Int. J. Devl Neuroscience 41 (2015) 80–91

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Sexual differences in long-term effects of prenatal chronic mild stress on anxiety-like behavior and stress-induced regional glutamate receptor expression in rat offspring

Yan Wang[a,1], Yuchao Ma[a,1], Wenwen Cheng[a], Han Jiang[b], Xinxin Zhang[c], Min Li[a], Jintao Ren[a], Xiaosong Zhang[a], Xiaobai Li[b,*,1]

[a] College of Humanities and Social Sciences, Applied psychology, China Medical University, Shenyang 110001, China
[b] Department of Psychiatry, The First Hospital of China Medical University, Shenyang 110001, China
[c] The Research Center for Medical Genomics, Key Laboratory of Cell Biology, Ministry of Public Health, Key Laboratory of Medical Cell Biology, Ministry of Education, China Medical University, Shenyang 110001, China

ARTICLE INFO

Article history:
Received 24 November 2014
Received in revised form 27 January 2015
Accepted 27 January 2015
Available online 29 January 2015

Keywords:
Prenatal chronic mild stress
Sexual difference
Prefrontal cortex
Hippocampus
Amygdala
Glutamate receptor

ABSTRACT

Severe adverse life events during pregnancy may increase the risk of anxiety disorders in the offspring. Glutamate receptors are neurobiological targets in anxiety disorders. In this study, we investigated the effects of prenatal chronic mild stress (PCMS) on anxiety-like behavior by using elevated plus maze (EPM), and evaluated the effects of PCMS and/or anxiogenic challenge on glutamate receptors in different brain regions. Our results showed that PCMS increased anxiety-like behavior in both male and female offspring. Moreover, compared with the male naïve rats, male EPM rats showed a significant reduction of mGluR2/3 in the prefrontal cortex, mGluR1 and mGluR2/3 in the hippocampus, and increments of mGluR5, NR1, NR2B and PSD95 in the amygdala. In contrast, compared with female naïve rats, female EPM rats showed decreased levels of mGluR5 in the hippocampus, and mGluR2/3 and mGluR5 in the prefrontal cortex, and increased levels of NR2B and PSD95 in the amygdala. Furthermore, PCMS seemed not to affect the baseline expression of glutamate receptors in adult offspring, but induced significant alterations of them triggered by anxiogenic challenge with a sex difference. These data strengthen the pathophysiological hypothesis that prenatal stress as a risk factor involves in the development of anxiety disorder in the offspring.

1. Introduction

Environmental adversity, either physical or emotional, experienced by the mother during pregnancy, may have an impact on the developing fetus, affecting its physical and mental wellbeing in later life. The majority of epidemiological and experimental studies have demonstrated the detrimental consequences of prenatal stress for the development of emotionality and cognition in the offspring, and have emphasized that prenatal stress imposed an elevated risk for depression, anxiety disorders (Darnaudery et al., 2004; King and Laplante, 2005; Rice et al., 2007), psychotic disorders (Bresnahan et al., 2005; Khashan et al., 2008), attention deficit/hyperactivity disorder (Li et al., 2010; Ronald et al., 2010) and conduct disorder (Beydoun and Saftlas, 2008; Graignic-Philippe and Tordjman, 2009; Wilson and Terry, 2013; Zohsel et al., 2013) in the offspring.

A large number of clinical and preclinical studies have demonstrated the important role of glutamate in the pathophysiology of anxiety disorders (Barbosa Neto et al., 2012; Herlenius and Lagercrantz, 2004; McQuillen and Ferriero, 2004; Van den Hove et al., 2013). For instance brain imaging analysis in patients with generalized social phobia demonstrated increased glutamate levels in the anterior cingulate cortex as well as a correlation between the magnitude of the glutamate signal and the severity of the phobic symptoms (Phan et al., 2005). The levels of glutamate and its associated amino acid glutamine are elevated in individuals with generalized social anxiety disorder relative to controls (Pollack

Abbreviations: BCA, bicinchoninic acid; EPM, elevated plus maze; GD, gestational day; iGluRs, ionotropic glutamate receptors; LSD, least significant difference; mGluRs, metabotropic glutamate receptors; PCMS, prenatal chronic mild stress; PKC, protein kinase C; PND, postnatal day; PSD, postsynaptic density protein; PVDF, polyvinylidene fluoride; SDS, sodium dodecyl sulfate; 11β-HSD-2, 11β-hydroxysteroid-dehydrogenase type 2.

