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Maternal restraint stress delays maturation of cation-chloride cotransporters and $GABA_A$ receptor subunits in the hippocampus of rat pups at puberty

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ABSTRACT

The GABAergic synapse undergoes structural and functional maturation during early brain development. Maternal stress alters GABAergic synapses in the pup's brain that are associated with the pathophysiology of neuropsychiatric disorders in adults; however, the mechanism for this is still unclear. In this study, we examined the effects of maternal restraint stress on the development of Cation-Chloride Cotransporters (CCCs) and the $GABA_A$ receptor α1 and α5 subunits in the hippocampus of rat pups at different postnatal ages. Our results demonstrate that maternal restraint stress induces a transient but significant increase in the level of NKCC1 (Sodium–Potassium Chloride Cotransporter 1) only at P14, followed by a brief, yet significant increase in the level of KCC2 (Potassium-Chloride Cotransporter 2) at P21, which then decreases from P28 until P40. Thus, maternal stress alters NKCC1 and KCC2 ratio in the hippocampus of rat pups, especially during P14 to P28. Maternal restraint stress also caused biphasic changes in the level of $GABA_A$ receptor subunits in the pup's hippocampus. $GABA_A$ receptor α1 subunit gradually increased at P14 then decreased thereafter. On the contrary, $GABA_A$ receptor α5 subunit showed a transient decrease followed by a long-term increase from P21 until P40. Altogether, our study suggested that the maternal restraint stress might delay maturation of the GABAergic system by altering the expression of NKCC1, KCC2 and $GABA_A$ receptor α1 and α5 subunits in the hippocampus of rat pups. These changes demonstrate the dysregulation of inhibitory neurotransmission during early life, which may underlie the pathogenesis of psychiatric diseases at adolescence.

1. Introduction

Early life stressors that shape the stress response in offspring have profound effects on mood and cognition in adulthood (Davidson & McEwen, 2012). Chronic exposure to glucocorticoids contribute to the dysfunction of the inhibitory network and impairment of rhythmic oscillations, which are critical for the regulation of brain activity and complex cognitive processes (Hu & et al, 2010). A dysfunctional GABAergic system is associated with the pathogenesis of neuropsychiatric diseases such as schizophrenia, anxiety and depression (Hines & et al, 2012).

During brain development, GABAergic synapses are formed prior to the formation of glutamatergic synapses and the activation of the $GABA_A$ receptor depolarizes immature neurons (Ben-Ari & et al, 2012; Ben-Ari, 2002; Ben-Ari & et al, 2007). Excitatory GABA transmission plays important roles in various neurodevelopmental processes including; neuronal migration, cell proliferation, neurite outgrowth and generating synchronized network activity (Cherubini & et al, 2011). Cation Chloride Cotransporter (CCC) is the key controlling factor in controlling the switch of the $GABA_A$ receptor function. CCCs control the reversal potential of the $GABA_A$ receptor-mediated current (EGABA), which is important for the modulation of the $GABA_A$ receptor function. There are two main types of CCCs; the outwardly directed Potassium-Chloride Cotransporter 2 (KCC2), and the inwardly directed Sodium–Potassium-Chloride Cotransporter 1 (NKCC1). In immature neurons, NKCC1 increases the chloride reversal potential thus it accumulates Cl^- inside the cell. KCC2, on the other hand, reduces the chloride reversal potential thus it extrudes Cl^- out of the cell and shifts the actions of the GABA from excitation to inhibition. Although the

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other chloride regulators channels and transporters also take part in this sequence (Blaesse & et al, 2009; Medina & Chudotvorova, 2006), the expression of KCC2 is thought to initiate the developmental switch of the $GABA_A$ receptor function from excitatory to inhibitory transmission (Ben-Ari, 2002).

In addition to the expression of Cation-Chloride Cotransporters, the $GABA_A$ receptor undergoes postnatal changes in its structure and function by the differential expression of different subunits' composition (Jacob et al., 2008). The presence of $GABA_A$ receptor α1 subunits mediates phasic inhibition by inducing a more rapid decay rate in $GABA_A$-mediated synaptic currents (Dunning & et al, 1999). In contrast, the $GABA_A$ receptor α5 subunits mediate tonic inhibition, which can be characterized by a slow decay rate of the synaptic current (Jacob et al., 2008). The α1 subunits are located at the synaptic sites and mostly found in mature neurons, while the α5 subunits are located at the extrasynaptic sites and found mostly in immature neurons prior to the formation of the inhibitory synapse (Jacob et al., 2008; Farrant & Nusser, 2005; Owens & Kriegstein, 2002). Thus, the maturation of the GABAergic function requires the precise expression of specific subunits of the $GABA_A$ receptor during postnatal brain development. The early expression of the $GABA_A$ receptor α5 subunit is required for the tonic inhibitory function of GABA, while the late expression of the α1 subunit is required for the phasic inhibition that indicates the maturation of the $GABA_A$ receptor function.

