Diacylglycerol kinase β knockout mice with impairment of spine conformation show an abnormal response on psychostimulant-induced behavioral change

1Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, Gifu, Japan
2Carna Biosciences, Incorporated, Kobe, Japan
3BioSignal Research Center, Kobe University, Kobe, Japan
4Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan
5Department of Social and Environmental Medicine, Graduate School of Medicine Osaka University, Suita, Japan

Objective

DGKβ Diacylglycerol kinase β (DGKβ): phosphorylates diacylglycerol (DG) to produce phosphatidyl ethanolamine (PE) within the G protein-coupled receptor (GPCR) signaling system, which regulates the effector ballet, second messenger, striatum and hippocampus.

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1. DGKβ expression rapidly increases after 14 days of age, which is coincident with the synapse formation in the brain (1).

2. In bipolar disorder patients, DGKβ protein displays a COOH-terminal truncation downstream of the catalytic domain (2).

Previously, we generated DGKβ-KO mouse (3) and investigated they exhibited lithium-sensitive behavioral changes.

Attention deficit hyperactivity disorder (ADHD) ADHD is a disease characterized by hyperactive motor movements, impulsivity and inability to pay attention to what is important. As a long-term treatment, methylphenidate (MPD) is an commonly used drug. MPD has a paradoxically effect on activity. That is, for normal person MPD shows locomotor-promoting (psychostimulant) effect. In contrast, MPD antagonizes for ADHD patient. However, the detailed mechanism of such a paradoxically effect is still unknown.

Methods

1. Cognitive functions and spine conformation

1.1. Open field test

Each mouse was placed at the end of one arm and allowed to move freely during an 8 min session. The sequence of arm entries was recorded manually. The alternation ratio was calculated as (actual alternation/maximum alternation) × 100.

1.2. Morris water maze

Mice were placed in the water facing the wall and trained with 4 trials per day for 5 days. Twenty-four hours after the last training trial, the mice were given a probe test without the platform. In this test, each mouse was placed in the pool once and allowed to search for 60 s.

1.3. Electrophysiology

Electrophysiology analysis was performed as described previously (4).

1.4. Gel shift

Each sample was further mixed in 3% agarose for 2-3 days. The tissue block was placed in 2% potassium dichromate for 2 days at 4°C and then in 2% silver nitrate solution for 3-4 days at 4°C in the dark. The block was cut into 60 µm-thick slices and fixed in distilled water. Finally, the sections were mounted onto slides, dried for 4 hours, dehydrated through 70% alcohol, 80% alcohol, clear in xylene and then placed in 2% potassium dichromate for 2 days at 4°C.

1.5. Primary culture of mouse hippocampal neurons

Fetuses were removed on embryonic 17-18 days. Hippocampi and CA1 and DG were dissected and prepared for culture in 24-well plates. Primary cultures were maintained in Dulbecco’s modified Eagle medium (DMEM) medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 µg/ml). All cells were cultured at 37°C in a humidified atmosphere containing 5% CO2. The 21-day-old culture was used for all experiments. The cultures were fixed with 4% paraformaldehyde for 20 minutes and then washed in PBS. The cells were permeabilized with 0.1% Triton X-100 and blocked with 5% normal horse serum (NHS). The cultures were incubated with the primary antibody (1:200) in 5% NHS overnight at 4°C. After washing, the cultures were incubated with secondary antibodies (1:200) for 1 hour. Finally, the cultures were mounted with Vectorshield (Vector Laboratories, Burlingame, CA) for observation under a fluorescent microscope.

2. ADHD like behaviors

2.1. Locomotor activity test

A mouse was placed in a transparent plastic cage (175 × 125 mm) with a sawdust bedding on floor. Locomotion was monitored every hour for 1 day using digital counter with infrared sensor (NS-2701; Neuroscience, Inc, Tokyo).

2.2. Open field test

Each mouse was placed in the periphery of the open field apparatus for 2 min. The total distance mice walked was recorded using EthoVision XT system (Noldus Information Technology, Wageningen, The Netherlands). The number of extra arm entries was manually counted for the first 10 min of test session in a blind manner by a single observer.

2.3. Elevated plus maze test

Each mouse was placed in the central platform, facing one of the open arms. During a 5 min session, mouse behavior was recorded using EthoVision XT.

2.4. Psychostimulant-induced hyperactivity test

Each mouse was placed in the periphery of the open field apparatus and left for 2 hours. After 30 min habituation, each mouse was administrated methylphenidate (30 mg/kg, dissolved in saline; i.p.), MK-801 (0.5 mg/kg, dissolved in saline; i.p.) or DMSO (control) i.p. twenty minutes after drug administration, we then measured mouse brain and separated it into striatum and hippocampus for a subset of Western blot analysis.

Cognitive function and spine conformation

DGKβ KO mice showed impaired cognitive function in the Y- maze test and Morris water maze test (Fig. 1).

DGKβ KO mice showed hyperactivity for ADHD patient. MPD has a paradoxically effect on activity. That is, for normal person MPD shows locomotor-promoting (psychostimulant) effect. In contrast, MPD antagonizes for ADHD patient. However, the detailed mechanism of such a paradoxically effect is still unknown.

DGKβ KO mice showed less anxiety (Fig. 2).

Impairment of spine conformation

1. DGKβ KO mice exhibited impaired cognitive function in the Y- maze test and Morris water maze test (Fig. 5).

2. The LTP in the hippocampal CA1 region of DGKβ KO mice was reduced in comparison with that of WT mice.

3. The hippocampal and cortical spine conformation of DGKβ KO mice was defect.

4. Overexpression of DGKβ enhanced the dendrite maturation in hippocampal primary neuron derived from DGKβ KO mice and SH-SY5Y cells.

Conclusion

DGKβ KO mice may show LTP reduction and consequently cognitive impairment by incompleteness of spine conformation. Furthermore, DGKβ KO mice showed the abnormal response on psychostimulant-induced behavioral change, suggesting that hyperactivity and careless behavior of DGKβ KO mice may elucidate the pathogenesis of ADHD.

References

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ADHD like behaviors

DGKβ KO mice showed an abnormal response on MPD induced hyperactivity

DGKβ KO mice showed normal response on MK-801 induced hyperactivity (Fig. 4).

DGKβ KO mice showed less anxiety (Fig. 3).

DGKβ KO mice showed an abnormal response on psychostimulant-induced behavioral change

1. DGKβ KO mice showed hyperactivity and less anxiety.

2. The psychostimulant effect of MPD was weaker in DGKβ KO mice than WT mice.

3. Using another psychostimulant MK-801 (noncompetitive inhibitor of NMDA receptors), DGKβ KO mice showed normal response.

4. After MPD treatment, activation of ERK1/2 was not observed in the stratum of DGKβ KO mice.

5. After MPD treatment, spectrin proteolysis was induced in the stratum of WT mice. On the other hand, the product of proteolysis was decreased in KO mice.

Disclosure

No potential conflict of interest.