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Research report

The change of spatial cognition ability in depression rat model and the possible association with down-regulated protein expression of TRPC6

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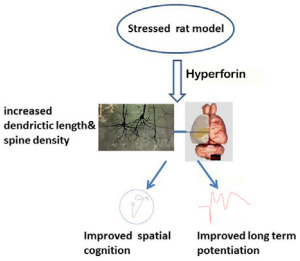
HIGHLIGHTS

- Impaired cognitive ability was observed in a chronic unpredictable stress rat model.
- TRPC6 decreased in hippocampus along with impairment of spatial cognitive ability.
- Hyperforin enhanced neurite spine density of hippocampal neurons.
- TRPC6 channels are critical for maintaining spine density and morphology.

GRAPHICAL ABSTRACT



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ABSTRACT

An increasing number of researches have focused on the cognitive changes in depression patients. Here, we observed impaired cognitive ability in a rat depression model along with down-regulated expression of canonical transient receptor potential 6 (TRPC6) protein. The cognitive defect could be rescued by treatment with hyperforin, which can invoke TRPC6 activation. This study was designed as following: rats were randomly divided into control, stressed and stressed + hyperforin groups. Chronic unpredictable stress combined with isolation rearing was applied on rats for three weeks, except for control group. Morris water maze was applied to evaluate spatial cognitive ability while long-term potentiation (LTP) was recorded to test the synaptic plasticity. Results showed that both spatial cognition and synaptic plasticity were impaired in stress group while improved after hyperforin treatment in stressed + hyperforin group, meanwhile, Western blot assay showed that TRPC6 expression was decreased in stressed group. The histological data also presented the decline of dendritic length, dendritic spine density and the number of excitatory synapses in stress group while they were increased in stressed + hyperforin group. These results suggest that there is a well potential of TRPC6 to become a new target for selecting promising new candidates as antidepressants with therapeutically effect on impaired cognition.

1. Introduction

Canonical transient receptor potential 6 (TRPC6) is a voltage-independent, Ca^{2+}-permeable cation channel involved in growth cone guidance [1], neurite outgrowth and excitatory synapse formation [2,3]. Studies have provided evidence of the involvement of TRPC channels in pathophysiology of many CNS diseases, such as subarachnoid hemorrhage [4], Alzheimer's disease [5] and epilepti-

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form activity [6]. A previous study showed that the down-regulated expression of TRPC6 led to altered neuronal morphology and function [7]. The observed neuronal phenotypes could then be rescued by the treatment with hyperforin. Hyperforin is a pharmacologically active component of the medicinal plant hypericum perforatum (St. John's wort), which can invoke TRPC6 activation [8]. Here, we focused on the association between TRPC6 and cognitive ability changes in a chronic unpredictable stress (CUS) rat model and observed whether or not there was any improvement after treatment with hyperforin.

People in modern society may suffer excessive stress, which can cause variety of psychiatric conditions, including depression, post-traumatic stress disorder, and other anxiety disorders [9]. Based on this observation, the chronic unpredictable stress (CUS) animal model has been developed to mimic the development and progress of clinical depression [10].

In many research, spatial memory is used as an assessment of cognitive function, and Morris water maze (MWM) is frequently applied to evaluate spatial memory in animal models. The reversal learning in MWM, which examines the cognitive flexibility in learning contingencies, is of particular interest in animal models [11,12]. In this research, MWM was applied to investigate the cognition of the CUS rat model.

For synaptic plasticity change, which is widely believed to comprise the cellular basis for learning and memory, we focus on the long-term potentiation (LTP) of hippocampus. LTP of synaptic transmission is one of the prime candidates for mediating learning and memory as well as many other forms of experience-dependent plasticity [13]. The LTP test in this study was recorded from perforant path (PP) to dentate gyrus (DG), one of three single synaptic pathways in hippocampus.

Some brain imaging studies in depressed patients revealed that the decreased hippocampal volume, and postmortem brain studies showed moderate apoptosis and atrophy in the CA1 region and the dentate gyrus [14–16]. Furthermore, the hypothesis that stress and depression are associated with the loss of hippocampal synapses and dendritic spines received recent attention [17,18]. So we applied Nissl staining, DiOlistic Labeling (DiI) and Golgi method to detect the morphological change of hippocampal neuron, and Western blot assay to observe changes of TRPC6 protein expression, in order to find the role of TRPC6 played in hippocampal pathologic change and impaired cognition ability in this study.

