Effect of exercise-induced fatigue on rat learning and memory ability and expressions of CaN in the brain

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Abstract—**Through establishing the rat model of exercise-induced fatigue, we detected the distribution and expression of calcineurin (CaN) in the cerebral cortex and hippocampus, and explored the role of CaN in fatigue-induced learning and memory disorder. Adult male SD rats were randomly divided into control group and fatigue group. Rats in fatigue group exercised to exhaust on treadmill for 7 days, and those in control group didn't exercise. Step-through test was used to detect the changes of learning and memory ability. Immunohistochemistry was used to observe the distribution and expression of CaN in the cerebral cortex and hippocampus. CaN-immunoreactive cells were also countered. The results indicated that rats with exercise-induced fatigue showed learning and memory disorder. In the cerebral cortex and hippocampus of rats in the fatigue group, the expressions of CaN increased obviously compared with that of the control group (P<0.05) . The present results suggest that the learning and memory disorder induced by fatigue might relate to the changes of the expression of CaN in the cerebral cortex and hippocampus.**

Keywords: exercise-induced fatigue; learning; memory; CaN

I. INTRODUCTION

The phosphatase calcineurin (CaN) are Ca^{2+}/calmodulin-binding proteins that are very abundant in the central nervous system. In brain, they mainly distribute in neurons of cerebral cortex, hippocampus and striatum [1]. CaN is composed of a catalytic subunit (CaN A) and a regulatory subunit (CaN B). CaN A has three isoforms ($α$, $β$ and $γ$), which are encoded by different genes, and CaN A $α$ is the most abundant form in the brain. CaN B has only one isoform [2] [3].

In recent 10 years, research has identified varietal functions of CaN in learning and memory. It is involved in depotentiation, long-term potentiation (LTP), long-term depression (LTD) of synaptic effect, cognitive memory, switch from short-term memory to long-term memory and brain aging et al [4]. Recent researches have identified that learning and memory damage is related to the change of CaN in Hippocampus or other brain regions, but there is no synergy to explore the brain regional changes in CaN in a stress injury, especially we have not found the effects of exercise-induced fatigue on rats' learning and memory

ability and expressions of CaN in the brain. So this study used Bedford incremental fatigue model to make the rats exhausted [5], Step-through test to detect the changes of learning and memory ability and immunohistochemistry to observe the distribution and expression of CaN in the brain. As a result provides experimental evidence to clarify the mechanism of fatigue-induced learning and memory impairment.

II. MATERIALS AND METHODS

A. Animals and Exercise Protocol

Male Sprague-Dawley rats weighing 250-300g were housed in polyethylene cages (4-5 per cage) with free access to food and water. Conditions in the animal room were kept constant at 25°C, 50% relative humidity and a 12/12 hr dark/light cycle with light on at 06.00 hr. Experiments were performed strictly according to the animal rights commission allowance.

Control group (CG) and fatigue group (FG) were formed with eight rats in each. FG rats ran on a treadmill at a speed of 8.2m/min (A1), 15 m/min (A2) and 20 m/min (A3) to exhausted for 7 days [5]. The CG rats were put on the treadmill without doing exercise for 7 days. Running exercises were performed on small animal treadmills between 9:00 a.m. and 12:00 p.m. The FG rats were adapted to the treadmill for 3 days. The time to exhaustion was recorded. The average exhaustion time was 150±11.5 min. The rats were sacrificed immediately post exercise in the FG by cervical dislocation under mild anesthesia. Brains were separated on ice-cold surface.

B. Step-through Test

In the present study, a two-compartment step-through type passive avoidance apparatus was used to measure learning and memory performance of rats. The box was divided into bright (25 cm × 25 cm × 30 cm) and dark (25 cm × 25 cm × 30 cm) compartments by a guillotine door. The bright compartment was illuminated by a white glow light. In the Train, a rat was placed in the illuminated compartment. After a habituation period in the illuminated compartment (30 s), the guillotine door was raised to allow the rat to enter the dark chamber. When the rat's hind legs entered the dark chamber, the guillotine door was closed and an electrical foot shock (0.25 mA, 20 V) was delivered

through the grid floor for 2 s. The time that elapsed prior to entry into the dark compartment (latency) was recorded. At 24 h after the train, the rats were submitted to a step-through-latency trial (STL). In the STL, the rat was placed in the bright compartment and the latency time for entry into the dark compartment was recorded for up to 300 s.

