Review

MOLECULAR METABOLISM

Interaction of glucose sensing and leptin action in the brain

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ABSTRACT

Background: In response to energy abundant or deprived conditions, nutrients and hormones activate hypothalamic pathways to maintain energy and glucose homeostasis. The underlying CNS mechanisms, however, remain elusive in rodents and humans.

Scope of review: Here, we first discuss brain glucose sensing mechanisms in the presence of a rise or fall of plasma glucose levels, and highlight defects in hypothalamic glucose sensing disrupt *in vivo* glucose homeostasis in high-fat fed, obese, and/or diabetic conditions. Second, we discuss brain leptin signalling pathways that impact glucose homeostasis in glucose-deprived and excessed conditions, and propose that leptin enhances hypothalamic glucose sensing and restores glucose homeostasis in short-term high-fat fed and/or uncontrolled diabetic conditions.

Major conclusions: In conclusion, we believe basic studies that investigate the interaction of glucose sensing and leptin action in the brain will address the translational impact of hypothalamic glucose sensing in diabetes and obesity.

Keywords Hypothalamus; Brain; Glucose metabolism; Glucose sensing; Leptin action; Lipid sensing; Hepatic glucose production; Hypoglycemia

1. INTRODUCTION

Diabetes and obesity are major public health problems. Obesity-associated type 2 diabetes is characterized by elevated blood glucose levels. While pancreatic-derived hormones regulate systemic glucose metabolism through endocrine action in peripheral organs, the brain emerges as a crucial target. In this perspective, we highlight that the hypothalamus of the brain senses changes in nutrient and hormone levels, and triggers negative feedback responses for glucose homeostasis. We examine the underlying mechanisms of glucose sensing or leptin action in the hypothalamus that maintain glucose homeostasis in the presence of a rise or fall in plasma glucose levels. We argue that a high-fat feeding, obesity, and/or uncontrolled diabetes disrupt hypothalamic glucose sensing and dysregulate hepatic glucose production. We suggest that the biochemical-signaling framework that mediates leptin enhances hypothalamic glucose sensing and restores hepatic glucose production and glucose homeostatic regulation in short-term high-fat fed or uncontrolled diabetic conditions. Lastly, the translational relevance of hypothalamic glucose sensing and leptin action is discussed, and we recommend further basic studies that investigate the interaction of glucose sensing and leptin action in the brain to address the translational impact of hypothalamic glucose sensing in diabetes and obesity.

2. GLUCOSE SENSING IN THE BRAIN

The brain preferentially utilizes glucose as its energy source and all six isoforms of the facilitated glucose transporters are found in the brain [1]. Transport of glucose across the blood—brain barrier is mediated by endothelial glucose transporter-1 (GLUT1) [2], and cellular glucose uptake by astrocytes and neurons is primarily accomplished through GLUT1 and glucose transporter-3, respectively [3]. While it is traditionally believed that glucose enters neurons directly and undergoes glycolysis to form pyruvate and provide neuronal fuel, it is alternatively demonstrated that L-lactate serves as an energy substrate in neurons [4,5].Precisely, glucose enters the astrocytes and undergoes lactate dehydrogenase-A (LDH-A; expressed in the astrocytes) dependent anaerobic glycolysis to form lactate. The release of lactate is shuttled and taken up by neurons via the astrocyte-neuron lactate shuttle, while lactate undergoes a subsequent LDH-B (expressed in the neurons) dependent neuronal pathway for fuel metabolism [6—9] (Figure 1). Although both glucose and lactate undergo metabolism to provide cellular neuronal energy, glucose and lactate sensing within the hypothalamus have also been tested in rats for their potential *in vivo* regulation on peripheral glucose homeostasis. First, direct infusions of 2 mM glucose into the mediobasal hypothalamus of rats increase hypothalamic glucose levels [10,11] to a comparable extent such as a doubling of plasma glucose levels [12]. Such a rise of hypothalamic

