Regulation of Energy and Glucose Homeostasis by the Nucleus of the Solitary Tract and the Area Postrema

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The central nervous system regulates feeding, weight and glucose homeostasis in rodents and humans, but the site-specific mechanisms remain unclear. The dorsal vagal complex in the brainstem that contains the nucleus of the solitary tract (NTS) and area postrema (AP) emerges as a regulatory center that impacts energy and glucose balance by monitoring hormonal and nutrient changes. However, the specific mechanistic metabolic roles of the NTS and AP remain elusive. This mini-review highlights methods to study their distinct roles and recent findings on their metabolic differences and similarities of growth differentiation factor 15 (GDF15) action and glucose sensing in the NTS and AP. In summary, future research aims to characterize hormonal and glucose sensing mechanisms in the AP and/or NTS carries potential to unveil novel targets that lower weight and glucose levels in obesity and diabetes.

Keywords: Area postrema; Solitary nucleus; GDF15; Glucose; Feeding; Energy metabolism; Glucose metabolism; Stereotaxic surgery

INTRODUCTION

The central nervous system is critical in maintaining metabolic homeostasis by integrating immediate and long-term nutrition-dependent signals related to energy intake, usage, storage, and glucose metabolism [1-3]. Specifically, the hypothalamus has been established to detect changes in circulating hormones such as insulin [4-7], leptin [8,9], glucagon [10,11], and glucagon-like peptide-1 (GLP1) [12,13], as well as nutrients, including glucose [14-16], fatty acids [4,17], and amino acids [18,19] to regulate feeding, weight, and glucose homeostasis.

Recently, an extra-hypothalamic region within the brainstem termed the dorsal vagal complex (DVC) has emerged as a significant regulator of energy and glucose metabolism [20-22]. The DVC consists of the nucleus of the solitary tract (NTS), the area postrema (AP), and the dorsal motor nucleus of the vagus. Upon refeeding, neurons in the DVC are activated [23], and the DVC neurocircuitry integrates hypothalamic and gastrointestinal vagal afferent circuits to control feeding and glucose homeostasis [22,24-29]. Direct sensing of the DVC may regulate metabolic homeostasis as well. For example, intranasal administration of leptin into rodents reaches the DVC and hypothalamus [30], while in humans, intranasal leptin or insulin administration reduces body weight [31,32] and glucose production...
GDF15 is a hormone from the transforming growth factor-β superfamily [44]. GDF15 is expressed in the kidney, liver, small intestine, adipose tissue, and muscle with the kidney and liver having the highest expression [45-47]. Injection of GDF15 lowers feeding and weight in high-fat fed rodents [48-51], while increasing energy expenditure [52,53], glucose tolerance [48,49], and insulin sensitivity [48,54].

GDF15 receptor, GDNF family receptor α-like (Gfral), is mostly expressed in the AP and NTS of the hindbrain [48-54]. Infusion of GDF15 activates Gfral-expressing cells within the AP and NTS [50], while AP and NTS lesions block the anorectic effect of peripheral GDF15 treatment [55,56]. Direct GDF15 administration into the lateral ventricle lowers feeding and weight in rodents as well [51,55]. In parallel, GDF15 improves glucose tolerance and insulin sensitivity, independent of changes in food intake and weight [54,57], and these effects were negated in Gfral knockout mice [54]. Although these collective findings suggest that the NTS and/or AP are the likely sites of GDF15 action, the direct impact of GDF15 on the AP versus the NTS remains elusive.