C. CaN Immunohistochemical Staining

After the 7 days load increasing treadmill exercise, the rats were anesthetized with pentobarbital (50 mg/kg) and perfused and fixed through the ascending aorta with ice-cold 0.1 M PBS and with 0.4% formaldehyde in 0.1 M phosphate buffer. Brains were removed and postfixed overnight in the same fixative solution and embedded in paraffin. The brain tissue containing the cerebral cortex and hippocampus were paraffin embedded and sections of 6 μm thickness prepared for immunohistochemistry. The streptavidin-biotion-peroxidase complex (SABC) method was performed to detect CaN protein expression in hippocampus and cerebral cortex. Slides were preincubation with 1 % H_2O_2-methanol for 30 min then immersed in pepsin solution (0.4 g in 100 ml of 0.1N HCl) at 37°C for 30 minutes, washed twice in PBS and placed in 10% common goat serum blocking reagent for 30 minutes. Sections were incubated overnight at 4 ℃ in a moist chamber with a panel of primary monoclonal antibodies diluted in 0.5% blocking reagent in TBS as follows: mouse anti-CaN (1: 200, Santa Cruz). Then put it in S-A/HRP reagent at 37°C for 2 hours. The peroxidase activity was made visible with diaminobenzidine (0.05% DAB/0.01% H_2O_2, 0.01 M PBS）and H_2O_2 in PBS for 5 to 10 minutes. 0.01 M PBS were used as negative controls in the assay. The nuclei of the cells were counterstained with Hemaeoxylin.

III. RESULTS

A. Step-through Test Result

Latency time of fatigue group was shorter (36.6 ± 17.7) than control group (258.4 ± 67.3), the number of errors was more (9.6 ± 2.5) than control group (1.6 ± 2.9), and this change was significant difference (P<0.01). This shows that the fatigue group rats' learning and memory ability were damaged.

B. Influence of exercise-induced fatigue on CaN expression

In the control group, the majority of pyramidal cells in the cerebral cortex (Fig.1A1) are not expressed, shorter processes; and hippocampus pyramidal cells color light, pale gray, the border is not clear, the processes are not clear yet, colorless vesicular nuclei were saturated (Fig.1B1). While in the fatigue group, pyramidal cells arrange turbulence and loosen, color dark gray, some are crinkle and strut even disintegrate (Fig.1A2, B2). Compared with the control group, Integrated OD Average (IOD) of CaN positive neurons are significantly increased in hippocampus and cerebral cortex in fatigue group（P<0.01）(Fig.2).

IV. DISCUSSION

Observed in this study, the rats' learning and memory ability after exercise-induced fatigue decreased significantly, and the expression of CaN increased significantly in the hippocampus and cerebral cortex. Suggesting that the overexpression of CaN may be the synaptic mechanism to this damage of learning and memory.

Sofa, et al. [1] used in situ hybridization and immunohistochemistry to observe the distribution of CaN A α in the mouse brain and found that CaN A α expressed high in the cerebral cortex and hippocampus, low in cerebellum, midbrain and brain stem. By measuring the overexpression of CaN inhibitor in mouse hippocampal neurons and the ability of non-LTP and spatial learning and memory, they found that the persistent of LTP and memory enhancement are parallel in the time point. Suggesting that the time-LTP is related to memory formation. Mnasuy IM, et al. [6] used the mice which overexpressed CaN and tested their spatial learning and memory ability by Barnes maze. Found that the mice which were trained once every day, their short-term memory had no difference to the control rats, but their long-term memory were damaged. CaN activity of SD rats decreased after the injection of CaN inhibitors FK506, and synaptic effect of hippocampal CA1 area increased. The expression and activity of CaN in the cytoplasm of hippocampal neurons also increased with age. Brion JP et al. [7] used immunohistochemistry to find that the expression of CaN was strong in the hippocampus and cerebral cortex neurons of Alzheimer's disease (AD) patients. Thomas C, et al. [8] has linked the changes of CaN activity in aging brain to intracellular Ca^{2+} concentration. They found that the CaN activity increased with aging process. One of the reasons was that the ability to block the brain L-type Ca^{2+} channel was weaken and result in the overload of intracellular Ca^{2+}, then Ca^{2+} started or accelerated the process of neural degeneration and cell death in different mechanisms which required the involvement of CaN.

