Molecules and Cells

Minireview

Role of Hypothalamic Reactive Astrocytes in Diet-Induced Obesity

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Hypothalamus is a brain region that controls food intake and energy expenditure while sensing signals that convey information about energy status. Within the hypothalamus, molecularly and functionally distinct neurons work in concert under physiological conditions. However, under pathological conditions such as in diet-induced obesity (DIO) model, these neurons show dysfunctional firing patterns and distorted regulation by neurotransmitters and neurohormones. Concurrently, resident glial cells including astrocytes dramatically transform into reactive states. In particular, it has been reported that reactive astrogliosis is observed in the hypothalamus, along with various neuroinflammatory signals. However, how the reactive astrocytes control and modulate DIO by influencing neighboring neurons is not well understood. Recently, new lines of evidence have emerged indicating that these reactive astrocytes directly contribute to the pathology of obesity by synthesizing and tonically releasing the major inhibitory transmitter GABA. The released GABA strongly inhibits the neighboring neurons that control energy expenditure. These surprising findings shed light on the interplay between reactive astrocytes and neighboring neurons in the hypothalamus. This review summarizes recent discoveries related to the functions of hypothalamic reactive astrocytes in obesity and raises new potential therapeutic targets against obesity.

Keywords: gliotransmitter, high-fat diet, hypothalamus, obesity, reactive astrocytes

INTRODUCTION

The major causes of metabolic disorders such as obesity are associated with the hypothalamus, which is crucial for bodyweight control (Thaler et al., 2012; Thorburn and Proietto, 1998). Hypothalamus consists of several small nuclei with various functions that communicate via different neurotransmitters and neuropeptides or hormones (Horvath et al., 2004; Quadt et al., 2018). It has been established that arcuate nucleus (ARC) of the hypothalamus firstly senses metabolic signals and hormones, which are then conveyed to the second-order hypothalamic regions of paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) of the hypothalamus (Bouret et al., 2004; Yaswen et al., 1999). ARC, PVN, LHA, ventromedial hypothalamus (VMH), and dorsomedial hypothalamus (DMH) contain most of the neurons involved in feeding and body weight control (Gold, 1973; Gooley et al., 2006; King, 2006; Schneeberger et al., 2014; Timper and Bruning, 2017; Waterson and Horvath, 2015).

In these hypothalamic regions, numerous types of neurons can be classified using various cellular markers with corresponding functional properties as follow: orexigenic (promoting appetite) properties of neuropeptide Y (NPY)-(Tatemoto et al., 1982) and Agouti-related peptide (AgRP)-positive neurons (Miltenberger et al., 1997) and anorexigenic (suppressing appetite) properties of pro-opiomelanocortin (POMC) (Cowley et al., 2001) and cocaine- and amphetamine-regulated transcript (CART) (Kristensen et al., 1998).

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In addition, CART neurons in the ARC decrease adiposity and increase energy expenditure (Lau et al., 2018). POMC/CART neurons secrete an α-melanocyte-stimulating hormone (α-MSH), which suppresses appetite via melanocortin 4 receptor (MC4R) (Baltatzi et al., 2008; Fan et al., 1997; Gantz et al., 1993; Huszar et al., 1997). In addition to ARC, PVN mediates many diverse functions via secretion of corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), oxytocin, and vasopressin (Konturek et al., 2005). Inputs from NPY neurons in ARC regulate energy intake via TRH secretion (Beck, 2006; Konturek et al., 2005; Nillni, 2010). Interestingly, ARC projections affect appetite through MC4R-expressing oxytocin neurons (Qin et al., 2018). Also, vasopressin cells are involved in brown fat thermogenesis (Hill, 2012). LHA occupies a large portion of the hypothalamus and contains melanin-concentrating hormone (MCH) which is an orexinergic peptide (Barson et al., 2013), and hypocretin/orexin. Hypocretin/orexin neurons in LHA increase food intake while regulating brown adipose tissue activity to enhance energy expenditure (Martins et al., 2016; Tupone et al., 2011). VMH also contains many distinct neuronal populations. Among them, steroidogenic factor 1 (SF-1) neurons have anorexigenic properties (Zhang et al., 2020). Lastly, DMH cholinergic neurons increase food intake (Jeong et al., 2017), whereas TrkB-expressing neurons suppress food intake (Liao et al., 2019). When animals are challenged by high-fat diet (HFD) feeding, neurons that are homeostatically regulated in the hypothalamus lose metabolic control (Moraes et al., 2009) and start to show dysfunctional firing patterns and distorted regulation by neurotransmitters and neurohormones (Beutler et al., 2020; Sa et al., 2022). It has been further reported that with chronic feeding of 20 weeks of HFD, markers of neuronal injury become evident in the neurons of the hypothalamus (Thaler et al., 2012).