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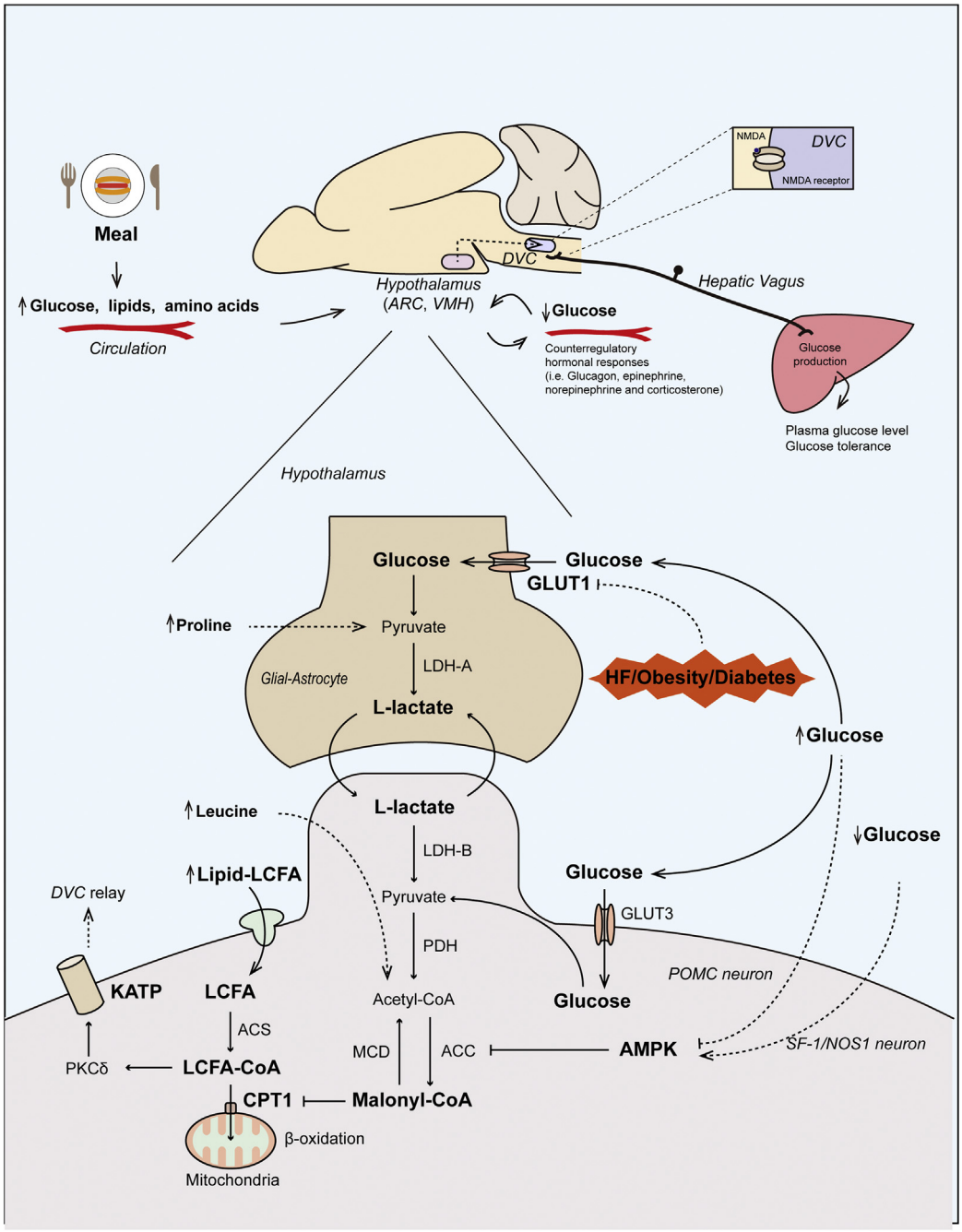

Figure 1: Schematic representation of the proposed hypothalamic glucose sensing mechanisms that regulate systemic glucose homeostasis in response to a rise or fall of glucose levels. Glucose, lipids and amino acids are absorbed into the circulation after a meal to provide energy for the body. Circulating glucose enters the hypothalamus containing the ARC and VMH to provide neuronal fuel. Hypothalamic glucose sensing also triggers a neuronal relay, via the NMDA receptor-dependent transmission in the DVC and the hepatic vagus, to lower hepatic glucose production/plasma glucose levels and increase glucose tolerance. With respect to the underlying hypothalamic mechanisms, an enhanced hypothalamic flux of glucose → L-lactate → malonyl-CoA is sufficient and necessary for glucose sensing to lower hepatic glucose production. The malonyl-CoA -> CPT-1 -> LCFA-CoA axis is also sufficient and necessary for glucose and lipid sensing to regulate hepatic glucose production via the activation of PKCδ and K_{ATP} channels. Amino acids such as proline and leucine converge with glucose-lactate sensing via formation of pyruvate in astrocytes and acetyl-CoA in neurons, respectively, while glucose enters neurons via GLUT3 and potentially influence POMC neurons to regulate glucose homeostasis. On the contrary, a fall of glucose levels in the hypothalamus not only relies on a direct neuronal rely to increase hepatic glucose production, but also indirectly via an increase in counter-regulatory hormones (i.e., glucagon, epinephrine, norepinephrine and corticosterone). Specifically, SF-1 and NOS1 neurons as well as AMPK in the VMH mediate hypoglycemia to activate counterregulation to increase hepatic glucose production and plasma glucose levels. Finally, high-fat fed, obese or diabetic conditions have been documented in both rats and humans to reduce brain glucose uptake and/or glial GLUT1 expression. We propose this could disrupt hypothalamic glucose sensing mechanisms. ARC-arcuate nucleus, VMH-ventral medial hypothalamus, NMDA-N-methyl-D-aspartate, DVC-Dorsal vagal complex, GLUT1-glucose transporter-1, LDH-A-lactate dehydrogenase-A, LDH-B-lactate dehydrogenase-B, PDH-pyruvate dehydrogenase, AMPK-AMP-activated protein kinase, ACC-acetyl-CoA carboxylase, MCD-malonyl-CoA decarboxylase, carnitine palmitoyl transferase-1 (CPT-1), long chain fatty acid (LCFA), malonyl-CoA decarboxylase (MCD), GLUT3-glucose transporter 3, PKCδ-protein kinase C, K_{ATP}-ATP-sensitive potassium channels. SF-1-steroidogenic factor-1, NOS1-nitric oxide synthase 1.

glucose levels gradually lowers plasma insulin and glucose levels in healthy rats when all hormones and metabolites are allowed to change without restriction [10]. Experiments show that when insulin levels are maintained at basal circulating levels during a pancreatic clamp, exogenous glucose infusion is required to prevent hypoglycemia in response to hypothalamic glucose infusion. The drop in plasma glucose levels during the clamp is due to a lowering of hepatic glucose production, and not an increase in glucose uptake [10]. However, it remains unknown whether hypothalamic glucose sensing in rats and mice alters glucose tolerance in response to an intravenous glucose tolerance test.

