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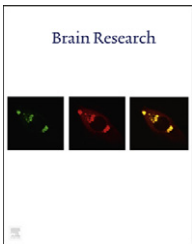

Research Report

Glutamic acid decarboxylase 65 and 67 expression in the lateral septum is up-regulated in association with the postpartum period in mice

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ABSTRACT

The postpartum period in mammals undergoes a variety of physiological adaptations, including metabolic, behavioral and neuroendocrine alterations. GABA signaling has been strongly linked to various emotional states, stress responses and offspring protection. However, whether GABA signaling may change in the lateral septum (LS), a core brain region for regulating behavioral, emotional and stress responses in postpartum mice has not previously been examined. In this study, we tested whether the expression of two isoforms of glutamic acid decarboxylase (GAD), GAD65 (GAD2) and GAD67 (GAD1), the rate-limiting enzyme for GABA synthesis, exhibits altered expression in postpartum mice relative to nonmaternal, virgin mice. Using microdissected septal tissue from virgin and age-matched postpartum females, quantitative real-time PCR and Western blotting were carried out to assess GAD mRNA and protein expression, respectively. We found both protein and mRNA expression of GAD67 in the whole septum was up-regulated in postpartum mice. By contrast, no significant difference in the whole septum was observed in GAD65 expression. We then conducted a finer level of analysis using smaller micro-dissections and found GAD67 to be significantly increased in rostral LS, but not in caudal LS or medial septum (MS). Further, GAD65 mRNA expression in rostral LS, but not in caudal LS or MS was also significantly elevated in postpartum mice. These findings suggest that an increased GABA production in rostral LS of the postpartum mice via elevated GAD65 and GAD67 expression may contribute to multiple alterations in behavioral and emotional states, and responses to stress that occur during the postpartum period. Given that rostral LS contains GABA neurons that are projection neurons or local interneurons, it still needs to be determined whether the function of elevated GABA is for local or distant action or both.

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Abbreviations: AH, anterior hypothalamus; BNST, bed nucleus of the stria terminalis; CycA, Cyclophilin A; GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase; GAD65 (GAD2), glutamic acid decarboxylase 65; GAD67 (GAD1), glutamic acid decarboxylase 67; LH, lateral hypothalamus; LS, lateral septum; LSc, caudal lateral septum; LSr, rostral lateral septum; MS, medial septum; PVN, paraventricular nucleus; qPCR, quantitative real-time polymerase chain reaction; Ywhaz, tyrosine 3-monooxygenase/tryptophan, 5-monooxygenase activation protein, zeta polypeptide.

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1. Introduction

The postpartum period in mammals is associated with the emergence of a number of maternal behaviors, including offspring care and offspring protection (Lonstein and Fleming, 2002; Numan and Insel, 2003) as well as changes in emotional state and stress reactivity (Carter et al., 2001; Neumann, 2001). Behavioral responses are altered during the postpartum period. In rodents, maternal behaviors are initiated following birth whereby a dam builds a nest, retrieves the pups to the nest site, licks the pups, and adopts a nursing posture over them to permit suckling (Dollinger et al., 1980; Rosenblatt and Lehrman, 1963). The dams can also display high levels of protective behaviors. In contrast, virgin females do not exhibit this suite of behaviors and can even act antagonistically towards pups (Fleming and Luebke, 1981; McCarthy, 1990). Gene expression changes in specific brain regions support such physiological adaptations that occur during the postpartum period (Broad et al., 1993; Brunton et al., 2008; Ottinger et al., 1995; Smith, 1993). The inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), has been strongly linked to aggressive behavior, emotional state, including anxiety, depression, and reactivity to stressors (Cole and Sawchenko, 2002; Cullinan, 1998; de Almeida et al., 2005; Earnheart et al., 2007; Lonstein, 2007; Sanders and Shekhar, 1995). GABA is also linked to offspring protection as the activation of $GABA_A$ receptor with various benzodiazepines ($GABA_A$ receptor agonists) elevated protective behavior (Lee and Gammie, 2007; Mos and Olivier, 1989; Yoshimura and Ogawa, 1991). Further, antagonism of $GABA_A$ receptor with bicuculline prominently disrupted maternal aggression while leaving other components of maternal behavior relatively intact in lactating females (Hansen and Ferreira, 1986; Lee and Gammie, 2009, 2010). GABA is synthesized from its immediate precursor glutamate by the rate-limiting enzyme glutamic acid decarboxylase (GAD). The adult brain expresses two isoforms of GAD, GAD65 (GAD2) and GAD67 (GAD1), which are encoded by two independently regulated genes and differ in cellular expression and synthesis of GABA (Erlander et al., 1991; Soghomonian and Martin, 1998). GABA-positive neurons containing both local interneurons and projection neurons are abundantly expressed in the lateral septum (LS) (Castaneda et al., 2005; Garrido Sanabria et al., 2006; Panula et al., 1984). Further, expression of GAD is an accurate reflection of GABA synthesis and activity (Lindefors, 1993; Mason et al., 2001; Segovia et al., 1990).

