Alterations of GABAergic neuron-associated extracellular matrix and synaptic responses in Gad1-heterozygous mice subjected to prenatal stress

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論文題目

Alterations of GABAergic neuron-associated extracellular matrix and synaptic responses in *Gad1*-heterozygous mice subjected to prenatal stress

（胎生期ストレスを受けた *Gad1* ヘテロ接合体マウスにおける GABA 細胞関連細胞外マトリックスとシナプス反応性の変化）

論文の内容の要旨

[Introduction]

Mutations to GABA synthesizing enzyme glutamate decarboxylase (GAD) 67 and environmental insults such as prenatal stress (PS) are risk factors associated with a spectrum of psychiatric disorders. Incidentally, findings from human studies corroborate the essentiality of the dysfunction of GABAergic network and specifically a deficit in the parvalbumin (PV)-expressing interneuron mediated inhibition in the medial prefrontal cortex (mPFC). Using GAD67-GFP knock-in ($GAD67^{+/GFP}$) mice with reduced GABA production by 50%, we previously reported that the application of restraint PS through mother mice suppressed the neurogenesis of GABAergic neurons in the medial ganglionic eminence (MGE) of $GAD67^{+/GFP}$ embryos. Furthermore, this resulted in the reduced density of PV+- neurons in the medial prefrontal cortex (mPFC) of postnatal $GAD67^{+/GFP}$ mice (HT-PS). Perineuronal nets (PNNs) are proteoglycan based extracellular matrix (ECM) structures found enwrapping the soma and proximal neurites of PV neurons in a lattice-like fashion. Decrements of PNNs in multiple brain regions were found in subjects with schizophrenia, suggesting that PNNs might play a role in its pathogenesis. Another component of ECM, dystroglycan, is a central member of the dystrophin glycoprotein complex (DGC) and has been implicated in the maintenance of mature inhibitory synapses. Here, we tested the hypothesis that our gene–environment interaction model causes abnormalities in the ECM and functions of GABAergic interneurons in the mPFC of HT-PS mice.

[Materials and Methods]

All procedures using animals were approved by Committee for Animal Care and Use, Hamamatsu University School of Medicine (No. 2016029). In the present study, female ($GAD67^{+/+}$) mice were mated with male $GAD67^{+/GFP}$ (>9 weeks) mice. Detection of a vaginal plug was marked as embryonic day (E) 0. Maternal restraint-and-light stress was performed during E15.0 to E17.5. Newborns were raised by naive surrogate mothers until postnatal day 21(P21). Using P21 male mice, 25 μm coronal cryosections containing mPFC were obtained. Immunostaining were performed for quantification of

PNN densities around PV+ interneurons. Immunostaining for aggrecan (ACAN) based PNNs were also performed to understand the assemblies of PNNs affected by gene environment interaction. The glycosylation pattern of α-dystroglycan (α-DG) was evaluated to ascertain the functionality of the DGC associated with inhibitory synapses. Quantitative polymerase chain reaction (PCR) was used for mRNA expression analysis of fukutin (*Fktn*), a glycosyltransferase implicated in altered glycosylation pattern of α-DG. The impact of ECM changes on the inhibitory synaptic output to layer V pyramidal neurons of mPFC were studied using slice patch clamp electrophysiology. In brief, using 350 μm thick slices whole cell voltage clamp recordings were performed for evoked inhibitory postsynaptic currents (eIPSC) and their properties were estimated. Furthermore, spontaneous IPSC (sIPSCs) events were recorded and distribution of amplitude, decay tau and interstimulus intervals (ISI) were estimated. Appropriate statistical tests were used for comparison with confidence intervals set at 95%.

[Results]

Consistent with our previous study, numbers of PV+ interneurons were significantly less in the mPFC of HT-PS. Reduction rate of PNN densities surrounding PV+ cells showed positive correlation with loss of PV+ cells indicating lowering of PNN density was due to loss of PV+ cells. ACAN+ PNN densities were also reduced only in HT-PS group. Densities of PNN were observed in groups without gene-environment interaction were unaltered as were intensities of PNN and ACAN staining surrounding PV+ and PV- cells. Decrease in the glycosylation of α-DG estimated as cluster density was observed selectively in HT-PS. This reduction was paralleled by reduced *Fktn* mRNA levels. Analysis of eIPSCs indicated lowered stimulus threshold and increased amplitude with prolonged decay (τ_{fast} and τ_{slow}) in layer V pyramidal neurons in the mPFC of HT-PS mice. Relative amplitudes corresponding to different phases of eIPSC decay were also significantly different. However paired pulse ratios were not different. Genotype alone didn't affect eIPSC parameters. Analysis of sIPSC events indicated increased frequency and slower decay kinetics. Lower and higher amplitude fraction were apparently increased and decreased in stressed mice.

[Discussion]

Recapitulating a neurodevelopmental approach to the pathogenesis of psychiatric diseases, we observed that PV+ neurons associated with PNN and ACAN+ PNNs were reduced in the mPFC of HT-PS. This reduction is correlated to reduced neurogenesis of GABAergic interneurons in MGE. This might cause substantial recapture of plasticity on the function of inhibitory networks. In addition, reduced glycosylation of α-DG may affect the ligand-binding and structure of the extrasynaptic space around GABAergic synapses. Remodeling of putative cholecystokinin (CCK) + inhibitory axon terminals

are possible. Evaluation of eIPSC and sIPSC properties indicate increased excitability of inhibitory network coupled with both pre and post synaptic changes affirming the substantial impact of ECM changes in functionality of mPFC in HT-PS mice.

[Conclusion]

In conclusion, *Gad1* abnormalities as a genetic risk factor may interact with environmental risk factor such as PS to induce loss of PV neurons and alteration of ECM. These alterations may underlie the observed changes in synaptic inhibition in the layer V of mPFC thereby affecting their functional output essential for higher cognitive functions.