To begin addressing whether hypothalamic lactate sensing is sufficient and necessary for glucose sensing (as postulated by the astrocyte-neuron lactate shuttle) to regulate peripheral glucose homeostasis and hepatic glucose production in unclamp or clamp conditions; lactate was directly infused into the hypothalamus of healthy rats. It was found to recapitulate the effect of hypothalamic glucose sensing in lowering circulating glucose and insulin levels in unclamp conditions. The lowering-effect of plasma glucose and insulin levels induced by both hypothalamic lactate and glucose infusion is prevented by the chemical inhibition of hypothalamic LDH-A and B [10]. Hypothalamic lactate infusion (like glucose) lowers hepatic glucose production during pancreatic-clamping [10]. Chemical inhibition of hypothalamic LDH-A and B also negates the hypothalamic lactate and glucose administration effect of lowering glucose production [10]. A recent study equally indicated that selective molecular knockdown of hypothalamic LDH-A expression negates the glucose sensing effect of lowering glucose production during pancreatic clamping in healthy rats as well [13]. These studies collectively demonstrate that hypothalamic LDH-A-dependent lactate metabolism in astrocytes is sufficient and necessary for glucose sensing to regulate glucose homeostasis and hepatic glucose production in healthy rats, and further strengthen the *in vivo* metabolic impact of the astrocyte-neuronal lactate shuttle in the hypothalamus (Figure 1).

Notably, these findings evidently do not cancel out the fact that glucose-sensing neurons in the brain could monitor local and systemic changes of glucose levels to activate negative feedback pathways to regulate glucose homeostasis as well [14–18]. Indeed, the underlying mechanisms of glucose sensing within specific neurons are reviewed elsewhere in details [19], and the relative contribution of glucose vs. lactate-sensing neurons in the brain in *in vivo* regulation of systemic glucose homeostasis remains elusive. However, whether by the astrocyte-glucose -> lactate neuronal shuttling pathway or the direct neuronal glucose uptake and sensing, subsequent conversion to pyruvate in neurons is a necessary biochemical step for hypothalamic glucose/lactate sensing. This is because direct activation of hypothalamic pyruvate dehydrogenase is sufficient to enhance the pyruvate neuronal flux to acetyl-CoA and recapitulate the glucose production-lowering effect induced by hypothalamic glucose-lactate sensing [10] (Figure 1). As is expected, glucose administration not only elevates lactate and pyruvate metabolism [8,20], but also malonyl-CoA levels in the hypothalamus [21]. The elevated malonyl-CoA is derived from acetyl-CoA via acetyl-CoA carboxylase within the neurons [22,23], while acetyl-CoA carboxylase is inhibited by AMP-activated protein kinase (AMPK) [22] (Figure 1).Moreover, central infusion of glucose [24] or lactate [25] suppresses hypothalamic AMPK, while non-metabolizable analogs of glucose increase AMPK in rats and mice [24,26]. These independent but yet parallel findings suggest that hypothalamic AMPK-mediated conversion of acetyl-CoA to malonyl-CoA (via acetyl-CoA carboxylase) is necessary for glucose-lactate-pyruvate sensing to regulate glucose homeostasis.

Undeniably, molecular activation of hypothalamic AMPK (i.e., that will inhibit acetyl-CoA carboxylase and reduce malonyl-CoA formation) not only negates the ability of hypothalamic glucose and lactate sensing to lower glucose production in rats [27], but also negates the ability of hypothalamic glucose or lactate injection to lower feeding in rats and mice [24–26,28].Furthermore, direct molecular inhibition of hypothalamic AMPK (i.e., that will activate acetyl-CoA carboxylase and increase malonyl-CoA formation) is sufficient to lower glucose production [27] and food intake [24] in the absence of hypothalamic glucose or lactate administration in both rats and mice (Figure 1). Alternatively, when hypothalamic malonyl-CoA level is lowered by over-expressing hypothalamic malonyl-CoA decarboxylase (i.e., an enzyme that converts malonyl-CoA back to acetyl-CoA) (Figure 1), both hyperphagia and a disruption in hypothalamic nutrient sensing in regulating glucose homeostasis have also been documented in rats and mice [29,30]. Together, these findings strongly demonstrate that an accumulation of hypothalamic malonyl-CoA levels is necessary for hypothalamic glucose-lactate sensing and sufficient to regulate glucose and energy homeostasis.

Malonyl-CoA inhibits carnitine palmitoyl transferase-1 (CPT-1) [31]. Long-chain fatty acids (LCFA) are esterified by acyl-CoA synthetase to form LCFA-CoA, and CPT-1 mediates the entry of LCFA-CoA into mitochondria where it undergoes beta-oxidation [32] (Figure 1). In fact, the overexpression of hypothalamic malonyl-CoA decarboxylase lowers not only malonyl-CoA levels but also LCFA-CoA levels given that the lowering of malonyl-CoA would relieve the inhibition on CPT-1 [29]. In parallel, the molecular and chemical inhibition of hypothalamic CPT-1 is sufficient to elevate hypothalamic LCFA-CoA levels and lower hepatic glucose production and food intake [33] (Figure 1). Given that the hypothalamic malonyl-CoA — CPT-1 axis not only regulates glucose-lactate metabolism (as discussed above) but also LCFA-lipid sensing, the malonyl-CoA — CPT-1 axis represents a point of convergence for both hypothalamic glucose and lipid sensing in regulating glucose homeostasis.

As per the underlying mechanism of hypothalamic lipid-LCFA sensing, a direct blockade of (i) hypothalamic LCFA-CoA accumulation via acyl-CoA synthetase inhibition [34], and (ii) hypothalamic protein kinase C-δ [35], is sufficient to negate LCFA-sensing in lowering glucose production (Figure 1). The link between hypothalamic glucose and lipid sensing is however further supported by the fact that hypothalamic increase of glucose alters LCFA metabolism in an AMPK-dependent fashion [36] and that both glucose [10] and lipids [37] activate (i.e., open) the hypothalamic ATP-sensitive potassium (K_{ATP}) channels to lower plasma glucose levels in unclamp and glucose production in clamp settings in rats. Interestingly, direct acute chemical activation of hypothalamic K_{ATP} in rats is also sufficient to lower plasma glucose levels (unclamp settings) and glucose production(pancreatic clamp settings) [38] (Figure 1), while the clinical relevance of these findings will be discussed below.

