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Obesity induces neuroinflammation mediated by altered expression of the renin–angiotensin system in mouse forebrain nuclei

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HIGHLIGHTS

- High-fat diet intake causes proinflammatory responses in mouse forebrain nuclei.
- Increased microglia size and number in forebrain nuclei follows high-fat diet intake.
- High-fat diet consumption leads to increased GFAP expression and staining.
- Some of these responses are reversed upon deletion of AT1a within the PVN.

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ABSTRACT

Obesity is a widespread health concern that is associated with an increased prevalence of hypertension and cardiovascular disease. Both obesity and hypertension have independently been associated with increased levels of inflammatory cytokines and immune cells within specific brain regions, as well as increased activity of the renin–angiotensin system (RAS). To test the hypothesis that high-fat diet (HFD) induced obesity leads to an angiotensin-II (Ang-II)-dependent increase in inflammatory cells within specific forebrain regions that are important for cardiovascular regulation, we first assessed microglial activation, astrocyte activation, inflammation and RAS component gene expression within selected metabolic and cardiovascular control centers of the forebrain in adult male C57BL/6 mice given either a HFD or a low-fat diet (LFD) for 8 weeks. Subsequently, we assessed the necessity of the paraventricular nucleus of the hypothalamus (PVN) angiotensin type-1a (AT1a) receptor for these responses by using the Cre/lox system in mice to selectively delete the AT1a receptor from the PVN. These studies reveal that in addition to the arcuate nucleus of the hypothalamus (ARC), the PVN and the subfornical organ (SFO), two brain regions that are known to regulate blood pressure and energy balance, also initiate proinflammatory responses after the consumption of a diet high in fat. They further indicate that some, but not all, of these responses are reversed upon deletion of AT1a specifically within the PVN.

1. Introduction

Obesity is an epidemic health concern that is associated with an increased prevalence of hypertension, as well as an enhanced risk for cardiovascular morbidity and mortality [1–6]. Due to their high incidence of co-morbidity, determining effective strategies that combat both obesity and hypertension are in high demand and both the renin–angiotensin system (RAS) and inflammatory cells within the brain have been implicated as promising targets in this regard. Obesity and hypertension are independently acknowledged as mild inflammatory conditions that are often accompanied by elevated RAS activity [7–13]. In rodents, high-fat diet (HFD) induced obesity leads to increased inflammatory factors and immune cells in peripheral tissues and in brain regions that are essential for maintaining of energy balance [13–16] and it is possible that this accumulation of astrocytes and microglia (the resident immune cells of the brain) in these specific brain regions then plays an integral role in the dysregulation of energy balance [17]. During hypertension, a similar scenario occurs; however, in these instances, the elevated levels of inflammatory cells and factors have primarily been localized to brain regions and peripheral tissues that are important for the regulation of cardiovascular function [10,18–22].

Despite the similarities between obesity and hypertension-related CNS immune cell activation, in many instances, the proposed mechanisms contributing to the shift toward a proinflammatory state within

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the brain during these conditions differ. In the case of obesity, several studies have implicated the proinflammatory effects of free fatty acids [23], leptin [24], and gut microbiota [25] as contributing mechanisms to the activation of CNS immune cells, while during hypertension, the proinflammatory actions of angiotensin-II (Ang-II) have received much attention [10,18,19,26]. Of relevance, obesity is also associated with increased RAS activity [8] and although the proinflammatory effects of Ang-II have been characterized [10,18,19], whether obesity-related inflammation within specific brain regions depends on angiotensin signaling is not clear. Furthermore, the effect of HFD feeding and other obesity-related factors on microglial activation, inflammation and RAS activity in cardiovascular and other metabolic control centers in the mouse forebrain is largely unknown.

Here we hypothesized that an important link between HFD-induced metabolic and cardiovascular dysregulation is a RAS-dependent increase in inflammation within specific forebrain regions that regulate energy balance and blood pressure. It is recognized that obesity leads to an accumulation of immune cells within ARC [13,17,27], a brain region that is critical for the regulation of energy balance [28]. The present studies sought to determine whether HFD consumption also leads to proinflammatory responses within the paraventricular nucleus of the hypothalamus (PVN) and the subfornical organ (SFO), two brain regions that regulate blood pressure and energy balance. We further evaluated whether deletion of angiotensin type-1a (AT1a) receptors within the PVN impacts microglial and astrocytic activation during HFD-feeding.

