



Role of the glucagon-like-peptide-1 receptor in the control of energy balance

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ABSTRACT

The peripheral and central glucagon-like-peptide-1 (GLP-1) systems play an essential role in glycemic and energy balance regulation. Thus, pharmacological targeting of peripheral and/or central GLP-1 receptors (GLP-1R) may represent a potential long-term treatment option for both obesity and type-II diabetes mellitus (T2DM). Uncovering and understanding the neural pathways, physiological mechanisms, specific GLP-1R populations, and intracellular signaling cascades that mediate the food intake inhibitory and incretin effects produced by GLP-1R activation are vital to the development of these potential successful therapeutics. Particular focus will be given to the essential role of the nucleus tractus solitarius (NTS) in the caudal brainstem, as well as the gut-to-brain communication by vagal afferent fibers in mediating the physiological and behavioral responses following GLP-1R activation.

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1. Introduction

The incidence of obesity and type 2 diabetes mellitus (T2DM) has risen dramatically in the United States over the last two decades. Promising results from a range of clinical and animal studies focus attention on the merits of glucagon-like-peptide-1 (GLP-1) physiology and pharmacology in offering positive treatment outcomes for both diseases. GLP-1, a posttranslational product of proglucagon, is a neuropeptide that is endogenously released principally from two distinct sources: 1) “L” cells in the gastrointestinal (GI) tract following nutrient entry [1–3]; and 2) neurons of the nucleus tractus solitarius (NTS) in the caudal brainstem that project to GLP-1 receptors (GLP-1R) both locally and throughout the brain [4–6]. Exogenous stimulation of either peripheral or central GLP-1Rs engages a set of physiological responses that include reduced food intake [7–11], inhibition of gastric emptying [7,12], and increased glucose-stimulated insulin secretion [13–15]. However, our understanding of the neural pathways, physiological mechanisms, specific GLP-1R populations, and intracellular signaling cascades that mediate the food intake inhibitory and incretin effects produced by endogenous or exogenous GLP-1R activation is limited. One neuronal population within the CNS clearly stands out in mediating the intake inhibitory effects of both the peripheral and central GLP-1 systems: the NTS [6,16–20]. The goal of this review is to elucidate the neuroendocrine mechanisms mediating the intake inhibitory effects

that follow activation of both peripheral and central GLP-1Rs, with special emphasis focused on the role of the NTS and the gut-to-brain communication by vagal afferent fibers in GLP-1R-mediated physiological and behavioral responses.

2. Distribution and metabolism of endogenous GLP-1

A single gene encodes proglucagon (precursor to GLP-1) in mammals, with identical mRNA produced in the GI tract, pancreas, and the NTS of the caudal brainstem [4,21,22]. Differences in the proglucagon cleavage products in these tissues are due to tissue-specific posttranslational processing of proglucagon (for review see [22,23]). GLP-1(7–36) amide, a posttranslational product of proglucagon in the GI tract and the NTS, has a sequence that is 100% preserved in all mammals [24]. In the periphery, the majority of intestinal proglucagon-derived peptides, including GLP-1, are secreted from L cells in the distal small intestine (i.e., from proximal jejunum to distal ileum) [22,25]. Interestingly, recent identification of GLP-1-positive/ α -gustducin-positive cells expressing T1R3, a subunit of both the sweet and umami taste receptors, have also been identified in the oral cavity, as well as the small intestine [23,26,27]. Additionally, a very small percentage of GLP-1 is also secreted from the pancreas following ingestion of a meal; although the amino acid sequence differs from intestinally derived GLP-1 (1–36 amide vs. 7–36 amide for pancreas vs. intestine, respectively) [22,28]. In contrast to fairly distributed sources within the periphery for endogenous GLP-1-secreting cells, NTS neurons represent the only known central source of endogenous GLP-1, with GLP-1-immunoreactive NTS neurons projecting to numerous GLP-1R-expressing neurons within the brain nuclei

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relevant to energy balance, including the paraventricular nucleus (PVN) and dorsal medial nucleus of the hypothalamus (DMH), as well as caudal brainstem structures such as the parabrachial nucleus (PBN), area postrema (AP), and endemically within the NTS [4].

The metabolism and degradation of endogenous GLP-1 by the enzyme dipeptidyl-peptidase-4 (DPP-IV) is very rapid. It is estimated that <25% of GLP-1 secreted from the GI tract enters the portal vein in an intact, active form prior to reaching the liver. Further degradation occurs rapidly within the liver (40–50% of the remaining GLP-1 not initially degraded by DPP-IV). Thus, at most, ~10–15% of endogenously secreted GLP-1 reaches systemic circulation in the intact form. This rapid degradation continues via circulating DPP-IV, and limits the total half-life of GLP-1 to the order of 1–2 min (see for review [22]). Given that DPP-IV is expressed heavily in both the enterocyte brush border and the endothelial cells lining the capillaries of the lamina propria of the small intestine, it is logical to assume that like other gut-peptides [e.g. cholecystokinin (CCK)], intestinally derived GLP-1 may act in a paracrine-like fashion, preferentially activating GLP-1Rs located adjacent to the site of release, rather than more distal destinations (see [29] for review of paracrine action of gut-peptides). The limited penetration of biologically active peripheral GLP-1 into the central nervous system (CNS) [22,30] and rapid degradation rate of endogenous peripheral GLP-1 have led to two separate emerging pharmacological approaches taking advantage of the peripheral GLP-1 system to treat T2DM and obesity: DPP-IV inhibitors (e.g. Sitagliptin [Januvia]) and DPP-IV-resistant GLP-1R analogues (e.g. Exendin-4 [Byetta], Liraglutide [Victoza]). Both of these clinical strategies target the treatment of T2DM by improving glycemia control, and preliminary clinical evidence suggests that long-acting GLP-1R agonists (Liraglutide and Exendin-4) may also produce weight loss as a consequence of reduced food intake [31–33].

