学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　宋 寧
（Degree conferred: Doctor of Philosophy）　（Name of recipient:　Song Ning　）

学 位 論 文 題 名
（Title of dissertation）

Involvement of CaMKIV in neurogenic effect with chronic fluoxetine treatment
(慢性 Fluoxetine 投与による神経細胞新生における CaMKIV の関与)

[Background and Objective]

Selective serotonin reuptake inhibitors, such as fluoxetine (FLX), are the most commonly prescribed drugs for the treatment of depression and anxiety. Chronic (>2-4 weeks) but not acute treatment with fluoxetine increases neurogenesis of subgranular zone (SGZ) of adult hippocampus in not only rodents but also non-human primate, and this time course parallels with clinical antidepressant responses. Behavioral studies suggest that antidepressant treatments exert their therapeutic effects via both neurogenesis-dependent and -independent pathways.

Ca^{2+}/calmodulin dependent protein kinase IV (CaMKIV) is a multifunctional, serine-threonine protein kinase that is activated in the presence of increased intracellular calcium. The tissue distribution of CaMKIV is restricted primarily to discrete region of brain, T-lymphocytes, and post meiotic germ cells. In the brain, CaMKIV is expressed in the parietal cortex, cerebellum and hippocampus. CaMKIV requires Ca^{2+}/calmodulin to initiate kinase activity and consequently phosphorylated by Ca^{2+}/calmodulin dependent protein kinase kinase (CaMKK) on Thr^{200} in human CaMKIV (Thr^{196} in the mouse) is necessary for maximal CaMKIV activity. CaMKIV is detected predominantly in the nuclei of neurons and plays a role in the activity-dependent phosphorylation of cAMP-response element binding protein (CREB). As the most characterized transcription factor, CREB regulates the expression of genes involved in neurogenesis, and chronic antidepressant treatments increase CREB activity within the hippocampus.

Given that CaMKIV participates in phosphorylation of nuclear CREB after chronic FLX treatment and the pivotal role of CREB in neurogenesis, we hypothesize that CaMKIV might regulate some aspects of adult neurogenesis with chronic FLX treatment. Experiments were therefore designed to assess the effects of chronic FLX treatment on cell proliferation and survival in SGZ of adult hippocampus between CaMKIV knockout (KO) mice and their wild type (WT) littermates. Meanwhile, the expression of CREB and phosphorylation of CREB was detected by RT-PCR and Western blotting. Furthermore, the behavioral effects of fluoxetine in KO and WT mice were examined in novelty suppressed feeding test (NSF test), which reflects neurogeneis-dependent actions of chronic fluoxetine.

[Materials and Methods]

Animal, agent and schedule

Wild type (WT) male C57BL/6 mice and CaMKIV knockout (KO) male mice, 8～12 weeks old, were group-housed 3-4 per cage and maintained in air-conditioned rooms at 22 ± 1 ℃ and a 12 h light/dark (6:00/18:00) cycle with free access to food and water. All procedures were in compliance with the Guide for the Care and Use of Laboratory Animals and approved by Hokkaido University School of Medicine Animal Care and Use Committee. Fluoxetine was dissolved in distilled water and delivered by gastric gavage at a volume of 18mg/kg once a day.

For experiment measuring proliferation, mice were injected with fluoxetine for 2 weeks and bromodeoxyuridine (BrdU) was singly injected intraperitoneally after last fluoxeitine administration. The mice were sacrificed after 4 weeks of BrdU injection. For experiment measuring survival, mice was singly injected with BrdU and followed by 4 weeks fluoxetine treatment. The mice were sacrificed on the second day of last fluoxetine injection. For novelty suppressed feeding test, mice were injected with fluoxetine for 3 weeks. After 24h of last injection, behavior test was done and mice were sacrificed on second day: hippocampus were quickly excised and prepared for RT-PCR and Western bloting.

Experiment-1

Adult neurogenesis (cell proliferation and survival) was investigated by immunohistochemistry.

Experiment-2

Expression and phosphorylation of CaMKIV and CREB were investigated by RT-PCR or Western blotting, respectively.

Experiment-3

Novelty suppressed feeding test, a neurogenesis-dependent behavior test, was used to investigate the behavioral consequence.

[Results]

Result-1

For cell proliferation, chronic FLX significant increased the BrdU-positive cells in WT (vehicle: 659.3 ± 97.78, n = 8; *vs* fluoxetine: 3101 ± 1000, n = 6; $p < 0.01$) but not in KO mice (vehicle: 632.3 ± 99.77, n = 8; *vs* fluoxetine: 820.5 ± 354.2, n = 6). Baseline of BrdU-positive cell number in vehicle-treated WT and KO mice were similar. For cell survival, fluoxetine significantly increased the BrdU-positive cells in WT (vehicle: 693.9 ± 80.94, n = 10; *vs* fluoxetine: 1716 ± 177.6, n = 11; $p < 0.001$) and in KO mice (vehicle: 648.0 ± 70.26, n = 7; *vs* fluoxetine: 1238 ± 243.6, n = 7; $p < 0.05$). Baseline of BrdU-positive cell number in vehicle-treated WT and KO mice were similar.

Result-2

Chronic FLX treatment has no effect on mRNA expression on CaMKIV and CREB. Phosphorylation of CaMKIV was increased in WT mice ($t = 2.522$, $df = 8$; $p < 0.05$). FLX increased the phosphorylation of CREB in WT but not KO mice ($p < 0.05$)

Result-3

Chronic FLX treatment significantly shortened the latency to feed in NSF test both in WT ($p < 0.01$) and KO mice ($p < 0.05$). There was no effect on the home cage feeding.

[Discussion]

Over the past decade, studies have shown that psychosocial stress impaired adult hippocampal neurogenesis and antidepressant treatment reversed the effect of stress. Meanwhile, the anti-depressive behavioral effect of fluoxetine in some animal models is dependent on hippocampal neurogenesis. To elucidate the pivotal components that participate in adult neurogenesis will contribute to our better understanding of depression and the development of new drugs. In present study, CaMKIV knockout impaired the FLX-induced cell proliferation, but not cell survival; meanwhile, the baseline of BrdU-positive cells between vehicle-treated WT and KO was not influenced. Previous *in vitro* researches showed that CaMKIV/CREB was involved in proliferation of neural stem/progenitor cell, and CaMKIV was shown having pro-survival effect in culturing isolated cerebellar granule cells; meanwhile, the phosphorylation of CREB was impaired in KO mice. These results confirmed the role of CaMKIV/CREB in cell proliferation *in vivo*, and indicated that CaMKIV are not involved in physiological or antidepressant induced cell survival. Finally, the behavior performance in NSF test showed that FLX decreased latency to feed in both types, paralleling with the result of cell survival, which suggested that cell survival might contribute to the performance in this behavior model.

[Conclusion]

Present data show that CaMKIV involved in neurogenic effect of chronic FLX treatment, and the downstream transcriptional factor CREB play an important role for this phenomenon. The role of CaMKIV in other depressive models needs to be further studied, which will deepen our understanding of depression.