Molecular and Cellular Neuroscience 80 (2017) 52–57

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Identification of a fatty acid binding protein4-UCP2 axis regulating microglial mediated neuroinflammation

Cayla M. Duffy [a,b], Hongliang Xu [c], Joshua P. Nixon [a,b], David A. Bernlohr [c,d], Tammy A. Butterick [a,b,d,*]

[a] Minneapolis Veterans Affairs Health Care System, Research 151, One Veterans Dr, Minneapolis, MN 55417, USA
[b] Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave, St. Paul, MN 55108, USA
[c] Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, 321 Church St SE, Minneapolis, MN 55455, USA
[d] Minnesota Nutrition and Obesity Center, University of Minnesota, 1334 Eckles Ave, St. Paul, MN 55108, USA

ARTICLE INFO

Article history:
Received 23 September 2016
Revised 7 February 2017
Accepted 13 February 2017
Available online 16 February 2017

Keywords:
Neuroinflammation
Reactive oxygen species
Palmitic acid
Obesity

ABSTRACT

Hypothalamic inflammation contributes to metabolic dysregulation and the onset of obesity. Dietary saturated fats activate microglia via a nuclear factor-kappa B (NFκB) mediated pathway to release pro-inflammatory cytokines resulting in dysfunction or death of surrounding neurons. Fatty acid binding proteins (FABPs) are lipid chaperones regulating metabolic and inflammatory pathways in response to fatty acids. Loss of FABP4 in peripheral macrophages via either molecular or pharmacologic mechanisms results in reduced obesity-induced inflammation via a UCP2-redox based mechanism. Despite the widespread appreciation for the role of FABP4 in mediating peripheral inflammation, the expression of FABP4 and a potential FABP4-UCP2 axis regulating microglial inflammatory capacity is largely uncharacterized. To that end, we hypothesized that microglial cells express FABP4 and that inhibition would upregulate UCP2 and attenuate palmitic acid (PA)-induced pro-inflammatory response. Gene expression confirmed expression of FABP4 in brain tissue lysate from C57Bl/6J mice and BV2 microglia. Treatment of microglial cells with an FABP inhibitor (HTS01037) increased expression of *Ucp2* and *arginase* in the presence or absence of PA. Moreover, cells exposed to HTS01037 exhibited attenuated expression of inducible nitric oxide synthase (iNOS) compared to PA alone indicating reduced NFκB signaling. Hypothalamic tissue from mice lacking FABP4 exhibit increased UCP2 expression and reduced iNOS, tumor necrosis factor-alpha (TNF-α), and ionized calcium-binding adapter molecule 1 (Iba1; microglial activation marker) expression compared to wild type mice. Further, this effect is negated in microglia lacking UCP2, indicating the FABP4-UCP2 axis is pivotal in obesity induced neuroinflammation. To our knowledge, this is the first report demonstrating a FABP4-UCP2 axis with the potential to modulate the microglial inflammatory response.

1. Introduction

Saturated fatty acids (SFAs) such as palmitic acid (PA) contribute to the onset of metabolic inflammatory diseases, including obesity, in part through hypothalamic dysregulation and degeneration (Cai, 2013; Karmi et al., 2010; Thaler et al., 2012; Valdearcos et al., 2014). In the hypothalamus, dietary PA activates microglia (immune cells of the brain) via a nuclear factor kappa B (NFκB)-mediated pathway to release pro-inflammatory cytokines and contribute to damage of neurons responsible for regulating body weight (Thaler et al., 2013; Wang et al., 2012).

Microglia are sensitive and highly dynamic in response to changes in the surrounding microenvironment. As microglia respond to the surrounding environment, they are activated to either a pro-inflammatory (M1) or anti-inflammatory, protective (M2) phenotype, depending on external stimuli. For example, PA activates microglia via a toll like receptor (TLR)-4 and induces the release of pro-inflammatory cytokines and factors such as tumor necrosis factor-alpha (TNF-α) and inducible nitric oxide synthase (iNOS) (Duffy et al., 2015; Valdearcos et al., 2014). Conversely, microglia activated with the anti-inflammatory cytokine interleukin (IL)-4 polarize to an M2 protective state, characterized by the release of anti-inflammatory cytokines and factors such as arginase-1 (Fumagalli et al., 2011). The polarization of microglial cells is a highly energetic process and dependent upon mitochondrial integrity and activation. Uncoupling protein 2 (UCP2) has been implicated in mediating energetic processes of microglial activation states (Emre and Nubel, 2010). Microglia activated to an M1 phenotype have reduced UCP2

Abbreviations: FABP, Fatty acid binding protein; UCP2, uncoupling protein 2; PA, palmitic acid; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor alpha; Iba1, ionized calcium-binding adapter molecule 1; SFA, saturated fatty acids; NFκB, nuclear factor kappa B.

