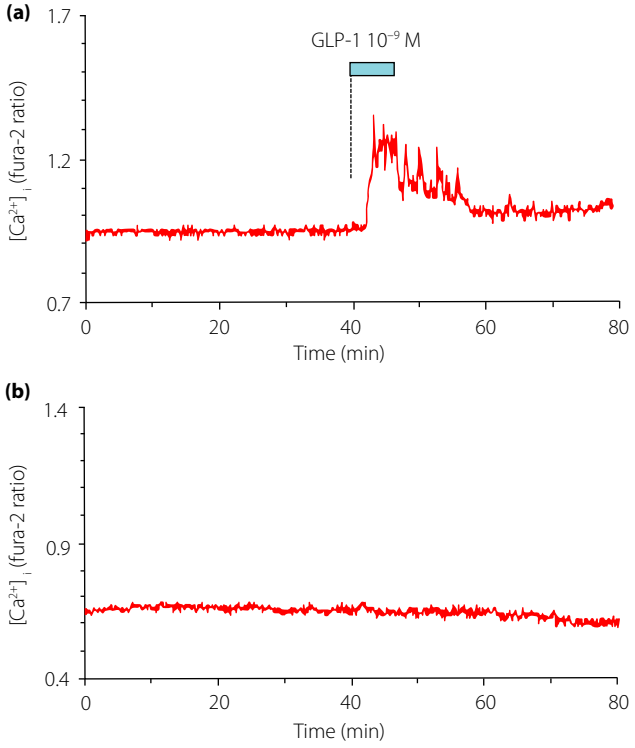

Figure 2 | (a) Glucagon-like peptide-1 (GLP-1) at 10^{-9} mol/L increases cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single nodose ganglion neurons isolated from rats. (b) As a control, $[Ca^{2+}]_i$ is stable in nodose ganglion neurons without GLP-1 administration.

(Figure 2)[7]. Vagal afferents also directly sense cholecystokinin[8,9], peptide YY_{3-36}[10], leptin[11], oxytocin[11] and nesfatin-1[12], the hormones regulating feeding and metabolism. Intraperitoneal co-injection of GLP-1 and leptin[13] or co-injection of GLP-1 receptor agonist exendin-4 and peptide YY_{3-36}[14] synergistically suppresses food intake. These hormones might act cooperatively on vagal afferents and regulate feeding behavior.

Injection of GLP-1 into the portal vein enhances insulin secretion, and this effect is attenuated by hepatic vagotomy[15], suggesting that the insulinotropic effect of GLP-1 is mediated not only by direct action on the pancreas, but also through vagal afferents, being recognized as a neuroincretin effect. A recent study by Krieger *et al.*[16] used the bilateral nodose ganglion injection technique to deliver a lentiviral vector to knock down the GLP-1 receptor specifically in the vagal afferent neurons of rats, and found that post-meal glycemia was elevated and insulin release was blunted in GLP-1 receptor knockdown rats. These results suggest that the vagal afferent GLP-1 receptor is a physiological contributor to the neuroincretin effect after meals. Furthermore, they found GLP-1 receptor knockdown increases meal size and accelerates gastric emptying, whereas it has little effect on the long-term maintenance of food intake and weight in normal eating conditions. These results highlight a crucial role for the vagal afferent neuron in mediating the effects of endogenous GLP-1 on glycemia and food intake.

PHYSIOLOGICAL ROLE OF BRAIN-DERIVED GLP-1

GLP-1 neurons in the NTS widely project in the central nervous system including the hypothalamus: the paraventricular nucleus (PVN), supraoptic nucleus (SON), arcuate nucleus (ARC) and hypothalamic dorsomedial nucleus (DMH), and the brainstem – the parabrachial nucleus, reticular formation of medulla and dorsal vagal nucleus, thalamus and intermediolateral cell column[2,17–21] – where GLP-1 receptors are expressed[22–25].