**NTS or AP site specificity**

A 26-gauge bilateral guide cannula or a 26-gauge single guide cannula can be inserted into the NTS or AP of rats, respectively, using stereotoxic surgeries to clarify their role in GDF15 metabolic regulation. The NTS is located within the DVC at the following coordinates in approximately 300 g male Sprague-Dawley rats: 0.0 mm on the occipital crest, ±0.4 mm lateral to the midline and 7.9 mm below the skull surface (Fig. 1A) [38]. Conversely, the AP of rats can be targeted via the following coordinates: 0.3 mm posterior to the occipital crest, 0.0 mm lateral to the midline, and 7.9 mm below the cranial surface (Fig. 1A) [58]. Of note, the NTS of 9- to 11-week-old mice can be stereotaxically targeted with bilateral steel guide cannulae positioned 2.0 mm above the NTS using a cannula holding bar in a 10-degree rostrocaudal angle at the following coordinates relative to the occipital suture: 0.5 mm anterior, 3 mm ventral, and ±0.4 mm lateral to the midline [20,59,60]. However, to the best of our knowledge, no study has implanted a chronic cannula into the AP of mice. For viral infections, the obex in mice, a landmark of the DVC may be used to target the NTS and AP. Viral infusions using the following coordinates relative to the obex: 0.2 mm posterior, ±0.2 mm lateral, and 0.2 mm ventral have been used to inject 100 nL of virus at a rate of 5 to 30 nL/min into the NTS of mice to virally and selectively infect the NTS [59,61,62]. Viral infusions directly into the obex of mice will target the AP using 35 nL of virus at a 2 nL/sec infusion rate [63-65].

Infusing GDF15 into the NTS has minimal effects on food intake and weight gain in rats after 23 to 24 hours of refeeding [45,66], while the same dose of GDF15 administered into the AP markedly decreases food intake and weight gain, highlighting a significant contrast in the response elicited by these two regions [45,66]. To validate cannula placement in the AP and NTS in rats, bromophenol blue injection was used [45,58]. Alternatively, radioative tracer [3-3H] glucose may be infused into the NTS or AP to measure radioactivity in anatomical locations of interest [38,67].

To study the biological role of Gfral expressing neurons, Gfral-Cre mice were crossed with a Cre-inducible hM3DqTg background to express activating designer receptors exclusively activated by designer drugs (DREADD) in Gfral neurons, which are activated by clozapine N-oxide (CNO) [66]. CNO activates Gfral expressing neurons which are mostly in the AP but not the NTS of mice [66], consistent with the fact that Gfral is mainly expressed in the AP and not the NTS of rats [45]. More importantly, CNO activates Gfral expressing neurons to lower feeding and weight [66], strongly suggesting that AP Gfral expressing neurons mediate GDF15 action to lower feeding and weight. GDF15’s role in the central regulation of weight holds potential as a target for anti-obesity treatments but is complicated by its associated side effects, such as nausea and/or emesis [46,68,69].

Chemogenetic activation of Gfral-expressing neurons (mainly expressed in the AP), activates calcitonin gene related peptide
Fig. 1. Surgical targeting and metabolic impacts of the area postrema (AP) and nucleus of the solitary tract (NTS). (A) Schematic of rat skull and brain anatomy to provide reference for NTS and AP stereotaxic surgery (top). Images show coronal sections of the rat brain with the AP and NTS highlighted, with corresponding surgical coordinates (bottom). (B) Schematic detailing the specific metabolic effects of growth differentiation factor 15 (GDF15) and glucose sensing in the AP and NTS. In the AP, GDF15’s feeding and weight-lowering effects partly stem from the GDNF family receptor α-like (Gfral)-calcitonin gene-related peptide (CGRP) pathway. These effects may not be dependent on glucagon-like peptide-1 receptor (Glp1r) activation, despite the co-expression of Glp1r with Gfral in AP cells. GDF15-Gfral axis in the AP enhances glucose tolerance and insulin sensitivity and inhibits hepatic glucose production as well. Lastly, Gfral-expressing cells in the AP and leptin receptor (Lepr)-expressing cells in the NTS synergistically reduce feeding and weight. In the NTS, Glp1r and Lepr activation lower feeding and weight. With respect to glucose sensing, both the NTS and AP share a glucose transporter 1 (GLUT1) and pyruvate kinase-dependent glucose sensing mechanism that regulates peripheral glucose metabolism. Images adapted from BioRender. PBN, parabrahchial nucleus; PDH, pyruvate dehydrogenase; DCA, dichloroacetate; HFD, high-fat diet.
(CGRP) neurons to induce nausea [66], while CGRP neurons in the parabrachial nucleus are sufficient to cause visceral malaise [70]. Conversely, silencing CGRP parabrachial neurons by injecting Cre-dependent AAV with doubled floxed tetanus toxin:green fluorescent protein (GFP) into CalcaCre:GFP mice negates GDF15-induced nausea [66]. Together with the fact that activation of Gfral expressing neurons selectively in the AP induces nausea [63], these findings collectively suggest that GDF15-induced weight loss is linked to nausea, evidenced by increased kaolin consumption induced by GDF15 [68,69,71].

