Brief Communication

MOLECULAR METABOLISM

Astrocyte IKKβ/NF-κB signaling is required for diet-induced obesity and hypothalamic inflammation

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ABSTRACT

Objective: Obesity and high fat diet (HFD) consumption in rodents is associated with hypothalamic inflammation and reactive gliosis. While neuronal inflammation promotes HFD-induced metabolic dysfunction, the role of astrocyte activation in susceptibility to hypothalamic inflammation and diet-induced obesity (DIO) remains uncertain.

Methods: Metabolic phenotyping, immunohistochemical analyses, and biochemical analyses were performed on HFD-fed mice with a tamoxifen-inducible astrocyte-specific knockout of IKKβ (*Gfap*^{CreER}*Ikbkb*$^{fl/fl}$, IKKβ-AKO), an essential cofactor of NF-κB-mediated inflammation.

Results: IKKβ-AKO mice with tamoxifen-induced IKKβ deletion prior to HFD exposure showed equivalent HFD-induced weight gain and glucose intolerance as *Ikbkb*$^{fl/fl}$ littermate controls. In *Gfap*^{CreER}TdTomato marker mice treated using the same protocol, minimal Cre-mediated recombination was observed in the mediobasal hypothalamus (MBH). By contrast, mice pretreated with 6 weeks of HFD exposure prior to tamoxifen administration showed substantially increased recombination throughout the MBH. Remarkably, this treatment approach protected IKKβ-AKO mice from further weight gain through an immediate reduction of food intake and increase of energy expenditure. Astrocyte IKKβ deletion after HFD exposure—but not before—also reduced glucose intolerance and insulin resistance, likely as a consequence of lower adiposity. Finally, both hypothalamic inflammation and astrocytosis were reduced in HFD-fed IKKβ-AKO mice.

Conclusions: These data support a requirement for astrocytic inflammatory signaling in HFD-induced hyperphagia and DIO susceptibility that may provide a novel target for obesity therapeutics.

Keywords Obesity; Astrocytes; Inflammation; Metabolism; Hypothalamus; Energy homeostasis

1. INTRODUCTION

Obesity and excessive dietary consumption promote an inflammatory state in peripheral metabolic tissues driven by ingress and activation of immune cells [1,2]. Likewise, the central nervous system (CNS) responds to high fat and sugar-rich diets with rapid upregulation of the master inflammatory NF-κB pathway in important brain regions including the hypothalamus, a critical site of energy homeostasis regulation [3–7]. High-fat diet (HFD) consumption drives hypothalamic cytokine production, neuronal stress, and insulin/leptin resistance that together promote excess weight gain and food intake [4,8–10]. Reducing neuronal inflammatory capacity through deletion or inhibition of NF-κB pathway intermediates (e.g. IKKβ, MyD88, IκBα) restores hypothalamic control of energy balance [5,6,11], resulting in reduced susceptibility to diet-induced obesity (DIO) and glucose intolerance. Thus, identifying mechanisms that regulate the hypothalamic inflammatory process can advance our understanding of obesity pathogenesis and assist with development of new treatment targets.

Recent evidence suggests that neuronal inflammation may be a downstream event during DIO, with the recruitment and activation of hypothalamic glial cells being a more proximal response to HFD exposure [3,10,12–14]. This gliosis process is characterized by the accumulation and proliferation of activated microglia and astrocytes in the region of the mediobasal hypothalamus (MBH) [10,12–17]. While several studies have implicated microglia in the generation of diet-induced inflammatory signals and metabolic dysfunction [17,18], a similar role for astrocytes remains unclear. One study demonstrated a modest contribution of astrocyte inflammation to caloric intake on the first day of HFD feeding, but no analysis of DIO susceptibility was reported [19].

