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Diet-induced cellular neuroinflammation in the hypothalamus: Mechanistic insights from investigation of neurons and microglia

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ABSTRACT

Diet-induced obesity can lead to detrimental chronic disorders. The severity of this global epidemic has encouraged ongoing research to characterize the mechanisms underlying obesity and its comorbidities. Recent evidence suggests that saturated fatty acids (SFA) in high-fat diets rapidly generate inflammation in the arcuate nucleus of the hypothalamus (ARC), which centrally regulates whole-body energy homeostasis. Herein, we will review the roles of hypothalamic neurons and resident microglia in the initiation of SFA-induced hypothalamic inflammation. Particularly, we focus on neuronal and microglial free fatty acid-sensing and capacity to produce inflammatory signaling. We also outline a potential role of peripherally-derived monocytes in this inflammation. And finally, we explore synaptic plasticity as a mechanism through which hypothalamic inflammation can modulate ARC circuitry, and thus disrupt energy homeostasis.

1. Introduction

Obesity represents an alarming pandemic, prevalent in not only developed nations but also in emerging nations (Swinburn et al., 2011). Evidence indicates that obesity is associated with metabolic syndrome, a cluster of risk factors for threatening conditions such as heart disease and stroke (Monteiro and Azevedo, 2010). Obesity is marked by the accumulation of excess body fat, and is caused by energy imbalance, particularly the consumption of fat-rich diets (Swinburn et al., 2011). Investigating the pathogenesis of obesity, it was discovered that a high-fat diet triggers inflammation in the hypothalamus (Thaler et al., 2012). This finding spurs particular concern considering: 1) the well-described role of inflammation in the development of peripheral insulin resistance; and 2) the central role of the hypothalamus in maintaining energy homeostasis. Nuclei in the hypothalamus, for example the ventromedial nucleus, paraventricular nucleus, and arcuate nucleus, integrate peripheral signals and tightly regulate feeding behavior and energy expenditure (Schwartz et al., 2000). Such nuclei are composed of a heterogeneous complement of cells, including neurons and microglia, among other relevant cell types. At present, however, we do not fully understand the roles of neurons and microglia in HFD-associated hypothalamic inflammation. This review aims to consolidate a picture of the mechanisms through which high-fat diet may cause hypothalamic inflammation.

2. The arcuate nucleus of the hypothalamus (ARC) dictates energy homeostasis

The arcuate nucleus (ARC), a region of the mediobasal hypothalamus (MBH) adjacent to the third ventricle, is a key player in regulating energy homeostasis (Könner et al., 2009; Gao and Horvath, 2008). Specifically, two ARC neuronal populations integrate peripheral and paracrine cues, and signal to second order neurons to modulate food intake and energy expenditure (Schwartz et al., 2000; Barsh and Schwartz, 2002). Namely, Agouti-related peptide/neuropeptide Y/γ-aminobutyric acid neurons (AgRP/NPY/GABA neurons) secrete the neuroendocrine peptides AgRP and NPY, and the neurotransmitter GABA to increase food intake and reduce energy expenditure (Schwartz et al., 2000; Barsh and Schwartz, 2002; Aponte et al., 2011; Tong et al., 2008; Wu and Palmiter, 2011). In contrast, proopiomelanocortin (POMC) neurons

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secrete the neuroendocrine peptide α-melanocyte-stimulating hormone (α-MSH) to decrease food intake and induce energy expenditure (Barsh and Schwartz, 2002). This hypothalamic circuitry is essential for maintaining normal energy homeostasis; whereas disruption of this network, for example in genetic deficiency of the α-MSH receptor melanocortin 4 receptor (M4CR), can result in an obesity phenotype (Farooqi et al., 2003; Santini et al., 2009). In addition, non-neuronal cells, including astrocytes (also astroglia) and microglia, also reside in the ARC (Lemus et al., 2015). These glial cells modulate neuronal function by mediating synaptic remodeling and central nervous system (CNS) immune responses (Bernardinelli et al., 2014; Tremblay et al., 2011).

3. Fenestrated vascularization allows ARC neurons and glia to sense peripheral signals

The ARC is vascularized by fenestrated vessels conducting peripheral signaling molecules into the hypothalamus (Ciofi, 2011; Ciofi et al., 2009). This "leaky blood-brain barrier (BBB)" at the ARC allows AgRP/NPY/GABA and POMC neurons to rapidly and directly sense peripheral regulatory signals such as ghrelin (Schaeffer et al., 2013). Moreover, this permeability allows ARC cells to sense and respond to nutrient signals such as levels of plasma free fatty acids (FFAs) (Lam et al., 2005a,b).

Increased FFA levels in the plasma are associated with increased FFA levels in the hypothalamus. For example, intravenous infusion of intralipid, a lipid emulsion composed of long-chain FFAs (LCFAs), increases levels of esterified LCFAs in the MBH (Lam et al., 2005a,b). Moreover, in the context of diet-induced obesity, it has been long proposed that high-fat diet (HFD) raises plasma FFAs, thereby also raising FFA levels in the hypothalamus. Recently, it has been demonstrated that after four weeks, a HFD rich in the saturated fatty acid (SFA) palmitate specifically increases palmitate levels in the hypothalamus (Posey et al., 2009; Valdearcos et al., 2014). Further, it was also recently demonstrated that enteral administration of palmitate-rich milk fat leads to rapid accumulation of palmitate in the ARC (Valdearcos et al., 2014). From these observations, we may speculate that ARC cells sense not only chronic elevations in FFA levels associated with prolonged HFD but also acute, postprandial elevations derived from "fatty" meals. Thus, it may be relevant to study the effects of rapid and prolonged high FFA levels on ARC cells. In brief, the vascularization of the ARC allows AgRP/NPY/GABA neurons, POMC neurons, and microglia to rapidly and directly sense peripheral signals such as acute or chronic changes in plasma FFA levels.