Nonetheless, although the neurocircuitry of hypothalamic nutrient sensing remains elusive, a few long-term genetic modified rodent studies are noteworthy. First, life-long expression of constitutively active K_{ATP} channels in the POMC neurons in mice disrupts glucose sensing and impairs whole-body glucose tolerance as assessed by an intraperitoneal glucose tolerance test [39]. In parallel, knocking out AMPK in both POMC and AgRP neurons of mice disrupts electrophysiological responses that are normally triggered by a reduction in cellular glucose levels, but the glucose production regulation was not assessed [40]. Further, knocking out LKB1 (upstream kinase of AMPK) in POMC neurons impairs circulating hyperinsulinemia to lower glucose production but yet these neurons responded normally to a

cellular drop in glucose levels [41]. Thus, the neuronal populations as well as the downstream neurocircuitry involved in both short and long-term glucose production and homeostatic regulations in response to hypothalamic glucose sensing mechanisms warrant future investigation in rats and mice.

As per the downstream neuronal relay of hypothalamic nutrient sensing, the NMDA receptor-mediated transmission in the dorsal vagal complex of the hindbrain as well as the hepatic vagal innervation are necessary for hypothalamic glucose-lactate-AMPK and lipid sensing to lower hepatic glucose production in rats [42–44] (Figure 1). As a matter of fact, in a non-clamped physiological setting, nutrient sensing activated by a fasting-refeeding protocol in rats fails to maintain peripheral glucose homeostasis when NMDA receptor transmission in the dorsal vagal complex is inhibited *in vivo* as well [44] (Figure 1).

Refeeding of a chow diet not only elevates plasma glucose and lipids but also amino acids levels. Moreover, the hypothalamus senses amino acids in order to regulate glucose homeostasis [45], and hypothalamic amino acid sensing partly converges with lactate and lipid sensing as well. For example, proline needs to be converted into pyruvate and lactate in the astrocytes by LDH-A in order to lower glucose production in rats [46]. Similarly, hypothalamic leucine sensing is disrupted when regulating glucose production in the presence of hypothalamic chemical AMPK activation or molecular overexpression of malonyl-CoA decarboxylase in rats [47] (Figure 1). Whether amino acid sensing requires a similar anatomic relay to the hindbrain for glucose regulation remains to be investigated, although a hypothalamic-dorsal vagal complex axis has been demonstrated to mediate leucine sensing to regulate feeding [48]. Nevertheless, future basic studies aimed at investigating the relative contribution of glucose, lipid, and amino acid-sensing hypothalamic–relay pathways in regulating systemic glucose homeostasis during refeeding conditions will be crucial in understanding the physiological impact of hypothalamic nutrient sensing (Figure 1).

The studies discussed above highlight the mechanistic and *in vivo* impact of hypothalamic glucose sensing generally in healthy rats and mice. However, are disruptions in hypothalamic glucose sensing mechanisms responsible for the dysregulation of hepatic glucose production and systemic glucose homeostasis in rodents with diabetes? More importantly, is the hypothalamic glucose sensing mechanism relevant in humans? Moreover, in both humans and rats, a rise of circulating glucose levels that activate glucose sensing *per se* during the pancreatic (basal insulin)-euglycemic clamps lowers glucose production in healthy but not in diabetic conditions [49,50]. To date, the mechanisms responsible for disruption of glucose sensing in diabetes remain unclear. However, both basic and clinical evidence suggest that a defect in hypothalamic glucose sensing could dysregulate hepatic glucose production and glucose homeostasis in diabetes. For example, hypothalamic glucose sensing in healthy rats contributes to the ability of a systemic rise of glucose to lower hepatic glucose production [10] (Figure 1), suggesting that a defect in hypothalamic glucose sensing in rats and humans could impair circulating glucose from lowering glucose production. In fact, direct glucose infusion into the hypothalamus fails to lower glucose production in high-fat fed or uncontrolled diabetic rats [11,13]. The defect in hypothalamic glucose sensing in lowering glucose production in diabetic rats parallels a failure for hypothalamic glucose infusion to elevate local hypothalamic glucose levels and a reduction of GLUT1 expression in the hypothalamic glial (non-neuronal) cells [11] (Figure 1). More importantly, a direct rescue of hypothalamic reduced glial GLUT1 expression in diabetic rats via overexpressing glial GLUT1 is sufficient to rescue the ability of hypothalamic glucose infusion to elevate hypothalamic glucose levels and lower hepatic glucose production in uncontrolled diabetic rats [11]. These findings have two important implications. First, it confirms that glucose sensing in hypothalamic astrocytes (non-neuronal cells) regulates glucose homeostasis (as discussed above). Second, downstream hypothalamic lactate sensing is intact in rodents with diabetes. In fact, direct infusion of lactate into the hypothalamus (unlike glucose) remains intact in lower plasma glucose levels and hepatic glucose production in high-fat fed or uncontrolled diabetic rats [51] (Figure 1).