3. Peripheral GLP-1

The food intake inhibitory and incretin effects of peripheral GLP-1 are mediated, at least in part, by a neural pathway involving the vagus nerve [20,22]. Once secreted, GLP-1 activates GLP-1R-bearing vagal afferent neurons both in a paracrine-like fashion (local to the site of release in the small intestine) and in an endocrine-like fashion at GLP-1Rs in the portal vein, liver, and upper GI tract [20,34]. GLP-1R-mediated vagal afferent signals are processed by CNS neurons which then drive neuroendocrine, behavioral, and physiological responses that result in improved glycemic control and reduced feeding. One such neuroendocrine response to vagal afferent activation by GLP-1 is the subsequent vagal efferent neural transmission to the pancreatic β -cell, resulting in insulin secretion [22,35,36]. Support for the GLP-1R-mediated vago-vagal incretin response comes from findings showing that blockade of either afferent or efferent vagal transmission eliminates the GLP-1-induced augmented insulin response seen in glucose-treated rodents, indicating that both sensory and motor components of the vagus are essential to mediate GLP-1R-induced insulin secretion from the β -cell [36,37].

It remains unclear whether endocrine-like GLP-1 signaling in the hepatportal region [34,38] or paracrine-like GLP-1 signaling within the intestine represent the primary site of GLP-1R activation for energy balance and glycemic control. Moreover, it is unknown whether GLP-1Rs expressed on *central* axon terminals of vagal afferent fibers in the medulla also mediate GI vagal signals involved in glycemic and food intake control, as recent literature focuses solely on the role of GLP-1Rs expressed on *peripheral* terminals of vagal afferents in mediating GLP-1 effects. That is, vagal afferent fibers express GLP-1Rs and other gut-peptide receptors on the central terminals (axon) of the vagus, prior to the synapse with the NTS [39]. That activation of GLP-1Rs on these central terminals may modulate vagal transmissions to the NTS in a presynaptic fashion.

4. Intake inhibitory effects following peripheral GLP-1R activation

It is well established that systemic administration of exogenous GLP-1(7–36) or GLP-1R analogues (e.g. Liraglutide, Exanatide) reduces food intake in a dose-dependent manner in rodents, non-human primates and humans [11,16,40–42]. This intake inhibitory effect of peripheral pharmacological GLP-1R activation is sustained in obese humans even in the presence of T2DM [43,44], and has therefore prompted research evaluating the efficacy of DPP-IV-resistant GLP-1R ligands as candidates for obesity treatment. Indeed, systemic administration of either Liraglutide or Exanatide has been shown to reduce body weight in both animal models and humans (see for review [22,45]). Interestingly, it appears that pharmacological activation of systemic GLP-1Rs (2 \times daily administration of Byetta [Exanatide]) produces the greatest magnitude of weight loss in the most morbidly obese individuals compared to weight loss observed in overweight or lean humans [31]. While the significant reduction in body weight for these obese patients following ~2 years of Byetta treatment was approximately 11 lb, the slope of weight loss was sustained over the treatment period [31]. It is possible that chronic GLP-1R activation following systemic DPP-IV resistant GLP-1R treatments continues to produce intake inhibitory- and body weight suppressive-responses with a lack of “resistance” (e.g., diminution of response when ligand levels are chronically elevated). These findings highlight the need for further evaluation of the GLP-1 systems in treating not only T2DM but also obesity through the careful design of more effective pharmacological treatments that chronically target the peripheral (and perhaps central) GLP-1 system.

The strength of the peripheral GLP-1 system as a candidate for obesity treatment is highlighted by research employing GLP-1 antagonist treatment to assess the endogenous role of this system in intake control. Recent evidence by Williams et al. [30] suggest that endogenous peripherally secreted GLP-1 plays a physiological role in food intake suppression by showing that intraperitoneal (IP) administration of the GLP-1R antagonist, Exendin-9 (9–39), attenuates the intake suppressive effects that follow voluntary consumption and intragastric infusion of a liquid meal in rats. Further, a recent report by Reimer et al. [46] showed that mice treated with the DPP-IV inhibitor NVP DPP728 in the drinking water decreased weekly food intake when maintained on either standard rodent chow or high fat diet.