LS is a core brain region linked to emotional state (e.g., anxiety, fear and depression) and stress reactivity (Sheehan et al., 2004; Singewald et al., 2011). For example, exposure to a variety of stressful and anxiogenic stimuli increases the activity of LS neurons (Cullinan et al., 1995; Silveira et al., 1993, 1995) and stimulation of neural activity in LS reduces fear and anxiety (Thomas, 1988; Thomas and Evans, 1983). Site-directed injection of a $5\text{-}HT_{1A}$ receptor agonist into the LS produces antidepressant-like effects (Martin et al., 1990; Schreiber and De Vry, 1993). LS has also been linked to offspring protection. The magnitude of oxytocin receptor binding in the LS positively correlates with the intensity of maternal defense (Caughey et al., 2011) and recent site directed injection studies, including GABA and norepinephrine manipulations, indicate a critical role for LS in protective behavior (D'Anna and Gammie, 2009; Lee and Gammie, 2009; Scotti et al., 2011). Although LS is linked to emotional state and aspects of maternal care, it is still not known whether changes in GABA occur in LS in association with the postpartum period.

In this study, we first used quantitative real-time PCR (qPCR) and Western blotting approaches to determine whether changes occur in the expression of GAD65 and GAD67 in the whole septum (lateral and medial) during the postpartum period. Because the whole septum includes LS and medial septum (MS), and because LS has subdivisions with different known actions and projections, we extended this study by also evaluating expression changes of GAD65 and GAD67 in rostral LS (LSr), caudal LS (LSc) and MS.

2. Results

2.1. GAD65 and GAD67 gene expression in the whole septum by qPCR analysis

Expression of GAD67 mRNA in the whole septum was up-regulated in lactating versus virgin mice ($p = 0.031$). By contrast, no significant difference was observed in GAD65 mRNA expression (Fig. 1).

2.2. GAD65 and GAD67 protein expression in the whole septum by Western blot analysis

In parallel with the expression changes of mRNA, GAD67 protein was increased ($p = 0.025$, Fig. 2C and D), while GAD65

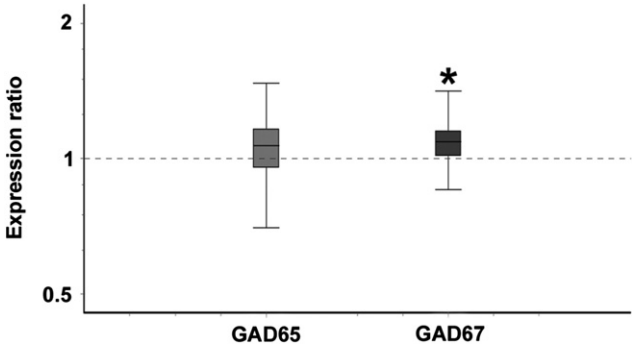

Fig. 1 – Quantitative real-time PCR analysis of GAD65 and GAD67 gene expression in the whole septum. Relative expression distribution of mRNA (Y-axis) represented as a ratio of lactating versus virgin mice ($n = 10$/group), was normalized against two reference genes CycA and Ywhaz, and shown by box-and-whisker plot as medians (solid lines), interquartile range (boxes) and ranges (whiskers). Ratios over one indicate genes that are more highly expressed in lactating than in virgin mice. Note that GAD67 mRNA in the whole septum was up-regulated in lactating mice compared to virgin females, while no significant change was observed in GAD65 mRNA. $*p < 0.05$ lactating versus virgin control.

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