* Corresponding author at: Department of Psychiatry, The First Hospital of China Medical University, 155 Nanjing Street, Heping District, Shenyang 110001, PR China. Tel.: +86 24 83282184; fax: +86 24 83282184.
E-mail address: xbli@mail.cmu.edu.cn (X. Li).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijdevneu.2015.01.003
0736-5748/

et al., 2008). Furthermore, the change in glutamate function could be reversed by anxiolytic drugs treatment (Pollack et al., 2008). In addition, a number of clinical studies have demonstrated the utility of glutamate-active drugs in anxiety disorders, including memantine (Sani et al., 2012), riluzole (Lapidus et al., 2013) and pregabalin (Barenbaum and Rilla Manta, 2004). Otherwise, basic research in animals has established a strong connection between anxiety and glutamatergic system (Amiel and Mathew, 2007; Aroniadou-Anderjaska et al., 2012; Busse et al., 2004; Cortese and Phan, 2005; Nordquist et al., 2008). Thus, it is thought that metabotropic and ionotropic glutamate receptors (mGluRs and iGluRs) are neurobiological targets in anxiety and stress-related disorders (Harvey and Shahid, 2012; Riaza Bermudo-Soriano et al., 2012).

NMDA receptors belong to one group of iGluRs that play a central role in both emotion and cognition. They are mainly situated postsynaptically, where they form large complexes with diverse proteins (enzymes, receptors, scaffolding proteins). The NMDA receptor requires co-activation by two ligands: glutamate (acting on NR2) and glycine (acting on NR1), and interaction with PSD95 (a scaffold protein that accumulates at the postsynaptic membrane) that mediates their stabilization and activity-dependent trafficking (Sturgill et al., 2009). Preclinical and clinical studies have shown that NMDA receptor antagonists can exert antidepressant effects (Inta et al., 2009). D-serine, which acts as a co-agonist for NR1, produces antidepressant-like effects in rodent behavioral despair models (Malkesman et al., 2012). Pharmacological blockade of NR2B receptor induces antidepressant-like effects lacking psychotomimetic action and neurotoxicity in the perinatal and adult rodent brain (Lima-Ojeda et al., 2013), and NR2B receptor antagonist was effective in treatment-resistant depression patients (Preskorn et al., 2008).

In contrast, mGluRs can be subdivided into group I (mGluR1 and 5), group II (mGluR 2 and 3) and group III (mGluR 4, 6–8) subforms, which are widely expressed throughout the mammalian CNS (Harvey and Shahid, 2012; Niswender and Conn, 2010; Swanson et al., 2005). The group I mGluRs play a major role in regulating postsynaptic excitability, and they may enhance the NMDA current through the branches of G_q-mediatied signaling (activation of PKC cascades and Ca^{2+} release) as well as a G-protein independent pathway (Inta et al., 2012; Kotecha et al., 2003; Kotecha and MacDonald, 2003). Group II and III metabotropic receptors are present largely on presynaptic membranes and on glial cells where they are believed to modulate glutamatergic neurotransmission by sensing glutamate spillover and regulating transmitter release (Sanacora et al., 2008).

