Prenatal Stress Impairs Neuroligin 1-dependent Neurogenesis through Suppressing Astrocytic FGF2-Neuronal FGFR1 Interaction

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Abstract

Exposure to maternal stress irreversibly and permanently impairs neurogenesis of offspring through inducing life-long effects on interaction between neurons and glia under raging differentiation process, culminating in cognitive and neuropsychiatric abnormalities in adulthood. Therefore, we identified how prenatal exposure to a major stress-responsive hormone glucocorticoid impedes synapse formation and subsequent neurogenesis using human induced neural stem cell (NSC) and ICR mice. First, we observed that maternal corticosterone exposure during embryonic day 14 triggered both depression/anxiety-like behaviors and spatial memory dysfunction in littersmates at postnatal day 23. We observed that phenotypes of differentiated astrocytes from NSC were changed A1-like astrocytes, which lose their neurotrophic functions. Cortisol-treated astrocyte conditioned media (ACM) then specifically downregulated AMPA-mediated glutamatergic synapse formation and transmission in differentiating neurons, especially via decreasing localization of ionotropic glutamate receptor (Glur) 1/2 in synapse. Data from RNA sequencing, antibody array, and subcellular fraction revealed that downregulated astrocytic fibroblast growth factor 2 (FGF2) and nuclear fibroblast growth factor receptor 1 (FGFR1) of neurons are key factors for reducing glutamatergic synapse formation. We further confirmed that cortisol-treated ACM specifically decreased binding of neuronal FGF2 to the LGN1 promoter among the synaptogenic genes, but reversed by FGFR1 restoration in differentiated neurons. Upreregulation of neuroligin 1, an important molecule to scaffold GluR1/2 into the postsynaptic compartments, eventually normalized glutamatergic transmission and neurogenesis. Consistent with these results, FGF2 pretreatment of a prenatal corticosterone-exposed mouse elevated neuroligin 1 expression and trafficking of GluR1/2 into postsynaptic compartment, improving the outcome of a spatial memory task and depression/anxiety-like behaviors. In conclusion, our results identified the restoration of astrocytic FGF2 and its downstream neuronal nuclear FGFR1 as critical targets of prenatal stress-induced glutamatergic synapse formation through regulating neuroligin 1 and demonstrated its function in controlling both neurogenesis and hippocampal-related behaviors including spatial memory and mood formation.

Introduction & Aim

Stress is a high risk factor that suppresses development and differentiation of both embryonic and adult neural stem cell (NSC). However, prenatal or early-postnatal stress continues to adversely affect neurogenesis, followed by dysregulation in memory function and mood formation during lifetime through permanently downregulating neurogenic or neurotrophic factors released by astrocytes whereas stress effect on neural tissue is usually reversible [1 - 4]. Therefore, we determined the effect of glucocorticoid on neuron-astrocyte communications and ensuing neurogenesis dysfunction including synaptic homeostasis dysregulation or behavior impairments using human induced neural stem cell (hNSC) and prenatal stress mouse model.

Materials & Methods


Results

Figure 1. Maternal stress induces memory impairment in its littersmates
3 week-old mouse model exposed to maternal corticosterone at E14 were used. (A) Mice underwent open field test to examine anxiety behavior. Mice with maternal corticosterone were likely to less explore open field, show activity at peripheral region, and lay less fecal balls. (B) Forced swim test were applied to detect depression-like behavior. Mice with maternal corticosterone exhibited immediate activity more than control mice. (C) Y-maze test was performed to detect spatial memory task. Mice with corticosterone exposure showed reduced memory task. (D) Desmethyline suppression test in dark cycle was done to determine hypothalamus-pituitary-adrenal (HPA) axis regulation. Mice with maternal corticosterone were more insensitive to suppression in desmethyline. (E) Doublecortin (DCX) positive and Neun (green) were immunostained to observe the immature neuron ratio to mature neuron in subgranular zone (SGZ) of dorsal ventral hippocampus.

Figure 3. Reduction in astrocytic FGF2 suppresses its downstream FGFFR- mediated transcription and subsequent glutamatergic synapse formation (A) Astrocyte conditioned media (ACM) exposed to cortical treatment for 5 days were administered to differentiated neurons and FM-14 dye release was detected. (B) ACM from astrocytes were treated in neurons. ACM showed downregulation of excitatory synapse formation immunostaining vGlu1 (green) and PSD95 (red). (C) AMPA-mediated glutamatergic transmission was impaired by cortisol-treated ACM. (D) RNA sequencing was done. ACM were exposed to maternal corticosterone to determine which neurotrophic factor-dependent signaling and subsequent depression in glutamatergic transmission are altered. (E) Subcellular fraction was done to determine which factors of FGF2 and FGF1 are changed in cortisol-exposed ACM. (F) Co-localization of FGFR2 and FGFR1 nucleus was determined with immunostaining. (G) FM-14 kinetics were detected to confirm glutamatergic transmission was mainly impaired by nuclear FGFR1 of neurons.

Figure 4. Glucocorticoid suppresses glutamatergic synapse formation and neurogenesis via downregulating neuroligin (A-B) Real-time PCR and CHIP assay were performed to determine which factor among synapsin, fibroblast growth factor 2, or FGF2 receptor 1 (FGFR1) was downregulated in cortex of glucocorticoid. (B) FGF2 kinetics were detected to observe the recovery effect of ACM expression on FGFR1 expression and null effect of ACM on FGF2 expression. (C) FGFR1 Kinetics were detected to observe the recovery effect of ACM expression on FGF2 expression and null effect of ACM on FGFR1 expression. (D) Real-time PCR and CHIP assay were performed to detect the recovery effect of ACM expression on FGF2 expression and null effect of ACM on FGFR1 expression.

Conclusion

Prenatal stress exposure reduces neuroligin 1-dependent AMPA trafficking into synapse and subsequent neurogenesis of mouse hippocampus/human induced-NSC, mainly induced by downregulated astrocytic FGF2 release and its downstream nuclear FGFR1 of neurons.

References