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Original article

Hyperactivity, memory deficit and anxiety-related behaviors in mice lacking the p85α subunit of phosphoinositide-3 kinase

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Abstract

We previously reported that knockout mice lacking the p85α regulatory subunit of phosphoinositide-3 kinase (PI3K) ($p85\alpha^{-/-}$ mice) significantly showed spatial learning-deficits, restlessness and motivation-deficit in water maze tests. It was also shown in the report that decline of PI3K activity in several brain areas related to losses of synapses and myelinated axons. However, any other behavioral patterns have not been elucidated, the aim of the present study was to observe behavioral natures of in $p85\alpha^{-/-}$ mice using several behavioral tests. In order to examine behaviors, a novel object recognition test, an open field test, an object exploring test, a hole-board test and an elevated plus maze test were carried out in $p85\alpha^{-/-}$ mice. The $p85\alpha^{-/-}$ mice significantly showed a deficit in object recognition memory, less exploring novel objects, and anxiety. Hyperactivity was observed in $p85\alpha^{-/-}$ mice in male-specific manner. The present results suggest that deficiencies in PI3K activity result in hyperactivity, memory deficit and an increase in anxiety. Therefore, these behavioral phenotypes can be analyzed in a relationship with losses of synapses and myelinated axons in the brain, which resulted from deficiencies in PI3K activity.

Keywords: Phosphoinositide-3 kinase; Recognition memory; Anxiety; Hyperactivity; Attention

1. Introduction

Phosphoinositide-3 kinase (PI3K) is a key enzyme of various signal transduction pathways that regulate cell survival and growth, immune function, metabolism, and cardiac function. Further, recent studies have demonstrated that the PI3K cascade may also regulate axonal specification and growth in cultured embryonic hippocampal neurons [1,2] and may regulate formation of axons, but not dendrites, in cultured embryonic cortical neurons [3].

Experiments *in vivo* showed that intracerebroventricular (i.c.v.) injection of wortmannin [4] or injection of LY294002 into the hippocampal CA1 [5] results in impaired memory in rats, which suggests that modulation of PI3K activity may result in learning- and memory-deficits. We previously clarified using gene knockout mice lacking the class I_A PI3K, p85α regulatory subunit-deficient ($p85\alpha^{-/-}$ mice) that PI3K-deficiency caused losses of the formations of axons, synapses and myelin *in vivo* and that the absence of PI3K results in abnormalities in learning-, motivation-related behaviors [6].

The $p85\alpha^{-/-}$ mice were originally generated for studies of diabetes [7] and the immune system [8], and their use has helped to elucidate important physiological functions of PI3K. Although remarkable

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spatial learning-deficit was observed in a water maze test in our previous report [6], other behavioral patterns have not been characterized. Therefore, the present study utilized a novel object recognition test, an open field test, an object exploring test, a hole-board test and an elevated plus maze test for behavioral characterization in p85$\alpha^{-/-}$ mice. Subsequent results provide p85$\alpha^{-/-}$ mice show hyperactivity, low object exploring and anxiety in addition to memory- and motivation-deficits which were previously clarified yet.

2. Materials and methods

2.1. Animals

Mice that lack the p85α regulatory subunit of PI3K (p85$\alpha^{-/-}$ mice) were kindly supplied by Dr. T. Kadowaki (University of Tokyo) and Dr. S. Koyasu (Keio University). Male or female p85$\alpha^{-/-}$ mice (BALB/c background) and wild-type BALB/c mice were used. All mice were maintained under specific pathogen-free conditions in our animal facilities. All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Sugitani Campus of University of Toyama.

2.2. Novel object recognition test

Identical two objects (plastic transparent bottles) were placed at a fixed distance within in a square field ($33 \times 28 \times 18$ cm). A mouse was then placed at the center in the field, and the number of times it made contact with the two objects was recorded during a 3-min period (training session). Mice were then placed back into the same field 24 h after the training session, in which one of the familiar objects used during the training session was replaced with a novel object (a white ball). The mice were then allowed to explore freely for 3 min and the number of times they made contact with each object was recorded (test session). A preference index, defined as the ratio of the number of times a mouse made contact with any of the objects (training session) or the novel object (test session) over the total number of times the mouse made contact with both objects, was used to measure cognitive function.

2.3. Open field test

Mice were released for 5 min in a circular space (diameter, 58 cm; height, 26.5 cm), and their paths were tracked by a digital camera. Moved distance and velocity for 5 min were analyzed with EthoVision 3.0. Testing was carried out in a dimly illuminated room.

2.4. Object exploring test

Object A (a black vase) was placed at 18 cm from the center of a circular space (diameter, 58 cm; height, 26.5 cm). Mice were released for 5 min. Exploring behaviors against the object (nosing, putting forelimbs) were counted by an experimenter. To confirm reproducibility of the exploratory behaviors between wild-type and p85$\alpha^{-/-}$, object B (a yellow toy block) was placed at another position instead of object A after 90 min interval. Testing was carried out in a dimly illuminated room.

2.5. Hole-board test

This modified hole-board test was originally designed by us. On a half part of a circular black board (diameter, 66 cm), there were three inner holes (diameter, 3 cm; 7 cm apart from the center) and four outer holes (diameter, 3 cm; 29.5 cm apart from the center). The board was placed in a circular field (wall height 6.5 cm). Mice were released for 5 min on the hole-board. Numbers of head-dipping into holes were counted by an experimenter, and their paths were tracked by a digital camera and analyzed with EthoVision 3.0. The reason why holes are located on a half area of the board is to observe whether mice prefer a half area with holes to another half without holes. We confirmed that all mice spent their trial time on the half area with holes. Testing was carried out in a dimly illuminated room.

2.6. Elevated plus maze test

The elevated plus maze was made out of black PVC and consisted of a plus-shaped platform elevated 40 cm above the floor, with two open (30×6 cm) and two closed ($30 \times 6 \times 15$ cm) arms and a connecting central zone (6×6 cm). Mice were placed onto the central compartment facing the closed arm. During a 10 min exposure, the following parameters were recorded/calculated by EthoVision 3.0. Distances traveled, frequency of entries into open arms, and duration in open arms were measured. Testing was carried out in a dimly illuminated room.

2.7. Statistical analysis

Statistical comparisons were performed with the Student's *t*-test or paired *t*-test. Values of $p < 0.05$ were considered statistically significant. Data are represented as means with SEM.

3. Results

Visual recognition memory was assessed using a novel object recognition test. Male mice (16 weeks old)