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Research report

High-fat diet suppresses the astrocytic process arborization and downregulates the glial glutamate transporters in the hippocampus of mice

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ABSTRACT

Metabolic disorders induce adverse effects on brain functions. The hippocampus is one of the most vulnerable regions to metabolic disorders. Disrupted neuroplasticity is a major cause of hippocampus-related behavioral impairments, including memory loss, anxiety, and depression. Astrocytes support processes of neuroplasticity. However, whether metabolic disorders induce changes in astrocytes and their roles in affective disorders is relatively unclear. To answer this question, we fed 8-week-old male C57BL/6 mice with a high-fat diet (HFD) for 12 weeks to induce metabolic disruption and then examined their performance of hippocampus-related memory, and anxiety- and depression-like behaviors. The morphology of astrocytes and the expression of astrocytic neuroplasticity-related proteins in the hippocampus were also assessed. The results showed that HFD led to obesity, systemic insulin resistance and dysregulated lipid metabolism in mice. HFD induced depression-like behaviors, but not anxiety or memory impairment. Furthermore, HFD increased the expression of GFAP, shortened the processes of $GFAP^+$ cells, and downregulated the expression of astrocytic neuroplasticity-related protein, GLAST, GLT-1, and connexin-43 in the hippocampi. In conclusion, HFD disturbs the function of hippocampal astrocytes and induces depression-like behaviors in mice. A decrease of hippocampal glutamate transporters may play a critical role in the pathogenesis of metabolic disorder-related depression.

1. Introduction

Metabolic disorders are emerging as one of the major medical and public health problems worldwide and are known to induce adverse effects on brain function (Arnold et al., 2018; Moheet et al., 2015; Raji et al., 2010). The hippocampus is one of the most vulnerable brain regions to systemic metabolic abnormalities (Biessels and Reagan, 2015; Spinelli et al., 2017). The hippocampal dysfunctions are implicated in the development of memory deficits and mood disorders, such as anxiety and depression (Bird and Burgess, 2008; Campbell and Macqueen, 2004; Engin and Treit, 2007). Interestingly, cognitive impairments and emotional illness are potential complications of metabolic disorders (Marazziti et al., 2014; Yaffe, 2007). The disrupted neuroplasticity has been known to be a major cause of these hippocampus-related behavioral impairments (Fuchs et al., 2004; Pittenger and Duman, 2008; Pittenger, 2013). Processes of neuroplasticity are supported by glial cells, including microglia, oligodendrocytes, and astrocytes (Allen, 2014). Among them, astrocytes have been shown to regulate both short-term and long-term synaptic plasticity, maintain the homeostasis of synaptic scaling and modulate the neurotransmission in the hippocampus (Andersson and Hanse, 2011; Chung et al., 2015; Ota et al., 2013).

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Astrocytes are critical for the neurotransmitter clearance, recycling, and synthesis. In the hippocampus, a high concentration of glutamate is released into the synaptic cleft during active neurotransmission and then taken up by the astrocytic glutamate transporters, i.e., GLT-1 and GLAST, which are estimated to remove 80% of released glutamate (Lopez-Bayghen and Ortega, 2011) which is subsequently converted to glutamine by glutamine synthetase (GS) in the astrocytes. The glutamine is released back to the extracellular space and then taken up by the neighboring neurons (Uwechue et al., 2012). Functionally, these astrocytic glutamatergic transmission-mediating proteins are linked to memory deficits and depression. It has been demonstrated that inhibition of GS activity hampers the consolidation of memory for passive avoidance task in chicks (Gibbs et al., 1996). Blocking of GLT-1 impairs the memory performance in rats (Bechtholt-Gompf et al., 2010), while the hippocampal levels of the GLT-1 complex are positively correlated with the memory function in mice (Heo et al., 2012). Furthermore, expressions of EAAT1 and EAAT2 (alternative terms of GLAST and GLT-1 in humans) are decreased in the hippocampi of patients with depression (Medina et al., 2013). These findings suggest that astrocytic functions are intimately associated with cognition and affective behaviors.

The astrocyte is an active player in brain energy delivery, production, utilization, and storage (Belanger et al., 2011). During the neuronal firing, a large portion of glucose entering the glycolytic pathway in astrocytes is released as lactate into the extracellular space via monocarboxylate transporters 1 and 4 (MCT1 and 4). The glycolysis-generated lactate is then taken up by neurons as an energy fuel to overcome the energy demand required for synaptic activity via MCT2 (Bouzier-Sore and Pellerin, 2013; Pellerin and Magistretti, 1994). Moreover, astrocytic lactate also diffuses into adjacent astrocytes via gap junction channels, i.e., connexin-30 and connexin-43 (Cx-30 and Cx-43) which link neighboring astrocytes to form an astrocyte metabolic network (Escartin and Rouach, 2013; Rouach et al., 2008). It has been demonstrated that the lactate produced by astrocytes is required for memory formation in rodents (Suzuki et al., 2011). Moreover, the peripheral administration of lactate produces antidepressant-like effects in mice (Carrard et al., 2018). These results suggest that the astrocytes play an essential role in regulating the neurotransmission and energy supply. It has been demonstrated that metabolic disorders affect the structure and function of astrocytes in the hypothalamus, the central metabolic center. For example, short-term high-fat diet (HFD) feeding increases the population of astrocytes (Balland and Cowley, 2017). Long-term HFD feeding induces a shrinkage of astrocyte processes and subsequently weakens the GABA reuptake ability of these astrocytes (Sharif and Prevot, 2017). However, whether metabolic disorders perturb astrocytes in the hippocampus remain relatively unclear.