Before hypothalamic neurons show dysfunctional firing patterns and markers of neuronal injury, hypertrophic and hyperplasic astrocytes are concurrently observed in the hypothalamus even 1 day after HFD feeding (Buckman et al., 2013; Thaler et al., 2012). Astrocytes normally participate in brain energy metabolism by controlling glycogen storage, sensing glucose, and supplying fuel to neurons under physiological conditions (Belanger et al., 2011; Choi et al., 2012; Fuente-Martin et al., 2012; Garcia-Caceres et al., 2016; Timper et al., 2020). In addition, astrocytes secrete chemokines, cytokines, and neurotrophic factors to promote neuronal development, neuroplasticity, and synaptic plasticity (Casse et al., 2018; Jo et al., 2014). Reactive astrocytes appearing in short-term HFD act to reduce HFD overload and return to normal (Thaler et al., 2012), whereas reactive astrocytes appearing in chronic HFD produce inflammatory factors and elevate inflammatory signals such as IκB kinase-β (IKKβ)/nuclear factor κB (NF-κB) signaling (Douglass et al., 2017; Zhang et al., 2008; 2017). Furthermore, reactive astrocytes have been reported to release gliotransmitters such as vascular endothelial growth factor (VEGF) and γ-aminobutyric acid (GABA) under DIO (Gruber et al., 2021; Zhang et al., 2017). VEGF inducese hypothalamic angiopathy and systemic hypertension. However, little is known about the effect of GABA from reactive astrocytes on the surrounding hypothalamic neurons in DIO. Increasing lines of evidence suggests that elevated levels of GABA from reactive astrocytes act as a common molecular mechanism in other neuroinflammatory diseases, such as Alzheimer's disease, Parkinson's disease, white matter stroke, inflammation-induced anxiety, and epilepsy (Chun et al., 2020; Heo et al., 2020; Jo et al., 2014; Nam et al., 2020; Pandit et al., 2020; Shim et al., 2019). Recently, GABA from hypothalamic reactive astrocytes under chronic HFD has been shown to contribute to the pathology of obesity by inhibiting the excitability of neighboring neurons facilitating energy expenditure (Sa et al., 2022). In this review, we summarize recent discoveries related to the causes of reactive astrogliosis and the consequences of reactive astrocytes in the hypothalamus in an attempt to gain insights into the interplay between reactive astrocytes and neurons under DIO.

REACTIVE ASTROCYTES IN THE HYPOTHALAMUS

Astrocyte hypertrophy has been recognized as an almost universal sign of central nervous system (CNS) pathology (Escartin et al., 2021). Neuroglial proliferation has been thought to accompany CNS lesions (Escartin et al., 2019). Astrocyte reactivity is observed in various pathological contexts under acute or chronic conditions and, in many situations, is reversible (Escartin et al., 2019; 2021). Reactive astrocytes are defined as astrocytes that undergo morphological, molecular, and functional remodeling in response to injury, disease, or infection of CNS, including neurodegenerative and demyelinating diseases, epilepsy, trauma, ischemia, infection, cancer, and obesity (Bedner et al., 2015; Brusilow et al., 2010; Buckman et al., 2013; Escartin et al., 2019; 2021; Garcia-Caceres et al., 2019; Michetti et al., 2019; Sa et al., 2022; Verkhratsky et al., 2017; Xu et al., 2010). Glial fibrillary acidic protein (GFAP) is a major protein constituent of astrocytic intermediate filaments and the most widely used marker of reactive astrocytes (Ben Haim et al., 2015; Eng and Ghirnikar, 1994). As summarized in Table 1, numerous lines of evidence point to the involvement of hypothalamic reactive astrocytes in the pathogenesis of DIO (Fig. 1).

Reactive astrocytes have been observed in various hypothalamic regions in rats and mice after HFD feeding (Buckman et al., 2015; Lee et al., 2013; Thaler et al., 2012). Among them, most studies on reactive astrocytes after HFD have focused on ARC (Table 1). This is probably because ARC provides a positional advantage at the ventral border of the hypothalamus for rapid nutrient sensing. Therefore, most studies have investigated reactive astrocytes in ARC after HFD feeding (Gonzalez-Garcia et al., 2017; Miyata, 2015; Moulle et al., 2014; Myers et al., 2009). When mice are fed HFD for approximately 1 week, the observed reactive astrocytes in ARC tend to be reversible (Buckman et al., 2015; Thaler et al., 2012). However, after chronic HFD feeding over 8 months, the severe reactive astrocytes are observed in the mediobasal hypothalamus, as similarly observed in obese humans (Thaler et al., 2012). These reactive astrocytes in ARC after HFD feeding appear to be the fastest players to be observed, yet their functional roles remain unclear. It has been proposed that reactive astrocytes under acute HFD act to re-establish homeostasis, whereas severe reactive astrocytes under chronic