Previous studies revealed that prenatal stress significantly reduced the excitatory amino acid transporter 2 (EAAT2) mRNA levels in the hippocampus, striatum, and frontal cortex. Significant reduction of EAAT3 mRNA expression was also found in the hippocampus. The decreased EAATs level may result in the decrease of glutamate reuptake and then potentially induce the accumulating of glutamate in the synaptic cleft, which might be a risk factor for increases of synaptic glutamate levels (Zhang et al., 2013). Furthermore, recent work has reported that prenatal stress produced an impairment of both ionotropic glutamate receptors (iGluRs)- and metabotropic glutamate receptors (mGluRs)- dependent synaptic plasticity (Berger et al., 2002; Burt et al., 2013; Holloway et al., 2013; Jia et al., 2010; Matrisciano et al., 2012; Owen and Matthews, 2007; Sun et al., 2013; Tavassoli et al., 2013). In addition, glutamate receptors were involved in neural stem cell self-renewal, proliferation, differentiation and survival (Duan et al., 2013; Inta et al., 2009; Xiao et al., 2013). There was compelling evidence reported that prenatal stress significantly inhibited hippocampal neurogenesis (Belnoue et al., 2013; Li et al., 2014), increased caspase-3 activity in hippocampus (Ladefoged et al., 2004), and increased fos expression in fetal paraudio videoentricular nuclei (PVN) and induced increased vulnerability to cell death, including apoptosis (Fujioka et al., 1999), suggesting glutamate receptors were involved in the often reported deleterious effects of prenatal stress on behavioral responses to stress.

Recent studies demonstrated that the effects of prenatal stress exposure in rats seemed to be sex-dependent (Charil et al., 2010; Glover, 2011; Weinstock, 2008). However the data on sex differences is still in an ambiguous state because of the various results and the imbalanced research status quo that many more studies have focused on males. For example, some studies found more anxiety- and/or depression-like behavior induced by prenatal stress especially in male rats rather than female rats (Chung et al., 2005; Sun et al., 2013; Szymanska et al., 2009), but other studies have failed to find a similar enhancement in male rats (Bogoch et al., 2007; Estanislau and Morato, 2005; Zagron and Weinstock, 2006, 2011). Furthermore, the neural underpinnings that underlie these sex differences induced by prenatal stress are largely unknown, but glutamatergic system is likely involved. Though prenatal stress had been found to affect the mGluRs and iGluRs in the prefrontal cortex, hippocampus and amygdala in male offspring (Biala et al., 2011; Chutabhakdikul and Surakul, 2013; Fumagalli et al., 2009; Laloux et al., 2012), only a few studies have considered the sex difference. It has been demonstrated that prenatal stress induced an opposite activity of mGluR1/5 or NR1 in the hippocampus between male and female rats (Biala et al., 2011; Zuena et al., 2008), but Sun et al. (2013) found no sex difference of NR1 activity induced by prenatal stress in the hippocampus. Fumagalli et al. (2009) found an opposite expression of NR2B in male and female prefrontal cortex induced by prenatal stress; whereas there is a paucity of research examining sex difference induced by prenatal stress in both the mGluRs and iGluRs in the amygdala which plays a major role in the pathophysiology of mood disorders. Otherwise, the different effects of prenatal stress on offspring at the level of behavioral and neurobiological mechanisms may depend on the stressors of prenatal stress paradigms. The most common type among these is the prenatal restraint stress model, but this way of inducing physical stress cannot simulate the psychopathology characteristic of depression or anxiety disorders well. Furthermore, it may potentially cause trauma to the fetus through physical pressure (Yum et al., 2012). In addition, repeated restraint stress (which is typically administered several times daily until delivery) can lead to habituation of the stress response (Melia et al., 1994). It should be noted that the variety of mild stressors used in chronic mild stress procedures (Abdul Aziz et al., 2012; Cabrera et al., 1999; Hougaard et al., 2005), in which a variety of relatively mild stressors are presented on a random schedule, should more accurately reflect the type of stress that many women are likely to experience during pregnancy. It may increase the translational potential and relevance of the experimental results (Willner, 2005).

In the present study, we used prenatal chronic mild stress (PCMS) to examine the effects of maternal stress on anxiety-like behavior in the offspring. Then, we analyzed the sex differentiation of PCMS and/or anxiogenic challenge on the expression profiles of mGluRs and iGluRs in adult offspring in different brain regions.

2. Materials and methods

2.1. Animals

Fifty-two nulliparous female Sprague-Dawley rats weighing 250–270 g aged 3–4 months (the Laboratory Animal Center of China Medical University, Shenyang, Liaoning, China) were group-housed (4 females per cage) for 1 week before mating. Afterwards, each female rat was housed with a male rat (280–300 g) for mating (1:1). A positive vaginal smear of sperm was designated as day 0