4. High-fat diet (HFD) rapidly induces hypothalamic inflammation through poorly understood mechanisms

Recently, a major study by Thaler et al. described the time course of hypothalamic injury over four weeks of HFD. This and other studies suggest that there is an initial acute, adaptive response, followed by a more pronounced reaction upon longer-term exposure to SFAs (Fig. 1). It was demonstrated that HFD rapidly induces hypothalamic inflammation and neuronal injury: one to three days of HFD increased expression of pro-inflammatory genes such as *Interleukin-1β* (*Il1b*) and *Inhibitor of nuclear factor kappa-B kinase* (*Ikbkb*), as well as a marker of neuronal injury, heat shock protein 72 (HSP72) (Thaler et al., 2012). This inflammation is followed by reactive gliosis, that is, microglial and astrocytic activation and accumulation. However, the early inflammatory response subsides, suggesting it is an adaptive response to overcome neuronal injury. And ultimately, with prolonged HFD, hypothalamic inflammation returns (Thaler et al., 2012; Berkseth et al., 2014). Despite these detailed observations, we do not completely understand the mechanisms that generate the initial, transient hypothalamic inflammatory response to HFD. In particular, we do not know which hypothalamic cell populations mediate this response.

Reactive gliosis only occurs after hypothalamic neuronal injury and inflammation has already been established (Thaler et al., 2012). Thus, the initial hypothalamic inflammatory response to HFD may be generated by hypothalamic neurons that sense elevated FFA levels.

5. Hypothalamic neurons may sense and initiate adaptive responses to high free fatty acid (FFA) levels

Neurons can sense circulating FFAs and generate adaptive responses to high FFA levels; thus, neurons may mediate the initial hypothalamic inflammatory response to HFD (Fig. 2). Neurons may directly sense FFAs through the expression of toll-like receptors (TLR) such as the TLR4 and TLR2, which mediate the pro-inflammatory effects of FFAs in peripherally-derived monocytes and macrophages (Lee et al., 2001; Lee et al., 2004; Shi et al., 2006; Snodgrass et al., 2013). Moreover, some studies have suggested that CNS-expressed TLR4 may mediate HFD-induced hypothalamic inflammation and diet-induced obesity (DIO) (Kleinridders et al., 2009; Milanski et al., 2009, Moraes et al., 2009). Whole-body TLR4-defective C3H/HeJ mice are protected from DIO, and in these mice, *i.c.v.* FFA injection does not elicit hypothalamic pro-inflammatory gene expression (Milanski et al., 2009). In spite of this, nonetheless, TLR4-defective mice on HFD demonstrate increased apoptotic programming in ARC neurons (Moraes et al., 2009). In addition, nestin (CNS)-targeted knockout of the TLR4 adaptor molecule MyD88 dampens HFD-induced activation of pro-inflammatory NF-κB signaling in the ARC (Kleinridders et al., 2009). These findings may support a neuron-specific role for TLRs in light of evidence that neurons express TLR2 and TLR4. Specifically, TLR4 expression has been determined in primary cortical neurons (Tang et al., 2007), paraventricular hypothalamus (PVN) neurons (Masson et al., 2015), and the rHypoE-7 (Wellhauser and Belsham, 2014), and N43/5 (Choi et al., 2010) murine, immortalized hypothalamic neuronal cell lines. Moreover, TLR2 expression has been demonstrated in mouse ARC neurons *in vivo* and in the mHypoE-42 (N42) hypothalamic neuronal cell line (Shechter et al., 2013). And interestingly, in N42 neurons, exposure to the native TLR2 ligand peptidoglycan induces *c-fos* expression, an indicator of neuroendocrine cell activity (Hoffman et al., 1993). Overall, hypothalamic neurons can express FFA-activated TLRs and may therefore directly sense FFAs. Nonetheless, FFA sensing may not require cell-surface receptors such as TLR4, and may instead be dependent on intracellular FFA metabolism.

FFAs are chemically unreactive; FFA metabolism to form lipid signaling molecules or oxidative metabolites is preceded by the esterification of FFAs into fatty acid acyl-coenzyme A (coA) (Schmelz and Naismith, 2009). It has been demonstrated that pharmacologically inhibiting FFA esterification in the MBH abolishes the ability of FFAs to repress hepatic glucose production (Lam et al., 2005a,b), suggesting that FFA sensing in the hypothalamus requires FFA metabolism. Additionally, inhibiting hypothalamic carnitine palmitoyltransferase 1 (CPT), a key enzyme regulating FFA oxidative metabolism, reduces food intake. Of notice, CPT1 is expressed by AgRP/NPY neurons and primary hypothalamic neurons (Andrews et al., 2008; Zammit and Arduini, 2008; McFadden et al., 2014), and hypothalamic CPT1 inhibition diminishes ARC *Npy* and *Pomc* mRNA expression (Obici et al., 2003). The Belsham lab has also recently demonstrated that in the mHypoA-GnRH/GFP hypothalamic gonadotropin-releasing hormone (GnRH) neuronal cell line, palmitate increases *Gnrh* mRNA expression, and this effect is independent of TLR4 signaling and may instead be mediated by