Brief communication

MOLECULAR METABOLISM

Evidence for a novel functional role of astrocytes in the acute homeostatic response to high-fat diet intake in mice

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ABSTRACT

Objective: Introduction of a high-fat diet to mice results in a period of voracious feeding, known as hyperphagia, before homeostatic mechanisms prevail to restore energy intake to an isocaloric level. Acute high-fat diet hyperphagia induces astrocyte activation in the rodent hypothalamus, suggesting a potential role of these cells in the homeostatic response to the diet. The objective of this study was to determine physiologic role of astrocytes in the acute homeostatic response to high-fat feeding.

Methods: We bred a transgenic mouse model with doxycycline-inducible inhibition of NFkappaB (NFκB) signaling in astrocytes to determine the effect of loss of NFκB-mediated astrocyte activation on acute high-fat hyperphagia. ELISA was used to measure the levels of markers of astrocyte activation, glial-fibrillary acidic protein (GFAP) and S100B, in the medial basal hypothalamus.

Results: Inhibition of NFκB signaling in astrocytes prevented acute high-fat diet-induced astrocyte activation and resulted in a 15% increase in caloric intake ($P < 0.01$) in the first 24 h after introduction of the diet.

Conclusions: These data reveal a novel homeostatic role for astrocytes in the acute physiologic regulation of food intake in response to high-fat feeding.

Keywords Astrocyte; Glia; High-fat diet; Nuclear factor-kappaB; Food intake; Hypothalamus

1. INTRODUCTION

Obesity is a leading public health problem worldwide and is caused by dysregulation of energy homeostasis, the balance between food intake and energy expenditure. The central nervous system (CNS) controls body weight by homeostatic regulation of food intake and energy expenditure. Genetic and pharmacologic studies in rodents have identified a number of CNS circuits critical for the regulation of energy homeostasis [1,2]. However, to date the vast majority of research on the central regulation of energy homeostasis has focused on the contribution of neurons, with the potential contribution of non-neuronal CNS cells, including glia, only beginning to be appreciated [3–6].

Studies from our laboratory and others demonstrate that in rodents high-fat feeding resulting in obesity causes CNS inflammation [5,7,8] and astrocyte activation, known as reactive astrogliosis [5,9–11]. Astrocytes are the most abundant glial cell type in the CNS and are essential for regulation of the CNS microenvironment and neuronal synaptic plasticity [12]. In the arcuate nucleus of the medial basal hypothalamus (MBH) chronic obesity-associated reactive astrogliosis has been implicated in modulating synaptic organization of the melanocortin circuitry [10], thus contributing to obesity by modulating the tone of melanocortin neurons. In addition to being elevated by chronic high-fat feeding, CNS inflammation and astrocyte activation are also evident in the rodent hypothalamus at only 24 h after the introduction of a high-fat diet [5]. During the initial 24 h period after the introduction of a highly-palatable high-fat diet mice undergo a period of voracious food intake, known as hyperphagia, before homeostatic mechanisms prevail to restore energy intake to an isocaloric level. The increase in

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Abbreviations: CNS, central nervous system; GFAP, glial-fibrillary acidic protein; HFD, high-fat diet; MBH, medial basal hypothalamus; NFκB, nuclear factor kappa B

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hypothalamic astrocyte activation during this period suggests a potential contribution of these cells in this homeostatic response; however, the physiologic significance of acute astrocyte activation after high-fat feeding is not clear.

The objective of this study was to determine the contribution of astrocytes to the acute homeostatic response to high-fat feeding. In other CNS disorders, inflammation is both protective and detrimental depending on the context [13]. We hypothesized that high-fat diet-induced activation of inflammatory signaling pathways in astrocytes is part of a protective homeostatic response, which restrains food intake in response to the diet. If this hypothesis is correct then inhibiting inflammatory signaling in astrocytes should attenuate this homeostatic response resulting in increased food intake during the initial acute hyperphagic response to the high-fat diet. In CNS injury, the nuclear-factor kappa B (NFκB) transcription pathway in astrocytes plays a key role in the production of proinflammatory cytokines and the development of reactive astrogliosis [14,15]. To determine the contribution of astrocytes to the acute homeostatic response to high-fat feeding we bred a mouse model with doxycycline-inducible inhibition of the NFκB transcription pathway under the control of the astrocyte specific glial-fibrillary acidic protein (GFAP) promotor. Using this model we examined how preventing astrocyte activation by inhibiting the NFκB transcription pathway specifically in astrocytes of adult mice altered the acute homeostatic response to a high-fat diet.

