Title: The CNS glucagon-like peptide-2 receptor in the control of energy balance and glucose homeostasis

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Abbreviation: GLP-1/2: glucagon-like peptide-1/2; Glp2r (gene) or Glp2r (protein): GLP-2 receptor; Glp1r (gene) or Glp1r (protein): GLP-1 receptor; Mc4r (gene) or Mc4r (protein): melanocortin receptor 4; LepR (gene) or LepR (protein): leptin receptor; InsR (gene) or InsR (protein): insulin receptor; PI3K: phosphatidylinositol-3-kinase; CNS: central nervous system; RYGB: Roux-en-Y gastric bypass; HGP: hepatic glucose production; FI: food intake; GE: gastric emptying; GI: gastrointestinal; icv: intracerebroventricular; PPG: preproglucagonergic; POMC: pro-opiomelanocortin; αMSH: α-Melanocyte-stimulating hormone; ARC: arcuate nucleus of the hypothalamus; DMH: dorsomedial nucleus of the hypothalamus; NTS: nucleus tractus solitarius in the brainstem; DMV: dorsal motor nucleus of the vagus; PBN: parabrachial nucleus of the brainstem; DVC: dorsal vagal complex of the brainstem (including NTS plus DMV)
The CNS glucagon-like peptide-2 receptor in the control of energy balance and glucose homeostasis

Abstract  The gut-brain axis plays a key role in the control of energy balance and glucose homeostasis. In response to luminal stimulation of macronutrients and microbiota-derived metabolites (secondary bile acids and short chain fatty acids), glucagon-like peptides (GLP-1/2) are co-secreted from endocrine L cells in the gut and co-released from preproglucagonergic neurons in the brainstem. Glucagon-like peptides are proposed as key mediators for bariatric surgery-improved glycemic control and energy balance. Little is known about the GLP-2 receptor (Glp2r)-mediated physiological roles in the control of food intake and glucose homeostasis, yet Glp1r has been studied extensively. This review will highlight the physiological relevance of the CNS Glp2r in the control of energy balance and glucose homeostasis, and focuses on cellular mechanisms underlying the CNS Glp2r-mediated neural circuitry and intracellular PI3K signaling pathway. New evidence (obtained from Glp2r tissue-specific KO mice) indicates that the Glp2r in POMC neurons is essential for suppressing feeding behavior, gastrointestinal motility and hepatic glucose production. Mice with Glp2r deletion selectively in POMC neurons exhibit hyperphagic behavior, accelerated gastric emptying, glucose intolerance and hepatic insulin resistance. GLP-2 differentially modulates postsynaptic membrane excitability of hypothalamic POMC neurons in Glp2r- and PI3K-dependent manners. GLP-2 activates the PI3K-Akt-FoxO1 signaling pathway in POMC neurons by Glp2r-p85α interaction. icv GLP-2 augments glucose tolerance, suppresses glucose production and enhances insulin
sensitivity, which require PI3K (p110α) activation in POMC neurons. Thus, the CNS Glp2r plays a physiological role in the control of food intake and glucose homeostasis. This review will also discuss key questions for future studies.

Keywords: Glucagon-like peptides; Glucose homeostasis; Food intake; Gastric emptying; Gut–brain axis; Insulin sensitivity

Abbreviation:
GLP-1/2: glucagon-like peptide-1/2
Glp2r (gene) or Glp2r (protein): GLP-2 receptor
Glp1r (gene) or Glp1r (protein): GLP-1 receptor
Mc4r (gene) or Mc4r (protein): melanocortin receptor 4
LepR (gene) or LepR (protein): leptin receptor
InsR (gene) or InsR (protein): insulin receptor
PI3K: phosphatidylinositol-3-kinase
CNS: central nervous system
RYGB: Roux-en-Y gastric bypass
HGP: hepatic glucose production
FI: food intake
GE: gastric emptying
GI: gastrointestinal
icv: intracerebroventricular
PPG: preproglucagonergic
POMC: pro-opiomelanocortin
Introduction
Among the greatest threats to public health are increasing rates of obesity and diabetes. Obesity promotes the development of more than 50 chronic disorders and costs ~9% of all U.S. health care expenditures(22). Thus, it is imperative to identify novel therapeutic targets and develop effective approaches to prevent and treat obesity and diabetes. Bariatric surgery (particularly Roux-en-Y gastric bypass, RYGB) has proven to be the most effective therapy for morbid obesity and diabetes(19; 26; 111), in which the gut-brain axis is highly involved in mediating beneficial effects on food intake, glycemic control and weight loss.

The gut–brain axis (i.e. the bidirectional communication between the gut and the brain) plays key roles in the regulation of energy/glucose homeostasis. Gut-derived signals (for nutritional, neural, immune, and endocrine information) are input from the gut to the brain, meanwhile autonomic and neuroendocrine messengers are output from the brain to the gut. The hypothalamus, a primary region of central integration for homeostatic signals, plays crucial roles in the regulation of energy balance and glucose homeostasis(7; 104; 114). In the arcuate
nucleus (ARC) of the hypothalamus, neurons expressing pro-opiomelanocortin (POMC neurons, a key component of the central melanocortin system) are positioned to integrate hormonal/ nutritional signals reflecting energy availability(25). Activation of POMC neurons enhances energy expenditure and suppresses food intake yet neuroendocrine mechanisms are not fully established. The gastrointestinal (GI) tract plays a critical role in digestion, absorption and utilization of nutrients(32), thus controlling energy intake at the first pass. Through the gut-brain axis, gut hormones play a crucial role in the regulation of energy balance (including food intake) and glucose homeostasis(27; 50). Recently, it has been shown that infusion of glucose or lipid into the rat jejunum improves glucose homeostasis in uncontrolled diabetes, suggesting that nutrient sensing by the gut-brain axis may play a key role in the control of glucose production(14; 31), yet little is known about the nutrient sensor identity and intracellular signaling network.