While the effects of peripheral GLP-1 agonist and antagonist treatment support a strong role for this system in the inhibitory controls of intake and body weight regulation, models of GLP-1R deficiency in mice have not been consistent with this interpretation. For instance, the GLP-1R knockout mouse is surprisingly lean, exhibits unaltered meal patterns, and does not develop obesity with aging or after several months of high fat diet maintenance [47,48]. These findings, which seem to be very different from the profile of responses observed in humans, non-human primates, and rats, have certainly raised many questions regarding the role of GLP-1R signaling in food intake and body weight regulation [11,16,40–42]. In fact, clear species differences have also been reported between mice and rats for GLP-1R-mediated control of visceral illness; in particular in LiCl-induced anorexia [49]. Likewise, species differences between the mouse and rat have also been reported with regard to the regulation of central GLP-1-immunoreactive neurons in the NTS by the adiposity hormone leptin [50]. It was reported that leptin induced phosphorylation of signal transducer and activator of transcription-3 (pSTAT3) in 100% of GLP-1 cells in the caudal brainstem of mice, whereas in rats a complete absence of pSTAT3 was observed in NTS GLP-1-positive neurons following leptin treatment [50]. Moreover, this same report showed that in mice, proglucagon mRNA was reduced by food deprivation, and this was prevented by leptin administration; whereas proglucagon mRNA was unaffected by either fasting or leptin treatment in rats [50]. The take home message here is two-fold: 1) caution should be taken when making generalizations between the mouse and rat regarding

the role of peripheral GLP-1 in energy regulation, and 2) the question of which species (rat or mouse) represents the appropriate model to understand the normal physiology and pathophysiology of human diseases is not straight forward, and will always depend on the physiological system under investigation.

The aforementioned report by Williams et al. [30] directly addressed the ongoing debate of whether administration of systemic GLP-1R ligands produce their intake inhibitory response through activation of peripheral or central GLP-1Rs. They reported that central blockade of GLP-1R attenuated only the intake suppression by central GLP-1 administration, whereas the intake suppression following peripheral (IP) GLP-1 administration was only attenuated following peripheral administration of the GLP-1R antagonist Exendin-(9–39) [30]. These findings support the notion that while the direction and profile of responses are similar for peripheral and central application of GLP-1R agonists, the two populations of receptors may in fact be considered the mediators of separate (peripheral vs. central) GLP-1 systems. Further support for this perspective comes from a wide variety of studies detailed below.

A number of findings support a role for vagal afferent fibers in mediating the intake suppressive and glycemic responses to exogenous systemic GLP-1R ligand administration. For instance, elimination of vagal afferent signaling via surgical or chemical deafferentation of the vagus attenuates GLP-1R-mediated suppression of food intake and gastric emptying, and inhibits GLP-1R-mediated increases in gastric acid secretion and glucose-induced insulin secretion (see [22] for review). CNS processing of GLP-1R-mediated vagal afferent activation has been shown to stimulate pancreatic-projecting vagal efferents that enhance insulin secretion [51]. Thus far, systemic GLP-1R-mediated control of glycemia has been attributed to either GLP-1R-expressing vagal afferent nerve terminals in the hepatic portal bed [14,34,38,52], or to direct activation of GLP-1R expressed on pancreatic β -cells (see for review [22]). This conclusion rests on the observation that intraportal infusion of a GLP-1R antagonist produced a hyperglycemic response following intragastric glucose infusion in anesthetized rodents, whereas, delivery of the same dose of the GLP-1R antagonist to the jugular vein did not alter the plasma glucose or insulin response following intragastric glucose infusion. However, a focused examination of the physiological role of GLP-1R signaling on GI-innervating vagal afferent fibers in glycemic control is needed. This notion is supported by findings from Rüttimann et al. [53] showing that the satiating effect of IP, but not intravenously (intrajugular or intrahepatoportal) administered GLP-1, requires vagal afferent signaling. Thus, an IP route of administration may represent a more physiological profile of action for the GLP-1R, taking advantage of the putative paracrine-like profile of endogenous GLP-1R activation in the small intestine. It is interesting to consider that general intravenous administration of GLP-1 (femoral vein infusion) can increase vagal afferent mass activity [54], and yet this electrophysiological response does not appear to be required for the suppression in meal size by IV infusion of GLP-1, while vagal activation is required for response production by IP GLP-1 [20]. The speculative conclusion of this discrepancy is a cautionary comment: that neuronal excitability of the vagus in an anesthetized preparation does not always equate to a CNS-dependent behavioral response (e.g. suppression of ongoing food intake).

The finding of Rüttimann et al. [53] suggests that GLP-1R expressed on vagal afferents innervating the hepatoportal region may not be required for mediating the intake suppressive effects of GLP-1. Instead, the finding that intraportal infusion of a GLP-1R antagonist produces a hyperglycemic response following intragastric glucose infusion [34] suggests that for vagal afferent GLP-1R populations in the periphery, the control of glycemic responses may be dissociable from the food intake inhibitory responses. Equally likely, however, is that endogenous GLP-1 signaling acting in a paracrine fashion on adjacent GLP-1Rs expressed on vagal afferents innervating the GI tract controls both intake and glycemia, while GLP-1Rs expressed on hepatoportal vagal afferents only control glycemic responses. Future analysis is certainly needed to

determine which populations of peripheral GLP-1Rs are required for the intake suppressive- and incretin-mediated effects by systemic GLP-1.

