Glucagon-like Peptides, the Central Nervous System, and the Regulation of Energy Homeostasis

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Abstract: The proglucagon-derived peptides glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) are released from gut endocrine cells in response to nutrient ingestion, and regulate gastric acid secretion, gastrointestinal motility, nutrient absorption, glucose homeostasis and cell proliferation and survival. GLP-1 and GLP-2 are also synthesized in the central nervous system, predominantly in neurons of the nucleus of the solitary tract in the brainstem, and to a lesser extent in the hypothalamus. Intracerebroventricular (ICV) administration of GLP-1 inhibits food intake in rodents and ICV GLP-1 activates neuroendocrine circuits, the sympathetic nervous system, and pathways coupled to transduction of an interoceptive stress response. Interruption of GLP-1R signaling in the rodent brain attenuates the development of a conditioned taste response and fos-activation following exposure to noxious agents such as lithium chloride. Although peripheral administration of GLP-1 to human subjects is associated with inhibition of gastric emptying, feelings of satiety, and reduction of food intake, chronic treatment of diabetic subjects with GLP-1R agonists prevents weight gain, but is not associated with major weight loss over 8-12 week treatment periods. Although ICV injection of GLP-2 also inhibits food intake, GLP-2 is much less anorexic compared to GLP-1. Both GLP-1 and GLP-2 exert direct cytoprotective and regenerative actions, suggesting that activation of glucagon-like peptide receptor signaling may attenuate cellular injury in the CNS. As peripheral administration of GLP-1R agonists activate CNS GLP-1R systems, the biology of glucagon-like peptides in the brain is directly relevant to pharmaceutical use of glucagon-like peptide agonists for the treatment of human disease.

Keywords: Food Intake, Glucagon-like peptides, Glucagon, GLP-1, GLP-2, satiety, weight loss, stress

INTRODUCTION

GLP-1 is a potent endogenous insulinotropic peptide that stimulates insulin secretion from the pancreatic β-cell in response to nutrient ingestion. GLP-1 also inhibits glucagon secretion and gastric emptying and enhances islet mass via regulation of β-cell proliferation, islet neogenesis and inhibition of β cell apoptosis [1, 2]. The physiological importance of GLP-1 action for glucoregulation is exemplified by studies demonstrating elevation in levels of blood glucose following interruption of GLP-1 action in rodent and human studies [3-6]. Furthermore, the demonstration that pharmacological administration of both native GLP-1 and related analogs lowers blood glucose in both normal and diabetic human subjects [7-10] has fostered considerable interest in the use of GLP-1R agonists for the treatment of patients with diabetes mellitus.

GLP-2 is a 33-amino acid peptide co-secreted with GLP-1 (Fig. **1**) from gut endocrine cells within minutes following meal ingestion [11]. GLP-2 also regulates gastric acid secretion and intestinal motility [12, 13] and stimulates intestinal hexose transport via enhancement of glucose transporter expression and activity [14, 15]. Exogenous GLP-2 administration in rodents stimulates mucosal growth [16, 17], increases epithelial integrity [18, 19], and reduces permeability in the gut mucosa [20]. The expansion of the gut mucosal epithelium following GLP-2 administration is mediated through inhibition of cell death [21, 22] and stimulation of cell proliferation in the enterocyte and crypt compartments [16, 21]. These findings have led to assessment of whether GLP-2 administration may be useful for improvement of nutrient absorption and energy retention in human patients with short bowel syndrome [23].

The glucagon-like peptides (Fig. **1**) are also synthesized in the central nervous system (CNS), predominantly in the nucleus of the solitary tract [24-26], and transported throughout the brain to the thalamus, cortex, hippocampus, amygdala, brainstem, parabrachial nucleus and spinal cord. The CNS region exhibiting the greatest extent of GLP-1- and GLP-2-innervation is the hypothalamus [25-28]. GLP-1 and GLP-2 were originally shown to activate adenylate cyclase in rat hypothalamic and pituitary membrane preparations [29]. Consistent with these observations, RNA transcripts for the GLP-1 [28, 30, 31], and GLP-2 receptors [32] were subsequently localized to hypothalamic centres that control feeding behaviour and appetite. Furthermore, a considerable body of more recent data from human studies demonstrates that central or peripheral GLP-1 administration decreases energy intake and meal size in both normal and diabetic subjects [33].