A reduction in brain glucose uptake has been observed in people with type 2 diabetes and obesity [52], people with type 1 diabetes [53], and high-fat fed rodents [54] as well (Figure 1). The reduction in brain glucose uptake in people with type 2 diabetes and obesity is even correlated inversely with an elevation of plasma free fatty acids levels [52], supporting the observation in rats that high-fat feeding disrupts the ability of hypothalamic glucose infusion to lower glucose production [13]. As is expected, the rise of free fatty acids in people with type 2 diabetes disrupts glucose sensing *per se* in lowering glucose production as well [55] (Figure 1). Although the underlying mechanisms responsible for the disruption of glucose sensing in regulating glucose production in lipid-enriched environments remain unclear in both rodents and humans, we propose (and clearly remains to be tested) that a disruption in hypothalamic glucose sensing contributes to the dysregulation of hepatic glucose production and glucose homeostasis in humans and rats with obesity and diabetes.

Finally, the clinical relevance of the central regulation of glucose production is also implicated at the level of the K_{ATP} channels given that the oral intake of the channel activator diazoxide lowers glucose production during pancreatic clamping in healthy humans and rats [56]. Consistent with the fact that hypothalamic K_{ATP} channels are necessary for glucose sensing to regulate glucose production in rats (see discussion above), the extra-pancreatic glucose-lowering effects of oral diazoxide in rats are shown to be centrally-mediated. While this extra-pancreatic glucose-lowering effect of oral K_{ATP} channel activator is lost in type 2 diabetic patients as well as in Zucker Diabetic Fatty rats [57], direct activation of K_{ATP} channels in the dorsal vagal complex of the brain in non-diabetic but centrally insulin resistant, high-fat fed rats remains sufficient to lower glucose production [58]. These findings collectively indicate that both high fat feeding and/or diabetes disrupts CNS-dependent mechanisms of hepatic glucose production regulation, but the molecular defect(s) may lie up and/or downstream of K_{ATP} channels, depending on the progression and stages of the metabolic diseases. Nonetheless, the clinical relevance of central regulation of glucose homeostasis remains elusive and awaits advancements in experimental tools to address the relevant shortcomings.

3. GLUCOSE DEPRIVATION IN THE BRAIN

The impact of central glucose sensing not only lies in its systemic glucose-lowering effects, but also in its ability to sense hypoglycemia and trigger counterregulatory responses [59]. Specific counterregulatory responses include lowering insulin and increasing glucagon, activating the hypothalamo-pituitary-adrenal axis, as well as heightening sympathetic nervous activity by stimulating the release of epinephrine, norepinephrine and corticosterone [60]. While peripheral glucose sensing exists [61], an early study indicates that the brain of dogs detects a drop in glucose levels in response to insulin infusion and triggers the release of glucagon, epinephrine, norepinephrine and cortisol [62]. This is supported by the fact that preventing central detection of hypoglycemia via glucose infusion towards the brain reduces glucose production during insulin-induced systemic hypoglycemia [62,63]. In rats, infusing the non-metabolizable glucose analog 2-deoxyglucose into

the ventral medial hypothalamus induces local glucopenia and activates counterregulatory responses [64], while direct glucose infusion into the ventral medial hypothalamus blunts the counterregulatory responses during insulin-induced hypoglycemia [65,66]. Thus, these subsequent rat studies have extended the early findings in dogs and pointed out the hypothalamus as a site that senses a drop in glucose levels and triggering counter-regulatory responses (Figure 1).

Similar to hyperglycemic conditions, AMPK also mediates hypothalamic gluco-deprived detection and triggers counterregulatory responses [67]. First, hypoglycemia increases hypothalamic AMPK activity in rats, while chemical activation of hypothalamic AMPK increases plasma glucose levels, glucagon and corticosterone [68]. Chemical activation of hypothalamic AMPK even rescue the blunted cortisol and glucagon release as observed in recurrent hypoglycemia (i.e., hypoglycemia-associated autonomic failure) [69]. Along the same vein and during insulin-induced hypoglycemic clamp conditions, hypothalamic activation of AMPK increases glucose production, but interestingly, independent of changes in glucagon, epinephrine and norepinephrine levels in healthy rats [70], while molecular inhibition of hypothalamic AMPK impairs counterregulatory responses by inhibiting glucose production in parallel to impaired glucagon, epinephrine and norepinephrine secretion [68,71]. It remains to be assessed whether during hypoglycemic conditions, hypothalamic AMPK regulates glucose production via hormonal-dependent or -independent mechanisms (Figure 1). It is worth noting that inhibiting hypothalamic AMPK in rats is sufficient to lower glucose production during the pancreatic-euglycemic clamp conditions independent of changes in plasma hormonal levels [27] via hepatic vagal innervation. Thus, future studies should investigate the hormonal-dependent or independent mechanisms in mediating hypothalamic AMPK-dependent mechanisms to regulate glucose production during a fall or rise of plasma glucose levels.

Nonetheless, direct suppression of hypothalamic acetyl-CoA carboxylase heightens counterregulatory responses [72], further supporting the downstream regulation of the malonyl-CoA-CPT1 axis (as discussed above) in facilitating glucose homeostasis in the context of nutrient depletion (Figure 1). Together, these findings (at least in rats) demonstrate that bidirectional changes of AMPK activity in the hypothalamus facilitate systemic glucose production, thereby acting as a critical regulator for whole-body glucose homeostasis under conditions of glucose-excess or deprivation.

With respect to the neurocircuitry involved in hypothalamic control of glucose counter-regulation, recent findings report that bidirectional changes of hypothalamic ventromedial neurons manipulated by optogenetic tools alter the homeostatic response to hypoglycemia [73,74] (Figure 1). In particular, direct inhibition of steroidogenic-factor 1 (SF-1) neurons expressed in the ventromedial hypothalamus disrupts counter-regulatory responses of insulin-induced hypoglycemia [73], while inhibiting NOS1 neurons expressed in the ventromedial hypothalamus also prevents the rise of glucagon induced by insulin-induced hypoglycemia [74]. These findings are consistent with the early observation that genetic disruption of glutamate release from the hypothalamic SF-1 neurons disrupts the glucagon release induced by insulin-induced hypoglycemia [75] (Figure 1).