Table 1. Reactive astrocytes across hypothalamus in response to HFD feeding

Region	Species and age	Sex	Feeding period	Diet	Proposed mechanism	Reference
ARC DMH	C57BL/6J mice (4 weeks old)	Male	>2 months	Research Diets (#D12451, 45 kcal% fat)	DIO induces functional astrocytic leptin receptors.	Hsuchou et al., 2009
ARC	Sprague-Dawley rats (7 months old) and C57BL/6J mice (6 weeks old)	Male	Rats: 3 months Mice: 20 or 37 weeks	Rat: Altromin (C1057, 60 kcal% fat) Mouse: Research Diets (#D12451, 45 kcal% fat)	The structure of the BBB and POMC and NPY cell bodies and dendrites became less accessible to blood vessels.	Horvath et al., 2010
ARC	Long-Evans rats and C57BL/6J mice (adult)	Male	From 1 day to 8 months	Research Diets (#D12492, 60 kcal% fat)	Both reactive gliosis and markers suggestive of neuronal injury were evident in the ARC.	Thaler et al., 2012
PVN SCN ARC DMH VMH LHA	C57BL/6J mice (12 to 17 weeks old)	Female	20 weeks	Research Diets (#D12532, 60 kcal% fat)	Differential GFAP immunoreactivity between lean and obese animals across hypothalamus	Buckman et al., 2013
ARC	C57BL/6J mice (8 to 10 weeks old)	Male	21 weeks	Research Diets (#D12492, 60 kcal% fat)	Hypothalamic gliosis in mice with DIO	Lee et al., 2013
ARC VMH	Wistar rats (adult)	Male	1 to 14 days	Research Diets (#D12492, 60 kcal% fat)	HFD causes rapid, non-apoptotic cleavage of caspase-3 in astrocytes.	Guyenet et al., 2013
ARC	C57BL/6J mice (6 weeks old)	No information	6 to 8 weeks	Research Diets (#D12451, 45 kcal% fat)	The absence of leptin receptors in astrocytes attenuated pSTAT3 signaling, induced reactive gliosis and DIO.	Wang et al., 2015
ARC VMH	C57BL/6J mice (8 to 10 weeks old)	Male	24 h	Research Diets (60 kcal% fat)	Inhibition of astrocytic NFκB signaling reactive astrogliosis in the mediobasal hypothalamus and resulted in hyperphagia.	Buckman et al., 2015
ARC LHA	C57BL/6J mice (8 weeks old)	Male	2 or 6 months	Specialty Feeds (SF04 - 001, 43 kcal% fat)	The effect of HFD exposure on neuronal number and volume of ARC NPY and POMC neurons, and orexin neurons	Lemus et al., 2015
ARC	hGFAP-CreERT2:IRf/f mice (6 weeks old)	Male	12 weeks	Research Diets (HFHS, high-fat and high-sugar; 58 kcal% fat w/sucrose)	Conditional deletion of astrocytic insulin receptor in HFHS results in no difference in body weight or food intake.	Garcia-Caceres et al., 2016
ARC	C57BL/6J mice	No information	8 months	Research Diets (#D12331, 58 kcal% fat w/ sucrose)	Postnatal ablation of LPL in GFAP-expressing astrocytes induced exaggerated body weight gain and glucose intolerance in HFD.	Gao et al., 2017
ARC	C57BL/6J mice (adult)	Male	6 weeks	Research Diets (#D12492, 60 kcal% fat)	Conditional deletion of astrocytic IKKβ in HFD mice results in reduced astrogliosis and inflammation, which prevent further weight gain.	Douglass et al., 2017
ARC	C57BL/6J mice (2 to 3 months old)	Male	10 days or 20 weeks	Specialty Feeds (SF04 - 001, 43 kcal% fat)	Short-term HFD increases the presence of astrocytes without altering leptin sensitivity.	Balland and Cowley, 2017

Table 1. Continued

Region	Species and age	Sex	Feeding period	Diet	Proposed mechanism	Reference
ARC VMH PVN	C57BL/6J mice (adult)	Male	3 or 5 months	Research Diets (#D12492, 60 kcal% fat)	During overnutrition, astrocytic activation via IKKβ/NF-κB seems to modify astrocytic morphology within the mediobasal hypothalamus.	Zhang et al., 2017
ARC	Sprague-Dawley rats (6 to 7 weeks old)	Male	12 weeks	Harlan (TD 0.88137, 42 kcal% fat)	Central leptin signaling occurs via neuron-astrocyte interactions in the ARC and contributes to the exaggerated sympathoexcitation.	Liu and Zheng, 2019
ARC PVN	C57BL/6J mice	No information	8 or 16 weeks	Research Diets (#D12492, 60 kcal% fat)	Astrocyte-specific Myd88 KO mice displayed ameliorated hypothalamic reactive gliosis and were resistant to DIO.	Jin et al., 2020
ARC	C57BL/6J mice (8 to 10 weeks old)	Male	1, 3, or 6 h	Safe (#U8954P V0100, 40.9 kcal% fat)	Dietary fat exacerbates postprandial hypothalamic inflammation involving GFAP-positive cells.	Cansell et al., 2021
PVN ARC DMH VMH LHA	Wistar Kyoto (WKY) rats and C57BL/6J mice (adult)	Male and female	Rats: 4 weeks Mice: 2 or >20 weeks	Research Diets (#D12331, 58 kcal% fat and sucrose)	During DIO, profound remodeling of the gliovascular interface results in arterial hypertension. This process is driven by HIF1α-VEGF signaling in astrocytes.	Gruber et al., 2021
LHA	C57BL/6J mice (6 weeks old)	Male	6 to 23 weeks	Research Diets (#D12492, 60 kcal% fat)	In DIO, reactive astrocytes releasing GABA, synthesized by MAOB, tonically inhibit GABRA5-positive neurons.	Sa et al., 2022