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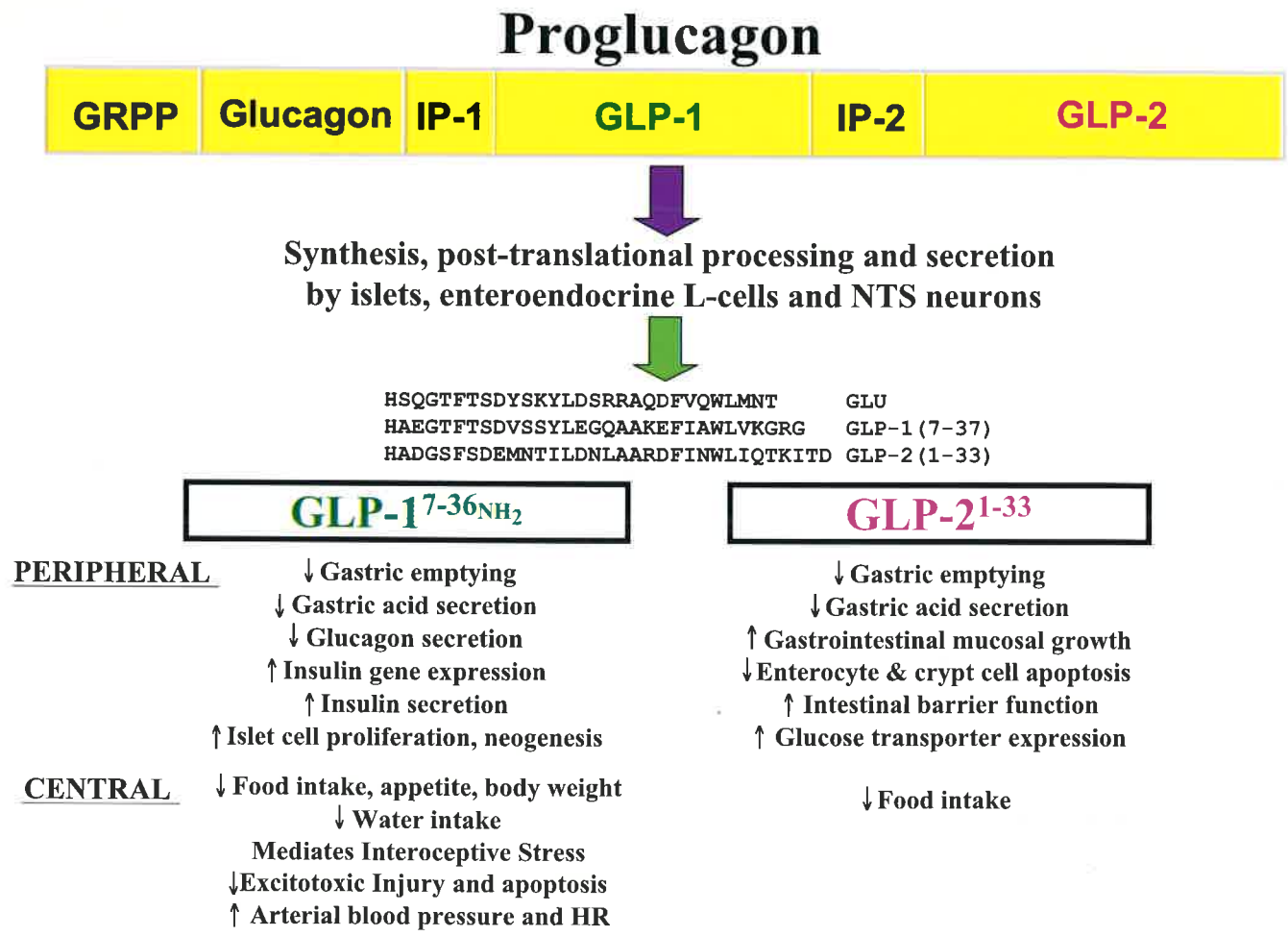

Fig. (1). The glucagon-like peptides GLP-1 and GLP-2 are derived from a common larger precursor prohormone, proglucagon. Glucagon is produced in islets and brain, whereas GLP-1 and GLP-2 are produced in the gut and brain. The amino acid sequences of human glucagon, GLP-1(7-37), and GLP-2 are shown. The actions of GLP-1, present as either GLP-1 (7-36)amide or GLP-1 (7-37) and GLP-2 in the periphery and central nervous system are indicated. CNS, central nervous system; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2; IP-1, intervening peptide-1; IP-2, intervening peptide-2; NTS, nucleus of the solitary tract.