Thus, the appetite-suppressing effects of GDF15 through AP Gfral-expressing cells may involve triggering malaise via an AP-Gfral-CGRP pathway (Fig. 1B).

A subset of cells in the AP, but not the NTS, co-express Gfral with the Glp1r [63,72], which also regulates weight. Interestingly, the effectiveness of GDF15 in reducing food intake remains intact in Glp1r-null mice, while Glp1r agonist iraglutide remains effective in GDF15- and Gfral-null mice [73]. It would appear Glp1r agonism and GDF15 inhibit food intake and body weight through different mechanisms [74]. Of note, combining GDF15 with Glp1r agonists in normal rodents further reduces food intake and weight loss without increasing malaise in rats [74]. Despite the co-expression of Glp1r with Gfral in AP neurons, most Glp1r-expressing cells do not co-express Gfral [63,72], indicating that GDF15 and Glp1r agonists do not target identical neuronal populations in the AP. Together, this suggests that GDF15 and Glp1r act through distinct anorectic neural circuits (Fig. 1B).

GDF15 and leptin interact as leptin-deficient ob/ob mice and wild-type mice treated with leptin receptor antagonists are less responsive to GDF15 leading to diminished weight loss and adiposity reduction [75]. Co-administration of subcutaneous leptin and GDF15 amplified weight and adiposity reduction by reducing food intake compared to the GDF15 treatment alone [75]. Although leptin receptor and Gfral are not co-expressed on the same neurons, synaptic connections between synaptophysin-leptin receptor and Gfral-expressing neurons are identified between the NTS and AP using immunofluorescence (Fig. 1B) [75]. The interconnection of GDF15 and leptin action in the NTS and/or AP warrants future investigation.

Glucose regulation

GDF15 infusion into the AP improves glucose tolerance and insulin sensitivity, leading to decreased hepatic glucose production [76]. These effects occur independently of any changes in food intake and weight, signifying a broader metabolic impact of GDF15 [76]. Subcutaneous administration of GDF15 in rats fed high-fat diets also exhibit decreased hepatic glucose production and increased insulin sensitivity in the liver, brown and white adipose tissue [54]. These changes occurred through increased β-adrenergic receptor signaling mediated by Gfral, and were independent of weight loss (Fig. 1B) [54]. In Gfral global knockouts and pharmacological inhibition of β-adrenergic receptors, the GDF15 insulin-sensitizing effect was nullified [54].

Knocking down Gfral, specifically in the AP, by infusing 2 μL of lentivirus harboring shRNA of Gfral over 15 minutes [45], disrupted glucose tolerance in the presence of GDF15 administration [76]. To verify gene knockdown specificity, reverse transcription polymerase chain reaction (qRT-PCR) assays were done to assess transcriptional levels of the targeted gene in concatenated and adjacent tissues [58,76]. If the infusion rate is too high, such as 3 μL of virus infused over 30 seconds [38], leakage into adjacent structures will result in non-specific knockdown of genes in the rest of the DVC when targeting only the AP [45]. Disrupted glucose tolerance in AP Gfral knockdown rats suggests that Gfral in the AP is essential for mediating the glucoregulatory and metabolic effects of GDF15.