We reported that endogenous GLP-1 in the brain targets PVN to restrict feeding behavior, in which the projection from NTS GLP-1 neurons and activation of corticotropin-releasing hormone and nesfatin-1 neurons might be implicated (Figures 3 and 4)[26]. NTS GLP-1 neurons are activated by both central signals and peripheral signals from vagal afferents. As a central signal, oxytocin neurons in PVN project to the NTS GLP-1 neurons, and central infusion of oxytocin activates c-Fos expression in NTS GLP-1 neurons[27]. It is established that peripheral signals, such as gastric distension[28], and intraperitoneal injection of lithium chloride that leads to conditioned taste aversion and nausea[29,30] cause c-Fos expression in NTS GLP-1 neurons. In addition, using brain slices of the transgenic mice expressing the yellow fluorescent protein under control of the preproglucagon promoter, the electrical properties of NTS GLP-1 neurons were reported. That study showed that leptin directly depolarizes NTS GLP-1 neurons, and cholecystokinin modulates noradrenergic or glutamatergic neurons and thereby secondarily depolarizes NTS GLP-1 neurons. In contrast, NTS

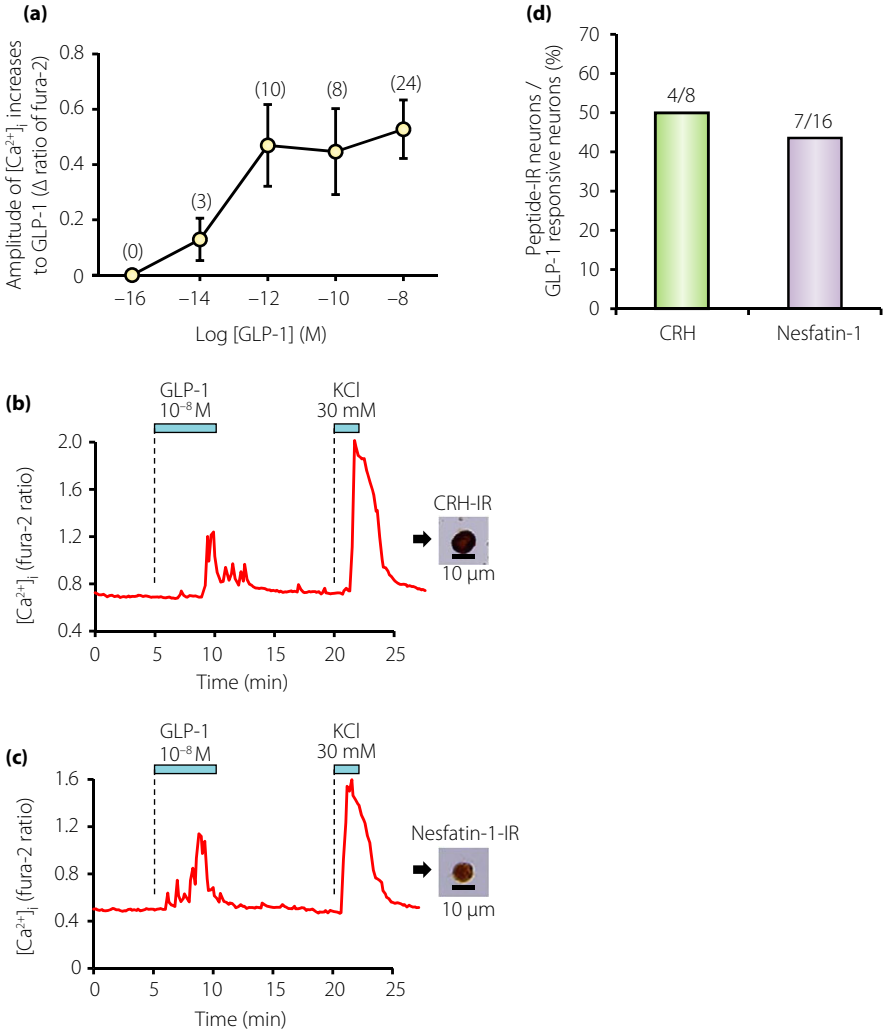

Figure 3 | Glucagon-like peptide-1 (GLP-1) concentration-dependently increases $[Ca^{2+}]_i$ in corticotropin-releasing hormone (CRH) and nesfatin-1 neurons of paraventricular nucleus (PVN). (a) Amplitude of $[Ca^{2+}]_i$ responses to GLP-1 in GLP-1 responsive neurons. Numbers around each point indicate number of neurons examined. Values are expressed by mean ± standard error of the mean. (b) GLP-1 at 10^{-8} mol/L increased $[Ca^{2+}]_i$ in a single neuron isolated from PVN (left panel), and this neuron was subsequently shown to be immunoreactive (IR) to CRH (right panel). This neuron also responded to 30 mmol/L KCl with an increase in $[Ca^{2+}]_i$. (c) GLP-1 at 10^{-8} increased $[Ca^{2+}]_i$ in a single PVN neuron that was subsequently shown to be IR to nesfatin-1. Superfusate contained 1 mmol/L glucose and the bars above the tracings indicate the periods of administration of agents in (b) and (c). (d) Incidence of the neurons that express CRH or nesfatin-1 over those responded to GLP-1 in the PVN. The numbers above the bars indicate the number of neurons IR to CRH or nesfatin-1 over that responded to GLP-1 in the range of 10^{-14}–10^{-8} mol/L.