SCN, suprachiasmatic nucleus; LPL, lipoprotein lipase; pSTAT3, phosphorylated signal transducer and activator of transcription 3; HIF1α, hypoxia-inducible factor 1-α.

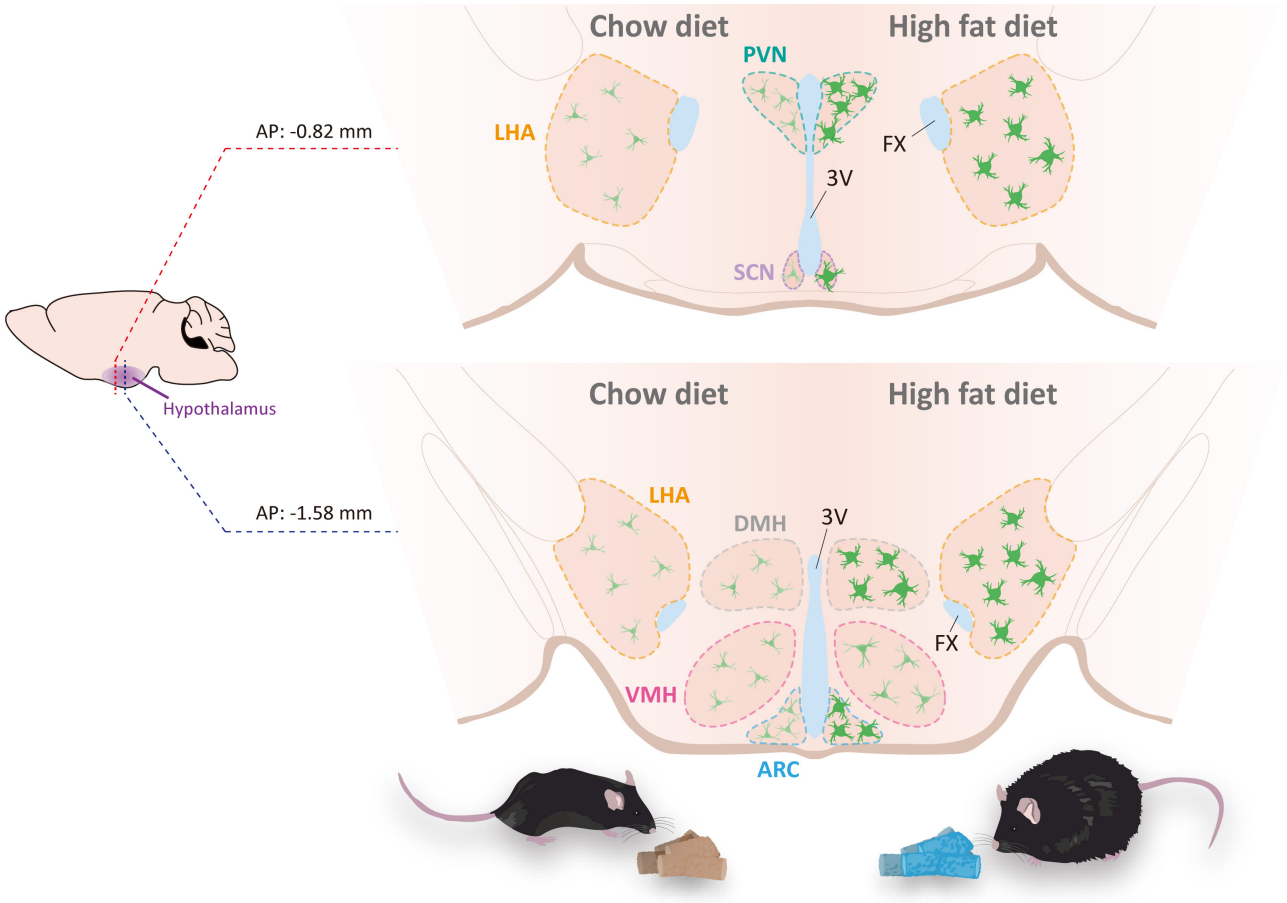

Fig. 1. Regional reactive astrocytes in hypothalamus. Distribution of reactive astrocytes in coronal sections of hypothalamus along the AP axis in chow diet versus high fat diet-fed rodents. AP, anterior-posterior; 3V, third ventricle; FX, fornix.

HFD act to further exacerbate the dysregulated homeostasis (Buckman et al., 2015; Thaler et al., 2012).

