学 位 論 文 の 要 旨

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学 位 論 文 名　Normalizing Hyperactivity of the Gunn Rat With Bilirubin-induced Neurological Disorders Via Ketanserin

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論 文 内 容 の 要 旨

INTRODUCTION

Severe neonatal hyperbilirubinemia has been known to cause the clinical syndrome of kernicterus, as well as the milder syndrome of Bilirubin-Induced Neurologic Dysfunction (BIND). BIND clinically manifests itself after the neonatal period in the form of developmental delay, cognitive impairment, disordered executive function, and such behavioral and psychiatric disorders as attention deficit hyperactivity disorder (ADHD), schizophrenia and autism spectrum disorder (ASD) . In neonatal hyperbilirubinemia, neurotoxic bilirubin in peripheral blood crosses the blood-brain barrier and binds to such brain regions as the brain stem, basal ganglia, hippocampus and cerebellum to adversely affect neuro-developmental processes including neurogenesis, myelination, and synaptogenesis during the early developmental stage. Mechanism-based elucidation, however, of the linkage from hyperbilirubinemia in peripheral blood to those disturbances in brain function has still to be carried out.

MATERIALES AND METHODS

Nine-to-ten-week-old male homozygous Gunn rats and male Wistar rats were used in this study. Liver UDP-glucuronosyl transferase (UGT: an enzyme responsible for bilirubin conjugation and excretion) activity of the rat is very low, caused by a single guanosine base deletion in the common-region exon 4 of UGT1A1. The Gunn rat is a model of BIND, and it demonstrates abnormal behavior. We investigated serotonergic dysfunction in Gunn rats by pharmacological analyses and *ex vivo* neurochemical analyses. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

We used 41 rats (18 Gunn rats and 23 Wistar rats) for behavioral tests, 12 rats (6 Gunn rats and 6 Wistar rats) to perfuse brain tissue for immunohistochemistry, and 38 rats (19 Gunn rats and 19 Wistar rats) to collect fresh brain tissue for experiments of serotonin (5-HT), dopamine and metabolite assay, real time PCR, and western blot. According to general model animal studies, significant differences can be detected with N=4-6<. Thus, we experimented with 5-8 animals per group.

Statistical analysis of the data was carried out using SPSS software. The data are presented as mean ± SEM. Comparisons of two groups were performed by ANOVA, followed by the appropriate multiple Student's *t*-test or Mann–Whitney *U*-test on the basis of the *F*-test or Dunnett's test. In all cases, differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

We report that 9-10-week-old Gunn rats having been subcutaneously (s.c.) injected with ketanserin 30min before open field tests showed normalized hyperlocomotion and serotonergic dysfunction. Ketanserin is a quinazoline derivative and 5-HT2AR antagonist, and a study of [3H]ketanserin binding showed that after its injection (i.v.), the highest accumulation area was the frontal cortex and striatum in rats. Then, by *ex vivo* neurochemical analyses, we compared the concentrations of serotonin (5-HT) and 5-HIAA (5-hydroxyindoleacetic acid, main metabolite of 5-HT) in the frontal cortices and striata of Gunn and control Wistar rats. Both 5-HT and 5-HIAA in the frontal cortex of the former were higher in concentration than in the latter, although in the striatum of the two groups neither of the them were statistically different from each other. The 5-HT2AR mRNA expression and protein abundance were studied, respectively, by RT-PCR and western blotting to show downregulation of the mRNA expression without alteration of the protein abundance in the frontal cortex, but not in striata, of Gunn rats compared with control. Of note was that serotonergic dysfunction was not found in the striatum, but was found in the frontal cortex of the Gunn rat. These results suggest that serotonergic dysfunction (or increase in serotonergic transmission) in the frontal cortex, but not by dopaminergic dysfunction in the striatum, potentially contribute to hyperlocomotion of the Gunn rat.

We examined the cause of hyperactivity in Gunn rats showing congenital hyperbilirubinemia, and our data demonstrate that hyperactivities in Gunn rat result in serotonergic dysfunction mainly

in PFC. Our findings may help in the prevention and treatment of neurodevelopmental disorders with a post-birth history of hyperbilirubinemia.

In terms of psychiatric objectives, the pathophysiological mechanism of neuropsychiatric disorders, such as schizophrenia and neurodevelopmental disorder, has yet to be defined. Previous studies suggested that there are many clinical cases of neuropsychiatric disorders complicated by idiopathic unconjugated hyperbilirubinemia (Gilbert's syndrome: GS). In particular, schizophrenia accompanied by GS (20.6%) is significantly increased compared with other mental disorders (mood disorders: 2.8%, neurosis: 4.2%). These data indicate that we can discuss these issues in relation to the role of hyperbilirubinemia in the pathophysiological mechanism of schizophrenia. In this study, we demonstrated the effect of ketanserin on hyperactivity in model rats of congenital hyperbilirubinemia. It is expected that 5-HT2AR antagonists will treat clinical symptoms observed in schizophrenia, and most notably when complicated by hyperbilirubinemia.

CONCLUSION

Our results suggest that higher serotonergic transmission voided the downregulation of 5-HT2AR mRNA expression and left the 5-HT2AR protein abundance unchanged in the frontal cortex of the Gunn rat, from which then developed the hyperactive phenotype of the rat. Although our experimental observations were limited to the frontal cortices and striata of 9-10-week-old Gunn and Wistar rats, it would be of value to be able to postulate that a therapeutic strategy for the BIND disorders would include restoration of the brain regions affected by serotonergic dysfunction to normal operation to prevent before, or to normalize after onset of the BIND manifestations.