In response to luminal stimulation of macronutrients and microbiota-derived metabolites (secondary bile acids and short chain fatty acids), glucagon-like peptides (GLP-1/2) are co-secreted from endocrine L cells in the gut(59; 100; 102; 106; 108; 123; 124); and assumedly co-released from preproglucagonergic (PPG) neurons in the brainstem as well. GLP-1/2 are key neuroendocrine signals for the gut-brain axis to control food intake and glucose homeostasis(92); and proposed as important mediators for bariatric surgery-improved glycemic control and insulin sensitivity (37; 47; 73; 74; 99; 121). In fact, GLP-1/2 receptor agonists are used clinically for the prevention and treatment of diabetes and digestive diseases(92). By means of neural and endocrine pathways, gut-derived GLP-1/2 signals are transduced to the brain to coordinate food intake and glucose homeostasis(14; 130), although their physiological roles are largely unknown. Thus, elucidation of GLP-1/2 intracellular signaling and metabolic
action in the control of energy balance may reveal novel targets for the treatment of intestinal dysfunctions, obesity and diabetes(92).

Through acting on a specific G protein-coupled receptor (GLP-2R), GLP-2 promotes intestinal epithelial homeostasis & function, enhances intestinal nutrient absorption & blood flow, and assists gut immune defense; and improves outcomes of short bowel syndrome(9; 47; 57). Except for its enterotrophic role, GLP-2-mediated metabolic action has not been fully recognized. Without incretin effect, GLP-2 was considered insignificant in glucose homeostasis. Recent studies reveal that central infusion of GLP-2 suppresses food intake and increases glucose tolerance (118; 121; 122). Importantly, GLP-2 has been proposed as a neurotransmitter in the control of feeding behavior(121; 122) and may mediate PPG neuron-induced synaptic transmission linking the hypothalamus and the brainstem(121). The Glp2r is not only expressed in nutrient-sensing endocrine cells (such as enteroendocrine cells and pancreatic α-cells), but also neurons (such as enteric neurons, vagal sensory neurons, and central neurons)(30; 45; 91; 95; 131; 138), suggesting complex modes for GLP-2 action via endocrine, paracrine or neuroendocrine mechanisms. Thus, GLP-2 may act as a crucial neuroendocrine signal (for temporary energy availability) to the hypothalamus in the control of feeding behavior (food intake) and glucose homeostasis. It seems that bariatric surgery-improved glucose tolerance is independent of Glp1r activation in rodents(85; 133; 136). Notably, bariatric surgery not only improves glucose tolerance, but also restores insulin sensitivity. Glp2r/Glp1r (two G protein-coupled receptors) play distinct roles, yet GLP1/2 are co-secreted. Glp2r/Glp1r in postprandial glycemic control has not been determined. Thus, it is still crucial to determine if Glp2r is a key contributor to improving glycemic control and insulin sensitivity after bariatric surgery.
To decode the GLP-2 physiologic role and signaling pathway, my laboratory has generated a
Glp2r floxed mouse line that has provided a powerful, genetic tool to dissect Glp2r metabolic
and mitogenic action in a tissue (or cell)-specific manner. Using Glp2r tissue-specific knockout
mouse models, we show that Glp2r is a key satiety signal for the physiological control of food
intake (FI) and gastric emptying, and this may contribute to the homeostatic control of energy
balance and body weight(46). Moreover, Glp2r in hypothalamic POMC neurons is required for
promoting hepatic insulin sensitivity and glycemic control(118). Through the gut-brain-stomach
(or-liver) axis, GLP-2 plays key physiological roles in the control of gastric emptying and hepatic
glucose production (HGP)(46; 118). In this review, we will focus on the physiological
significance and cellular action of the CNS GLP-2 in the control of feeding behavior and glucose
homeostasis, and propose that GLP-2 as an endocrine and neural signal may transmit intestinal
nutrient sensing to the hypothalamus-brainstem to suppress feeding behavior and glucose
production in a negative-feedback manner.

1 GLP-2R is expressed in the hypothalamus and brainstem

The rodent Glp2r mRNA is not only expressed in the hypothalamus (DMH and ARC), but also
localized in the hippocampus and the brainstem (DMV and PBN)(81; 121; 131). Using
fluorescence in situ hybridization and immunohistochemistry (using validated Glp2r
antibody(45)), we further confirmed that Glp2r mRNA and protein are expressed in neurons in
the ARC and dorsomedial nucleus (DMH) as reported (118; 121). Moreover, the Glp2r protein is
expressed in the POMC neurons(118). Intracerebroventricular (icv) infusion of GLP-2 increases
POMC mRNA expression captured from the mouse ARC region(46). These data suggest that
Glp2r in POMC neurons may play an important, physiological role in the control of feeding behavior and glucose homeostasis.

2 The CNS GLP-2 suppresses feeding behavior

Is the CNS GLP-2 a key satiety signal for the physiological short-term control of feeding behavior? It was debated that icv GLP-2 suppresses food intake, but peripheral GLP-2 does not (80; 121; 122). The hypothalamus plays pivotal roles in the regulation of energy balance and glucose homeostasis(7; 46; 104; 105; 114). It was unknown if the CNS GLP-2 plays any physiological role in the control of energy homeostasis (e.g. feeding behavior). A key experiment was warranted to address if GLP-2 in targeted neurons of the hypothalamus modulates feeding behavior and gastric emptying. Two groups of neurons in the arcuate nucleus of the hypothalamus control feeding: Satiety POMC\textsuperscript{ARC} neurons are activated by PACAP\textsuperscript{VMH} neurons while hunger AgRP\textsuperscript{ARC} neurons are activated by SIM1\textsuperscript{PVH} neurons(69). Since POMC neurons play a critical role in the homeostatic control of energy balance(36), we hypothesized that Glp2r in POMC neurons plays an important, physiological role in the control of energy balance, specifically feeding behavior. Using the Cre-LoxP system, we generated POMC-Glp2r KO mice with Glp2r deletion selectively in POMC neurons(46). The POMC-Glp2r KO mice display hyperphagic behavior (indicated by increased food intake) and late-onset obesity, suggesting a physiological role of the CNS GLP-2 in the control of feeding behavior(46).