GLP-1 and the Regulation of Food Intake: Insights from Rodent Experiments

The suppressive actions of GLP-1 on feeding behaviour were first demonstrated by Turton and O'Shea as intracerebroventricular (ICV) administration of GLP-1 decreased food and water intake in fasted rats in a dose-dependent manner [34]. Conversely, central infusion of the GLP-1 receptor antagonist, exendin(9-39) reversed the appetite-suppressive effects of GLP-1 and exendin(9-39) alone increased food intake in satiated rats, suggesting that endogenous GLP-1 regulates feeding behaviour specifically through central GLP-1 receptors. ICV GLP-1 also induced c-fos expression in the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala [34]. These findings sparked intense interest into the physiological relevance of GLP-1 receptor signaling systems in the control of appetite and body weight regulation.

Subsequent studies confirmed that ICV GLP-1 administration produced potent short term inhibition of both food and water intake [35-38]. Nevertheless, the anorectic effects of single ICV GLP-1 injections were transient, and not consistently associated with significant reductions in body weight in lean or obese rats [39]. Furthermore, in some studies repeated daily ICV GLP-1 administration was associated with progressive reduction in and ultimately loss of the inhibitory effect on food intake in rats [39]. Similarly, although central administration of exendin-4 for 8 days produced significant early reductions in food intake and body weight in both lean and obese rats, maximal suppression of food intake was observed by days 3-4, yet less inhibition of food intake was observed by the end of the study period [40]. In contrast, other studies have shown more sustained anorectic effects of GLP-1 administration, as twice daily or continuous ICV GLP-1 administration (30 ug twice daily) significantly reduced daily food intake each day over a six day period in rats, resulting in body weight loss but no evidence for desensitization [41].

The demonstration that acute or repeated administration of the GLP-1 receptor antagonist exendin (9-39) increases

food intake and body weight suggested that endogenous GLP-1 receptor signaling contributes to the physiological regulation of body weight [34, 42]. In contrast, mice with genetic disruption of GLP-1 receptor signaling were lean, and did not exhibit abnormalities in short or long term control of food intake or body weight [3]. Furthermore, prolonged exposure of GLP-1R-/- mice in the CD1 genetic background to a high fat diet for 18 weeks did not result in more weight gain than age-and sex-matched CD1 control mice [43]. Nevertheless, more recent experimentation suggests an important influence of genetic background on GLP-1R-dependent weight regulation; hence whether endogenous GLP-1R signaling is essential for murine weight regulation requires further study.

GLP-1 and the Inhibition of Food Intake: Mechanisms

The observations that both GLP-1 and leptin inhibited food intake, together with the demonstration of leptin receptors on GLP-1+ brainstem neurons [44, 45], prompted assessment of whether anorexic effects observed following leptin administration reflected activation of inter-dependent GLP-1R signaling systems. Although c-fos activation is observed following ICV administration of both peptides, a markedly different regional pattern of neuronal activation was observed in the rat CNS following leptin compared to GLP-1 administration [46]. Conversely, infusion of GLP-1 in normal human volunteers had no effect on levels of circulating leptin [47]. Furthermore, although the anorectic effects of a single leptin injection were sustained for up to 16 hours, GLP-1 produced only transient inhibition of feeding behavior [48]. Although leptin increases the levels of GLP-1 content in the rat hypothalamus [49], the anorectic action of leptin is enhanced, not attenuated, in mice with complete disruption of GLP-1 receptor signaling [50]. Furthermore, the control of feeding and body weight regulation is similar in GLP-1R-/- mice with leptin deficiency compared to ob/ob mice alone, demonstrating that the presence or absence of an intact GLP-1 receptor signaling system does not modify the phenotype of leptin deficiency *in vivo* [51].

An alternative explanation for the anorexic actions of GLP-1, namely the activation of condition taste aversion (CTA) responses, was proposed following experiments demonstrating dose-dependent development of a CTA response in saccharin-treated rats following ICV GLP-1, but not leptin administration [48]. Although several studies failed to confirm the development of a GLP-1-induced CTA response [35, 52], a considerable body of evidence now implicates GLP-1 receptor signaling systems as an essential component of the rodent CNS response to aversive stimulation [53]. Central infusion of the GLP-1 receptor antagonist des His^1Glu^9 exendin-4 attenuated lithium chloride induced c-fos activation in rat brainstem nuclei, including the area postrema, the nucleus of the solitary tract, and the lateral parabrachial nucleus [54]. Similarly, suppression of food intake following peripheral lithium chloride administration was significantly reduced by pre-treatment of rats with ICV GLP-1R antagonist exendin(3-39) [55] and administration of a GLP-1R antagonist completely blocked the lithium chloride-induced reduction of food intake [56]. Moreover administration of lithium chloride, lipopolysaccharide, or cholecystokinin activated c-fos in GLP-1-immunopositive brainstem neurons [57] suggesting that noxious stimulation may activate central signal transduction systems in part through GLP-1R-dependent mechanisms [53].