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Abbreviations: Agrp, Agouti-related peptide; ARC, arcuate nucleus; *Bdnf*, brain-derived neurotrophic factor; DIO, diet-induced obesity; *Ccl2*, C–C motif chemokine ligand 2; *Cart*, cocaine- and amphetamine-regulated transcript; DMH, dorsomedial hypothalamus; GFAP, glial fibrillary acidic protein; GSIS, glucose-stimulated insulin secretion; GTT, glucose tolerance test; IKKβ, inhibitor of kappa B kinase beta; HFD, high-fat diet; Iba1, ionized calcium binding adaptor molecule 1; IHC, immunohistochemistry; ir, immunoreactivity; ITT, insulin tolerance test; *Il*, interleukin; LPS, lipopolysaccharide; MBH, mediobasal hypothalamus; *Npy*, neuropeptide Y; NF-κB, nuclear factor kappa B; *Pomc*, proopiomelanocortin; RER, respiratory exchange ratio; TMX, tamoxifen; *Tnfa*, tumor necrosis factor α; VMN, ventromedial nucleus

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Astrocytes are abundant throughout the CNS and involved in many fundamental processes including synaptic transmission, neurovascular coupling, and blood–brain barrier maintenance [20]. In addition, astrocytes participate in CNS immune responses, adopting an activated phenotype with increased glial fibrillary acidic protein (GFAP) expression and release of proinflammatory cytokines that can enhance neurotoxicity and neurodegenerative disease progression [20–22]. Thus, astrocytes have the potential to impact energy homeostasis regulation in health and disease. Indeed, MBH astrocytes modulate feeding behavior when pharmacologically activated [23,24] and show dynamic responses to circulating signals of nutrient availability such as insulin and leptin [25–28]. In addition, MBH astrocytes become activated with obesity and HFD feeding in rodents and humans [10,29], raising the possibility that astrocyte inflammation disrupts hypothalamic regulation of energy balance and promotes DIO. To address this hypothesis, we developed a mouse model with an inducible astrocyte-specific deletion of IKKβ. Using this approach, we demonstrate that reduction of astrocyte inflammatory signaling protects mice from HFD-induced hypothalamic inflammation and reduces susceptibility to DIO and glucose intolerance. These results highlight the important role of non-neuronal cells in obesity pathogenesis and suggest the possibility of new cellular targets for therapy.

2. MATERIAL AND METHODS

2.1. Animals

All mice used in the experiments were male and on a C57BL/6J background. The reporter strain was generated by crossing *Gfap*^{CreER} mice (strain #012849, Jackson Laboratory) with ROSA26-stop$^{fl/fl}$-tdTomato mice (Ai14, strain #007914, Jackson Laboratory). The *Ikbkb*$^{fl/fl}$ mice were obtained from the laboratory of Dr. Michael Karin [30] and mated with *Gfap*$^{CreER/wt}$ mice. Breeding in our facility generated the littermates used in the experiments that were *Gfap*$^{CreER/wt}$*Ikbkb*$^{fl/fl}$ (IKKβ-AKO) and *Gfap*$^{wt/wt}$*Ikbkb*$^{fl/fl}$ (Ctl). Genotyping was performed by PCR using ear genomic DNA and the following primers: *Ikbkb* floxed allele (forward-CCT TGT CCT ATA GAA GCA CAA C; reverse-GTC ATT TCC ACA GCC CTG TGA); GFAP-CreERT2 allele (forward-GCC AGT CTA GCC CAC TCC TT; reverse-TCC CTG AAC ATG TCC ATC AG). All mice, including controls, were administered 2 subcutaneous injections (48 h apart, 4 mg dissolved in 200 μl warm purified corn oil) of tamoxifen (TMX; Sigma, T5648) to induce CreER-mediated recombination. Mice were housed with *ad libitum* access to water and diet in a temperature-controlled room with a 12 h:12 h light:dark cycle under specific-pathogen free (SPF) conditions. After weaning, all mice were fed standard rodent chow (5001; 13% (kcal) fat, LabDiet, St. Louis MO) or were switched to 60% HFD (D12492; Research Diets, Inc.; USA). One cohort ($n = 6–9$ per group) received TMX at 6 wks of age (TMX-HFD) while the other ($n = 5–7$ per group) received 6 wks of HFD prior to TMX treatment (HFD-TMX). All procedures were performed in accordance with NIH Guidelines for Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee at the University of Washington.