Notably, in rats that are diabetic or have been exposed to chronic hypoglycemia, counterregulatory responses specifically triggered by the hypothalamus are impaired [76]. Although an impairment of counterregulatory responses to hypoglycemia is also observed in people with diabetes [77], whether glucose sensing or deprivation in the hypothalamus is a source of impairment, or even necessary for counterregulatory responses in healthy humans, remains unclear. The experimental challenges in addressing the relative contribution of CNS/hypothalamic-mediated mechanisms in whole-body metabolic regulation in the context of hypoglycemia is apparent and clearly analogous to the difficulties (or limitation of experimental tools/approaches) one faces in addressing the clinical relevance of glucose sensing in the context of a glucose rise in the brain in regulating glucose production and glucose homeostasis (see previous section). However, due to the fact that changes in brain glucose uptake and metabolism are reported in rats and humans in high-fat fed, obese, type 2 and/or type 1 diabetic conditions [52—54], and that brain sensing mechanisms have been attributed and/or demonstrated to regulate (or dysregulate) glucose production and homeostasis in rats and humans (see discussion above), future investigations continually aimed at addressing the basic and clinical relevance of CNS-glucose sensing/deprivation mechanisms in regulating whole-body glucose homeostasis in healthy, obese and diabetic conditions, remain an urgent necessity.

4. LEPTIN ACTION IN THE BRAIN

While the mechanisms underlying hyperglycemia and hypoglycemia regulations by the brain remain to be elucidated in rodents and humans, leptin replacement has been documented to not only lower blood glucose levels in humans with leptin deficiency and severe lipodystrophy [78], but also enhance intranasal insulin administration-dependent hypothalamic mechanisms to regulate food intake and whole-body insulin sensitivity in congenital leptin deficient human [79]. These studies implicate that leptin triggers CNS-mediated pathways to restore metabolic homeostasis. Indeed and consistently, more than 20 years ago, direct leptin administration into the brain of rats and mice was known to regulate glucose homeostasis [80—82], while mice with leptin receptor gene mutation develop diabetes and obesity [83].

Once activated, leptin receptors recruit the tyrosine kinase, Janus kinase 2, to phosphorylate tyrosine residues (Tyr985, Tyr1077, Tyr1138) in the cytoplasmic domain [84,85]. Phosphorylation of Tyr985 results in the activation of the extracellular signal regulated and recruitment of suppressor of cytokine signaling (SOCS)-3 that inhibits leptin signaling [86,87]. As such, hyperleptinemia in obesity *per se* may trigger brain leptin resistance via SOCS3 activation [88].Besides, partial reduction of the plasma leptin level restores hypothalamic leptin sensitivity and increases glucose tolerance in high-fat fed mice [89], while mutating Tyr985 of leptin receptors increases leptin sensitivity and reduces body weight and food intake in mice [90]. On the other hand, high fat feeding increases hypothalamic SOCS-3 via inflammation and induces leptin resistance [91]. Phosphorylation of Tyr1077 activates the signal transduction and activator of transcription (STAT)5 [85], and mutating Tyr1077 of leptin receptor increases body weight and food intake in mice [92]. The phosphorylation of Tyr1138 induces the activation of STAT3 [93]. Mice bearing a mutation at Tyr1138 of the leptin receptor exhibit the phenotype of increased body weight, food intake and decreased energy expenditure [94] as well as hyperglycemia [95] and hepatic insulin resistance [96]. Conversely, short-term molecular and chemical inhibition of hypothalamic STAT3 not only negates the ability of leptin administration to activate hypothalamic STAT3 but also dysregulate glucose homeostasis and food intake in rats [96]. These findings collectively point out the necessity of hypothalamic STAT3 in leptin's glucoregulatory effect in rats and mice, although STAT3-independent effect leptin in regulating glucose is also apparent in rodents [95] (Figure 2).

The leptin—leptin receptor complex promotes the interaction of Janus kinase 2 and SH2—B, leading to the activation of the IRS1/2 dependent phosphatidylinositol-3-OH kinase (PI3K) pathway and a potential cross-link with insulin action [97]. In fact, restoring leptin receptor

expression in the hypothalamus of leptin receptor-deficient Koletsky rats [98] or mice [99] enhances insulin sensitivity in lowering blood glucose levels and hepatic glucose production by activating hypothalamic PI3K [98,100], consistent with the fact that insulin activates hypothalamic PI3K to lower hepatic glucose production in healthy rats [101] (Figure 2). In light of the observed glucose-lowering effect of leptin replacement [78] that is due in part to secondary enhancement of brain insulin action in congenital leptin deficient human [79], and the fact that brain-targeted insulin delivery increases whole-body insulin sensitivity and lower glucose production in healthy but not in individuals with obesity [102,103], future mechanistic studies are necessary to unravel the central glucoregulatory mechanisms of leptin. In this regard, hypothalamic AMPK seems like a reasonable target of central leptin action as expressing the constitutively activated AMPK in the hypothalamus blocks the effect of leptin to lower food intake and body weight in mice [24,104]. In addition, molecular inhibition of hypothalamic AMPK is not only sufficient to reproduce the food intake-lowering effect of leptin in mice [24,105], but is also sufficient to inhibit hepatic glucose production in rats [27]. However, life-long knockout of AMPK in the POMC/AgRP neurons nor LKB1 in POMC neurons affect leptin's effect on neuronal firing [40,41]. But the lack of such evidence to-date clearly does not withstand a potential link of leptin and glucose sensing in the brain.