2. MATERIALS AND METHODS

2.1. Animals

Animal studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Vanderbilt University. Animals were housed at 21 ± 2 °C and fed a standard chow diet (13% kcal from fat; Purina 5010, PMI Nutrition International, MO) unless stated otherwise. Male C57BL/6J mice (Stock # 000664) were purchased from the Jackson Laboratory (Bar Harbor, ME). Double transgenic mice with astrocyte targeted expression of a tetracycline-inducible inhibitor of NFκB signaling were generated by crossing IκBα-dominant negative mice [16–21] on the FVB background with C57BL/6J mice expressing the reverse tetracycline-controlled transactivator under the control of the human glial-fibrillary acidic protein (GFAP) promotor [GFAP-rtTA*M2; Jackson Laboratory, ME (stock number 014098)]. Hereafter these animals will be referred to as IκB-DN^{+} mice. Single transgenic littermates expressing only the IκBα-dominant negative transgene (IκB-DN^{-}) were used as controls. F1 hybrid mice were used for all studies with transgenic animals. Male mice (8–10 weeks of age) were used for all feeding studies and, to reduce wastage, female mice were used for verification of transgene expression. The tetracycline-inducible IκBα-dominant negative mouse has been extensively characterized and has previously been successfully used in studies examining the importance of NFκB transcription pathway in disease pathology in mouse models of cancer [16,19,20] and lung inflammation [17,18].

2.2. Acute high-fat feeding studies

Mice were individually housed and their food intake measured daily for 7 days to obtain basal caloric intake before being switched to a high-fat diet (HFD; 60% of total calories from fat; Research Diets Inc., NJ). Food intake was measured every 24 h for the next 7 days. For the molecular studies (including immunohistochemistry), the animals were euthanized 24 h after introduction of the HFD. For the studies with the IκB-DN^{+} mice, the animals were switched to water containing 2 g/L doxycycline hyclate (Sigma–Aldrich, MO) and 20 g/L Splenda® brand sweetener (to mask the bitter taste of the doxycycline) 7 days before the introduction of the HFD to induce expression of the transgene.

2.3. Tissue collection for molecular analyses

Animals were deeply anesthetized and transcardially perfused with 0.9% saline. The brain was removed and sectioned using a 1-mm acrylic brain block. The MBH was dissected from a single 2 mm coronal slice Coordinates for the MBH slice were: +1.0 to +3.0 mm interaural, as referenced by Paxinos and Franklin [22], with the medial basal section of the slice corresponding to the MBH being dissected. All tissues were rapidly frozen on dry ice and placed at −80 °C until used for molecular analyses.

2.4. *Ex vivo* molecular analyses

2.4.1. Primary cell culture

Primary neural cells were isolated from hypothalamii dissected from adult IκB-DN^{-} and IκB-DN^{+} mice using a trypsin based neural tissue dissociation kit, according to the manufacturer's instructions (Miltenyi Biotech Inc., CA). The culture procedure was modified from one described in the literature for culture from adult animals [23]. Cells isolated from one hypothalamus were distributed equally across three wells of a 6-well culture dish containing poly L-lysine (Sigma–Aldrich, MO) coated glass coverslips. Cells were maintained in an incubator at 37 °C in 5% CO_2 in culture media [Dulbecco's Modified Eagle Medium (DMEM), high-glucose, containing 1% penicillin-streptomycin and fetal bovine serum (FBS)]. For the first 1-week after culture the cells were maintained in culture media containing 20% FBS before being switched to media containing 15% FBS in week 2 and 10% FBS in week 3. After 2-weeks of culture, 1 μg/ml doxycycline hyclate (Sigma–Aldrich, MO) was added to the culture media to induce transgene expression. The cells were maintained in culture for a total 3-weeks before use.

2.4.2. *In vitro* cell stimulation and immunocytochemistry

Primary neural cells were switched to culture media containing 1% FBS 24 h prior to stimulation with 5 μg/ml lipopolysaccharide (LPS), a potent activator of NFκB signaling. After 1 h of stimulation, the media was removed and the cells fixed with cold 100% methanol. After washing in 0.01M phosphate buffered saline (PBS; pH 7.4) cells were incubated with 1.5% FBS diluted in PBS containing 0.01% Triton-X100 (PBS-T) for 1 h at room temperature to block non-specific binding. The cells were then incubated overnight at 4 °C with antibodies against the p65 subunit of NFκB (cat # sc-372; Santa Cruz Biotechnology Inc., CA) and GFAP (cat # MAB360; Millipore Inc, MA), diluted 1:200 and 1:1,000 respectively, in 1.5% FBS in PBS-T. After washing with PBS the primary antibody binding was detected after incubation with the following secondary antibodies for 1 h at room temperature: donkey anti-rabbit Alexa 488 (p65) and donkey anti-mouse Alexa 594 (GFAP) (Life Technologies, CA), both diluted 1:500 in PBS-T. After washing with PBS, the coverslips were mounted onto glass slides with mounting media containing the nuclear marker DAPI (Pro-long Gold, Life Technologies, CA) and the staining visualized under fluorescence using a Zeiss AxioImager Z1 (Zeiss, Germany). Activation of NFκB signaling was assessed by the ability of LPS to induce translocation of p65-immunoreactivity from the cytoplasm to the nucleus. The images shown are representative of two independent experiments.

2.4.3. Verification of transgene induction using RT-PCR

RNA was extracted from brain, liver, and pancreas using Trizol according to the manufacturer's instructions (Life Technologies, CA).