An additional interesting piece of evidence with regard to GLP-1's site-of-action comes from the finding that the meal size suppressive effects produced by jugular GLP-1R ligand administration do not require vagal afferent mediation [53]. This suggests that GLP-1R expressed on splenic fibers may be mediating this response, or that GLP-1 infusion in the jugular vein, at levels above what would normally be seen under endogenous circumstances [22], are producing their intake inhibitory response through direct activation of GLP-1Rs-expressed in the brain, likely at nuclei classified as or adjacent to circumventricular organs (CVO). The extremely short half-life (1–2 min) and minimum penetration through the blood brain barrier by GLP-1 makes direct action in the CNS negligible under endogenous circumstances [22]. Clearly the brain CVO, e.g. AP and subfornical organ (SFO), plays a role in responses generated by peripheral endocrine hormones acting in the CNS [55]. However, a very recent report shows that ablation of both the AP and the SFO does not attenuate the intake inhibitory effects that follow IP administration of the GLP-1R agonist, Exendin-4 [56]. Thus, splenic mediation seems to be the more likely mediator of the intake inhibitory response to GLP-1R ligands present in general circulation (i.e. jugular vein), particularly when considering recent findings that suggest a dissociation between the mechanism through which peripheral vs. central GLP-1R activation produces an intake inhibitory response.

It is generally well accepted that peripheral GLP-1 ligand administration (IP, IV, or subcutaneous) reduces food intake through a reduction in meal size [20,42], and has even been recently categorized as a satiation signal [30]. However, a recent preliminary report shows that hindbrain activation of brain GLP-1Rs reduces food intake by reducing meal number (thus increasing the inter-meal-interval), not through an alteration in meal size [57]. This finding further highlights the strength of the GLP-1 systems as potential candidates for obesity treatment, as future treatments designed to target both peripheral and central GLP-1 systems would potentially offer an avenue to decrease not only the size of the meals being consumed, but potentially the number of meals and/or snacks taken in a day.

5. Efficacy of gastric bypass: a role for GLP-1?

It is an unfortunate reality that to date, the only long-term effective treatment for morbidly obese patients involves the surgical rewiring of the GI tract. Even more unfortunate is the realization that these risky surgical procedures are not effective for everyone, with 20–30% of patients failing to reach the typical post-operative weight loss or begin to regain large amounts of weight within the first years [58–61]. And yet, despite this occurrence, the vast majority of obese individuals undergoing gastric bypass achieve drastic, life-changing reductions in their total adiposity and morbidity profile. Perhaps even more remarkable is that obese patients with T2DM who have undergone Roux-en-Y-Gastric Bypass exhibit an extremely rapid amelioration of their diabetes [59,62–64]. This drastic improvement in T2DM by gastric bypass, which seemingly occurs within days after the surgery, has promoted a wealth of research aimed at elucidating the mechanisms that surround this occurrence. Leading candidate ideas including malabsorption, a behavioral change by the patient regarding the composition of food ingested, as well as an overall reduction in food consumed, have proved to be negligible contributors to this phenomenon (see [65]). Instead, current thinking is now focused on understanding what impact gastric bypass has on the neuroendocrine controls that govern food intake and glycemic regulation – including both homeostatic and non-homeostatic peripheral and CNS systems [62,65,66]. Prevalent among this new emerging research is the idea that GI-derived incretin hormones, such as GLP-1, serve an integral role in regulating blood glucose values in post-operative gastric bypass patient [67]. Indeed, previous studies have shown that obese individuals who have undergone Roux-en-Y-Gastric Bypass show a rapid and

sustained increase in postprandial GLP-1 secretion that is greater in magnitude than that seen in either obese patients who have undergone gastric banding or obese controls with no surgical intervention [67]. This result raises a number of questions which are now being heavily investigated: 1) What are the physiological mechanisms that may account for such an altered postprandial GLP-1 response following Roux-en-Y GI surgery? 2) Could this dramatic increase in postprandial GLP-1 secretion contribute to the amelioration of T2DM and reduction in overall food intake in patients who have undergone Roux-en-Y-Gastric Bypass? 3) Finally, if GLP-1 and other GI-derived hormones that are also altered by gastric bypass (e.g. PYY) are the main contributing force behind the improvements seen in blood glucose/insulin profiles and potentially food intake inhibition, could pharmacological tools aimed at targeting the GLP-1 system circumvent the need for obese patients to undergo surgery?

