学 位 論 文 の 要 旨

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学 位 論 文 名　Electroconvulsive Shock Attenuated Microgliosis and Astrogliosis in The Hippocampus and Ameliorated Schizophrenia-like Behavior of Gunn Rat

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論 文 内 容 の 要 旨

INTRODUCTION

Electroconvulsive therapy (ECT) has been used as a treatment for mental disorder since the 1930s because of its effectiveness and the fast action in several psychiatric disorders such as bipolar disorder, major depression, and schizophrenia accompanied by catatonia, extreme depression, mania, and other affective components. However, the exact mechanism of therapeutic action of ECT remains unknown.

Recent studies indicate that ECT affects the immune-related cells, such as microglia, astrocytes, and lymphocytes. Moreover, a change in immune system functions associated with glial activation may be involved in the pathogenesis of schizophrenia. In fact, microglial activation and astrocytic activation have been implicated in postmortem brains of schizophrenia patients. Therefore, it is tempting to determine the effect of ECT on the activation of microglia and astrocytes.

It is believed that there is a relationship between hyperbilirubinemia and schizophrenia. Schizophrenic patients have a significantly higher frequency of hyperbilirubinemia relative to patients with other psychiatric disorders and to the general healthy population. Gunn rats, a mutant of the Wistar strain, have a genetic deficiency in glucuronyl transferase. This deficiency leads to high levels of unconjugated bilirubin in their blood and various tissue, including the brain. Our previous studies have revealed that Gunn rats show a behavioral abnormality similar to schizophrenia with deficits in prepulse inhibition (PPI). Furthermore, we have shown that microglia are activated in the hippocampal dentate gyrus (DG) of Gunn rats. Based on these

findings, the present study evaluated the effects of electroconvulsive shock (ECS), an animal model of ECT, on schizophrenia-like behavior, as well as on microgliosis and astrogliosis in the hippocampus of Gunn rats.

MATERIALS AND METHODS

Six-week-old male homozygous (j/j) Gunn rats and male Wistar rats (Japan SLC, Inc., Japan) were used in this study. The rats were divided into 4 groups, i.e., Wistar sham group (WS), Wistar ECS group (WE), Gunn sham group (GS), and Gunn ECS group (GE). The ECS group received an ECS treatment once daily for 6 consecutive days. The sham-treated control groups were handled identically to the ECS groups including anesthesia except that no current was delivered. Subsequently, PPI test was performed.

After the behavior tests, animal underwent deep intraperitoneal anesthesia and were perfused transcardially. The brains were quickly removed and were fixed in a solution of 10% formalin at room temperature for 4 hours. The brain were immersed overnight in a cold solution of 20% sucrose and then were cut into 40-μm-thick serial sections using a sliding microtome. Immunohistochemistry analysis was carried out to determine the activation degree of microglia and astrocytes in the hippocampus by using anti-CD11b and anti-GFAP antibody, respectively.

GFAP- or CD11b-label glial cells images were captured from three areas within the hippocampus, namely the dentate gyrus (DG), the cornu ammonis (CA)1, and the CA3. The intensity of astroglial and microglial immunoreactivity was measured by a computer-assisted image analysis program (Image J 1.47v). The software automatically converted all immunolabeled element beyond the threshold range into pure black pixels and converted the rest of the image into pure white pixels. The software then calculated the percentage of pure black pixels for statistical analysis. All the data are presented as the mean $\pm$ standard error of the mean. Differences among the groups were evaluated by using one-way ANOVA followed by the post hoc Fisher's least significant different test. This analysis was performed with SPSS software. A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

There were three major findings in the present study. First, ECS administration significantly ameliorated the schizophrenia-like behavior in Gunn rats. Second, ECS inhibited microglial activation in the hippocampi of Gunn rats, as shown by the decreased immunoreactivity of CD11b. Third, ECS also attenuated astrocytic activation in the hippocampi of Gunn rats, as indicated by the reduced expression of GFAP. There have been several animal studies which evaluated the effects of ECS on glial cells in the normal brain. However, to our

knowledge, there have been only a few studies which examined the effects of ECS on glial cells in the pathological brain. The present study determined the effect of ECS on the microglial activation and astrocytic activation in the diseased brain by using Gunn rats.

The present study showed that CD11b expression was significantly increased in the hippocampal DG, CA1 and CA3 areas in Gunn rats compared to Wistar rats. The finding that microglia in the hippocampus of Gunn rats are activated is in line with our previous studies. Gunn rats have high levels of unconjugated bilirubin (UCB) in their blood. Although UCB entrance into the brain is prevented by the blood-brain barrier (BBB), the free fraction of UCB still diffuses into the brain through the BBB and causes glial activation. After ECS, we found a significant decrease in CD11b expression in the DG, but not in the CA1 and CA3. Based on these findings, abnormal behavior similar to schizophrenia may be associated with microglial activation in the DG. Furthermore, ECS may inhibit microglial activation in the pathological brain and this inhibitory effect on activated microglia may be a part of the therapeutic action of ECS.

Not only microglia, but astrocytes are also activated by UCB. Astrocytes, like microglia, are activated in a response to injury or other pathological processes in the CNS and have either a neuroprotective or a neurotoxic role. In the present study, the level of GFAP expression in the hippocampi of Gunn rats was significantly increased compared to Wistar rats in the DG and CA1. After the ECS administrations, the GFAP expression was significantly decreased in the DG and CA1. The abnormal behavior in Gunn rats may be caused by high levels of UCB which may precede chronic inflammation and neurodegeneration in the Gunn rat brain. Therefore, it is presumed that activated astrocytes may play a neurotoxic role in Gunn rats and that ECS may exert therapeutic effect through inhibition of such activation of astrocytes.

Our results showed that ECS significantly suppressed the CD11b expression only in the DG, not in the CA1 and CA3. In addition, the significant inhibitory effect of ECS on the GFAP expression has been observed in the DG and CA1, but not in the CA3. Based on these findings, it is tempting to presume that the response to ECS treatment is regionally selective and the mechanism of ECS to improve PPI deficit may be related to the DG and CA1 rather than the CA3.

CONCLUSION

In conclusion, our findings indicate that ECS on Gunn rats ameliorates schizophrenia-like behavior and attenuates microgliosis in the DG and astrogliosis in the DG and the CA1 of Gunn rats. Accordingly, therapeutic mechanism of ECT may be exerted in part by inhibition of glial activation. These results may also provide crucial information to elucidate the role of activated glia in the pathogenesis of schizophrenia and to determine whether future therapeutic interventions should attempt to up-regulate or down-regulate glial functions.