Melanocortin receptor 4 (Mc4r, a G-protein coupled receptor) binds \(\alpha\)-melanocyte stimulating hormone (\(\alpha\)-MSH) and play a central role in the regulation of body weight, glucose homeostasis, energy intake and expenditure. The brainstem DVC is a critical site of Mc4r action.
for POMC-derived α-MSH input and gut signals to inhibit food intake(10; 25; 71; 110; 125). GLP-2 is produced from PPG neurons in the brainstem NTS while Glp2r is expressed in the brainstem DMV and PBN (unpublished data). We speculated that the brainstem may be an additional site of GLP-2 action on feeding behavior. Interestingly, GLP-2 infusion (into the 4th ventricle) suppressed food intake and gastric emptying, while GLP-2-mediated effects were abolished in the Mc4r KO mice(46).

This study indicates that Glp2r in POMC neurons plays an important, physiological role in the control of feeding behavior, and this is mediated through the melanocortin system(46). Thus, the CNS Glp2r is a physiological, satiety signal.

3 The CNS Glp2r decelerates in vivo gastric emptying

Gastrointestinal motility is fine-tuned by metabolic, neuronal and hormonal signals, and plays a crucial role in the control of digestion and absorption, influencing the intake rate of net energy. Specifically, gastric emptying (GE) is a critical process for the short-term control of food intake; and maybe contributes to the long-term maintenance of energy homeostasis(46; 86; 134; 141). Gastric emptying is enhanced with overeating(34) and obesity(18; 134). The overall impact of faster gastric emptying results in shorter satiety period, increasing meal frequency and energy intake(56). Thus, gastric emptying may be a target for controlling appetite(34; 56).

Peripheral GLP-2 induced the gastric relaxation in vitro in rodents (3), but showed inconsistent gastric emptying in humans(93). Glp2r is expressed in both excitatory and inhibitory enteric neurons(24; 45). Thus, peripheral GLP-2 inhibits the gastrointestinal motility by decreasing cholinergic neurotransmission and/or increasing NO or VIP release(2; 4). Little was known
about the physiological relevance of the CNS GLP-2 in the regulation of gastric emptying. To determine if Glp2r in POMC neurons modulates gastric motility to control intake of net energy, we quantified the mouse gastric emptying under the conscious condition using $^{13}$C-octanoic acid breath test(46). Both half-excretion time ($T_{1/2}$) and lag phase ($T_{lag}$) for liquid meal were dramatically shorter, while gastric emptying coefficient was higher in the POMC-Glp2r KO mice than those in the WT mice. Thus, the new evidence that Glp2r deletion in POMC neurons accelerated rates of gastric emptying not only explains the hyperphagic behavior, but also supports the notion of ileal break that GLP-1/2 secreted from the endocrine L cells in the ileum slow down gastric emptying to suppress food intake at least in part via the gut-brain-stomach axis. Moreover, the Mc4r signaling is required for icv GLP-2-mediated suppression of both food intake and gastric emptying(46). Inhibition of Mc4r-positive cholinergic neurons in the DMV would alleviate excitatory input to the gastrointestinal tract, decreasing gastric emptying(42; 110). The CNS Mc4r signaling is important for the tonic control of gastric emptying. Further studies are warranted to determine if GLP-2 acts on Mc4r-positive neurons in the brainstem through presynaptic input (from POMC-neurons in the NTS); and if Glp2r deletion in POMC neurons impairs nutrient absorption by the gut per se.

4 The CNS Glp2r promotes postprandial glycemic control and hepatic insulin sensitivity

Increased hepatic glucose production (HGP, mainly gluconeogenesis) is a major factor in the pathogenesis of hyperglycemia in type 2 diabetes. GLP-1/2 signals are proposed to contribute to improving metabolic outcomes (including glycemic control & insulin sensitivity) after gastric bypass surgery(37; 73; 74), which may be mediated via a neuroendocrine mechanism instead of incretin action. Except for pancreatic islets, the brain regulates glucose homeostasis(115). Direct action of insulin and leptin on POMC neurons is required to maintain glucose
homeostasis via the control of HGP(52; 76; 96; 135). Except for insulin action in the liver, HGP is also suppressed by enhanced hepatic vagal input(43; 67; 71; 98; 105). Moreover, leptin signaling in POMC neurons is sufficient to correct diabetes in Lepr$^{db/db}$ mice(58), while insulin receptor (InsR) activation in POMC neurons promotes HGP (76; 96), pointing to the central control of POMC neurons in glucose homeostasis.

Peripheral GLP-2 regulates glucose homeostasis through promoting intestinal absorption of glucose and pancreatic secretion of glucagon(47; 83). Due to its enterotrophic action, GLP-2 is highlighted as a key signal to drive intestinal reprogramming of glucose metabolism to contribute to glycemic improvement after RYGB(113). The CNS GLP-2, like insulin and leptin, may play a crucial role in the control of glucose homeostasis and insulin sensitivity (i.e., suppressing HGP) via enhanced hepatic vagal input(43; 67; 98; 105). \textit{Glp2r} global deficiency shows severer hyperglycemia and impaired glucose tolerance in Lep$^{ob/ob}$ mice(8), suggesting that Glp2r activation is required for glucose homeostasis in obese mice(8). Leptin can stimulate GLP-1/2 secretion from L cells(5). Leptin directly depolarizes PPG neurons in the brainstem(55), suggesting that the CNS GLP may act as key downstream neurotransmitters of brainstem leptin receptor (LepR) in the control of food intake and glucose homeostasis. PPG neurons innervate primarily autonomic regions (including hypothalamic ARC, DMH and PVN; and brainstem DMV) where GLP-2R is expressed(46; 77; 118; 121). Our recent data indicate that Glp2r is required for postprandial glycemic control. It is difficult to interpret these data since Glp2r is expressed not only in endocrine cells (such as enteroendocrine cells and pancreatic $\alpha$-cells), but also in neurons (such as enteric neurons, vagal sensory neurons, and the CNS neurons)(45; 131). To dissect the physiological relevance of tissue-specific Glp2r in glycemic control, a site-specific inducible Glp2r knockout mouse model is greatly needed. As we
reported(118), mice lacking Glp2r selectively in POMC neurons display impaired postprandial glucose tolerance and hepatic insulin resistance (by increased gluconeogenesis). icv infusion of GLP-2 increases glucose tolerance and insulin sensitivity, and this requires Glp2r activation in POMC neurons(118), pointing to the physiological significance of GLP-2 neural action in glycemic control. Considering 50% of glycemic control through insulin-independent mechanisms(115), it will be important to quantify the extent to which GLP-2 can distinctly normalize glycemia under insulin/leptin resistance.