One speculative reason for the rapid and sustained postprandial GLP-1 secretion observed following Roux-en-Y surgery could be that the main population of GLP-1 secreting L cells in the jejunum and ileum are being exposed to intractable nutrients sooner and in a much greater concentration following food ingestion. Support for this idea comes from the well established fact that the enterochromaffin cells of the small intestine, such as the L-class cells, are directly responsive to intraluminal nutrients and thus, secrete specific neuropeptides and neurotransmitters (see for review [29,68,69]). Of course, the exact mechanisms that account for this increase in postprandial GLP-1 secretion following Roux-en-Y-Gastric Bypass warrants further investigation, and the consequences of such an effect are just beginning to be examined in both humans and animal models [62,65,66,70–73].

6. CNS processing of the energy balance effects following peripheral and central GLP-1R activation

Stimulation of central GLP-1Rs results in many of the same responses (e.g. inhibition of food intake, increased insulin secretion) that are observed following peripheral GLP-1R ligand administration [7–9,14]. The effects of GLP-1R stimulation on feeding, gastric emptying and energetic responses involve behavioral, sympathetic and parasympathetic effector pathways that are downstream of CNS processing [74–78]. CNS structures in the ascending visceral afferent pathway, including include nuclei in the caudal brainstem (NTS; PBN), hypothalamus (lateral hypothalamus, LH; PVN), and basal forebrain (bed nucleus of stria terminalis, BNST; central nucleus of the amygdala) [79,80], may thereby play a role in mediating responses triggered by peripheral GLP-1R agonist treatment. Central GLP-1R ligand administration activates neurons (Fos-LI) in many of these same structures that show GLP-1-binding [5] and/or express GLP-1R mRNA [6]. Moreover, many of the aforementioned nuclei in the visceral afferent pathway project to other GLP-1R-expressing structures involved in energy balance control (AP, ventral tegmental area, arcuate nucleus, medial preoptic area) [see [5,6]]. Until recently, it was often asserted that hypothalamic/forebrain processing is critical for mediating the effects of peripheral GLP-1R stimulation, as well as for central agonist delivery [8–10,81–83]. This perspective was recently challenged using a complete supracollicular transection of the neuraxis (i.e. chronic supracollicular decerebrate (CD) rat [84]) to eliminate both the ascending forebrain projecting limb of the ascending visceral afferent pathway and the descending projections from the hypothalamus and basal forebrain to hindbrain, thereby blocking forebrain–caudal brainstem communication. This strategy was used to directly investigate the importance of hypothalamic/forebrain and caudal brainstem processing in the mediation of behavioral, sympathetic, and parasympathetic responses generated by peripheral GLP-1R agonist treatment and, in separate experiments, by hindbrain-delivered GLP-1R ligand, Exendin-4 [7]. The magnitude and duration of responses observed in CD and control rats were comparable. That is, peripheral administration of Exendin-4 sup-

pressed food intake and reduced gastric emptying and core body temperature to a similar magnitude in both control and CD rats. Hindbrain ventricular delivery of Exendin-4 also produced similar intake, emptying, and thermal responses in CD and neurologically intact controls. These data provide clear support for the hypothesis that central processing restricted to the caudal brainstem is sufficient for the generation of energy balance responses triggered by exogenous peripheral GLP-1R stimulation and also by central GLP-1R ligand delivered to the caudal brainstem. In addition, these data argue for the need for further research aimed at elucidating the physiological mechanism(s), mediating neuropeptides, and signaling pathways by which caudal brainstem processing is contributing to these coordinated behavioral and physiological effects.

Despite abundant research showing that feeding is suppressed by exogenous activation of central GLP-1R expressing nuclei, and the recent finding that caudal brainstem processing is sufficient to mediate the intake suppressive effects of hindbrain-directed GLP-1R ligands, whether or not endogenous hindbrain GLP-1R activity is required for the normal control of energy balance and glycemia is a question that is just beginning to be addressed [17]. To understand the physiological role of endogenous CNS GLP-1 in food intake and body weight regulation, previous research administered the GLP-1R antagonist Exendin-(9–39) into the forebrain ventricles (i.e. 3rd or lateral icv) [85,86]. While results indicate that this treatment can increase food intake, the paradigms employed were not physiological. For instance, in one study satiated rats were injected with a very large Exendin-(9–39) dose (100 µg icv) [85], while in another, the antagonist was applied over multiple days at the 100 µg (icv) dose in rats that have restricted access to food (6 h/day) [86]. Furthermore, forebrain ventricular delivery of large doses of Exendin-(9–39) leaves unresolved the nuclei-site-of-action mediating the effects of the antagonist, as cerebrospinal fluid flows in a caudal direction, allowing forebrain icv injectates to access both forebrain and caudal brainstem nuclei. Thus, previous studies have not sought to identify the contribution of endogenous hindbrain GLP-1R activation to intake control.

