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Down-regulation of fatty acid binding protein 7 (Fabp7) is a hallmark of the postpartum brain

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ABSTRACT

Fatty acid binding protein 7 (Fabp7) is a versatile protein that is linked to glial differentiation and proliferation, neurogenesis, and multiple mental health disorders. Recent microarray studies identified a robust decrease in Fabp7 expression in key brain regions of the postpartum rodents. Given its diverse functions, Fabp7 could play a critical role in sculpting the maternal brain and promoting the maternal phenotype. The present study aimed at investigating the expression profile of Fabp7 across the postpartum CNS. Quantitative real-time PCR (qPCR) analysis showed that Fabp7 mRNA was consistently down-regulated across the postpartum brain. Of the 9 maternal care-related regions tested, seven exhibited significant decreases in Fabp7 in postpartum (relative to virgin) females, including medial prefrontal cortex (mPFC), nucleus accumbens (NA), lateral septum (LS), bed nucleus of stria terminalis dorsal (BnSTd), paraventricular nucleus (PVN), lateral hypothalamus (LH), and basolateral and central amygdala (BLA/CeA). For both ventral tegmental area (VTA) and medial preoptic area (MPOA) levels of Fabp7 were lower in mothers, but levels of changes did not reach significance. Confocal microscopy revealed that protein expression of Fabp7 in the LS paralleled mRNA findings. Specifically, the caudal LS exhibited a significant reduction in Fabp7 immunoreactivity, while decreases in medial LS were just above significance. Double fluorescent immunolabeling confirmed the astrocytic phenotype of Fabp7-expressing cells. Collectively, this research demonstrates a broad and marked reduction in Fabp7 expression in the postpartum brain, suggesting that down-regulation of Fabp7 may serve as a hallmark of the postpartum brain and contribute to the maternal phenotype.

1. Introduction

Postpartum female rodents exhibit a wide array of behaviors that promote the survival and development of their offspring. The manifestation of these maternal behaviors has been associated with alterations in circulating hormones (Bridges, 1984; Numan and Insel, 2003), and neurosignaling pathways, such as oxytocin, enkephalin, and GABA (Gammie, 2005; Insel, 1990; Petraglia et al., 1985; Qureshi et al., 1987; Young et al., 1997). However, it is likely that other unidentified genes are contributing to the reconstruction of the maternal brain that regulates postpartum behavioral phenotype. Recent microarray studies have identified hundreds of genes that display altered expression in postpartum females compared to virgin controls. However, the extent and functional significance of these alterations in gene expression have yet to be clarified.

Microarray studies from our laboratory and others have identified that one gene, fatty acid binding protein 7 (Fabp7), also referred to as brain lipid binding protein (BLBP), has altered expression in several regions of postpartum brain. Fabp7 expression was found to be decreased in arcuate nucleus of postpartum rats (Xiao et al., 2005), and in

Abbreviations: BLA/CeA, basolateral and central amygdala; BnSTd, bed nucleus of stria terminalis dorsal; Fabp7, fatty acid binding protein 7; Gfap, glial fibrillary acidic protein; KO, knockout; LH, lateral hypothalamus; LS, lateral septum; LSD, dorsal part of lateral septum; LSI, intermediate part of lateral septum; LSV, ventral part of lateral septum; mPFC, medial prefrontal cortex; MPOA, medial preoptic area; NA, nucleus accumbens; PVN, paraventricular nucleus; qPCR, quantitative real-time polymerase chain reaction; VTA, ventral tegmental area; WT, Wild-type

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mouse hypothalamus (Gammie et al., 2005). These initial findings have been complemented by recent microarray studies in the medial prefrontal cortex (mPFC) (Eisinger et al., 2014), medial preoptic area (MPOA) (Driessen et al., 2014a), nucleus accumbens (NA) (Zhao et al., 2014), and the septum of two different strains of mice (Eisinger et al., 2013b; Zhao et al., 2012b). Combined, these studies indicate that there is overall a consistent down-regulation of Fabp7 in postpartum females, though verification of microarray findings were only conducted for the septum microarrays (Eisinger et al., 2013b; Zhao et al., 2012b).

Fabp7 is primarily expressed in neural progenitor cells and astrocytes (Kurtz et al., 1994), and has long been involved in glial differentiation and proliferation, neurogenesis and multiple behavioral processes. For example, Fabp7 knockout (KO) mice displayed aberrant dendritic morphology and decreased spine density (Ebrahimi et al., 2016), increased anxiety and fear memory compared to wild-type controls (Owada et al., 2006). Several lines of research have also indicated the involvement of Fabp7 in maternal phenotype. Inhibition or deletion of cannabinoid receptor 1 (Cnr1) disrupts maternal behaviors (Schechter et al., 2012, 2013), and Fabp7 binds to anandamide, an endocannabinoid (Berger et al., 2012; Kaczocha et al., 2009). This suggests that Fabp7 may modulate maternal behavior via acting on endocannabinoid signaling pathway, although this effect has not been tested. Moreover, bioinformatics studies have indicated that alterations in genes associated with development and mental health disorders may contribute to the maternal phenotype by promoting neural remodeling and sociability in the maternal female (Driessen et al., 2014a; Eisinger et al., 2013a,b, 2014). Correspondingly, Fabp7 has been linked to both CNS development and mental health disorders, including schizophrenia, autism, bipolar disorder, and depression (Ayalew et al., 2012; Iwayama et al., 2010; Kumar et al., 2011; Uriguen et al., 2008; Watanabe et al., 2007; Ziats and Rennert, 2011). The association of altered Fabp7 with CNS development and mental health disorders suggests the involvement of Fabp7 in maternal behavioral phenotypes. Postpartum depression in human studies is correlated with decreased levels of free fatty acids (Liu et al., 2010; Storch and Corsico, 2008). Increased free fatty acids or a high fat diet can reduce postpartum depression in rats (Feng et al., 1994; Owada et al., 1996), indicating that Fabp7 levels in mothers could contribute to a healthy maternal phenotype.