In other hypothalamic regions such as PVN and DMH, reactive astrocytes are readily found after chronic HFD feeding for 20 weeks in female DIO mice, whereas comparatively fewer reactive astrocytes are seen in the VMH (Buckman et al., 2013). Most studies on the DIO mouse model have been conducted with C57BL/6J mouse, as it is the most susceptible mouse line to obesity and obesity-related phenotypes (Montgomery et al., 2013). Additionally, male mice have been used in most studies (Table 1). This is because they are more susceptible to weight changes during the same period than females (Arcones et al., 2019; Hong et al., 2009). However, the previous study comparing GFAP immunoreactivity in the hypothalamus between chow-fed and HFD-fed mice has been performed with females (Buckman et al., 2013). Therefore, reactive astrocytes in PVN, DMH, and VMH need to be re-examined in male DIO mice. Moreover, functional studies on reactive astrocytes in these areas are very few so future studies are needed.

Astrocytic VEGF

Chronic HFD over 20 weeks induces reactive astrocytes that affect the structure of blood-brain barrier (BBB), which makes it difficult for POMC and NPY cell bodies and dendrites to access blood vessels (Horvath et al., 2010; Yi et al., 2012). POMC neurons have been shown to lose excitatory synapses in DIO with a significantly greater number of inhibitory inputs and increased glial coverage (Horvath et al., 2010). Astrocytes in ARC secrete VEGF under chronic HFD feeding, and this VEGF signaling increases BBB permeability (Argaw et al., 2012; Lee et al., 2020). This profound remodeling of gliovascular interface after chronic HFD has been reported to be driven by elevation of HIF1α-VEGF signaling and leptin levels (Gruber et al., 2021).

Astrocytic NF-κB

In ARC, astrocytic leptin receptors have been associated with the reactivity of astrocytes in DIO (Liu and Zheng, 2019). After short-term HFD feeding for 10 days, reactive astrocytes have been observed without altered leptin sensitivity (Balland and Cowley, 2017), whereas chronic HFD feeding over 2 months induces upregulation of leptin receptor-positive reactive astrocytes (Hsuchou et al., 2009). In contrast, astrocyte-specific deletion of leptin receptors attenuates hypothalamic pSTAT3 signaling, but still induces astrogliosis

and promotes the development of DIO (Wang et al., 2015), suggesting that astrocytic leptin receptors might not be the cause of astrocytic reactivity and that pSTAT3 signaling may not mediate the astrocytic reactivity. Therefore, the involvement of leptin receptors and pSTAT3 signaling in reactive astrogliosis of ARC remains controversial in DIO.

Unlike that of astrocytic leptin receptors, the involvement of astrocytic NF-κB signaling has been more clearly demonstrated. Inhibition of NF-κB signaling in astrocytes prevents reactive astrogliosis after HFD feeding (Buckman et al., 2015). Further, conditional deletion of astrocytic IKKβ in mice after 6 weeks of HFD reduces the reactivity of astrocytes and neuroinflammation in ARC, which results in a reduction in food intake and an increase in energy expenditure (Douglass et al., 2017). Furthermore, astrocytic reactivation via IKKβ/NF-κB signaling modifies the astrocytic morphology and elevates extracellular GABA level with decreased BDNF expression within mediobasal hypothalamus after 5 months of HFD feeding (Zhang et al., 2017). However, how elevated extracellular GABA level leads to a reduction in food intake and an increase in energy expenditure is still unknown. Conditional deletion of astrocytic myeloid differentiation primary response 88 (Myd88), which can activate intracellular inflammatory signaling cascades such as NF-κB pathways (de Git and Adan, 2015; Santamarina et al., 2018), ameliorates the reactive astrogliosis and neuroinflammation induced by chronic HFD and results in resistance to DIO (Jin et al., 2020). Based on these findings, it has been proposed that activation of neuroinflammatory signaling pathways such as Myd88 and IKKβ/NF-κB signaling disrupt the leptin signaling pathways, thereby hampering the sensing of metabolic signals (Cai and Liu, 2011; Lee et al., 2020). However, further investigations are needed to better understand the involvement of leptin signaling in reactive astrogliosis under various neuroinflammatory conditions.