GLP-1 neurons failed to show electrical responses to peptide YY, GLP-1 and ghrelin[31].

The role of brain-derived GLP-1 in glucose metabolism has been reported[32]. Intragastric glucose infusion, compared with intragastric water infusion, increased muscle glycogen synthesis and c-Fos expression in the NTS, and decreased c-Fos expression in the neuropeptide Y-positive neurons in the ARC. These effects were diminished with simultaneous central infusion of the GLP-1 receptor antagonist exendin(9-39), and abolished in GLP-1 receptor knockout mice. Because intragastric glucose infusion does not affect the blood levels of glucose, insulin and GLP-1, enteric glucose might send signals to the brain through vagal afferents and activate a GLP-1 receptor-dependent signal in the brain to control peripheral glucose metabolism (Figure 4).

CENTRAL EFFECTS OF ENDOGENOUS GLP-1 VS GLP-1 RECEPTOR AGONISTS

Several GLP-1 receptor agonists have recently been used to treat type 2 diabetes patients. GLP-1 receptor agonists have DPP-4-resistance and a longer half-life of several hours or more because of their structural modification. Unlike the gut-derived endogenous GLP-1 that is inactivated within 2 min by DPP-4,

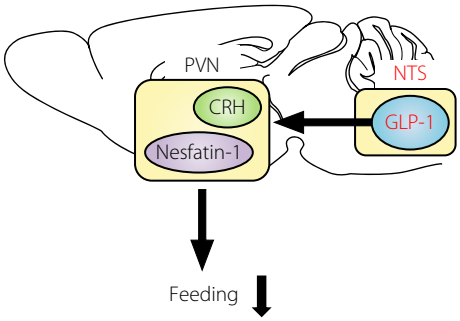

Figure 4 | Model for feeding suppression by endogenous brain-derived glucagon-like peptide-1 (GLP-1). Nucleus tractus solitarius (NTS) GLP-1 neurons project to paraventricular nucleus (PVN), and activate corticotropin-releasing hormone (CRH) and nesfatin-1 neurons to suppress feeding, and possibly regulate glucose metabolism.

GLP-1 receptor agonists administered in the periphery are stable and highly likely to act on the brain through the humoral pathway in addition to the neural pathway. In addition, GLP-1 receptor agonist could remain in the brain for several hours, and hence exert effects similar to those induced by the brain-derived endogenous GLP-1.

Indeed, it was reported that the GLP-1 receptor agonist, liraglutide, administered in the periphery can reach the brain and exert central effects[33]. That study investigated the distribution of fluorescence-labeled liraglutide in the mouse brain after peripheral administration. Fluorescence-labeled liraglutide was observed in all CVOs, the zona interna of the median eminence (ME), the area postrema (AP), the sobfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT), and also in hypothalamic regions, the ARC, PVN and SON. These signals of fluorescence-labeled liraglutide in specific brain areas were blunted in GLP-1 receptor knockout mice. Interestingly, no fluorescence-labeled liraglutide was observed in the NTS, suggesting that peripheral liraglutide could not directly interact with the NTS GLP-1 neurons. This study also showed that peripheral liraglutide-induced feeding suppression was abolished by infusion of GLP-1 receptor antagonist exendin(9-39) into the ARC, but not the PVN. Neither the ablation of the AP nor deafferentation of the subdiaphragmatic vagal afferent affected the peripheral liraglutide-induced feeding suppression. Furthermore, electrophysiological measurements of murine brain slices showed that GLP-1 directly stimulates pro-opiomelanocortin/cocaine- and amphetamine-stimulated transcript neurons, and indirectly inhibits neurotransmission to neuropeptide Y/Agouti-related peptide neurons through gamma-aminobutyric acid-dependent signals. Taken together, it was concluded that peripheral liraglutide directly acts on the ARC, most likely pro-opiomelanocortin/cocaine- and amphetamine-stimulated transcript neurons, through the humoral pathway to suppress feeding.