More recent data provides evidence for functionally distinct GLP-1R+ neuronal populations which contribute to the differential regulation of food intake or induction of visceral illness. Injection of GLP-1 into the lateral or 4^{th} ventricle produced a reduction of food intake at comparable doses, whereas only lateral ventricular GLP-1 administration elicits a CTA response. Furthermore, direct administration of GLP-1 into the central nucleus of the amygdala, produced a strong CTA response without producing anorexia. Similarly injection of a GLP-1R antagonist into the central nucleus of the amygdala diminished lithium chloride-induced CTA [58]. Hence, these findings implicate the presence of anatomically separate GLP-1R populations exhibiting differential responsivity to anorexic vs CTA-inducing actions of GLP-1.

GLP-1 and the Control of Hypothalamic, Pituitary, and Neuroendocrine Systems

ICV administration of GLP-1 produces diverse effects other than control of food intake, including a lowering of body temperature [59]. Acute GLP-1 injection in rats has also been shown to modulate energy expenditure and metabolic rate [60], although the effect of GLP-1 on energy expenditure independent of food intake has not been extensively studied. Consistent with the localization of GLP-1 binding sites to the hypothalamus and pituitary, GLP-1 stimulates LHRH release from the GT_1-7 cell line and ICV GLP-1 increased the levels of circulating plasma luteinizing hormone in male rats [61]. Similarly, GLP-1 stimulated cAMP formation from rodent thyrotrope α-TSH cells, and increased TSH release from dispersed rat anterior pituitary cell cultures [62].

Central GLP-1 administration in freely moving non-stressed rats has also been associated with stimulation of vasopressin and corticosterone but not oxytocin secretion, and 80% of the CRH-positive neurons in the hypophysiotropic medial parvicellular paraventricular nucleus co-expressed c-fos following GLP-1 administration [63]. Conversely examination of neuroendocrine parameters in GLP-1R-/- mice revealed normal levels of gonadotropins, estrogen, progesterone, testosterone and thyroid hormones, but an exaggerated corticosterone response to stress [64]. Hence, GLP-1R signaling does not appear to be essential for intact hypothalamic-pituitary function in mice [65].

Studies from several laboratories have demonstrated that enhanced GLP-1R signaling in rodents produces sympathetic nervous system activation and increases in heart rate and blood pressure. Systemic infusion of GLP-1, but not GLP-2, in rats increased systolic and diastolic blood pressure and heart rate and pre-treatment with either reserpine, propranolol, or phentolamine did not abrogate the pressor or chronotropic effects of GLP-1 *in vivo* [66]. Although the effects of GLP-1 and exendin-4 on blood pressure and heart

rate are blocked by the GLP-1R antagonist exendin(9-39), peripheral infusion of exendin(9-39) alone had no effect on baseline cardiovascular parameters in rats [67]. Evidence for the importance of the central nervous system in the GLP-1R-dependent control of cardiovascular responses emerged from studies demonstrating that ICV GLP-1 also increased heart rate and blood pressure. These effects were blocked by ICV but not peripheral administration of exendin(9-39) [68]. In contrast, the increase in heart rate and blood pressure observed following intravenous administration of GLP-1 was blocked by both ICV and peripheral exendin(9-39). Furthermore, bilateral vagotomy blocked the effects of ICV GLP-1 on heart rate and blood pressure and prevented the attenuation of GLP-1-activated cardiovascular responses following ICV but not intravenous exendin(9-39) administration [68].

More recent studies have implicated GLP-1R dependent signaling in the activation of the rodent sympathetic nervous system. Administration of GLP-1 or exendin-4 to rats induced c-fos expression in the adrenal medulla and activated neurons in autonomic control regions including medullary catecholamine neurons providing input to sympathetic preganglionic neurons [69]. GLP-1R agonists also activated tyrosine hydroxylase transcription in brainstem catecholamine neurons. These findings implicate a functional link between brainstem GLP-1 neurons, sympathetic outflow and the CNS regulation of cardiovascular responses [69].