Astrocytic MAOB-dependent GABA

LHA occupies a relatively large portion of the hypothalamus and polysynaptically innervates adipose tissues, indicating that it is an important region for energy balance and fat storage. However, it has received little attention from the perspective of reactive astrocytes in DIO. From the neuronal perspective, it has been reported that chronic HFD feeding reduces orexin-positive neuronal population with a selective loss of neurons with relatively large volumes (Lemus et al., 2015). Orexin-expressing neurons contribute to increased food intake (Baird et al., 2009), regulate brown adipose tissue activity, and enhance energy expenditure (Martins et al., 2016; Tupone et al., 2011). In Wistar rats fed with HFD for 8 weeks, it has been consistently found that apoptosis is significantly increased, whereas synaptic input is significantly decreased in LHA (Moraes et al., 2009). Moreover, neuronal dysfunction and decreased neuronal activity after chronic HFD exposure have been reported (Moraes et al., 2009; Sa et al., 2022). Recently, a unique population of GABRA5-positive neurons in LHA has been discovered, and these neurons display pacemaker firing activity, which is decreased after HFD feeding in LHA (Sa et al., 2022). These GABRA5-positive neurons are tonically inhibited by astrocytic GABA, which is synthesized by monoamine oxidase-B (MAOB) in reactive astrocytes (Lee et al., 2010; Sa et al., 2022; Yoon et al., 2014). Astrocyte-specific MAOB knockdown in LHA reduces weight gain without altering food intake after HFD feeding (Sa et al., 2022). Moreover, pharmacological inhibition of MAOB leads to significant weight loss without changing food intake in chronic HFD-fed mice and reduces reactive astrogliosis in LHA (Sa et al., 2022). These discoveries call for a drastic paradigm shift in molecular targets for the treatment of obesity towards reducing reactive astrocytes.

CAUSES OF REACTIVE ASTROCYTES

What causes reactive astrogliosis? One obvious candidate is the fatty acid itself in HFD, which acts as a triggering molecule. It is well known that chronic overnutrition increases peripheral fat levels, as well as free fatty acids from the plasma to the brain (Karmi et al., 2010; Wang et al., 1994). High levels of circulating saturated free fatty acids have been found to activate inflammatory signaling in cultured astrocytes (Gupta et al., 2012). In addition, enrichment of saturated fatty acids causes lipid accumulation in the hypothalamus (Borg et al., 2012; Giles et al., 2016; Posey et al., 2009). Among the various types of saturated fatty acids, palmitic acid (16:0), which is the predominant saturated fatty acid in the circulatory system and tissues, is the most common free fatty acid accounting for 21%-30% of human deposited fat (Bysted et al., 2005; de Almeida et al., 2002; Firl et al., 2013; Kingsbury et al., 1961; Liu et al., 2015). Notably, it has been demonstrated that palmitic acid treatment by intracerebroventricular cannulation induces reactive gliosis in the hypothalamus (Jin et al., 2020). Interestingly, long-term treatment of fatty acids increases GABA production in cultured hypothalamic astrocytes, indicating that astrocytes turn into reactive astrocytes (Lee et al., 2018). These studies raise a strong possibility that fatty acids in HFD can cause reactive astrogliosis.

What is the triggering mechanism of reactive astrocytes? In our previous studies, we have demonstrated how common molecular pathways such as MAOB-dependent putrescine degradation and GABA production are shared, even though the triggering factors are different (Chun and Lee, 2018; Chun et al., 2020; Heo et al., 2020; Jo et al., 2014; Nam et al., 2020; Pandit et al., 2020; Shim et al., 2019). We have reported that autophagic degradation pathway is commonly triggered by pathogenic molecules such as diphtheria toxin, Aβ, cytokines, damaged tissue debris, and viral infections, which usually accompany neuroinflammation (Chun et al., 2020; Ju et al., 2021). Under pathological conditions, astrocytes take up or internalize these toxic molecules to degrade them and subsequently turn on the urea cycle to convert the accumulating toxic ammonia to less toxic urea (Cohen, 1981; Ju et al., 2021; Meijer et al., 1990; Morris, 2002). The net consequence of this degradation and turning-on of the urea cycle is the production of putrescine, which further turns on MAOB-dependent production of GABA (Ju et al., 2021). Of note, a recent study has reported that MAOB, which is mainly expressed in astrocytes, is elevated in transcriptionally profiled hypothalamic cells of DIO mice (Rossi et al., 2019). In addition to GABA, excessive hydrogen peroxide (H_2O_2),

a reactive oxygen species (ROS) originating from MAOB in reactive astrocytes, has been shown to cause glial activation and neuronal death (Chun et al., 2020). It is highly likely that the same common molecular mechanism working in the hypothalamus causes reactive astrogliosis and the production of GABA and H_2O_2 in a MAOB-dependent manner (Chun et al., 2020; Sa et al., 2022). Therefore, elevated free fatty acids, acting as pathogenic molecules during chronic HFD, may produce putrescine by activating the urea cycle of astrocytes and MAOB produces GABA and H_2O_2 in astrocytes, the process by which astrocytes turn into reactive astrocytes (Fig. 2). This interesting hypothesis needs to be further investigated and validated in the future.

CONSEQUENCES OF REACTIVE ASTROCYTES

What are the functional consequences of reactive astrocytes? It is well known that chronic HFD induces metabolic damages in hypothalamic neurons. For example, POMC neurons in ARC have shown elevated autophagy, an independent marker of neuronal stress or injury, in mice fed HFD for 8 months (Thaler et al., 2012). In other hypothalamic regions, signs of neuronal injury, including long-lasting desensitization of neurons and a reduction in synaptic inputs, have also been observed in DIO (Beutler et al., 2020; Moraes et al., 2009). In response to chronic HFD, homeostatic circuits of neuronal activity are disrupted (Beutler et al., 2020). Moreover, chronic HFD impairs neuronal responses to nutrients and hormones in a way that is expected to promote weight gain, which lasts for weeks after mice have been returned to a low-fat diet and lost weight (Beutler et al., 2020).