Differences between GLP-1 and GLP-1 receptor agonist exendin-4 in their central anorectic effects have been reported. This is partly explained by their different sensitivity to DPP-4[34], but might also involve other factors. The anorectic effect of intracerebroventricular (i.c.v.) injection of GLP-1 was attenuated by i.c.v. injection of GLP-1 receptor antagonist before GLP-1. By contrast, the anorectic effect of i.c.v. injection of exendin-4 was not attenuated by i.c.v. injection of GLP-1 receptor antagonist before exendin-4, but was abolished in GLP-1 receptor knockout mice. In contrast, the anorectic effect of intraperitoneal (i.p.) injection of exendin-4 was completely blocked by i.p. injection of GLP-1 receptor antagonist before exendin-4. These data suggest a key difference between GLP-1 and exendin-4 in their interaction with the brain GLP-1 receptor. There might be a difference in the GLP-1 receptor properties between the brain and the periphery, which could include post-translational processing, binding to GLP-1 receptor antagonist, interaction with other peptides and signal transduction.

A recent report by Beiroa *et al.*[35] investigated the effects of specific injection of liraglutide in the various hypothalamic sites that regulate food intake and brown adipose tissue thermogenesis. Injection of liraglutide specifically into the hypothalamic ARC, PVN and lateral hypothalamic area (LHA), but not ventromedial nucleus (VMH), reduced food intake. Conversely, injection of liraglutide specifically into the VMH, but not ARC, PVN and LHA, stimulated brown adipose tissue thermogenesis. This effect depends on AMPK in the VMH, and involves expression of uncoupling protein 1 in the brown adipose tissue to higher levels.

CARDIOVASCULAR EFFECTS OF ENDOGENOUS GLP-1 VS GLP-1 RECEPTOR AGONISTS

Central GLP-1 and GLP-1 receptor agonists have cardiovascular actions. The i.c.v. infusion of GLP-1 and GLP-1 receptor agonist transiently increases blood pressure and heart rate in normal rodents. The possible underlying mechanisms involve central cholinergic system[36], neurohypophysial hormones[36,37] and medullary catecholamine neurons[38]. Regarding heart rate regulation, it was reported that exendin-4 inhibits neurotransmission to cardiac vagal neurons in the medullary nucleus ambiguus, leading to suppression of cardiac parasympathetic activity thereby increasing heart rate[39]. Studies in hypertensive patients with type 2 diabetes or obesity showed that long-term treatment with GLP-1 receptor agonists reduced blood pressure[40]. In animal studies, chronic peripheral injection of GLP-1 receptor agonists reduced blood pressure. The i.p. injection of exendin-4 twice daily for 12 weeks reduced blood pressure in *db*/*db* mice[41]. Furthermore, twice-daily exendin-4 attenuated the increase in blood pressure by angiotensin II infusion for 2 weeks in mice[41]. Underlying mechanisms for the chronic antihypertensive effect of GLP-1 receptor agonists has not yet been fully elucidated. It is postulated as possible mechanisms that GLP-1 receptor agonist directly acts on the kidney to induce natriuresis[41,42], and on the endothelial cells to induce vasodilatation[43]. Additionally or alternatively, chronic peripheral injection of GLP-1 receptor agonists would possibly inform the brain through humoral and/or neural pathways to activate

central GLP-1 receptors and/or neural circuits, thereby evoking these chronic antihypertensive effects.

These studies suggest that central GLP-1 or GLP-1 receptor agonists exert different actions depending on acute vs chronic paradigms, and on physiological vs pathological subjects. The result from a clinical trial of GLP-1 receptor agonist have provided cardiovascular safety for clinical use, although risk reduction of cardiovascular events has not been observed[44]. The follow-up period so far is approximately 3 years, and further investigations will be required to evaluate longer-term efficacy and safety.

CONCLUSION

In the past several years of clinical experience, incretin-based medicines have been shown to exhibit pleiotropic effects beyond glycemic control, which include the effects on feeding, weight, lipid metabolism, cardiovascular functions and cognitive functions. Central effects of GLP-1 contribute to these extrapancreatic effects, regulating systemic homeostasis in physiological states, and providing beneficial responses to pathological states. This review has shown that gut-derived GLP-1 and GLP-1 receptor agonist send their information to the brain through neural and humoral pathways. However, how their information is relayed to efferent nervous systems to control peripheral tissues and organs remains to be clarified. Further investigations in basic and clinical studies will provide new insights about central actions and the underlying mechanisms of incretin-based medicines.

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DISCLOSURE

The authors declare no conflict of interest.

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