Metformin-induced glucose, feeding, and weight regulation via brainstem GDF15 signaling

Metformin lowers plasma glucose, feeding and weight in rodents and humans [77-81]. Metformin was first associated with elevated plasma GDF15 levels in an epidemiological study [82] and was later confirmed in a randomized control trial, establishing a link between metformin-induced weight loss and elevated GDF15 levels [83]. The metformin-induced rise in plasma GDF15 results in reduced food intake, weight gain, and increased glucose tolerance and insulin sensitivity in high-fat fed rodents through activation of Gfral [76,83,84]. Metformin increases glucose tolerance and plasma GDF15 levels in high-fat fed rats within 2 hours of upper small intestinal infusion, independent of GDF15’s role in insulin secretion and weight changes [76]. However, AP-specific knockdown of Gfral expression negates the glucoregulatory and food intake-lowering effects of upper small intestinal metformin infusion, indicating that the GDF15-Gfral signaling axis is necessary for metformin to increase glucose tolerance and weight loss in rats fed a high-fat diet [76]. Contrasting reports highlight that metformin’s glucoregulatory effects may not always depend on GDF15. Coll et al. [83] found no significant glucose tolerance difference between GDF15-null and wild-type mice after a single metformin dose. However, Coll et al. [83] used a high dosage of metformin (300 mg/kg), potentially activating GDF15-independent pathways to promote glucose toler-
ance. This suggests a need for further research to elucidate the GDF15-Gfral signaling-dependent and independent pathways mediating metformin’s glucoregulatory actions. Collectively, these studies reveal that the weight-reducing and possible glucose-regulating effects of metformin are mediated through the GDF15-Gfral signaling pathway in the AP.

**BRAIN GLUCOSE SENSING**

Glucose sensing in the brain maintains whole-body glucose homeostasis [85], but site-specific mechanisms remain unclear. Glucose uptake in the brain [86,87] is facilitated by the expression of glucose transporter genes across multiple cell types: endothelial cells forming the blood-brain barrier (glucose transporter 1 [GLUT1]), astrocytes (GLUT1), and neurons (GLUT2/3) [88]. The extent and requirement for neuronal glucose metabolism remains unclear. The reliance on glucose as the sole energy source seems insufficient for maintaining energy stability, especially under high metabolic demand or limited oxygen availability. Supporting this idea, inhibiting glucose uptake in hippocampal neurons by deleting GLUT3 increases pyruvate-to-lactate conversion as a potential compensatory response [86]. The brain utilizes alternative fuels, such as lactate, produced through aerobic glycolysis by astrocytes and is referred to as the astrocyte-neuron lactate shuttle [89,90]. In response to excitatory neuronal activity, the uptake of glutamate by astrocytes via Na+-cotransport stimulates Na+-K+ ATPase activity, releasing lactate into the extracellular space. Neurons can uptake lactate through monocarboxylate transporters [91] and convert it to pyruvate by lactate dehydrogenase (LDH) isofrom B for fuel [92].

Lactate infusion mirrors the hypothalamic glucose-induced lowering effect in rats and mice on plasma glucose and insulin levels as well as hepatic glucose production [14]. Blocking lactate-to-pyruvate conversion through LDH chemical inhibitor, oxamate, or lentiviral-mediated knockdown of astrocytic LDH-A expression [93] impairs the glucose-lowering response of hypothalamic glucose/lactate [14,93]. These findings highlight the in vivo relevance of the astrocyte-neuron lactate shuttle hypothesis in hypothalamic regulation of glucose homeostasis. Not all areas of the brain preferentially utilize lactate; the cortex directly uptakes glucose via neurons during increased neuronal activity [87]. Nonetheless, impairments in glucose transporters are linked to disrupted central glucose sensing and metabolic disorders. In uncontrolled diabetic rats, sustained hyperglycemia impairs hypothalamic glucose sensing via a reduction in hypothalamic glial GLUT1 expression [94] while downstream lactate sensing remains intact [15]. Hypothalamic glucose, but not lactate sensing, fails to lower glucose production in high-fat fed rats as well [15,93], although the role of hypothalamic GLUT1 remains unclear.

To assess whether glucose sensing in the DVC regulates glucose homeostasis, rats underwent a pancreatic euglycemic clamp with glucose infused directly into the DVC [14]. Like hypothalamic glucose infusion [14], glucose infusion into the DVC of chow rats required more exogenous glucose to prevent hypoglycemia [67]. This glucose-lowering effect was due to a decrease in hepatic glucose production rather than an increase in glucose uptake [67].