* Corresponding author at: Minneapolis Veterans Affairs Health Care System, Research 151, One Veterans Dr, Minneapolis, MN 55417, USA.
E-mail address: butte017@umn.edu (T.A. Butterick).

http://dx.doi.org/10.1016/j.mcn.2017.02.004
1044-7431/

activity and expression, resulting in increased production of reactive oxygen species (ROS) (De Simone et al., 2015). Conversely, UCP2 activity is robustly increased following activation of an M2 protective phenotype (De Simone et al., 2015), indicating a potential target to manipulate microglial activation states.

UCP2 activity is regulated by fatty acid binding proteins (FABP), lipid chaperones regulating metabolic and inflammatory pathways in response to fatty acids (Hotamisligil and Bernlohr, 2015). Targeted deletion of the adipocyte FABP (FABP4, also known as aP2) is sufficient to prevent obesity induced insulin resistance, diabetes, atherosclerosis and asthma, (Boord et al., 2004; Furuhashi et al., 2007; Hotamisligil et al., 1996; Shum et al., 2006). Mice lacking FABP4 (also referred to as aP2 deficient mice) have been used to extensively characterize diabetes, atherosclerosis and asthma linking FABP4 signaling to important roles in metabolic homeostasis and immunometabolic diseases, as reviewed in (Hotamisligil and Bernlohr, 2015). In peripheral murine macrophages, the loss of FABP4 protects against the development of atherosclerosis and dyslipidemia (Makowski et al., 2001). The loss of FABP4 in macrophages via either molecular or pharmacologic means results in attenuated obesity-induced inflammation through a UCP2-redox based mechanism (Xu et al., 2016; Xu et al., 2015). While the role of the FABP4-UCP2 axis in peripheral macrophages has been extensively characterized, this axis has not been explored in the brain immune cells such as microglia.

We hypothesized that inhibition of FBAP4 in microglia would attenuate PA-induced pro-inflammatory response through a UCP2 mediated mechanism. Herein we demonstrate that mice lacking FABP4 have increased expression of UCP2 and reduced expression of TNF-α, iNOS, and ionized calcium-binding adapter molecule 1 (Iba1, a marker of microglial activation) in the hypothalamus. Moreover, pharmacological inhibition of FABP increases UCP2 expression and reduces PA-induced pro-inflammatory response and ROS production. Further, this effect is negated in microglia lacking UCP2, indicating the FABP4-UCP2 axis is pivotal in obesity-induced neuroinflammation.

2. Materials and methods

2.1. Cell culture and reagents

Immortalized murine microglial cells (BV2) and UCP2 knockdown BV2 microglia (UCP2kd) were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad CA USA) supplemented with 10% fetal bovine serum and 1% penicillin, streptomycin, and neomycin and maintained at 37 °C with 5% CO_2. Pan-FABP inhibitor (HTS01037; Cayman Chemical) was suspended in dimethyl sulfoxide (DMSO; Sigma Aldrich, St. Louis MO USA) and diluted to 30 μM in DMEM. Palmitic acid (PA; Sigma Aldrich) was conjugated to fatty acid free bovine serum (BSA; Sigma Aldrich) and diluted to 0.1 mM in DMEM. Cells were seeded in 6 well plates at 6×10^5 cells per well and grown to ~80% confluency. For gene expression studies, cells were pretreated with HTS01037 or vehicle control for 3 h and challenged with PA (or vehicle) for 12 h.

2.2. shRNA knockdown of UCP2 in microglia

BV2 microglia were transduced with a short hairpin RNA (shRNA) lentivirus as previously described (Curtis et al., 2010). Green fluorescent protein (GFP) scrambled and UCP2 targeting sequences were obtained from Open Biosystems (Pittsburgh, PA USA). The following were used UCP2 (GenBank accession number NM_011671) targeting sequence (UCP2 knockdown; UCP2kd) 5′-CCGGTCTCCCAATGTTGCCCGTAATCT CGAGATTACGGGCAACATTGGGAGATTTTTG-3′; scrambled sequence, 5′-AACGTACGCGGAATACTTCGA-3′. UCP2 expression knockdown is approximately 90% (Supplemental Fig. 1).