Here, we used HFD-induced obesity in the mouse as an animal model to investigate the effects of metabolic disorders on the performances of hippocampus-related behaviors and the hippocampal astrocytes. Body weight gain and systemic insulin sensitivity were used as indicators of metabolic disorders. The hippocampus-related spatial and non-spatial memories, and anxiety- and depression-like behaviors were examined in these mice. The effects of HFD on the morphological properties of astrocytes and the expression of astrocytic neuroplasticity-related proteins in the hippocampus were also analyzed.

2. Results

2.1. HFD induces metabolic disorders in mice

We fed 8-week-old mice with HFD for 12 weeks to induce metabolic disorders (Fig. 1A, left panel). Repeated measures two-way ANOVA revealed that the body weights of the HFD group were significantly larger than those of the Chow group [$F(1, 8) = 781.6$, $p < 0.0001$] (Fig. 1A, middle panel). Post-hoc analysis showed that the body weight differences between the two groups began after 4 weeks of HFD feeding (Fig. 1A, middle panel). At the end of the 12-week feeding, the body weights of the HFD group increased 50% more than those of the Chow group ($t = 9.6$, $d.f. = 8$, $p < 0.0001$, Student's t-test, Fig. 1A, right panel). Interestingly, the energy consumptions were comparable between Chow and HFD groups [$F(1, 8) = 3.1$, $p = 0.115$, repeated measures two-way ANOVA, Fig. 1B]. The HFD feeding induced insulin resistance in mice, including higher levels of fasting plasma glucose ($t = 2.7$, $d.f. = 8$, $p = 0.026$, Student's t-test), fasting plasma insulin ($t = 5.0$, $d.f. = 8$, $p = 0.001$, Student's t-test), and the HOMA-IR index ($t = 4.5$, $d.f. = 8$, $p = 0.002$, Student's t-test) (Fig. 1C). In addition, the levels of plasma glucose in the intra-peritoneal glucose tolerance test [IPGTT, $F(1, 8) = 10.1$, $p = 0.013$, repeated measures two-way ANOVA] and intra-peritoneal insulin tolerance test [IPITT, $F(1, 8) = 113.0$, $p < 0.0001$, repeated measures two-way ANOVA] of the HFD group were higher than those of the Chow group (Fig. 1C). Moreover, HFD also disrupted lipid metabolism in mice as evident by increased levels of circulating free fatty acid (FFA, $t = 7.6$, $d.f. = 8$, $p < 0.0001$, Student's t-test), high-density lipoprotein (HDL, $t = 4.6$, $d.f. = 8$, $p = 0.002$, Student's t-test) and low-density lipoprotein/very low-density lipoprotein (LDL/VLDL, $t = 6.0$, $d.f. = 8$, $p = 0.0003$, Student's t-test) (Fig. 1D). The levels of plasma triglyceride were not affected by HFD feeding ($t = 0.8$, $d.f. = 8$, $p = 0.442$, Student's t-test).

2.2. HFD does not affect the hippocampus-related memory in mice

After establishing the mice model with metabolic disorders, we further characterized the effects of HFD on the performances of hippocampus-related behaviors. The hippocampus-related spatial memory was examined using the Morris water maze (MWM) test (Shih et al., 2016), and the hippocampus-related non-spatial memory was examined using the object recognition test (ORT) (Lin et al., 2015). Results showed that HFD did not alter the swimming speed ($t = 0.6$, $d.f. = 8$, $p = 0.568$, Student's t-test) and the performance of mice in the MWM during the learning phase [$F(1, 8) = 0.0$, $p = 0.858$, repeated measures two-way ANOVA] or the memory retrieval phase (probe test, $t = 0.6$, $d.f. = 8$, $p = 0.581$, Student's t-test) (Fig. 2A). The performance of mice in the ORT was also unaffected by HFD ($t = 1.7$, $d.f. = 8$, $p = 0.128$, Student's t-test) (Fig. 2B).

2.3. HFD decreases the locomotor activity but does not induce anxiety in mice

The open field test (OFT) and elevated plus maze (EPM) test were used to examine the effects of HFD on the exhibitions of anxiety-like behaviors in mice (Tatem et al., 2014; Walf and Frye, 2007). In the OFT, HFD decreased the total travel distance ($t = 2.6$, $d.f. = 8$, $p = 0.032$, Student's t-test; Fig. 3A) of mice. Consistently, the HFD mice had higher immobilized time fraction during the OFT ($t = 2.9$, $d.f. = 8$, $p = 0.021$, Student's t-test; Fig. 3A). However, the time spent in the center zone was not statistically different between the Chow and HFD groups ($t = 1.6$, $d.f. = 8$, $p = 0.150$, Student's t-test; Fig. 3A). In the EPM, HFD did not alter the measured parameters, including the time spent in the open arms ($t = 0.2$, $d.f. = 8$, $p = 0.833$, Student's t-test), the number of entries into the open arms ($t = 1.0$, $d.f. = 8$, $p = 0.347$, Student's t-test), the time spent in the center zone ($t = 0.4$, $d.f. = 8$, $p = 0.701$, Student's t-test), and number of entries into the center zone ($t = 1.0$, $d.f. = 8$, $p = 0.359$, Student's t-test) (Fig. 3B).