Long-lasting damage to hypothalamic neurons could be a direct consequence of reactive astrocytes. Reactive astrocytes produce and release inflammatory factors including tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) in response to fatty acid treatment (Gupta et al., 2012). However, the

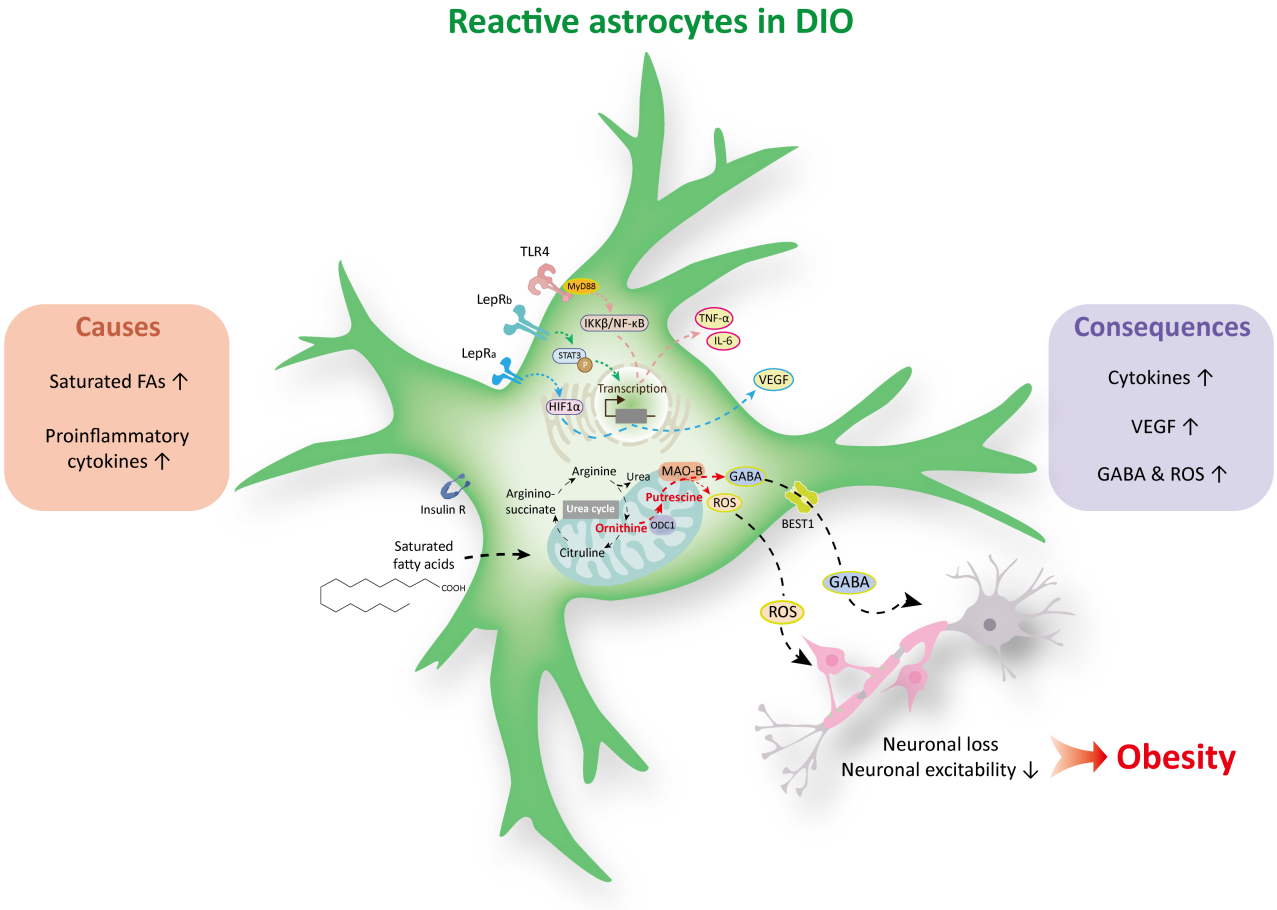

Fig. 2. Molecular mechanisms of causes and consequences of reactive astrocytes in DIO. Astrocytes express several receptors, such as toll-like receptor 4 (TLR4), leptin receptor (LepR), and insulin receptor (Insulin R). High levels of circulating fatty acids (FAs) in DIO can induce TLR4 activation. MyD88, an adaptor for TLRs, can activate IKKb/NF-κB pathways, which in turn trigger the downstream activation of cytokines such as TNF-α and IL-6. Long-form leptin receptor ($LepR_b$) activates pSTAT3 signaling, which triggers downstream transcriptions. Short-form leptin receptor ($LepR_a$) activates HIF1α to increase VEGF, which increases BBB permeability. Circulating FAs can be taken up by astrocytes, which can turn on the urea cycle. Putrescine, produced from ornithine via ornithine decarboxylase 1 (ODC1), is converted to GABA. Excessive GABA and H_2O_2 via MAOB can induce neuronal death and decrease neuronal excitability. These cascades eventually lead to obesity.