Given the above mentioned unknowns and inconsistencies our laboratory recently reexamined the relevance of endogenous CNS GLP-1 signaling to the control of food intake by focusing our attention in the caudal brainstem [87]. The role of endogenous NTS GLP-1R activation to intake control was the focus of these recent studies given that: 1) NTS neurons are the endogenous source of CNS GLP-1; 2) caudal brainstem processing is sufficient to mediate suppression of intake by hindbrain GLP-1R activation [7]; 3) the NTS receives and integrates both vagal afferent satiation and blood born energy status signals and issues output commands essential to energy balance control [29,88–93]; and 4) gastric distention activates GLP-1 containing neurons in the NTS [94]. Therefore, we examined a liquid meal preload paradigm along with two distinct sources of physiological within-meal GI satiation signals, gastric distension and intraduodenal nutrients for their individual intake suppressive contributions via hindbrain GLP-1R activation. We reported [87] that the intake suppressive effects that follow ingestion of a preload (9 ml of a nutritionally complete liquid meal, Ensure) require endogenous hindbrain GLP-1R activity, as both 4th icv and direct NTS delivery of Exendin-(9–39) increased food intake following ingestion of this preload. This result supports the interpretation that GLP-1R expressing NTS neurons contribute to the intake effect observed with 4th icv Exendin-(9–39) administration. Ingestion of the Ensure preload gives rise to an array of satiation signals from the GI tract, including stomach distension and intraduodenal nutrient contact, each of which excite vagal afferents projecting to NTS neurons (for review see [20]). Therefore, our laboratory subsequently tested whether endogenous hindbrain GLP-1R activity mediates suppression of intake from gastric distension or intractable nutrient infusion [87]. Blockade of hindbrain GLP-1R attenuated the suppression of intake by gastric distension but did not affect the intake suppressive effect of intraduodenal nutrient infusion. Taken together, these findings indicate that

endogenous NTS GLP-1R activity contributes to the endogenous control of food intake by mediating the satiating effects of gastric distension.

The abovementioned findings raise a number of critical unanswered questions. The first is to determine whether NTS GLP-1Rs that mediate suppression of intake by gastric distension are pre- or post-synaptic to the vagal afferent signaling from distension of the stomach. Vagal afferent fibers transport GLP-1R and other gut-peptide receptors to central (axon) terminals that synapse on NTS neurons [39]. Thus, it is equally possible that hindbrain GLP-1R mediation of gastric distension-induced vagal afferent signaling may be mediated by pre-synaptic GLP-1Rs expressed on central terminals of vagal afferents or may be mediated by post-synaptic activation of NTS GLP-1R-expressing neurons. To date, we are not aware of any experiments directly examining the role of GLP-1R on central vagal afferent terminals in mediating GI afferent signaling.

7. Intracellular signaling pathway mediating suppression of intake by hindbrain GLP-1R activation

The intake suppressive effects following hindbrain GLP-1R activation undoubtedly involve endemic intracellular signaling pathways that alter intracellular Ca^{2+} -influx and/or longer-term alterations in gene transcription and protein synthesis that integrate various anorectic signals in food intake control. In pancreatic β -cells, GLP-1R activation leads to stimulation of adenylate cyclase, an increase in cAMP, and the subsequent activation of protein kinase A (PKA) [95,96]. In neuronal cells, increased intracellular cAMP can increase phosphorylation of the p44/42 mitogen-activated protein kinase [p44/42 MAPK; also known as: extracellular signal-regulated kinase (ERK)-1/2] [97,98], presumably through a PKA-dependent pathway [83]. In fact, in pancreatic β -cells, GLP-1 stimulates p44/42 MAPK phosphorylation through a mitogen-activated protein kinase kinase (MEK)-dependent, but Raf/Ras-independent pathway that requires PKA activation, an influx of extracellular Ca^{2+} , and CAM kinase II activation [96]. Direct *in vivo* and *in vitro* evaluation of these plausible intracellular mechanisms in mediating the intake suppressive effects of hindbrain GLP-1R activation however, is still required.

In addition to the hypothesis that hindbrain GLP-1R activation leads to an increase in p44/42 MAPK phosphorylation via a PKA-dependent pathway, PKA activation is also known to inhibit calmodulin-dependent protein kinase kinase (CAMKK) [99]. CAMKK, together with LKB1, are considered the principal upstream kinases in mammalian tissue for the fuel sensing enzyme, AMPK by phosphorylation of the AMPK α catalytic subunit at Thr172 (for review see [100]). Thus, inhibition of CAMKK activity leads to decreased AMPK activation [99]. AMPK has recently been implicated in CNS control of energy balance [100–102], and its activity is increased by the sequelae of food deprivation [100,101,103] and by the energy reducing effects of insulin hypoglycemia or 2-DG cytogluopenia [104–106]. Additionally, Seo et al. [102] showed that an increase in AMPK mRNA in the hypothalamus following food deprivation was attenuated by GLP-1R activity. Recently the role of AMPK activity in NTS neurons to energy balance control has also been evaluated [107]. Similar to previous reports evaluating AMPK activity in various hypothalamic nuclei [100,101], it was found that food deprivation increases AMPK activity in NTS-enriched lysates. Also, as previously observed in the hypothalamus [100,101], NTS AMPK activity is inhibited by treatment with the adiposity hormone leptin, and by refeeding following a period of food deprivation [107]. Finally, the intake-reducing effects of hindbrain leptin delivery are mediated by AMPK signaling, as pharmacological-induced increases in hindbrain AMPK activity by 4th icv administration of AICAR, an AMP-mimicking promoter of AMPK activity, reversed the suppression of food intake by hindbrain leptin delivery. Together, these data support the notion that reduced AMPK activity in the hindbrain may mediate the suppression of intake that follows GLP-1R activation.

