論文の内容の要旨

農学生命科学研究科 獣医学専攻

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論文題目 Physical training regulates the serotonin 2A receptor sensitivity of neuronal cells in the central nervous system and contributes to alleviation of spasticity after spinal cord injury

（運動は中枢神経系における神経細胞のセロトニン2A受容体感受性を調節し、脊髄損傷後痙縮の改善に寄与する）

It is generally known that physical training (exercise) has numerous beneficial effects on the body system including the nervous system which leads psychological as well as neuro-functional improvements. Particularly, there is accumulating evidence that physical training shows its clinical effects on mental disorders including schizophrenia, anxiety and depression, which are relating to disorder of serotonergic functions in the central nervous system (CNS). In regard to serotonergic system, it is documented that exercise induces an elevation of serotonin (5-HT) levels and regulates the expression of 5-HT receptors. These reports indicate that physical activities by exercise could modulate 5-HT system in the CNS. On the other hand, related to 5-HT system, not only confined to mental disorders, physical training also shows beneficial effects on spinal disorders including spasticity and locomotive functions after spinal cord injury as in the sense of rehabilitation.

Spasticity is one of the common complications after traumatic spinal cord injury (SCI). Over 60% of human SCI patients present spasticity which lowers their quality of life. Spasticity is defined as a velocity dependent increase in the stretch reflex caused by hyperexcitability of spinal circuits. Hypersensitivity to 5-HT and up-regulation of 5-HT receptors below the injury site after SCI has been reported to be related to the hyperexcitability of spinal motor neurons, and consequent spasticity. It is generally recognized that physical training (e.g. treadmill training) is efficient to manage spasticity. However, it is not enough studied using proper spasticity models for evaluating the effect of physical training on the spastic behaviors *in vivo* in related to examining the 5-HT receptors. Moreover, it would be expected to administer combination therapy with medications and physical therapy, aiming

for an additive or synergistic therapeutic effect on spasticity. Since rehabilitation programs with medication targeting spasticity have not been extensively examined in both human and animal studies, there is a need to research the effect of combination therapies including physical training with using a proper animal model of spasticity. Taken together, it is assumed that regulation of 5-HT system plays an important role in therapeutic mechanisms of physical training after the CNS disorders and there is a need to know how physical trainings give beneficial effects not only for better understanding of therapeutic mechanism but for developing more effective and safer rehabilitative therapies.

Although it is reasonable to assume that beneficial effects of physical training on the CNS are produced via the regulation of both ligand and/or receptor sensitivity of 5-HT receptors which belong to G protein-coupled receptors (GPCRs) family, numerous reports have been focused the facts that physical trainings increase the level of 5-HT in the brain as well as spinal cord, while the effects of exercise on the regulation of 5-HT receptor sensitivity have not been paid much attention and still remained unclear. To find out how physical training exerts its effects on the CNS related to the regulation of 5-HT receptor sensitivity, I focused on mechanical components which induced by physical activity itself, such as generated forces, rather than a ligand stimulation or secreted biological factors. Recent studies suggest that the interstitial fluid, the fluid between cells, which is consisted of 20% of volume of the brain, could be changed its velocity or direction by physical activity. Also, it is reported that cerebral interstitial fluid diffuses naturally. Moreover, different direction and velocities of diffusion of cerebral interstitial fluid were observed at the different parts of the brain. When the fluid flows, fluid shear stress (FSS) is occurred on the surface of objects. It is proved that FSS can induce biological modulations on the cells and promote endocytosis of receptors, especially GPCRs. Thus, I assumed that physical training may generate FSS in the CNS and lead to changes in regulation of the 5-HT receptor sensitivity.

From described these backgrounds, I hypothesized that physical training has a role on the regulation of 5-HT receptor and the mechanical stress due to physical activities can be translated to a reason of the effect physical training on the CNS. To examine this hypothesis, first, I developed a novel method for evaluating spasticity after SCI and investigated the effect of physical training on spasticity. Next, I examine the combinational interventions with serotonergic drugs along with physical training. Last, I explored the reason of how the mechanical stress generated by physical activities could give an effect to the 5-HT receptor sensitivity. Overall, the purpose of the thesis is to elucidate whether and how physical training (exercise) regulates the 5-HT receptor sensitivity by using proper experimental models for better understanding about underlying mechanisms of the beneficial effects of exercise on spasticity and for contributing to develop novel therapeutic or rehabilitative strategies for treatment of spasticity as well as the other 5-HT receptor related disorders.