At present, synaptic plasticity is believed the basis of learning and memory in neurobiology [9]. Both of short-term and long-term synaptic plasticity requires the participation of Ca^{2+}. While LTP (long-term potentiation) is closely related to the process of learning and memory. People call LTP the "synaptic model of memory" [10]. Different types of learning and memory are mainly related to two signal pathways, the one is Ca^{2+}、CaN/NF-AT(nuclear factor of activated T cell) and the other is Ca^{2+}/CaM、CaN、PP1、CREB. Neurons can enhance synaptic connection by the positive feedback pathway Ca^{2+}、CaN、NF-ATc, and the pathway has an important role in learning and memory. While the pathway Ca^{2+}/CaM、CaN、PP1、CREB is a negative pathway, and mainly related to long-term depression (LTD) and weakening the synaptic effects [11]. Together with biochemical studies showing that IP3 and ryanodine receptors are phosphorylated by PKC and CaM-KII and dephosphorylated by CaN [12] and the

phosphorylation of IP3 receptors appears to increase their sensitivity to IP3 [13], we outline a scheme responsible for exercise-induced fatigue damaging learning and memory. The exercise-induced fatigue enhances the expression of CaN which decreases the ratio of active CaMKII and PKC to CaN and facilitates the action of CaM-KII and PKC in phosphorylating protein substrates that contribute to synaptic potentiation and Ca^{2+} decreases from intracellular stores by IP3 and ryanodine receptor channels. Decreased intracellular Ca^{2+} decreases the formation of Ca^{2+}/CaM complexes and further inactivation of CaM-KII and PKC. This network constitutes an amplification cascade (negative feedback) that is triggered by increasing CaN activity and may lead to limited potentiation of synaptic transmission. The mechanisms by which the CaN-dependent negative signaling pathway participates in the fatigue-induced impairment of memory at hippocampus excitatory synapses are thought to involve the CaN-dependent activation of PP1 by inactivating I-1 (Inhibitor-1), a PP1 inhibitor [14]. PP1 could dephosphorylate phosphor-CREB (cAMP responsive element binding protein) in nucleus and make it deactivated and finally down the learning and memory ability. So the pathway Ca^{2+}/CaM、CaN、PP1、CREB may play an important role on learning and memory damage of exercise-induced fatigue. This study examined the rats learning and memory ability by Step-through test and found that learning and memory ability was damaged through 7 days exhaustive exercise. This damage also may be related to other reasons, for example, CaN may inhibits PKA dependant LTP [15] and plays a key role on short-term memory to long-term memory [6].

V. CONCLUSION

This study used 7 days fatigue model of rats by treadmill exercise and Step-through test to establish a fatigue-induced impairment of memory model, and found that the rats' learning and memory ability after exercise-induced fatigue decreased significantly, and the expression of CaN increased significantly in the hippocampus and cerebral cortex may be the synaptic mechanism to this damage of learning and memory.

VI. ACKNOWLEDGMENT

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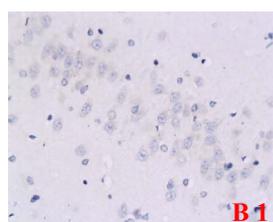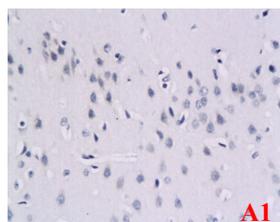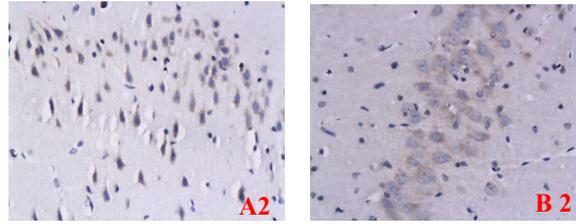
Figure 1.　Expression of CaN in cerebral cortex (A) and hippocampus (B) in control (1) and fatigue groups (2).　×400
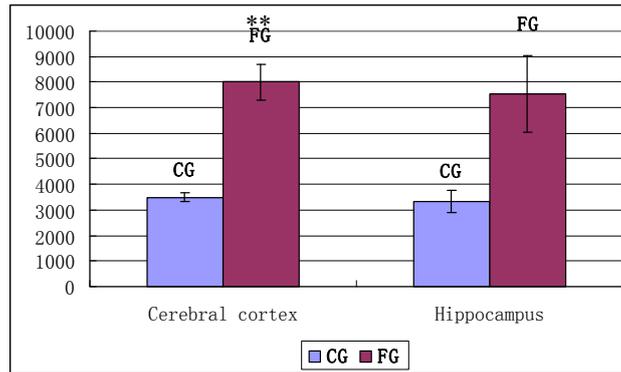

Figure 2.　The comparison of Integrated OD Average of CaN positive neurons in the cerebral cortex and hippocampus in groups

(** p<0.01)