The mammalian target of rapamycin (mTOR) is one of the downstream targets of AMPK (for review see [100]) and is implicated in food

intake regulation [108]. Activation of AMPK results in suppression of mTOR signaling, thereby suppressing anabolic processes such as protein synthesis and cAMP response element-binding protein (CREB)-mediated nuclear transcription [109] and simultaneously promoting ATP-producing catabolic processes. We hypothesize that hindbrain GLP-1R activation increases PKA activity, which inhibits CAMKK, leading to reduced AMPK activity, thereby promoting mTOR signaling. This intracellular cascade together with a direct, PKA-mediated pathway, would increase CREB-mediated nuclear transcription and protein synthesis [109]. Simultaneously, if our working model is correct (Fig. 1), PKA-mediated activation of p44/42 MAPK signaling also promotes CREB-mediated transcriptional effects [110]. This would indicate that caudal brainstem GLP-1R activation (presumably localized to the NTS), engaging CREB-mediated transcriptional effects, could potentially position NTS GLP-1R expressing neurons to integrate other various anorectic signals (e.g. GI vagal satiation signals and circulating energy status signals, such as leptin) into a coordinated longer-term control of meal taking. Collectively, our working model (Fig. 1) is that gastric distension-generated vagal afferent signaling activates hindbrain GLP-1R leading to a suppression in food intake. The intracellular signaling pathways mediating this intake suppression occur through a coordinated PKA-mediated suppression of AMPK activity and activation of p44/42 MAPK/MEK signaling by promoting Ca^{2+} -dependent depolarization of the GLP-1R expressing neurons and longer-term CREB-mediated transcriptional effects, thus integrating various anorectic signals involved in meal-to-meal food intake control. This working hypothesis of the intracellular signaling pathways mediating hindbrain GLP-1R activation requires further *in vitro* and *in vivo* analysis using a range of behavioral and molecular techniques.

8. Neuroendocrine interactions with peripheral and central GLP-1 in control of food intake

Consistent with the developing perspective that the neuroendocrine controls of food intake (and body weight regulation more broadly) involve redundant and overlapping interactions between various anorectic systems and regions of the peripheral and central nervous system [29,111–118], both peripheral and central GLP-1-mediated physiological and behavioral responses have been shown to involve interactions with various other anorectic systems. For example, Talsania et al. [119] have shown that the intake inhibitory effects following peripheral GLP-1R activation by Exendin-4 are synergistically enhanced by co-administration of peptide YY(3–36NH₂) (PYY(3–36)). Interestingly, PYY(3–36) is co-secreted from L cells of the small intestine with GLP-1(7–36) [22]. In a separate report [120], combined administration of low doses of PYY(3–36) and GLP-1(7–36) produced an additive intake suppressive effect in both humans and mice, as well as a significant increase in Fos-LI within the arcuate nucleus of the hypothalamus, whereas Fos-LI was absent following administration of either peptide alone in the rodent models examined. Although the report does not identify the neuronal pathways accounting for the enhanced neuronal activation in the arcuate by PYY(3–36) and GLP-1(7–36), the effects likely involve the visceral vagal afferent pathway [10] as discussed previously in this report. However, despite the enhanced suppressive effects on food intake [119,120] and an indication of common homeostatic nuclei activated [120], PYY(3–36) and GLP-1 appear to mediate intake via independent mechanisms, with the intake inhibition by Exendin-4 being mediated by sensory afferent GLP-1R expressing neurons, whereas the intake suppression for PYY(3–36) is mediated by a Y2-receptor pathway [119]. Further work is certainly required to determine the mechanisms accounting for the enhanced intake suppressive effects produced by combined GLP-1(7–36) and PYY(3–36) administration.

Finally, a separate interaction has been reported for both central and peripheral GLP-1 systems with the adiposity hormone, leptin [18,121–123]. Systemic administration of leptin increased hypothalamic GLP-1 peptide content and it has been proposed that central GLP-1 signaling in