To examine whether the astrocyte-neuronal lactate shuttle mediates glucose sensing in the DVC, like in the hypothalamus [14], lactate was first infused in the DVC to see whether it recapitulates the glucoregulatory effect of glucose. In contrast to the hypothalamus, DVC lactate infusion fails to alter the glucose infusion rate and glucose production during the pancreatic clamps [67]. Additionally, chemical inhibition of LDH in the DVC fails to disrupt the glucoregulatory effects of DVC glucose infusion, suggesting that lactate metabolism is neither sufficient and necessary for glucose sensing in the DVC to regulate hepatic glucose production. These findings highlight the DVC detects changes in glucose to regulate glucose homeostasis, but the glucose sensing mechanisms engaged in the DVC is different than the hypothalamus.

**NTS and AP glucose sensing mechanism**

Given that hypoglycemia induces cFOS (a proto-oncogene) expression in NTS and AP neurons [95,96], and AP lesions attenuate hyperphagic response to glucoprivation [97], both the NTS and/or AP within the DVC could sense glucose to regulate peripheral glucose metabolism. To investigate the specific contributions and mechanisms of glucose sensing in the NTS and AP, site-specific brain infusions were performed as described above for GDF15 studies. Bromophenol blue dye was infused through NTS or AP cannulas to confirm accurate delivery.

Direct glucose infusion into the NTS or AP (like DVC) lowers hepatic glucose production in chow-fed rats during clamps [58]. To investigate the role of GLUT1 (i.e., glucose uptake) as well as pyruvate kinase (i.e., pyruvate formation via glycolysis) within the NTS and/or AP for glucose sensing, site-specific, lentiviral-mediated knockdown of GLUT1 and pyruvate kinase was performed. This involved injecting 3 µL of lentivirus via bilateral cannulae over 10 minutes into the NTS or 2 µL of lentivirus over 15 minutes in the AP, respectively [58]. Knocking
down GLUT1 or pyruvate kinase expression in either the NTS or AP negated the glucose-regulating effects of glucose infusion into these regions, preventing the reduction in hepatic glucose production [58]. These findings indicate that glucose uptake and subsequent metabolism to pyruvate are necessary for glucose sensing in the NTS and AP to regulate hepatic glucose production. Importantly, high-fat feeding impairs NTS and AP glucose sensing in association with a reduction in GLUT1 expression, though pyruvate kinase levels remained unaffected [58]. Interestingly, administration of dichloroacetate, an agonist of pyruvate dehydrogenase (that converts pyruvate to acetyl-coenzyme A), into the NTS or AP mimicked the glucose-lowering effect of glucose sensing in chow-fed rats [58]. However, dichloroacetate in both NTS and AP failed to reduce glucose production in rats fed a high-fat diet, suggesting that high-fat feeding not only disrupt GLUT1-mediated glucose uptake but also downstream pyruvate metabolism in the NTS and AP that affected glucose sensing (Fig. 1B) [58]. Taken together, both the NTS and AP rely on a GLUT1 and pyruvate kinase-dependent glucose sensing mechanism to decrease glucose production, thereby highlighting the critical role of the NTS and AP in glucose sensing for whole-body glucose metabolic regulation.

CONCLUSIONS

Although the hypothalamus is an established center that senses changes in circulating hormones and nutrients to maintain whole-body metabolic homeostasis, the DVC containing the NTS and AP has received much attention recently that impacts energy and glucose homeostasis as well. Herein, we highlight the AP and NTS-targeted techniques used to characterize GDF15 action and glucose sensing in these specific regions. Both the NTS and AP are essential for glucose sensing, which is dependent on both GLUT1 and pyruvate kinase expression. Differences in function between the NTS and AP arise in the context of GDF15. GDF15 seems to specifically exert its anorectic and glucoregulatory effects through its receptor Gfrα1 in the AP. Future studies aim to advance mechanistic knowledge in understanding how hormones and nutrients activate respective signals in the NTS and/or AP to regulate weight and glucose levels could unveil therapeutic targets to lower weight and glucose levels in obesity and diabetes.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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NTS and AP Regulates Energy and Glucose Homeostasis


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