(1) Glp2r deletion in POMC neurons impairs postprandial glycemic control and hepatic insulin sensitivity: POMC-Glp2r KO mice display postprandial hyperglycemia and glucose intolerance after iv glucose tolerance test(118). Using hyperinsulinemic euglycemic clamp coupled with stable isotopic tracers ($^2$H$_2$O and 6,6-$^2$H$_2$-glucose), the author's laboratory has demonstrated that POMC-Glp2r KO mice had lower glucose infusion rate (representing whole-body insulin sensitivity), which was attributed majorly to higher HGP and gluconeogenesis since glucose disappearance rate (representing glucose disposal) was not altered. Thus, Glp2r deletion in POMC neurons leads to hepatic insulin resistance, suggesting that Glp2r in POMC neurons is required for insulin-mediated suppression of HGP (i.e., gluconeogenesis). It is noted that glucose tolerance and insulin sensitivity are reduced in obesity. To avoid any confounding effects of body fat mass, glucose tolerance test and hyperinsulinemic euglycemic clamp were performed in mice at the age of 12 weeks (i.e., prior to the onset of obesity). Thus, the metabolic phenotype of hepatic insulin resistance (characterized in POMC-Glp2r KO mice) cannot be attributed to hyperphagic obesity, indicating a distinct role of Glp2r in POMC neurons in glycemic control.
(2) icv GLP-2 promotes glycemic control and insulin sensitivity in POMC Glp2r- and PI3K-dependent manners: As reported, Glp2r activation in POMC neurons is required for both icv and iv GLP-2 to enhance insulin-mediated suppression of HGP (via gluconeogenesis)(118). PI3K signaling in POMC neurons is essential for leptin-induced activation and insulin-induced inhibition of POMC neurons(53). Increased PI3K activity in POMC neurons improves insulin sensitivity, whereas decreased PI3K signaling impairs glucose homeostasis. Thus, the PI3K signaling in POMC neurons is important for both energy balance and glucose homeostasis(54; 118). Importantly, icv infusion of GLP-2 augments glucose tolerance, suppresses basal HGP, and enhances insulin sensitivity, which are dependent on PI3K (p110α) signaling in POMC neurons(118). These data indicate that the CNS GLP-2 promotes glycemic control and insulin sensitivity via Glp2r-PI3K-mediated central melanocortin system, which is independent of incretin-mediated insulin action(118).

5 GLP-2 differentially modulates the excitability of POMC neurons in Glp2r- and PI3K-dependent manners

(1) GLP-2 modulates the excitability of two subgroups of POMC neurons differentially: GLP-2 acutely potentiates L-type, voltage-gated Ca^{2+} (Cav) channel activity in primary hippocampal neurons(131). Though Glp2r is expressed in POMC neurons(46), it was unknown if GLP-2 can directly modulate their excitability. To determine GLP-2-induced action on POMC neuronal excitability, we performed whole-cell patch-clamp recording on GFP-labeled POMC neurons in brain slices from POMC-Glp2r KO and WT mice(118). There are two subgroups of hypothalamic POMC neurons in response to acute GLP-2(118), i.e., GLP-2 (100 nM) acutely depolarized ~50% of POMC neurons with increased spontaneous firing rate; in contrast, GLP-2 (100 nM) acutely hyperpolarized ~50% of POMC neurons with decreased spontaneous firing
rate. Of note, leptin-responsive POMC neurons (i.e., acutely activated by leptin) are segregated from insulin-responsive POMC neurons (i.e., acutely inhibited by insulin)(132). In agreement with this new notion, we further identified that GLP-2-excited POMC neurons expressed leptin receptor (LepR), while GLP-2-inhibited POMC neurons presented insulin receptor (InsR) by single-cell RT-PCR(118). Currently, little is known about segregated subgroups of hypothalamic POMC neurons (such as neuronal signature, spatial projection & synaptic transmission, and distinct neural function & metabolic phenotype) in the control of energy balance and glucose homeostasis. It will be important to characterize GLP-2-differentially activated subgroups of POMC neurons, which will ultimately determine their distinct neuroendocrine actions.

(2) GLP-2 modulates the excitability of POMC neurons in Glp2r- and PI3K-dependent manners: Moreover, activation of Glp2r or PI3K is required to modulate the membrane excitability of POMC neurons(118). As shown, GLP-2 neither depolarized nor hyperpolarized POMC neurons with Glp2r deletion in the brain slices, indicating GLP-2 direct action on membrane potential (not attributable to synaptic transmission). Activation of PI3K signaling in POMC neurons is crucial for energy/glucose homeostasis (1; 52; 54; 67; 88; 103); and required for leptin and insulin to activate TRPC and K\textsubscript{ATP} channels, respectively, resulting in depolarization and hyperpolarization of POMC neurons to suppress food intake and HGP(11; 53; 68; 103; 109; 119). The PI3K-Akt signaling pathway (as a conserved central node) may underlie GLP-2-modulated membrane excitability. In the presence of PI3K inhibitor, POMC neurons do not respond to GLP-2 anymore. Interestingly, both leptin and insulin activate PI3K signaling in POMC neurons, resulting in depolarization (leptin) or hyperpolarization (insulin). In summary, GLP-2 directly modulates postsynaptic membrane excitability of POMC neurons in Glp2r and
PL3K-dependent manners; and this may be synchronized with leptin and insulin signaling in segregated subgroups of POMC neurons.