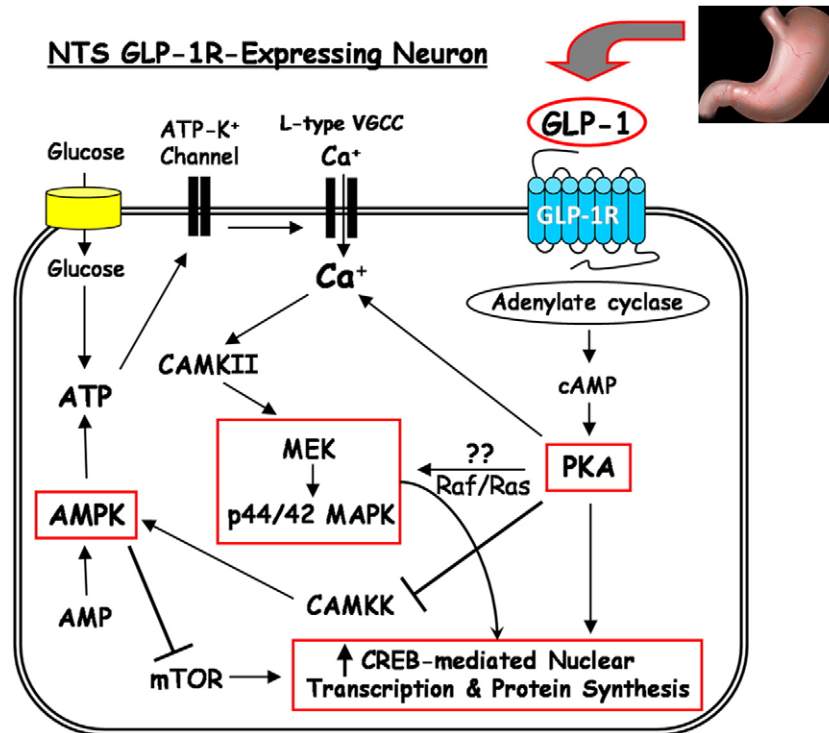


Fig. 1. Proposed intracellular signaling pathways in nucleus tractus solitarius (NTS) glucagon-like-peptide-1 receptor (GLP-1R)-expressing neurons mediating suppression of intake by GLP-1R activation. Gastric vagal afferent signaling increases endogenous NTS-derived GLP-1 that acts on endemic NTS GLP-1R-expressing neurons to engage a cyclic AMP (cAMP)-dependent increase in protein kinase A (PKA) activity. An increase in PKA activity drives simultaneous increases in the phosphorylation of p44/42 mitogen-activated protein kinase (MAPK) and decreases in adenosine monophosphate protein kinase (AMPK) activity. An increase in PKA and p44/42 MAPK activity together with a decrease in AMPK activity drives an increase in cAMP response element-binding (CREB)-mediated nuclear transcription and protein synthesis, thus allowing the NTS GLP-1R-expressing neurons to potentially integrate various anorectic signals involved in meal-to-meal food intake control. mTOR = mammalian target of rapamycin; CAMKK = calmodulin-dependent protein kinase kinase; CAMKII = calcium/calmodulin-dependent protein kinase II; VGCC = voltage gated calcium channel; MEK = mitogen-activated protein kinase kinase.

the CNS mediates, at least in part, the anorectic response to leptin [122]. It was recently reported [18] that a combination of an IP subthreshold dose of Exendin-4 produced an intake inhibitory response when combined with either an IP or 3rd ICV subthreshold dose of leptin. However, the CNS site of this interaction between leptin and GLP-1 signaling is not yet clear. Leptin has been shown to reduce food intake by potentiating the intake inhibitory effects of GI-derived satiation signals that require NTS processing [111,112], such as CCK [124–127], gastric distension [90] and systemic GLP-1R activation [18]. Consistent with this perspective is the fact that neurons of the NTS are the first CNS site that receive and process GI-derived vagal afferent satiation signals, and that these neurons also co-express and respond to activation of leptin receptors [90,127]. Furthermore, NTS neurons are the only CNS nuclei known to synthesize endogenous GLP-1 within the brain, and NTS neurons themselves express the GLP-1R. Collectively these findings indicate that the NTS is a plausible site mediating the anorectic interaction between leptin and GLP-1 receptor signaling. However, this postulation has not yet been validated. In addition it is unknown whether the processing and integration of leptin and GLP-1 anorectic signaling within the NTS is occurring in one population of NTS neurons or requires activation of multiple phenotypes of NTS neurons and secondary integration.

9. Conclusions

The energy balance and glycemic responses generated by activation of peripheral and/or central GLP-1R have great potential for the treatment of obesity and T2DM. The work summarized in this review has highlighted the fact that the GLP-1 sites of action for these GLP-1R mediated responses are widely distributed throughout the body. For the peripheral GLP-1 system, evidence suggests a potential role for GLP-1 acting in: 1) a paracrine-like fashion on GLP-1R expressed on peripheral terminals of vagal afferent fibers that innervate the GI tract, adjacent to

the site of GLP-1 release from the intestinal “L” cells, and 2) an endocrine-like fashion on GLP-1R expressed within the hepatoportal region. Furthermore, accumulating evidence supports the notion that peripheral endogenous GLP-1, as well as select exogenous GLP-1R analogues (e.g. Exenatide) activate GLP-1R within the periphery on vagal and splenic fibers which subsequently engage CNS processing, but do not elicit their behavioral and physiological responses by acting directly within the brain. Yet, similar, and in some cases distinct energy balance and glycemic responses are certainly mediated by central GLP-1R activation, specifically within the caudal brainstem. Moreover, these CNS caudal brainstem GLP-1-mediated responses are physiologically required for the normal control of food intake. Thus, this review has accentuated the critical role of the NTS in mediating the intake-reducing effects that follow activation of both peripheral and central GLP-1Rs. Future research examining the NTS-specific processing of central and peripheral GLP-1 systems may prove useful in the development of more effective pharmacotherapies aimed at treating obesity and T2DM.

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