2. Materials and methods

2.1. Animals

For the experiments conducted in adult male C57BL/6 mice, animals were obtained from Harlan laboratories (Tampa, FL). The Cre/lox system was used to generate mice with specific deletion of AT1a within the PVN (PVN AT1a KO) on a C57BL/6 mixed background as previously-described [29]. In brief, PVN AT1a KO (homozygous for AT_{1a} flox and expressing Sim1Cre) and littermate control mice (homozygous for AT_{1a}flox/flox) were generated by crossing AT1a flox mice (obtained from Dr. Alan Daugherty, University of Kentucky [30]) with Sim1Cre mice (generated by Dr. B. Lowell, Beth Israel Deaconess Medical Center and Harvard Medical School; [31]). It is important to consider that PVN AT1a KO mice and littermate control mice were born at the University of Florida animal facilities, while the cohorts of C57BL/6 wild-type mice were obtained from Harlan laboratories. As a consequence, these separate cohorts of mice were exposed to different prenatal and birthing conditions, which are known to impact physiology even during adulthood. In the present manuscript, direct comparisons are therefore only made between cohorts of mice subjected to the same breeding conditions. Unless otherwise noted, mice were 8–10 weeks of age at the initiation of all studies, were individually-housed, maintained on a 12 h light/dark cycle and were given ad libitum access to water and to a HFD (60% kcal fat; Research Diets, New Brunswick, NJ [D12492]) or a low-fat diet (LFD; 10% kcal fat; Research Diets, New Brunswick, NJ [D12450B]). All studies were approved by the University of Florida Institutional Animal Care and Use Committee and were in compliance with the animal welfare guidelines of the United States National Institute of Health.

2.2. Experimental design

Experiments conducted in wild-type mice were initiated one week after their arrival to the University of Florida animal facilities. At this time, mice were divided into two groups matched for body weight: those given HFD and those given LFD. Experiments conducted in PVN AT1a KO mice and controls were initiated when the mice were 8–9 weeks of age, at which time all PVN AT1a KO mice and controls were given ad libitum access HFD. In all cases, mice remained on HFD or LFD for 8 weeks, after which mice were fasted for 2 h and anesthetized using sodium pentobarbital. Mice were then either euthanized via decapitation to collect brains for gene expression analysis or perfused trans-cardially with 0.15 M NaCl followed by 4% paraformaldehyde. Brains were collected from perfused mice and post-fixed in 4% paraformaldehyde for 4 h. Afterwards, brains were stored in 30% sucrose at 4 °C until processing for immunohistochemistry (IHC). Brains collected for gene expression analysis were flash-frozen in dry ice-cooled isopentane and stored at −80C until processing.

2.3. Body and adipose mass

Body mass was assessed at the initiation of the study and weekly, thereafter. At the termination of the experiments, mice were euthanized and adiposity was evaluated by manually removing the epididymal, inguinal, mesenteric and retroperitoneal white adipose tissue pads and then weighing them on a calibrated scale.

2.4. Immunohistochemistry

Four series of 25 μm coronal brain sections were taken on a Leica CM3050 S cryostat and placed in cryoprotective solution for storage at −20 °C. For assessment of Iba-1 or GFAP immunoreactivity, sections were washed in 50 mM KPBS and then placed in a blocking solution (50 mM KPBS with 2% bovine serum albumin and 0.1% Triton-X) at room temperature for 2 h. Subsequently, sections were incubated in rabbit anti Iba-1 (1:3000; Wako Chemicals, Richmond, VA) or mouse anti GFAP (1:1500; EnCor Biotechnology, Gainesville, FL) at 4 °C in the blocking solution overnight. The following day, sections were brought to room temperature, rinsed, and incubated for 2 h in the secondary Ab (Cy3 anti-rabbit for Iba-1 and Alexa 488 anti-mouse for GFAP; Jackson ImmunoResearch; 1:500) made in blocking solution. Sections were then rinsed, sequentially mounted onto microscope slides and cover-slipped with polyvinyl alcohol mounting medium with DABCO.

2.5. Imaging and analysis

Brain regions of interest (ROI) were identified using anatomical landmarks and coordinates described by Franklin and Paxinos [32]. Fluorescence images were captured using a Zeiss AxioImager M2 microscope (Carl Zeiss, Thornwood, New York). Image capture and analysis was performed at 20× for Iba-1 staining of microglia and 10× for GFAP staining of astrocytes. For the assessment of microglial size and number, 12 μm z-stacks (containing 20 images) were taken through Iba-1 stained sections at 20× magnification. For assessment of GFAP immunoreactivity, 10× images were captured for all brain regions assessed.

Images of brain sections were analyzed for number of and size of Iba-1 positive cell-bodies or for the area fraction of GFAP staining within the brain regions of interest using ImageJ (NIH). In a subset of samples, the size of the microglia was manually verified using Axiovision. Captured images were converted into greyscale and binary formats and thresholds for black and white balance were adjusted to the same level for each ROI. Counts were taken from matched sections representing the ROI with reference to Franklin and Paxinos [32]. In the case of bilateral nuclei, counts were taken from both sides and averaged.

2.6. RNA extraction and cDNA synthesis

For gene expression analysis, the PVN, SFO, and mediobasal hypothalamus (MBH; containing the ARC, VMH and ME) were isolated using a cryostat and a micro-punch kit. Brains were sectioned at 100 μm and punches were taken from brain slices through the entirety of the regions of interest and submerged in RLT buffer containing β-