(3) Putative ion channels underlying GLP-2 neural action: It is unknown through which ion channels GLP-2 modulates membrane excitability of POMC neurons. Activation of PI3K-Akt signaling pathway potentiates L-type, voltage-dependent calcium channel (Ca\textsubscript{v}) activation and enhances Ca\textsubscript{v} currents in neurons\cite{13, 21, 126}. In addition, leptin (via the PI3K signaling) evokes L-type Ca\textsuperscript{2+} currents\cite{129} and transient receptor potential canonical (TRPC) currents in POMC neurons\cite{107}, exciting them. As discussed below, GLP-2 activates the PI3K signaling in POMC neurons\cite{118}. Moreover, Glp2r interacts \textit{in vitro} with calmodulin\cite{82}, a protein partner with TRPC channels\cite{96}. Using the whole-cell patch clamp, we show that GLP-2 acutely induces inward Ca\textsuperscript{2+} currents (i.e., increases intracellular Ca\textsuperscript{2+} level) through enhancing L-type, Ca\textsubscript{v} channel activity in primary hippocampal neurons\cite{131}. It will be important to identify if putative ion channels (Ca\textsubscript{v} and TRPC) are required for GLP-2 to excite LepR-expressing POMC neurons. ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels in the hypothalamus are essential for glucose homeostasis\cite{84}. Activation of K\textsubscript{ATP} channels in hypothalamic neurons is required for insulin- and leptin-mediated suppression of HGP\cite{97, 98, 104, 120}. Insulin-activated PI3K signaling (through PIP3) activates the Sur1/Kir6.2 K\textsubscript{ATP} channels in POMC neurons to decreases HGP\cite{1, 60, 70, 98, 103, 104}. Thus, we hypothesized that GLP-2 acutely enhances K\textsubscript{ATP} channel activity, inhibiting InsR-expressing POMC neurons. A pharmacological study indicates that K\textsubscript{ATP} channel activation is responsible for GLP-2-induced inhibition of POMC neurons\cite{118}.

In summary, GLP-2 directly modulates postsynaptic membrane excitability of POMC neurons in Glp2r- and PI3K-dependent manners. GLP-2 differentially depolarizes or hyperpolarizes POMC
neurons in the hypothalamus; and this paradox action may be synchronized with leptin and insulin signaling in segregated subgroups of POMC neurons. Moreover, \( K_{\text{ATP}} \) channel activation is responsible for GLP-2-induced inhibition of POMC neurons expressing InsR(118). Studies are warranted to characterize neural and metabolic phenotypes of functionally segregated POMC neurons; and to identify putative ion channels underlying GLP-2R-mediated excitation of POMC neurons expressing LepR.

6 GLP-2R-activated intracellular signaling pathways in the brain

How does GLP-2 act on ARC POMC neurons intracellularly? Little is known about how Glp2r activates intracellular signaling pathways in neurons or endocrine cells. In Glp2r-expressing cells, GLP-2 activates PI3K and PKA signaling pathways(17; 33; 75; 117; 118; 131; 137). The PI3K-Akt signaling pathway in hypothalamic neurons plays a critical role in the control of energy balance and glucose homeostasis(88; 103). Increased PI3K activity in POMC neurons improves insulin sensitivity, whereas decreased PI3K activity impairs HGP(54). As the PI3K major catalytic subunit(38; 61; 66), \( p110\alpha \) is required for leptin and insulin-induced PI3K activity in the hypothalamus(38; 67). Specifically, PI3K signaling in POMC neurons is essential for leptin-induced activation and insulin-induced inhibition of POMC neurons(1; 53). PI3K signaling is required for leptin and insulin to activate TRPC and \( K_{\text{ATP}} \) channels, respectively, resulting in depolarization and hyperpolarization of segregated POMC neurons to suppress food intake and glucose production. We proposed that GLP-2-activated intracellular signaling pathways are cell-specific, i.e., PI3K-dependent Glp2r action in POMC neurons.

(1) GLP-2 activates PI3K signaling by initiating Glp2r-p85\( \alpha \) binding: Through Glp2r primary sequence analysis, we noticed a putative p85\( \alpha \)-binding motif in the Glp2r amino acid sequence.
It has been shown using immunoprecipitation that Glp2r interacts with the PI3K regulatory subunit (p85α) in primary hippocampal neurons(118). This Glp2r-p85α interaction was further confirmed in HEK293 cells transfected with Glp2r-c-myc and p85α-GFP using antibodies against c-myc and GFP. Moreover, Glp2r is required for the basal level of Akt phosphorylation in POMC neurons(118).

(2) GLP-2 activates PI3K-Akt-FoxO1 signaling in POMC neurons: FoxO1 is a downstream target of the PI3K/Akt signaling pathway, and regulates energy balance (62; 65). The Cre-dependent expression and nuclear exclusion of FoxO1-GFP not only indicates transcriptional repression of FoxO1, but also reports signaling activation of PI3K-Akt pathway(39). The PI3K-Akt-FoxO1 pathway is considered a key universal pathway in hormonal, nutritional, and neural actions. GLP-2 rapidly phosphorylates Akt in primary hippocampal neurons in a PI3K-dependent pathway(117). Therefore, we proposed that GLP-2-activated PI3K-Akt signaling can be indicated by FoxO1 nuclear exclusion. As shown, GLP-2 enhances PI3K-dependent p-Akt signaling, facilitating FoxO1 nuclear exclusion in hypothalamic POMC neurons(118). It should be noted that PI3K deletion (i.e. p110α KO) or inhibition (e.g. LY294002 application) negates GLP-2-mediated effects, which would block the entire PI3K-PIP3-Akt signaling pathway(118). Importantly, PI3K does not phosphorylate Akt. PI3K activation phosphorylates membrane-bound PIP2 to generate PIP3. Through the binding of its PH domain to PIP3, Akt is translocated to the plasma membrane and phosphorylated at Thr308 by PDK1 and at Ser473 by mTORC2, respectively. It has been shown that GLP-2 facilitates Akt phosphorylation at the plasma membrane and induces Akt phosphorylation at Thr308 (which requires the intact p85α-binding site on Glp2r)(118). Moreover, GLP-2 increases nuclear exclusion of phosphorylated FoxO1 in a p110α (a catalytic subunit of PI3K)-dependent manner. However, these results do
not necessarily point to GLP-2-mediated, direct phosphorylation on Akt or FoxO1 per se. Interestingly, brainstem Glp1r-mediated suppression of food intake requires a PI3K-dependent decrease in phosphorylation of membrane-bound Akt(112). Therefore, GLP-2 activates the PI3K-Akt-FoxO1 signaling pathway via initiating an interaction of Glp2r and p85α. However, it is still unknown if GLP-2 induces PI3K-independent phosphorylation of Akt or FoxO1. 

