学 位 論 文 の 要 旨

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学 位 論 文 名: Electroconvulsive Shock Restores the Decreased Coverage of Brain Blood Vessels by Astrocytic Endfeet and Ameliorates Depressive-like Behavior

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論 文 内 容 の 要 旨

INTRODUCTION

Application of electroconvulsive therapy (ECT) is still considered to be superior, especially, in its rapid effect on the treatment of psychiatric disorders, such as, drug-resistant major depression, schizophrenia, mania and catatonia. Growing evidence indicates that glial cells are involved in response to ECT. However, the exact mechanisms of therapeutic effect of ECT have yet to be clarified. Glial cells have attracted attention as new promising targets for antidepressant action. These findings prompt us to investigate the mechanisms involving astrocytes in the therapeutic effect of ECT.

Aquaporin (AQP) 4 is a water channel and plays pivotal roles in the brain functions. Astrocytic endfoot processes, on which AQP4 is expressed abundantly, ensheath entire network of blood vessels in the brain and is involved in forming gliovascular units. It has been shown that AQP4 is required for the anti-depressive action via regulating adult hippocampal neurogenesis. A recent postmortem study on patients with major depression has revealed a decrease in the coverage of blood vessels by AQP4-immunostained endfeet of astrocytes in the prefrontal cortex. It is, therefore, tempting to elucidate the effect of ECT on the decreased coverage of blood vessels by AQP4-immunopositive endfeet, which appears to be involved in the pathogenesis of the major depression.

The Gunn rat, a mutant of the Wistar strain, has a genetic deficiency in glucuronyltransferase leading to high levels of unconjugated bilirubin in its blood and brain. Our previous study has revealed that Gunn rats with glial activation in the hippocampus present depressive-like behavior. In the present study, we investigated the morphology of gliovascular units with regard to the astrocytic coverage of blood vessels in the diseased brain of Gunn rat and determined the effect of electroconvulsive shock (ECS), an animal counterpart of ECT, on the astrocytic coverage of brain blood vessels of Gunn rats whose depressive-like behavior was ameliorated by ECS, to examine whether the change in morphology of gliovascular units is related to the therapeutic efficacy of ECS. Additionally, we also confirmed the change of hippocampal expression of AQP4 and tight junction molecule claudin-5.

MATERIALS AND METHODS

Eight-week-old male homozygous (j/j) Gunn rats and male Wistar rats were used in this study. The animals were assigned into four groups, i.e., Wistar Sham (WS), Wistar ECS (WE), Gunn Sham (GS) and Gunn ECS (GE) group. The ECS group received an ECS treatment once daily for 6 consecutive days. All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University.

Each individual was given the Y-maze test and the forced swimming test (FST). After the behavior tests, the animals were sacrificed by transcardial perfusion under deep intraperitoneal anesthesia. After perfusion, the brain was quickly removed and fixed in a solution of paraformaldehyde, subsequently, immersed in a cold solution of sucrose. The brains were cut at 40-μm thickness and then immunofluorescence was carried out to determine the AQP4 coverage of blood vessels by using lectin and anti-AQP4 antibody. Fluorescent images were captured with a 40x objective lens in the prelimbic (PrL) area and the infralimbic (IL) area of the medial prefrontal cortex (mPFC) and in the dentate gyrus (DG), the cornu ammonis (CA)1 and the CA3 of the hippocampal formation. First, the lectin-positive area was extracted and displayed red. Next, colocalization area, namely, the AQP4-positive area on the lectin-positive area was extracted and displayed yellow. The pixels of the yellow areas were calculated to determine the % of “coverage”, which was defined as area of co-localization/total area of blood vessels.

In addition, hippocampal tissues were collected from other rats to determine AQP4 and claudin-5 expression using Western blot. Individual expression levels of AQP4 or claudin-5 were normalized to the expression levels of β-actin and presented as percentages of the control group (Wistar sham). Statistical analyzes were conducted using two-way ANOVA, one-way ANOVA followed by the post-hoc Fisher's least significant different test, and Pearson correlation test.

RESULTS AND DISCUSSION

There were four major findings in the present study. First, the coverage of blood vessels by AQP4-immunoreactive endfeet of astrocytes in the mPFC and the hippocampal formation was significantly reduced in Gunn rats compared to that of Wistar rats. Second, ECS significantly reduced immobility time in the FST, suggesting that ECS ameliorated depressive-like behavior of Gunn rats. Third, ECS significantly improved deficits of working memory of Gunn rats as shown by normalized percentage of Spontaneous Alternation Behavior in the Y-maze test. The last, ECS significantly increased the reduced AQP4 coverage of brain blood vessels in Gunn rat.

Our observation showed that the coverage of brain blood vessels by AQP4-immunoreactive astrocytic endfeet was significantly diminished in Gunn rats presenting depressive-like behavior which were reversed by ECS. Moreover, the hippocampal expression of AQP4 and claudin-5, one of the tight junction molecule, showed the same trend. To our knowledge, this is the first study showing that antidepressant treatment, namely ECS administration in our study, restores the decreased coverage of brain blood vessels by astrocytic endfeet in the pathological brain. Based on these findings, the astrocytic-endfoot coverage of blood vessels may be negatively correlated with manifesting depressive symptoms. To verify this hypothesis, further studies to determine the effects of antidepressant treatment, especially neuropharmacological medication, on the astrocytic-endfoot coverage of brain blood vessels are

clearly warranted.

Our previous studies have demonstrated that Gunn rats possess astrocytic activation in the hippocampus. In addition, it has been demonstrated that ECS significantly attenuate the increased hippocampal expression of glial fibrillary acidic protein in Gunn rats, indicating ECS has inhibitory effects on activated astrocytes. Interestingly, it has been shown that astrogliosis or astrocytic activation was accompanied by a decrease in amount of AQP4-immunopositive endfeet colocalized with brain blood vessels in a mouse model of a cerebral small vessel disease with features of gliovascular disruption and cognitive deficits. Activated astrocytes may, therefore, contribute toward impaired gliovascular units with a decrease in the endfoot coverage of brain blood vessels. Considering our previous observation of the efficacy of ECS in inhibiting activated astrocytes in the hippocampus of Gunn rats, regenerating the impaired gliovascular units associated with activated astrocytes may be involved in the therapeutic mechanisms of ECT. In other words, the therapeutic effect of ECS may be exerted, at least in part, by increasing the decreased endfoot coverage of blood vessels, that is presumably related to activated astrocytes.

CONCLUSION

ECS increased the reduced coverage of blood vessels by astrocytic endfeet in the mPFC and the hippocampus and ameliorated depressive-like behavior in Gunn rats. Therefore, therapeutic mechanism of ECT may involve restoration of the impaired gliovascular units by increasing the astrocytic-endfoot coverage of blood vessels. These findings may provide crucial information to elucidate roles of gliovascular units in the pathogenesis of neuropsychiatric disorders and to develop future therapeutic interventions via modulating astrocytic functions.