As a matter of fact, leptin has been documented 20 years ago in rats and mice to inhibit the activation of the hypothalamic—pituitary—adrenal axis in response to stress by inhibiting the release of corticotropin-releasing hormone [106], although the underlying mechanisms remain unknown. Interestingly, cholecystokinin (CCK)-expressing neurons in the parabrachial nucleus (PBN) have been recently identified and found to project to the VMH SF-1 neurons (Figure 2). These CCK-expressing neurons are not only activated by hypoglycemia to trigger counter-regulatory responses in mice [107], but are also inhibited by leptin in a leptin receptor-dependent manner to blunt counterregulation [108] (Figure 2). A set of follow-up studies conducted in rats [109] indicate that leptin's ability to regulate counterregulatory responses to metabolic stress may also act within the hypothalamus as direct administration of leptin into the VMH significantly increases the exogenous glucose infusion rate required to maintain low plasma glucose levels (~50 mg/dl) during the hyperinsulinemic-hypoglycemic clamps. Collectively, future basic, mechanistic, and clinically-relevant studies are warranted to delineate central leptin actions in regulating glucose homeostasis in conditions of glucose deprivation.

5. INTERACTION OF LEPTIN ACTION AND GLUCOSE SENSING IN THE BRAIN

This final section addresses whether hypothalamic leptin action interacts and enhances hypothalamic glucose sensing mechanisms (in glucose-excess conditions) to regulate glucose homeostasis. In fact, hypothalamic leptin infusion has been documented in both rats and mice to lower plasma glucose levels and glucose production in insulin-deficient hyperglycemic (i.e., uncontrolled diabetes) conditions [110—113]. It is worth noting that in comparable insulin-deficient hyperglycemic rats, hypothalamic infusion of lactate [51], but not glucose [11], lowers plasma

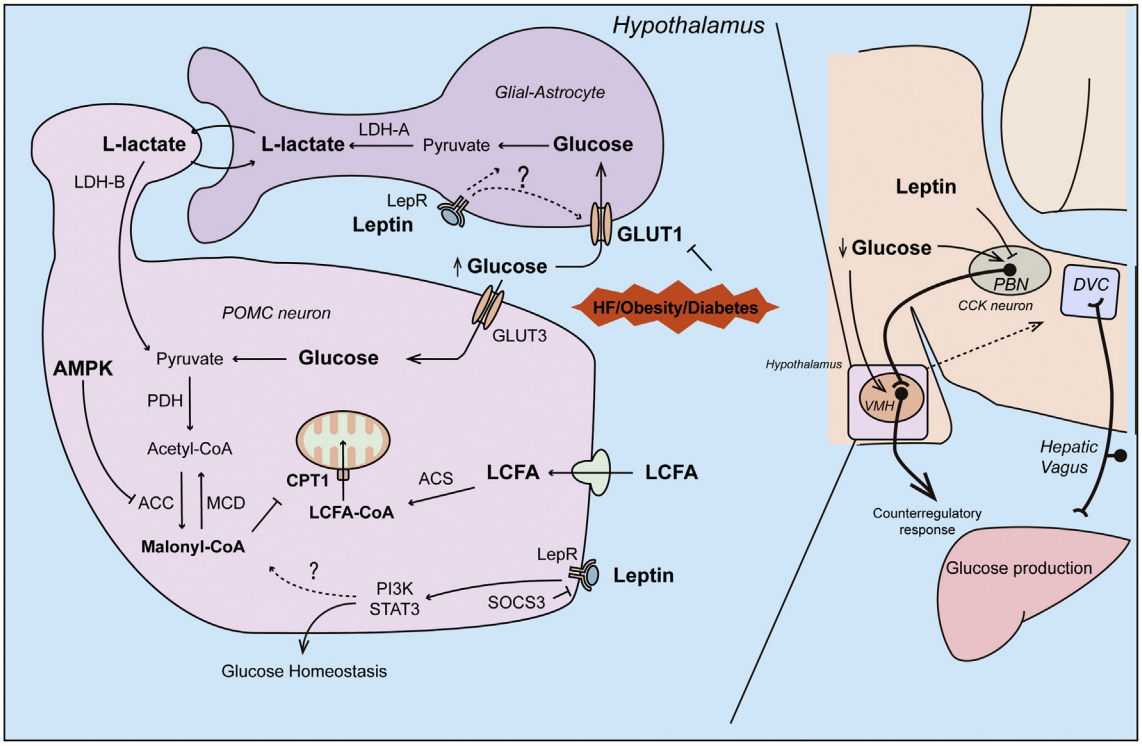

Figure 2: Proposed interaction of leptin action and glucose sensing in the brain. In high-fat fed and/or diabetic conditions, leptin action in the hypothalamus activates PI3K and/or STAT3 to regulate glucose homeostasis. In parallel, leptin enhances hypothalamic glucose flux into lactate via LDH-A. An enhancement of lactate metabolism is required for leptin to lower hepatic glucose production. In light of the convergence of lactate metabolism with lipid sensing pathways, leptin is postulated to activate a hypothalamic glucose -> lactate -> LCFA-CoA axis and DVC neuronal relay to lower hepatic glucose production. In response to a fall in plasma glucose levels, CCK-expressing neurons in the PBN are activated and projected to the VMH SF-1 neurons to trigger counterregulatory responses. Importantly, CCK neuronal cells in the PBN express leptin receptor and are inhibited by leptin. Thus, leptin blunts hypoglycemia-induced counterregulation via the inhibition of CCK-neurons in the PBN. *PBN-parabrachial nucleus, CCK-cholecystokinin.*