(3) PI3K in POMC neurons is required for GLP-2 to promote glucose homeostasis and insulin sensitivity: It appears that GLP-2 acts on POMC neurons through activating the PI3K-Akt-FoxO1 signaling pathway. Is the PI3K activation required for the CNS GLP-2 metabolic action? As a major catalytic subunit of PI3K, p110α is required for leptin- and insulin-induced PI3K activity in the hypothalamus(38; 61; 66). Thus, we generated POMC-p110α KO mice to address this question. icv GLP-2 promotes insulin sensitivity and glycemic control (indicated by increased glucose tolerance and suppressed basal HGP); and this was abolished in mice with p110α deletion selectively in POMC neurons. 

In summary, three lines of evidence indicate that GLP-2 activates the PI3K-Akt-FoxO1 signaling in POMC neurons: (a) Glp2r physically binds to p85α (a PI3K regulator subunit), enhances Akt phosphorylation, and activates PI3K-Akt-dependent nuclear exclusion of FoxO1 in POMC neurons(118). Moreover, GLP-2 rapidly activates the PI3K-Akt-mTOR pathway in primary hippocampal neurons(117). (b) PI3K signaling is required for GLP-2-mediated membrane excitation of POMC neurons (as discussed above). Of note, leptin excites POMC neurons via activation of TRPC channels in a PI3K-dependent manner(53; 88; 89). (c) p110α (a PI3K catalytic subunit) in POMC neurons is required for GLP-2-mediated glycemic control and insulin sensitivity(118). Thus, the CNS GLP-2 plays a key role in the control of HGP via PI3K-
dependent activation and nuclear transcription of POMC neurons. We speculate that Glp2r activation → POMC neuronal excitation (via PI3K-dependent activation of TRPC channels) → α-MSH release (as a slow-acting neurotransmitter) on postsynaptic Mc4r-expressing autonomic neurons → glycemic control. GLP-2R-induced intracellular signaling in hypothalamic POMC neurons is summarized in Figure 1.

7 Key questions need to be addressed in future studies

(1) Are the NTS/DMV direct targets for GLP-2-mediated control of autonomic outflow? Selective activation of distinct neurons in the DVC decreases HGP (mainly gluconeogenesis)(105; 142).

As an integrative center from ascending and descending signals, the NTS via postsynaptic GABA/ glutamate modulates the DMV that fine-tunes the autonomic outflow to discrete visceral areas(6; 29; 41). Importantly, NTS PPG neurons project widely to key central autonomic sites including the brainstem and hypothalamus(77; 78; 127), suggesting a potential role of downstream GLP-1/2 receptor signaling in the control of autonomic outputs(79). Specifically, PPG neurons innervate brainstem autonomic neurons including the dorsal motor nucleus of the vagus (DMV) where Glp2r is expressed (unpublished data)(77). Leptin directly excites PPG neurons(55), suggesting that GLP-1/2 may serve as downstream neurotransmitters for brain leptin action and relay vagal afferent signals. GLP-2 indirectly activates the NTS neurons through vagal afferents(95). We speculate that GLP-2 secretion (from enteroendocrine L cells) → Glp2r activation on vagal sensory neurons → presynaptic input to PPG neurons in the brain NTS → neuronal GLP-2 release → Glp2r activation in the hypothalamus and brainstem → autonomic control of peripheral glucose metabolism. Regardless of GLP-2 secreted from L cells
in the gut or released from PPG neurons in the brainstem, Glp2r activation on DMV neurons in
the brainstem may modulate vagal outflow, reprogramming peripheral glucose metabolism.

(2) How does GLP-2 act in brainstem DMV neurons? Studies show that the plasticity at the
NTS–DMV synapse depends on cAMP-PKA pathway(15). It is unknown about Glp2r-activated
intracellular signaling pathways in the brainstem, yet the 4th icv GLP-2 suppresses food
intake(46) and GLP-2 directly excites liver-projecting DMV neurons (unpublished data). GLP-2
activates cAMP-PKA signaling pathway(16; 81; 128; 137). As GLP-2 regulates mRNA
expression of hypothalamic genes in a PKA-dependent manner(28), GLP-2 potentiates L-type
Ca\(^{2+}\) channel activity in hippocampal neurons (131). Brainstem Glp1r activation suppresses
food intake and body weight through PKA-mediated suppression of AMPK and activation of
MAPK(48; 49). We speculate that GLP-2R activation \(\rightarrow\) DMV neuronal excitation (via PKA-
dependent long-term potentiation) \(\rightarrow\) Ach release (as a rapid-acting neurotransmitter) on
postganglionic neurons \(\rightarrow\) glycemic control. Thus we favor a model that the DMV Glp2r
activation initiates the PKA-dependent Ca\(^{2+}\)-triggered depolarization (long-term potentiation),
enhancing the synaptic transmission of preganglionic neurons in the DMV \(\rightarrow\) postganglionic
neurons \(\rightarrow\) glycemic control. However, this model for Glp2r action in the brainstem waits for
experimental support.