The purpose of the present study was to investigate in depth the alterations in Fabp7 expression in broad functional brain regions of postpartum females compared to virgin controls. Using quantitative real-time PCR (qPCR), we validated previous microarray findings and extended our investigation to include other brain regions associated with maternal behaviors. Further, we utilized fluorescent immunohistochemistry to examine the subregional distribution of Fabp7 in the lateral septum (LS) and confirm qPCR findings at the protein level.

2. Materials and methods

2.1. Animals

Nulliparous age-matched outbred hsd:ICR female mice (~70 days of age; Harlan, Madison, WI) were used for all experiments. One half of the females remained housed together (two mice per cage) while the remaining females were pair-housed with breeder hsd:ICR males (Harlan, Madison, WI). After two weeks of cohousing, all females were individually housed. This housing strategy minimized the isolation-induced stress (Brown and Grunberg, 1995; Palanza et al., 2001), and provided all females with a similar social environment. All mice were housed in polypropylene cages with nestlets and *ad libitum* access to water and breeder chow (Harlan, Madison, WI). On postpartum day 1, litters were culled to standardize litter size to 11 pups. Animals were housed in a 12:12 light/dark cycle with lights on at 6:00 h CST. All experimental procedures were in compliance with the guidelines of the National Institutes of Health Guide and Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the University of Wisconsin.

For verifying the specificity of Fabp7 antibody, C57BL/6 wild-type (WT) and Fabp7 knockout (KO) male mice of same genetic background (Owada et al., 2006) were used. The mice were 19 months old and maintained under a 12:12 light/dark cycle (lights on at 8:00 A.M.) with ad libitum access to standard food and water. All experimental protocols were performed according to the Guidelines for Animal Experimentation of the Tohoku University Graduate School of Medicine under the laws and notification requirements of the Japanese Government.

2.2. Tissue collection and slicing

On postpartum day 6 or 7 between 9:00 h and 12:00 h CST, brains were removed from lactating and age-matched virgin females. Estrous states for virgin females were determined using a vaginal lavage (Marcondes et al., 2002), and only diestrus naive females were used in subsequent experiments (Zhao and Gammie, 2015). For the qPCR analysis, females were lightly anaesthetized with isoflurane before brains were removed and flash frozen in methylbutane on dry ice. Brains were stored at −80 °C until sliced to a 200 µm thickness on a cryostat (Leica, CM1850, Bannockburn, IL, USA). Sections were mounted on gelatin coated glass slides and regions of interest were collected using the Brain Punch Set (Stoelting, Wood Dale, IL, USA) under a dissection microscope. The bregma coordinates for the collected brain regions are as follows according to The Allen Mouse Brain Atlas (reference atlas version 1, 2008): mPFC (1.98 mm–1.54 mm), NA (1.70 mm–0.98 mm), LS (1.10 mm–0.14 mm), BnSTd (0.26 mm–0.02 mm), MPOA (0.26 mm to −0.10 mm), PVN (−0.58 mm to −0.94 mm), LH (−0.70 mm to −1.06 mm), BLA/CeA (−0.82 mm to −1.70 mm), and VTA (−2.92 mm to −3.80 mm). These brain regions were selected because they are linked to maternal behavior, other socially motivated behaviors and mood state (D'Anna and Gammie, 2009; Febo et al., 2010; Fleming et al., 1980; Insel and Harbaugh, 1989; Li and Fleming, 2003; Numan and Numan, 1996; Numan et al., 2005; Zhao and Li, 2010). For fluorescent immunohistochemistry, females were lightly anaesthetized with isoflurane, injected with 0.15 mL sodium pentobarbital, then transcardially perfused with approximately 50 mL saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were postfixed overnight in 4% paraformaldehyde, then submerged in 30% sucrose in 0.1 M phosphate buffer for two days before being stored at −80 °C. Brains were sliced to a 30 µm thickness on a cryostat, then stored at −20 °C in anti-freeze cryoprotectant until labeling.

Preparation of brain sections from C57BL/6 wild-type (WT) and Fabp7 knockout (KO) mice for immunofluorescent labeling was made as described previously (Sharifi et al., 2011, 2013). Briefly, Fabp7 KO and WT mice brains were perfusion-fixed in 4% paraformaldehyde, postfixed overnight at 4 °C and cryoprotected in graded concentrations of sucrose. Coronal sections (30 µm) containing the LS were sliced, and stored in anti-freeze cryoprotectant until staining.

2.3. Western blotting

Western blot analysis was performed to characterize the specificity of Fabp7 antibodies according to our previously published protocol (Zhao et al., 2012a; Zhao and Gammie, 2015). Briefly, brain tissue from a postpartum female was homogenized with ice-cold RIPA buffer containing protease and phosphatase inhibitors. Following tissue homogenization, samples were centrifuged and the supernatant fraction was collected. Protein concentration was determined using BCA Protein Assay (Pierce Chemical Co.; Rockford, IL). Twenty µg of total protein was loaded and gel electrophoresis was run using a 4–20% Mini-PROTEAN TGX precast gel (Bio-Rad; Hercules, CA) and transferred to a PVDF membrane. Membrane was blocked for 1 h in 0.1 M TBST