glucose levels and glucose production as discussed above. The disruption of hypothalamic glucose sensing is secondary to a reduction in hypothalamic glial glucose uptake [11] and subsequent metabolism to lactate [51]. This is consistent with the observation that brain glucose uptake is decreased in people with type 1 diabetes [53] as discussed above, and suggests that leptin enhances (or restores) hypothalamic glucose metabolism to lactate and lowers glucose production in hyperglycemic conditions (Figure 2). In fact, hypothalamic leptin administration enhances glucose infusion to increase hypothalamic malonyl-CoA levels in mice [21], a downstream mediator of the hypothalamic AMPK-malonyl-CoA-CPT1 axis that is sufficient and necessary for glucose sensing to lower glucose production in rats as discussed above [27,114] (Figure 2). These studies collectively imply that during glucose excess conditions, leptin enhances hypothalamic glucose sensing mechanisms to lower glucose production and maintain whole-body glucose homeostasis in insulin-deficient conditions (Figure 2). Although such working hypothesis remains to be tested, evidence is already becoming apparent as leptin enhances hypothalamic glucose sensing in short-term high-fat fed rats with insulin resistance [13].

Consistent with the fact that a reduction of brain glucose uptake is observed in obese and type 2 diabetic humans [52] and a reduction of brain glucose uptake and GLUT1 expression is observed in high fat-fed mice [54], hypothalamic infusion of glucose fails to lower glucose production in short-term high-fat fed rats [13]. These findings indicate that high-fat feeding disrupts hypothalamic glucose sensing. But most importantly, a recent study documented that direct infusion of leptin into the hypothalamus of short-term high-fat fed rats rescues the defect of glucose sensing by enhancing the ability of hypothalamic infusion of glucose to lower glucose production during the pancreatic-euglycemic clamps [13]. Furthermore, even if all glucoregulatory hormones are allowed to change at will, hypothalamic leptin infusion increases glucose tolerance in response to an intravenous glucose tolerance test (i.e., glucose sensing is activated) in high-fat fed rats [13] (Figure 2). Thus, these findings collectively indicate that hypothalamic leptin action enhances hypothalamic glucose sensing to lower glucose production and increase glucose tolerance in high-fat fed rats. Unlike glucose sensing, it is noteworthy that hypothalamic lactate infusion lowers glucose production in the same short-term high-fat fed rats [51], indicating that high-fat feeding disrupts glucose sensing mechanisms that lie upstream of lactate metabolism [13]. Given that leptin restores the ability of glucose sensing to suppress glucose production [13], the disruption(s) that negate hypothalamic glucose metabolism to lactate induced by short-term high-fat feeding could be reversed by leptin [13] (Figure 2). Although the underlying molecular targets of leptin action on glucose sensing remain elusive, both molecular and chemical inhibition directed against LDH-A in the hypothalamus of high-fat fed rats negate the ability of hypothalamic leptin and glucose infusion to lower glucose production [13]. These findings strengthen the notion that hypothalamic lactate metabolism is necessary for glucose sensing to regulate glucose homeostasis (as discussed above), and that leptin potentially activate molecular targets that convert glucose to lactate to restore hypothalamic glucose sensing (Figure 2). Although these postulations could be relevant to short-term high-fat feeding and/or uncontrolled diabetic insulin-deficient conditions, future studies that assess the relevance of leptin's interactive action with glucose sensing in chronic obese conditions with sustained hyperleptinemia and/or leptin resistance are awaited.

Finally, the molecular mechanisms and the neurocircuitry involvement of leptin interaction with glucose sensing that impact whole-body glucose homeostasis *in vivo* remain elusive. Interestingly, leptin action within the POMC neurons is necessary for leptin to exert its anti-diabetic effect during insulin-deficient and uncontrolled 'hyperglycemic' conditions [115], suggesting perhaps leptin and glucose sensing in POMC neurons exert *in vivo* glucoregulatory impact to normalize glucose homeostasis in uncontrolled diabetic as well as high-fat fed/obese/diabetic conditions (Figure 2).

6. CONCLUSION

This perspective highlights recent advances in dissecting the underlying mechanisms and biological responses of hypothalamic glucose sensing. Studies highlight the relevance of hypothalamic glucose sensing mechanisms in detecting a rise or fall in local and/or systemic glucose levels, triggering minute-to-minute physiological responses (i.e., bi-directional changes of hepatic glucose production) to maintain glucose homeostasis in healthy conditions. Part of the hypothalamic glucose sensory pathways become defective in high fat fed, obese, and/or diabetic conditions, leading to a dysregulation of hepatic glucose production and systemic glucose homeostasis. It is apparent that future basic studies are highly needed to dissect and clarify the metabolic impact of hypothalamic glucose sensing, and more importantly, its potential interaction with hypothalamic leptin action as recent basic studies indicate that leptin regulates glucose homeostasis in glucose-deprived conditions. In short-term high-fat fed or uncontrolled diabetic conditions, hypothalamic leptin action enhances hypothalamic glucose sensing to lower hepatic glucose production and restore glucose homeostasis but the underlying mechanisms require intense future investigation. Finally, although the translational impact of brain glucose sensing and systemic glucose regulation in various metabolic conditions has been discussed and highlighted, future clinical studies are highly needed to accurately and directly assess the glucoregulatory impact of hypothalamic glucose sensing. In conclusion, we put forward the working hypothesis that studying the potential interaction of hypothalamic glucose sensing mechanisms with leptin action in rodents could shed light on the translational impact of hypothalamic glucose sensing in diabetes and obesity.

AUTHOR CONTRIBUTIONS

All authors contributed in writing the manuscript.

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CONFLICT OF INTEREST

None declared.

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