2.3. Real-time RT PCR

Whole hypothalamic tissue was dissected from fifteen week old FABP4/aP2 knockout (also referred to as AKO) and wildtype (WT) mice maintained on 60% high fat diet (HFD) for 12 weeks (Hertzel et al., 2006; Kotz et al., 2012). Mice were obtained from our breeding colony and the experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Minnesota. Total RNA was extracted from microglia and hypothalamic tissue with the aid of Trizol (Invitrogen) (Butterick et al., 2012; Chomczynski, 1993). A final concentration of mRNA was determined spectrophotometrically (Nanodrop ND-8000; ThermoFisher Scientific, Waltham MA USA). Real-time thermal cycling were carried out in a Roche LightCycler (Roche Diagnostics Corporation, Indianapolis, IN USA) by one-step RT-PCR using the general method as previously described (Duffy et al., 2015). Target gene expression (Table 1) was determined using SYBR Green detection normalized to GAPDH using the $^{\Delta\text{-}\Delta}$CT method (Livak and Schmittgen, 2001).

Amplification products were separated via electrophoresis on 3% agarose gels stained with SYBR green. qRT-PCR products were purified using a commercially available kit (MinElute PCR Purification, Qiagen Valencia CA USA) and validated using Sanger di-deoxyterminator sequence method at the University of Minnesota Genomics Center.

2.4. Reactive oxygen species assay

Intracellular ROS production was determined using Deep Red Fluorescence kit (Abcam, Cambridge GBR) as previously described (Duffy et al., 2016). Briefly, cells were pretreated with HTS01037 or vehicle for 3 h and then challenged with or without PA for 1 h (time points based on (De Simone et al., 2015)). Cells were then exposed to the ROS Deep Red Dye for 1 h in 5% CO_2 at 37 °C. Intracellular superoxide and hydroxyl radicals react with the deep red dye, producing a fluorescent signal which was measured using a spectrophotometer at 650_{Ex}/675_{Em} (SpectraMax-M5; Molecular Devices, Sunnyvale CA USA). Data are presented as relative fluorescence units.

2.5. Statistical analysis

Significant differences were determined by either a one- or two-way ANOVA followed by Holm-Sidak's correction for multiple comparisons using Graph Pad Prism 6 (GraphPad Software, Inc. La Jolla CA USA). Letters indicate significant differences between treatment groups (e.g. columns with the same letters do not differ from each other, while columns with different letters are significantly different).

3. Results

3.1. Microglial cells express FABP4 and FABP5, but not FABP7

To verify that microglial cells express FABP, real-time PCR products of FABP4 (Fig. 1A), FABP5 (Fig. 1B), and FABP7 (Fig. 1C) were separated via electrophoresis and visualized on an agarose gel. Similar to

Table 1
Real-time qPCR primer sequences.

Target	Forward primer (5′–3′)	Reverse primer (5′–3′)
Arginase	TAACCTTGGCTTGCTTCGGAAC	TCTGTCTGCTTTGCTGTGATGC
Gapdh	GACATCAAGAAGGTGGTGAAGCAG	AAGGTGGAAGAATGGGAGTTGC
Iba1	GTCCTTGAAGCGAATGCTGG	CATTCTCAAGATGGCAGATC
iNos	CCTACCAAAGTGACCTGAAAGAGG	TTCTGGAACATTCTGTGCTGTCCC
Tnf-α	AACACAAGATGCTGGGACAGTGAC	TGGAAAGGTCTGAAGGTAGGAAGGC
Ucp2	TCGGAGATACCAGAGCACTGTCG	GCATTTCGGGCAACATTGG
Fabp4	ATGAAATCACCGCAGACGACA	CATAAACTCTTGTGGAAGTCACGCC
Fabp7	TGGCAAGATGGTCGTGACTC	CCAGTGCTTCATTAGCTGGC
Fabp5	TCCCACCATGGCCAGTCTTA	ACCGTGATGTTGTTGCCATC