At *Chapter 1*, a novel method to assess spasticity using a swimming test was developed. I examined the feasibility of the swimming test and an EMG for assessing spasticity in a contusive SCI rat model. Sprague-Dawley rats received an injury at the 8th thoracic vertebra. Swimming tests were performed 3 to 6 weeks after SCI induction. I placed the SCI rats into spasticity-strong or spasticity-weak groups based on the frequency of spastic behavior during the swimming test. Subsequently, I recorded the Hoffman reflex (H-reflex) and examined the immunoreactivity of 5-HT and 5-HT_{2A} receptor in the spinal tissues of the SCI rats. The spasticity-strong group had significantly decreased rate-dependent depression of the H-reflex compared to the spasticity-weak group. The expression of 5-HT_{2A} receptor immunoreactivity was significantly increased in the spasticity-strong group. Thus, both electrophysiological and histological evaluations indicate that the spasticity-strong group presented with a more severe upper motor neuron syndrome. I also observed the groups in their cages for 20-hours. In consistent with other results, the spasticity-strong group showed more frequent spastic behaviors during the cage observation. The *Chapter 1* suggest that the swimming test provides a behavioral evaluation of spasticity in this contusive SCI model and the severity of spasticity is correlated to the expression of 5-HT_{2A} receptor on the spinal motor neurons.

At *Chapter 2*, I investigated the effect of physical training on spasticity using the method and animal model described in the *Chapter 1*. Along with treadmill training as physical training, I administrated serotonergic drugs to modulate 5-HT system. In this chapter, SCI rats were subjected to the swimming test described in chapter 1 4 weeks after SCI, and spasticity-strong SCI rats were selected and used. Rats were received combinatorial interventions for 2 weeks, i.e., either only treadmill training, treadmill training with fluoxetine (a selective serotonin re-uptake inhibitor), treadmill training with cyproheptadine (a 5-HT_2 receptor antagonist), only fluoxetine, or only cyproheptadine. I performed the swimming test to quantify the frequency of spastic behaviors. I also evaluated hind limb locomotor functions every week. At the end of the intervention, I examined the H-reflex test from the plantar muscle and the expression of the 5-HT_{2A} receptor in spinal cord tissues. While the treadmill training group and cyproheptadine-treated groups showed decreased spastic behaviors during swimming tests and reduction in spinal hyperreflexia, the fluoxetine-treated group showed the opposite effect even though it was combined with treadmill training. Moreover, treadmill training suppressed the expression of the 5-HT_{2A} receptor in the lumbar spinal motor neurons caudal to injured site, while cyproheptadine treatment did not change it. I did not observe any differences in locomotor functions between the groups. Taken together, the results of *Chapter 2* indicate that treadmill training and cyproheptadine significantly alleviated spastic symptoms, but did not show synergistic or additive effects. Also, the *Chapter 2* suggest that physical training may show its beneficial effect via the regulation of 5-HT receptor sensitivity.

Last, at *Chapter 3*, I explored how the physical training (exercise) affects the 5-HT receptor regulation in the CNS. To do so, I targeted the brain for better explanation in regards to the CNS and for using a proper model. I focused to dynamic head movement during exercise, and then, I conceived a simple and direct method to deliver mechanical stress to the brain: 'passive head motion' (PHM). PHM generates vertical accelerations at the head, which is similar to those during treadmill running, and can produce a reminiscent model to investigate the effect of mechanical stress by physical training on spinal neurons shown in the *Chapter 2*. Also, I utilized head-twitch response which is a hallucinogenic behavioral action that has been reported to represent the intensity of 5-HT_{2A} receptor signaling at the prefrontal cortex (PFC) of rodents. Using these system, I demonstrated that PHM in C57BL/6J mice recapitulates the suppressive effects of treadmill running on the head-twitch response and 5-HT signaling at the PFC in the brain via the internalization of 5-HT_{2A} receptor. By MRI analysis, I verified that PHM facilitates diffusion of cerebral interstitial fluid with the certain direction which is considered as generating fluid shear stress. Furthermore, applying of the fluid shear stress on the neuronal cell line induces internalization of 5-HT_{2A} receptor via activation of protein kinase C (PKC) which is known to mediate signaling involved in ligand-independent internalization of GPCRs expressed in various types of cells. PKC inhibition nullified the head-twitch response and the internalization of 5-HT_{2A} receptor at both in *vivo* and *in vitro* experiments. The *Chapter 3* demonstrated that the effects of physical activities may involve 5-HT receptor modulations in the CNS by generated optimal FSS on the neurons. Thus, the mechanical perturbation model used in this study suggested that the underlying mechanism of how physical training alleviates spasticity after SCI as well as how regulates the sensitivity of 5-HT receptor in the brain.

In conclusion, this study suggests a novel method for evaluating spasticity after SCI, reveals the correlation of 5-HT_{2A} receptor with spasticity and the effect of physical training. Moreover, my thesis proposes that the mechanical factor generated by physical movement can regulate the sensitivity of 5-HT_{2A} receptor in the CNS. The results in this study showed that the physical activities could desensitize 5-HT signaling toward hypersensitivity of 5-HT_{2A} receptor after spinal cord injury or attenuate the neuronal response of 5-HT receptor to overdose 5-HT agonist in the brain. Considering these points, my study may help to develop novel rehabilitation programs or therapies for not only spasticity, but the other 5-HT receptor related disorders, focusing to maintain the homeostasis of 5-HT signaling. In addition, given that mechanical regulation of the brain and spinal cord functions has not been extensively studied or even supposed so far, further related studies are expected.