GLP-1R Agonists and Body Weight Loss: Rodent and Human Studies

Although initial investigations demonstrated that the anorectic effects of ICV GLP-1 were transient and not consistently associated with weight loss, administration of GLP-1R agonists in rodents produces variable effects on feeding and body weight, depending on the experimental model and dose and frequency of peptide administration. Daily administration of exendin-4 to db/db mice produced only a transient reduction of food intake, but no significant weight loss over the 13 week study period [70]. In contrast, twice daily injection of Zucker rats with exendin-4 produced a sustained reduction in food intake and weight gain over the 56 day study, in association with a marked reduction in visceral fat deposition [71]. Similarly, even once daily subcutaneous administration of exendin-4 to obese Zucker rats reduced food intake and attenuated weight gain [72] and twice daily administration of the GLP-1R agonist NN2211 inhibited food intake and reduced body weight gain in normal and monosodium glutamate-treated rats [73]. In contrast, transgenic mice expressing a metallothionein promoter-exendin-4 transgene do not exhibit reductions in food intake or body weight regulation [74], and chronic treatment of db/db mice with exendin-4, NN2211, or CJC-1131, a long acting GLP-1 derivative, does not produce weight loss [70, 75, 76] .

The available information on the effects of GLP-1 administration on food intake and weight loss in human subjects is predominantly confined to short term studies, which have shown variable results [77]. Infusion of GLP-1 induced satiety but did not reduce food intake in obese men [78] yet a related study by the same investigators demonstrated significant GLP-1 effects on reduction of both hunger and food intake [79]. Similarly, GLP-1 administration reduced diet-induced thermogenesis [80], hunger and food intake in some [81, 82] but not all [81] studies of normal healthy subjects, obese subjects [83] and patients with type 2 diabetes [84, 85]. The modest effects of GLP-1 on appetite and food ingestion may be mediated via actions on CNS satiety centers, or through reductions in gastric emptying and consequent promotion of satiety [78, 86-89]. A meta-analysis of available human studies demonstrated a significant dose-dependent reduction of food intake in lean and obese subjects [33]. Although limited information is available on the effects of GLP-1R agonists on weight loss in chronic human studies, a six week infusion of native GLP-1 produced a mean 1.9 kg weight loss in 20 diabetic subjects [10]. Hence the available data suggests that GLP-1R analogue therapy of diabetic patients is unlikely to be associated with significant weight loss, however modest weight loss or prevention of weight gain are useful attributes of an agent with significant beneficial effects on glucose control and lowering of HbA1c [10, 90, 91].

Neurotrophic and Cytoprotective Actions of GLP-1 in the CNS

The actions of GLP-1R agonists on stimulation of β cell proliferation, islet neogenesis, and islet cell differentiation together with inhibition of β cell apoptosis [1] have received considerable attention due to the potential for enhancement or preservation of functional β cell mass in diabetic subjects [2]. The widespread expression of the GLP-1 receptor in the CNS, taken together with the demonstration that GLP-1 receptor expression is induced at the site of brain injury [92], further suggests that GLP-1R signaling likely subserves functions other than control of energy homeostasis. Intriguingly, GLP-1 may also exert cytoprotective or proliferative actions in neuronal or glial cell types [93]. Treatment of rat PC12 cells with exendin-4 promoted neurite outgrowth and NGF-initiated differentiation in a PI3-kinase, ERK MAP kinase-dependent manner [94]. Although exendin-4 co-treatment did not prevent cell death associated with NGF withdrawal, addition of exendin-4-after NGF withdrawal improved cell survival. Furthermore, both GLP-1 and exendin-4 significantly protected cultured rat hippocampal neurons against glutamate-induced apoptosis and reduced ibotenic acid-induced depletion of choline acetyltransferase immunoreactivity in basal forebrain cholinergic neurons [95]. Conversely, blockade of GLP-1R signaling by infusion of the antagonist exendin(5-39) prevented memory impairment and CA1 DNA fragmentation induced by administration of β amyloid protein (1-40) in male Wistar rats [96]. These findings implicate a role for endogenous GLP-1R signaling in the pathophysiology of β amyloid protein-associated brain injury. Whether peripheral infusion of GLP-1R agonists might also exert neuroprotective effects in the CNS remains unclear and merits additional experimentation.