It is well documented that stress increases glucocorticoid hormones and thereby potentiates excitotoxic damage in hippocampal GABAergic neurons (Elliott & Sapolsky, 1992; Stein-Behrens & Sapolsky, 1992). Early life stress exerts an effect on the hippocampal neurons and predisposes individuals to psychosis (Stumpf & et al, 1989; Tornello & et al, 1982; Zhang & et al, 1990; Scheller-Gilkey & et al, 2003). The hippocampus exhibits subtle alterations subsequent to neuropsychiatric diseases such as schizophrenia and mania depressive disorder (Benes, 1999). Previous studies in postmortem brains from schizophrenia patients have shown a decrease in hippocampal GABAergic activity that could potentiate excitotoxic damage to hippocampal interneurons, consistent with abnormal oscillatory rhythms and increased basal metabolic activity (Benes, 1999). It is still unclear how prenatal stress affects the development of GABAergic synapses in the hippocampus of the offspring. In this study we hypothesized that prenatal stress may affect the structural and functional maturation of the $GABA_A$ receptor in the hippocampus of rat pups. Therefore, the purpose of this study was to examine the effect of maternal restraint stress on the levels of NKCC1 and KCC2, as well as $GABA_A$ receptors α1 and α5 subunits in the hippocampus of the offspring to provide insights about the involved mechanisms of maternal stress as a cause of dysregulation of GABAergic synapses that are known to be associated with the pathogenesis of psychiatric diseases at adulthood.

2. Materials and methods

2.1. Animals

Pregnant Sprague Dawley rats and their offspring were used in this experiment. Pregnant rats were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Thailand. They were housed in a single housing condition with a temperature and humidity controlled environment and maintained on a 12 h light/dark cycle with free access to food and water. Each pregnant female rat was weighed on gestation day (GD) 7–21. In the morning of GD 21, each pregnant rat received nesting material, and thereafter, the cage was checked twice daily for the appearance of a litter. The day a litter gets discovered becomes designated as postnatal day 0 (P0), and the length of gestation was noted. All experiments were conducted according to the Guidelines for Care and Use of the Laboratory Animals and approved by the Experimental Animal Ethics Committee of the Institute of Molecular Biosciences, Mahidol University, Thailand (COA.MB-ACUC 2015/003). Every effort was taken to minimize the number of animals used and their suffering.

2.2. Maternal restraint stress

Pregnant rats were divided into two groups; 1) control group, 2) maternal restraint stress group (N = 4/group). For the restraint stress, each pregnant rat was put into a small Plexiglas cylindrical cage in which the length can be adjusted to accommodate the size of each animal. The restraint stress was performed during GD14-20, at four hours daily intervals during the light phase of the cycle as previously described (Surakul et al., 2011; Chutabhakdikul & Surakul, 2013). The control group was left undisturbed in their home cages. Gestation days 14–20 were selected because they represent the most sensitive period for the behavioral teratogenic effect of prenatal stress (Fride & Weinstock, 1984).

2.3. Tissue preparation

Whole hippocampal tissues were collected from rat pups at different postnatal days (P) from P7, P14, P21, P28 and P40, with n = 4/group. Brain tissues were then suspended in a lysis buffer composed of 50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% Sodium Deoxycholate, 1% SDS, 1 mM PMSF, 1% Triton-X-100 and supplemented with a complete protease and phosphatase inhibitor cocktail set (Calbiochem, Germany), then homogenized twice with a sonicator for 10 s each. The homogenized samples were centrifuged at 14,000 rpm, 4 °C for 15 min. The supernatant was collected for protein determination. The protein concentration from each sample was determined by a Bradford protein assay.

2.4. Western blot analysis

Cell lysates were mixed with a sodium dodecyl sulfate (SDS) sample buffer and boiled. Equal amounts (20 μg) of extracted protein samples were resolved in 10% SDS-PAGE and electrophoresis at 100 V for 150 min. The protein bands were then transferred to the PVDF membrane (Amersham, USA) at 100V for 135 min. The membranes were then incubated in a blocking solution containing 3% skimmed milk for NKCC1, KCC2 and $GABA_A$ receptor α1 and α5 subunits, and 5% skimmed milk for actin at room temperature for 60 min. Then the membranes were incubated with the following specific primary antibodies purchased from the available commercial sources. Polyclonal goat anti-NKCC1 (SC-21545; 1:500), polyclonal goat anti-KCC2 (SC-19420; 1:500), polyclonal goat anti-$GABA_A$ receptor α1 subunit (SC-7348; 1:500), polyclonal goat anti-$GABA_A$ receptor α5 subunit (SC-7357; 1:500), and monoclonal mouse anti-actin (SC-69879; 1:5000), all antibodies were purchased from Santa Cruz Biotechnology, USA. The membranes were then thoroughly washed 3 times using 0.1% Tween-TBS (TTBS) for 5 min each, and then incubated with an appropriate HRP-conjugated secondary antibody. After that, the membranes were washed 3 times using 0.1% TTBS for 5 min each, and then the signals were detected by an Enhanced Chemiluminescence System (ECL Prime, Amersham Biosciences, USA) and film exposure. The films were then scanned and digitally processed using Adobe Photoshop software. The intensities of the band were quantified using densitometry software (Image J, National Institutes of Health, USA). The immunoblot data were corrected for corresponding product of the β-actin extracted from the same tissue which serve as an internal control.