consequences of these pro-inflammatory factors are rather contradictory. It has been reported that high concentrations of TNF-α injection lead to weight loss in mice, whereas low concentrations of TNF-α injection result in sufficient changes to cause obesity (Arruda et al., 2011). Along with these conflicting results, mice with astrocyte-specific overexpression of IL-6 exhibit reactive astrocytes, but no weight change in response to HFD (Hidalgo et al., 2010). From these reports, it is possible to conclude that pro-inflammatory factors from reactive astrocytes might not directly cause long-lasting neuronal damage.

Intriguingly, mice with astrocytic IKKβ/NF-κB activation after chronic HFD feeding not only show increased expression of several pro-inflammatory genes such as TNF-α and IL-6 (Liu et al., 2017), but also a significantly elevated level of extracellular GABA in the hypothalamus (Gonzalez-Garcia and Garcia-Caceres, 2021; Zhang et al., 2008; 2017). Astrocytes release numerous neuroactive molecules, including the classical inhibitory neurotransmitter GABA (Garcia-Caceres et al., 2019; Lee et al., 2010; Yoon and Lee, 2014). Accumulating evidence suggest that elevated levels of GABA in reactive astrocytes act as a common molecular mechanism in various neuroinflammatory diseases (Chun and Lee, 2018), such as Alzheimer's disease (Chun et al., 2020; Jo et al., 2014), Parkinson's disease (Heo et al., 2020), recovery after stroke (Nam et al., 2020), stab-wound injury (Chun et al., 2022), epileptic seizure (Pandit et al., 2020) and inflammation-induced anxiety (Shim et al., 2019). Consistently, it has been reported that extracellular GABA in mediobasal hypothalamus (ARC and VMH) is elevated in DIO mice (Zhang et al., 2017). In our recent study, we have demonstrated that GABA from reactive astrocytes in LHA after chronic HFD feeding contributes to exacerbation of obesity (Sa et al., 2022). Mechanistically, MAOB-dependent GABA from reactive astrocytes tonically and strongly inhibits the excitability of the newly identified GABRA5-positive GABAergic pacemaker firing neurons that facilitate energy expenditure, resulting in increased fat mass and body weight in DIO mice (Sa et al., 2022). Along with elevated GABA production, MAOB-dependent production of H_2O_2 is the key common molecular switch that turns on a distinct state of severe reactive astrocytes, and the toxic level of H_2O_2 is sufficient for neurodegeneration (Chun et al., 2020). Taken together, these recent discoveries strongly suggest that the accumulation of toxic H_2O_2 in hypothalamic reactive astrocytes might directly contribute to the long-lasting impairment and loss of neighboring neurons in the hypothalamus (Fig. 2). This exciting possibility awaits future investigation.

CONCLUSIONS AND FUTURE QUESTIONS

We have comprehensively reviewed the role of hypothalamic reactive astrocytes (Table 1), the regional distribution of reactive astrocytes in the hypothalamus (Fig. 1), the triggering mechanisms of reactive astrocytes, and the consequences of reactive astrocytes in DIO (Fig. 2). Indeed, reactive astrocytes are actively involved in the pathogenesis of DIO. Mechanistically, elevated free fatty acids during chronic HFD feeding activate inflammatory signals via IKKβ/NF-κB pathway and produce putrescine possibly by turning on the urea cycle in reactive astrocytes. The putrescine produced is then degraded by MAOB to GABA in reactive astrocytes. This GABA is tonically released and inhibits the excitability of neighboring neurons, especially those of the newly identified GABRA5-positive neurons, which facilitate energy expenditure. This cascade of events leads to the exacerbation of obesity in DIO. In addition, the role of MAOB-dependent H_2O_2 is still unknown and further investigation is needed to clarify the relationship between toxic H_2O_2 from reactive astrocytes and the loss of hypothalamic neurons.

Although the presence of reactive astrocytes in the hypothalamus after HFD has been observed for a long time, the role of reactive astrocytes in DIO has only recently been investigated. Moreover, most studies have focused on reactive astrocytes in ARC in response to HFD. Since overall metabolic signals from ARC are conveyed to and act on other hypothalamic regions, it is necessary to deeply understand the role of reactive astrocytes in other regions of the hypothalamus, as has been recently discovered in LHA. These newly developed approaches and tools will be very useful for developing potentially effective therapeutic strategies to fight against obesity with minimal side effects.

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AUTHOR CONTRIBUTIONS

M.S. and C.J.L. discussed the contents of the manuscript and wrote it. M.G.P. made the table and figures.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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