GLP-2 and the Regulation of Food Intake

The actions of central and peripheral GLP-1 in regulating feeding behaviour are comparatively well established; however the physiological importance of GLP-2 for regulating satiety remains uncertain. Although exogenous peripheral GLP-2 administration inhibits gastrointestinal motility, enhances nutrient absorption and promotes intestinal growth (Fig. **1**), food intake is unaltered in rodents or humans following treatment with GLP-2 [23, 97, 98]. ICV injection of GLP-2 in ad-libitum fed rats produced a transient inhibition of feeding for two hours that was blocked by administration of the GLP-1R antagonist exendin(9-39) [32]. In contrast, although large amounts of ICV GLP-2 inhibited food intake in mice, exendin(9-39) enhanced, rather than inhibited the actions of GLP-2 on food ingestion. Similarly, the GLP-2-mediated inhibition of food intake was enhanced, rather then attenuated, in mice with disrupted GLP-1R signaling. The reasons for the differences in anorexic GLP-2 action in mice versus rats remain unclear and require further study [99]. The available limited data suggests that GLP-2, in contrast to GLP-1, is unlikely to be an important regulator of feeding behaviour.

INTERRELATIONSHIPS BETWEEN GLP-1 AND OTHER ANOREXIGENIC/OREXIGENIC PEPTIDES

The potential for interdependent relationships between GLP-1 and regulatory peptides with overlapping actions on hypothalamic feeding centers exists at the level of both GLP-1 secretion and GLP-1 action. For example, nutrients differentially modulate the release of multiple gut peptides with hypothalamic actions, including ghrelin, PYY, and GLP-2. The circulating levels of nutrient-stimulated ghrelin and GLP-1 are inversely related [100]. In contrast, the secretion of PYY, GLP-1, and GLP-2, peptides co-secreted from the same enteroendocrine cell which inhibit food intake [32, 99, 101], is stimulated by food ingestion. As discussed above, although leptin activates GLP-1R+ neurons, GLP-1 receptor signaling is not essential for the anorexic actions of leptin *in vivo* [44, 49, 50]. Intracerebroventricular GLP-1 reduces the stimulatory effect of NPY and melanin-concentrating hormone (MCH) in rats [34, 38], but GLP-1 has only modest inhibitory effects on orexin-induced feeding [102]. Central administration of GLP-1 activates corticotropin-releasing hormone+ (CRH+) neurons in the rat, leading to increased corticosterone secretion [63], and the anorectic effects of both GLP-1 and CRH were not attenuated by the melanocortin-4 receptor peptide antagonist Agrp(83-132) [103]. Hence, anorexic GLP-1R+ signaling pathways interact with and are modified by complex peptidergic networks which control food ingestion.

SUMMARY

It is becoming increasingly evident that the glucagon-like peptides coordinate not only the intake, absorption and utilization of nutrients following meal ingestion, but may also act as endogenous neurotransmitters regulating satiety and appetite. Importantly, this inference is derived not only from animal experiments but also from studies of GLP-1 administration in lean and obese healthy or diabetic human subjects. With the anatomical and functional delineation of distinct sub-populations of GLP-1 receptors in the CNS our appreciation for the diverse actions of GLP-1 in the brain is becoming increasingly refined. Although GLP-1 may gain direct access to the CNS by crossing the blood brain barrier [104], peripheral GLP-1 administration may be associated with direct vagal activation [105] which in turn provides ascending inputs to central GLP-1R signaling systems [86, 106]. The development of CNS-selective GLP-1R agonists and antagonists, and studies designed to specifically interrupt anatomically and functionally distinct CNS GLP-1R signaling systems will be useful for further refining our understanding of the physiological importance of glucagon-like peptide receptor signaling in the brain.

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ABBREVIATIONS

Agrp	=	Agouti-related peptide
CNS	=	Central nervous system
CRH	=	Corticotropin-releasing hormone
CTA	=	Conditioned taste aversion
GLP-1	=	Glucagon-like peptide-1
GLP-2	=	Glucagon-like peptide-2
GLP-1R	=	Glucagon-like peptide-1 receptor
HbA1c	=	Hemoglobin A1c
ICV	=	Intracerebroventricular
LHRH	=	Luteinizing hormone releasing hormone
NGF	=	Nerve growth factor
PYY	=	Peptide YY
RNA	=	Ribonucleic acid
TSH	=	Thyroid stimulating hormone

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