2. Experimental procedures

2.1. Animals and reagents

Adult male Wistar rats weighing 250 ± 20 g were purchased from the Laboratory Animal Center of Academy of Military Medical Science of People's Liberation Army, and were housed in standard rodent cages in Experimental Animal Room of Medical School, Nankai University. The environmental conditions were under controlled at 22 ± 2 °C, and 45–55% relative humidity, with 12:12 h light/dark cycle. Conventional food and drinking water were freely available during all phases of the experiment except model establishing and sucrose consumption phases. After five days' habituation to the environment, the rats were randomly divided into three groups: control group (Con), stressed group (Str) and stressed + hyperforin treated group (Str + Hyp). Rats in Con group were housed with four individuals in one cage while other rats were reared separately. The experiment was carried out in accordance with practices outlined in the Institutional Animal Care and Use Committee (IACUC). All efforts were made to minimize the number of animals used and their suffering.

Table 1
Chronic unpredictable stress experimental schedule.

	Week 1	Week 2	Week 3
1	Water deprivation	Cage tilt	Reversed light/dark cycle
2	Ice water swimming	Manufactured noise	Manufactured noise
3	Manufactured noise	Tail pinch	Ice water swimming
4	Tail pinch	Reversed light/dark cycle	Cage tilt
5	Cage tilt	Ice water swimming	Water deprivation
6	Reversed light/dark cycle	Food deprivation	Tail pinch
7	Food deprivation	Water deprivation	Food deprivation

Hyperforin (Dr. Willmar Schwabe, Karlsruhe, Germany) was bought from Jingwei medicamentarius. Rabbit polyclonal anti-TRPC6 antibodies (primary antibody, working dilution 1:1000, Abcam, Cambridge, MA, U.S.A); anti-postsynaptic density 95 (PSD95) antibody (primary antibody, working dilution 1:1000, Abcam, Cambridge, MA, U.S.A); Alexa 488-conjugated goat anti-rabbit IgG antibodies (secondary antibody, working dilution 1:1000, Invitrogen, San Diego, CA, U.S.A); Rabbit polyclonal anti-β-actin IgG (primary antibody, working dilution 1:500, Santa Cruz Biotechnology, Inc., CA, U.S.A); chemiluminescent HRP substrate (Immobilon western, Millipore Corporation, Billerica, MA, U.S.A); NeuroTrace™ Fluorescent Nissl Stains (N21480, working dilution 1:200, Molecular Probes Inc., Eugene, Oregon,U.S.A). CM-DiI (C7000, working concentration 1 mg/ml).

The rats received daily intragastric administration of normal saline in Con and Str group or hyperforin in Str + Hyp group for 21 days from 8 th to 28 th day counting from the beginning day of CUS. The dose of hyperforin 20 mg/kg/day in 2.0 ml/kg volume dissolved in saline solution was chosen on the basis of previous studies [19,20].

2.2. CUS procedure

The CUS procedure was based on the modification method of Quan [21]. The entire process lasted for 3 weeks. Seven types of stimulation were conducted every week. The details of the procedure are in Table 1. The rats of Con group were left undisturbed throughout the model establishing period except the sucrose consumption experiment.

2.3. Body weight measurement and sucrose consumption experiment

Body weights were measured every other day at 9 a.m. Four days before CUS, rats were provided 1% sucrose solution as substitute for normal water for 2 days to make them get used to the sucrose solution. Two days before CUS, sucrose consumption procedure began. In this procedure, all rats were kept off food and water for 23 h then they were provided with two bottles of water for 1 h, one bottle was the 1% sucrose solution and another was plain water. The sucrose solution consumption percentage = 100% × sucrose solution intake/the total water consumption. This procedure was repeated on the last two days of each week during the CUS period.

2.4. Morris water maze (MWM) experiment

Rats were trained and tested in MWM to evaluate their cognitive ability after CUS. The procedure was comprised of four phases: initial training (IT); initial probe trials (IPT); reversal training (RT) and reversal probe trials (RPT). It was performed as previously described in IT and IPT phases. The platform was placed in the