学 位 論 文 の 要 旨

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学 位 論 文 名　Theobromine Improves Working Memory by Activating the CaMKII/CREB/BDNF Pathway in Rats

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論 文 内 容 の 要 旨

INTRODUCTION

Coffee, cocoa, and chocolate are among the most frequently consumed substances in the world. Coffee has various beneficial effects on human health, as it appears to be cardio-protective, neuroprotective, hepatoprotective, and nephroprotective. Like coffee, the use of the seeds from the cacao tree (Theobroma cacao) to create beverages, dates back to the early formative period of Mesoamerican history (2000–1000 BC). In recent years, there has been a notable interest in the neuroprotective effects of flavonoids, with evidence emerging that they may lead to improvements in memory and learning by improving neuronal functioning, while also promoting neuronal protection and regeneration. Cacao beans, a very popular food worldwide, contains many flavonoids. Theobromine (TB) is one of the flavonoids found in products made from cacao beans. TB is a primary methylxanthine and generally contain approximately 1% in cacao bean products. From recent literature, both scientific and popular, TB has been implicated in the health benefits of cacao intake. We know that TB traverses the blood-brain barrier (BBB) and that this might induce effects on the brain, alter the cellular redox environment, modulate neuronal signaling pathways, and influence gene expression, as well as protein activity, perhaps in a manner similar to other flavonoids. In our previous study, we confirmed that mice fed TB performed better on learning tasks and TB acted as a phosphodiesterase (PDE) inhibitor, by enhancing the cAMP/cAMP-response element-binding protein (CREB)/brain-derived neurotrophic factor (BDNF) pathway. We also confirmed that TB supplemented chow inhibited mTOR signaling in the brain and liver. In the

current experiment, we want to assess the effects of TB on rats, using a prolonged feeding period of 73 days, and we have checked the rats using the behavioral test, radial arm maze (RAM) task, Y-maze test, and novel object recognition (NOR), for assessing the memory function, especially working memory. Working memory is the cognitive capacity to actively and temporarily maintain information for the purpose of task execution. The dorsolateral prefrontal cortex in primates, which is homologous to the medial PFC (mPFC) in rodents, is essential for working memory. Remarkably, many genes have been altered following CREB activation, including key proteins involved in neuronal plasticity, such as BDNF. This protein mediates neuronal development and synaptic function, which is critical for the differentiation and survival of neurons during development. Studies have shown that Ca^{2+}, acting as an important messenger via Ca^{2+}-calmodulin-dependent protein kinases (CaMKs), triggers phosphorylation of CREB. This phosphorylated CREB (p-CREB) activates BDNF transcription by binding to a cAMP response element within the gene. In this study, we found that TB fed rats appear to have improved working memories. As with the cAMP/CREB/BDNF pathway, which was established in our previous study for motor learning in mice, it appears that yet another novel molecular pathway, CaMKII/CREB/BDNF, may be responsible for working memory improvement in rats.

MATERIALS AND METHODS

Male Wistar rats (5 weeks old, 120–140 g body weight, $n = 44$) were maintained at an ambient temperature of 24 ± 0.1°C and relative humidity of 45% ± 5% under a 12 : 12-h light–dark cycle (lights on at 7:00 h), with food and water ad libitum. Rats were divided into two groups. The control group was fed by normal food, whereas the TB group was fed a 0.05% TB supplemented food for 73 Days. Three different behavior experiments (RAM, NOR test and Y-maze) were carried out to assess the memory functions of rats. After the administration period, the rats were anaesthetized using isoflurane and blood were collected, and brains were rapidly separated. Then, the hippocampus and mPFC were collected. Kidney and liver function tests were done from blood plasma, the mRNA and protein expression levels for BDNF and CREB and CaMKII phosphorylation levels in mPFC and hippocampus were analyzed using enzyme immuno-solvent assay, real time PCR and Western blotting, respectively.

All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSION

The long-term TB supplementation (73 days) did not show any adverse effects on body weight gain, food and water intake, and liver and kidney functions in rats. In the RAM task, TB rats made

fewer WMEs and lower latency, as the number of trials increased. TB also triggered a significant increase in the spatial working memory, as indicated by a higher spontaneous alteration ratio in the Y-maze test. TB not only ameliorated the RAM task- and Y-maze-determined working memory, but also significantly contributed to the enhancement of memory examined in NOR test. This was confirmed by increases in the DI and exploration time at both 1 h and 24 h of the post-familiarization test, which respectively can be referred to as short-term and long-term memory. Several studies proposed both cortical and hippocampal areas are related to different memory functions. So, the exact location of the neural circuitry of the memory is yet to be clearly elucidated. We hypothesized mPFC is responsible for working memory along with it other important functions. Many studies have suggested that CaMKII phosphorylates the transcription factor CREB, transforms it into active form p-CREB. p-CREB then initiates transcription and translation of proteins/receptors required for neuronal plasticity. In the current study, TB supplementation significantly upregulated CaMKII, as indicated by increased ratios of p-CaMKII/CaMKII in the cortical tissues. TB augmented the levels of p-CREB concomitantly, as compared to those of the control rats. We speculate that TB-induced increase in the levels of p-CaMKII, and p-CREB contributed to the improvement of neuronal plasticity, hence, the learning and memory of TB rats. Improvements of working memory were accompanied with an increased level of BDNF protein in the frontal cortex. Consistently, TB-fed rats, had also higher BDNF mRNA and protein levels in the mPFC, whereas hippocampal BDNF level did not differ between groups. Moreover, the improvements in learning and memory were positively correlated with the levels of memory-related substrates—p-CaMKII, p-CREB, and BDNF of mPFC. Therefore, it is conceivable that TB-instigated increases in the levels of p-CaMKII, p-CREB, and BDNF improved the learning and the memory of the rats.

CONCLUSION

This study demonstrated that the oral administration of TB for 73 days resulted in the upregulation of p-CaMKII and p-CREB in the mPFC, and found both BDNF mRNA expression level, and protein level upregulation in the mPFC of TB rats. These results clearly suggest that TB supplementation may facilitate the CaMKII/CREB/BDNF pathway in the mPFC. We also clearly observed a significant improvement in working memory in TB rats. These observations are also firmly supported by previous findings concerning the role of the CaMKII/CREB/BDNF pathway in working memory and learning in rats.