(3) Do GLP-2-sensitive ARC POMC neurons signal to brainstem DVC via \(\alpha\)-MSH-Mc4r
pathway? ARC POMC neurons project to selected target sites in the hypothalamus and
brainstem(35; 63). A brainstem dorsal vagal complex (DVC, including NTS and DMV) integrates
hypothalamic inputs and vagal afferent inputs to regulate the autonomic control of feeding
behavior and glucose homeostasis (71; 87; 116; 139; 140). As suggested, ARC DVC-projecting POMC neurons may represent an anatomically and functionally distinct subgroup of POMC neurons (63; 140). As the major α-MSH receptor, Mc4r plays a pivotal role in energy homeostasis and is a key target for the treatment of obesity and diabetes (143). Mc4r is heavily expressed in the brainstem DVC (42; 64; 90). The brainstem DVC is a critical site for ARC POMC-derived α-MSH input to suppress food intake and HGP (10; 25; 36; 44; 79; 110), yet little is known how the NTS-DMV plasticity is modulated to fine-tune hepatic vagal output, influencing HGP (125). Interestingly, local infusion of GLP-2 (2.5 nmol into the 4th ventricle) suppresses food intake and gastric emptying; but these effects are abolished in Mc4r KO mice (46). Activation of Mc4r-positive cholinergic neurons in the DMV would enhance excitatory input to the liver, suppressing HPG (42; 110). Thus, it is conceivable that GLP-2 activates POMC neurons to release α-MSH, activating Mc4r-positive ChAT neurons in the DMV that enhances hepatic vagal output to suppress HGP.

(4) Does GLP-2 directly modulate vagal afferents to improve glycemic control? Vagal afferent neurons convey primarily information from the viscera to the CNS. Vagal afferents may be a key neural pathway that regulates food intake, gastrointestinal motility and glucose homeostasis (23; 42; 42; 130). Activation of N-methyl-D-aspartate receptors in the DVC is sufficient to trigger a brain-liver axis to lower HGP (71; 72; 105). Moreover, gastrointestinal hormones excite neurons in the rodent nodose ganglia by closure of K⁺ channels (40; 51). GLP-2 activates vagal afferent pathway where Glp2r is expressed (95). Interestingly, GLP-2 mediates the gut-brain communication via activating vagal afferents (94; 95). We speculate that Glp2r activation on vagal afferents may influence neural inputs to a) preproglucagonergic (PPG) neurons in the brainstem NTS → GLP-2 release → Glp2r activation in the hypothalamic
ARC/DMH and the brainstem DMV →→ autonomic outputs; and b) POMC neurons in the brainstem NTS → α-MSH release → MC4R activation in the hypothalamic ARC/DMH and the brainstem DMV →→ autonomic outputs. However, GLP-2-modulated neural circuitries (including distinct neurons on vagal afferents) have not been identified. Of note, chemical/surgical vagotomy does not selectively inactivate distinct types of neurons(12). It will be important to reassess the physiological relevance of targeted neurons in vagus using the cre-dependent pharmacogenetic modulation; and determine whether GLP-2-induced activation of vagal afferent neurons impacts glucose homeostasis and insulin sensitivity.

(5) Does augmented GLP-2 signaling contribute to improving metabolic outcomes after bariatric surgery? Bariatric surgery (particularly RYGB) is the most effective therapy for morbid obesity and diabetes(19; 26; 111), in which the gut-brain axis is highly involved in mediating beneficial effects on food intake and glycemic control. A recent report indicates that metabolic reprogramming of glucose to support intestinal hypertrophy renders the gut itself a major tissue for glucose disposal, contributing to improved metabolic outcomes after RYGB(113). In fact, RYGB increases blood GLP-2 (by 200%) at postprandial status(73) and enhances proliferation rates of the intestinal crypt epithelial cells, suggesting that GLP-2 (as a potent enterotrohic factor) may be an important signal to drive intestinal adaptation. However, it is still unclear whether GLP-2 is a key neuroendocrine signal via the gut-brain axis to enhance insulin sensitivity and glucose homeostasis following RYGB.

(6) Limitations are not addressed in current GLP-2 studies. There are two major limitations in current studies. One is how to define the physiological significance of Glp2r in site-specific populations of POMC neurons. The POMC-cre-mediated recombination of floxed Glp2r allele
will result in Glp2r deletion in POMC neurons including hypothalamus, brainstem, and pituitary
gland. Thus, Glp2r metabolic action in extra-hypothalamic POMC neurons should be
considered as well. However, the metabolic phenotype of POMC-Glp2r knockout mice may be
largely attributed to the Glp2r deletion in POMC neurons in the hypothalamic ARC(118). There
are three lines of evidence to support this speculation: a) The POMC cre mouse line had little
cre expression in the brainstem NTS when crossed with a cre reporter mouse line (such as
Rosa26-tdTomatto). b) The Glp2r has not been shown to express in the brainstem NTS or
pituitary gland. c) POMC neurons in the ARC play important roles in energy/glucose
homeostasis(101). To further dissect the physiological role of Glp2r in site-specific populations
of POMC neurons, one should manipulate Glp2r deficiency in distinct sites by nucleus-specific
injection of cre-dependent, lenti-viral mediated Glp2r shRNA (to knockdown Glp2r expression).

Another one is how to distinguish the physiological significance of GLP-2 from distinct sources
in the control of energy/glucose homeostasis. GLP-2 can be originated from endocrine L cells
in the gut and preproglucagon (PPG) neurons in the brainstem NTS while GLP-2R is
expressed in endocrine and neural cells. Thus, GLP-2 action can be mediated via endocrine
and neural manners. In a majority of pharmacologic studies, administration routes are used to
distinguish so-called peripheral vs central action of neuropeptides. This is not appropriate
specifically for neuropeptides that can be across the blood-brain barrier via passive
permeability or active transport. We speculate that peripheral GLP-2 can be transported into
the hypothalamic ARC where the blood-brain barrier is semipermeable or taken up through the
median eminence. In addition, it is very challenging to quantify metabolic effects of endocrine
or neural GLP-2 (i.e. originated from endocrine L cells or PPG neurons). As a fact, there are no
genetic approaches available to distinguish the physiological significance of GLP-2 from
distinct sources. Instead, we propose to define the functional relevance of CNS GLP-2 by deleting its receptor only in central distinct neurons. It is demonstrated using tissue-specific Glp2r KO mouse model that Glp2r activation in POMC neurons is required for iv and icv GLP-2 to promote glucose homeostasis and insulin sensitivity; and for GLP-2 to modulate the excitability of hypothalamic POMC neurons ex vivo(118), pointing to the functional relevance of Glp2 action on POMC neurons regardless of the source of GLP-2.