2.2. Body composition and indirect calorimetry

In vivo body composition analysis of lean mass and fat mass from conscious, immobilized mice was performed by the NIDDK-funded Nutrition Obesity Research Center (NORC) Energy Balance and Glucose Metabolism (EBGM) Core, using quantitative magnetic resonance spectroscopy (QMR) (EchoMRI 3-in-1; Echo Medical Systems). For calorimetric analyses, mice were acclimated to metabolic cages 3 weeks after TMX treatment (wk 9 of HFD in the HFD-TMX model) after which energy expenditure was measured using a computer-controlled indirect calorimetry system (Promethion, Sable Systems, Las Vegas, NV) run by the EBGM Core. For each animal, O_2 consumption and CO_2 production were measured for 1 min at 10-min intervals. Respiratory exchange ratio (RER) was calculated as the ratio of CO_2 production to O_2 consumption. Energy expenditure was calculated using the Weir equation without normalization, since body weight and composition did not differ between groups at this time point. Light and dark cycle energy expenditure were determined using the average of all 72 data points per 12-h light cycle of 3 consecutive days, and these, in turn, were averaged to obtain total 24-h energy expenditure. Ambulatory activity was measured continuously with consecutive adjacent infrared beam breaks in the x-, y- and z-axes scored as an activity count that was recorded every 10 min as previously described. Data acquisition and instrument control were coordinated by MetaScreen v.2.0.0.9, and raw data was processed using ExpeData v.1.6.4 (Sable Systems) with an analysis script documenting all aspects of data transformation.

2.3. Glucose and insulin tests

For the glucose tolerance test (GTT), mice were fasted 4 h and then administered 2 g/kg body weight D-glucose i.p., and blood glucose from tail was measured by glucometer (Freestyle Lite, Abbot Diabetes Care Inc.). In a separate experiment that analyzed glucose-stimulated insulin secretion (GSIS), blood samples were taken at $t = 0$ and 15 min for measurement of serum insulin by ELISA (Crystal Chem Inc). For the insulin tolerance test (ITT), food was removed from mice 4 h prior to experiment and recombinant insulin (Humulin R, Eli Lilly & Co.) was administered i.p. at 1.25 U/kg. Area-under-curve (AUC) and area-over-curve (AOC) were calculated by the trapezoid rule.

2.4. Primary astrocyte culture

Control and IKKβ-AKO mice aged P1 to P4 were sacrificed by decapitation, meninges removed, and cortices isolated under a dissecting microscope. Four cortices per group were pooled and minced, and incubated in Hanks Buffered Salt Solution (HBSS) with 2.5% Trypsin/EDTA at 37 °C for 30 min with shaking. After centrifugation, the tissue was resuspended in media and further dissociated by pipetting into a single cell suspension. Mixed cortical cells were seeded onto a poly-D-lysine coated T75 flask with culture media (Dulbecco's Modified Eagle's Medium (DMEM), 4.5 g/L glucose, 10% fetal bovine serum, L-glutamine, 25 mM HEPES, and 1% penicillin/streptomycin) and incubated at 37 °C and 5% CO_2/air. After cells reached confluency, microglia were removed by shaking at 180 rpm for 30 min and discarding the supernatant, followed by further shaking at 240 rpm for 6 h to remove oligodendrocyte precursors. To induce recombination, the resulting astrocyte-enriched culture was incubated in culture media containing 5 μM 4-hydroxy tamoxifen (4-OHT, Sigma) 3 days prior to experiments.

2.5. Quantitative real time PCR (qRT-PCR)

Total RNA was extracted using RNeasy micro kit according to manufacturer's instructions (Qiagen) and reverse-transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Levels of transcripts were measured by quantitative real-time PCR on an ABI Prism 7900 HT (Applied Biosystems) using the standard curve method and specific primer sequences (Supplementary Table 1).

2.6. Histological analyses

Mice were perfused with PBS and then 4% paraformaldehyde (PFA). Brains were removed from the skull, post-fixed in PFA, and cryoprotected in 25% sucrose/phosphate buffered saline (PBS). Brain