8 Innovative approaches need to define the CNS control of feeding and metabolism

One major interest is to determine the CNS control of glucose homeostasis and feeding behavior. Increasing evidence indicates that the CNS neural circuitry is selective for glucose homeostasis or feeding behavior, in which subgroups of distinct neurons are segregated. To remotely modulate activation/ inactivation of targeted neurons in conscious mice, one innovative pharmacogenetic approach, namely designer receptors exclusively activated by designer drugs (DREADDs), has been employed to elucidate the CNS neural circuits for feeding behavior(69). It has been very challenging to genetically dissect metabolic actions of targeted neurons on glycemic control or insulin sensitivity, which are confounded per se by developmental, behavioral or body-adiposity factors. The DREADDs can remotely, acutely, and reversibly control circuit activity in vivo and thus will provide a powerful approach to dissect metabolic phenotypes of targeted neurons in defined spatial and temporal manners. Another state-of-the-art approach is in vivo stable isotopic tracer methodologies (in conjunction with oral glucose tolerance test and hyperinsulinemic euglycemic clamp) to quantify dynamic glycemic kinetics at postprandial and postabsorptive states, and assess tissue-specific insulin sensitivity in conscious mice(46). Thus, it will be instrumental to combine in vivo stable isotopic tracer methodologies (including MS-based targeted metabolomics) with the remote control of targeted
neurons approaches (such as DREADDs) to address the CNS control of glucose metabolism independent of body adiposity.

Another major interest is to map distinct hypothalamus-brainstem neural circuits underlying glycemic control. Optogenetic activation/inactivation of targeted neural circuitry has been used to establish how the neural circuits are wired functionally in the brain in vivo. Specifically, optogenetics has been successfully employed to define the hypothalamic neural circuitry controlling feeding behavior in rodents(20). Future studies are warranted to integrate optogenetic, electrophysiological and metabolic approaches to define the functioning neural circuits responsible for glucose homeostasis.

**Perspectives and Significance**

GLP-1/2 are co-produced from enteroendocrine L cells in the gut and preproglucagonergic neurons in the brainstem. Dietary macronutrients and gut microbiota-derived metabolites (secondary bile acids and short-chain fatty acids) function as effective secretagogus of GLP-1/2. Because of Glp2r expression in nutrient-sensing endocrine cells and neurons, GLP-2 acts as a key endocrine and neural signal to regulate energy balance and glucose homeostasis. In the gastrointestinal tract, GLP-2 promotes intestinal epithelial homeostasis & function, enhances intestinal nutrient absorption & blood flow, and inhibits gastrointestinal motility, thus controlling energy intake at the first place. In the hypothalamus and brainstem, Glp2r plays physiological roles in regulating food intake and glucose homeostasis, which are mediated through fine-tuning vagal outputs via the proposed neural circuits (**Figure 2**). New evidence underscores the CNS GLP-2-mediated promotion of glycemic control and insulin sensitivity via the PI3K-dependent, central melanocortin system. Importantly, the GLP-2 gut–brain axis may
be physiologically modulated by nutrition, gut microbiota, and bariatric surgery. Using Glp2r floxed mouse line in combination of state-of-the-art approaches (such as stable isotopic tracer methodologies and the remote control of targeted neurons), one will further dissect the GLP-2 gut-brain axis and identify novel targets for the treatment of gastrointestinal diseases, obesity and diabetes.

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51. Heldsinger A, Grabauskas G, Song I and Owyang C. Synergistic Interaction between Leptin and Cholecystokinin in the Rat Nodose Ganglia Is Mediated by PI3K and STAT3 Signaling Pathways:


**Fig. 1 GLP-2R-induced intracellular signaling in hypothalamic POMC neurons**

In hypothalamic POMC neurons, GLP-2 induces Glp2r-p85α protein interaction, activating the PI3K-Akt-FoxO1 signaling pathway to derepress POMC transcription. GLP-2-activated PI3K signaling depolarizes one subgroup of POMC neurons (expressing leptin receptor) via activating putative TRPC channels; and hyperpolarizes another subgroup of POMC neurons (expressing insulin receptor) via activating K\textsubscript{ATP} channels. This paradox action on membrane excitability may be synchronized with leptin and insulin signaling in segregated POMC neurons. GLP-2-activated POMC neurons are integrated with central autonomic control by enhancing vagal outflows: decelerating gastric emptying or suppressing hepatic glucose production. This figure is modified from the article(118).

**Fig. 2 GLP-2R-activated neural circuits in the hypothalamus and brainstem**

GLP-2 (secreted from endocrine L cells and PPG neurons) may fine-tune autonomic outputs via the proposed neural circuits: In addition to sympathetic outflow, Glp2r activation in POMC neurons in the hypothalamus and brainstem $\rightarrow$ α-MSH release $\rightarrow$ MC4R activation in the brainstem DVC $\rightarrow$ vagal outflow. Moreover, Glp2r activation on vagal afferents may influence neural inputs to (1) PPG neurons in the brainstem NTS $\rightarrow$ GLP-2 release $\rightarrow$ Glp2r activation in the hypothalamic ARC/PVH and the brainstem DMV $\rightarrow$ vagal outflows; and (2) POMC neurons in the brainstem NTS $\rightarrow$ α-MSH release $\rightarrow$ MC4R activation in the hypothalamic ARC/VMH and the brainstem DMV $\rightarrow$ vagal outflows. However, GLP-2-modulated neural circuitries have not been fully defined. BBB, blood–brain barrier; GI, gastrointestinal; HGP, hepatic glucose production.