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Research report

Kynurenine 3-monooxygenase is implicated in antidepressants-responsive depressive-like behaviors and monoaminergic dysfunctions

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HIGHLIGHTS

- Kynurenine 3-monooxygenase deficiency changes the contents of kynurenine pathways metabolites in the serum and hippocampus.
- Kynurenine 3-monooxygenase knockout mice show antidepressants-responsive depressive-like behaviors and monoaminergic dysfunctions.
- Kynurenine 3-monooxygenase knockout mice is good validities for mouse model of major depressive disorder.

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ABSTRACT

L-Tryptophan (TRP) is metabolized via serotonin and kynurenine pathways (KP). Several studies have demonstrated that abnormality of both pathways is involved in the pathogenesis of major depressive disorder (MDD). Kynurenine 3-monooxygenase (KMO), a pivotal enzyme in the KP, has been suggested to play major roles in physiological and pathological events mediated by bioactive kynurenine metabolites. In this study, we investigated the role of KMO in the emotional and cognitive functions by using KMO knockout (KO) mice. We measured contents of TRP and monoamines and their metabolites in the serum and hippocampus of KMO KO mice. Further, we investigated whether antidepressants improved the depressive-like behaviors in KMO KO mice.

KMO KO mice showed depressive-like behaviors such as decreased sucrose preference and increased immobility in the forced swimming test and high anxiety by decreased time spent in the center area of open field. But, there was no difference in spontaneous alternation in Y-maze test, counts of rearing or locomotor activity. Higher contents of TRP metabolites such as kynurenine (KYN), kynurenic acid (KA), anthranilic acid (AA), and 3-hydroxykynurenine (3-HK) in the serum and hippocampus and decreased serotonin turnover and higher content of normetanephrine (NM) in the hippocampus were observed in the KMO KO mice. Although both antidepressant attenuated increase of immobility, sertraline but not imipramine improved decrease of sucrose preference in the KMO KO mice.

These findings suggested that KMO KO mice show antidepressants-responsive depressive-like behaviors and monoaminergic dysfunctions via abnormality of kynurenine metabolism with good validities as MDD model.

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1. Introduction

Major depressive disorder (MDD) is a complex, serious and multifunctional disorder. There are approximately 120 million people worldwide with the possibility of at least one occurrence of MDD during lifetime [1], and this trend is anticipated to increase. A depressed mood results in fatigue and loss of energy, which are the core symptoms of MDD, and a diminished quality of life [2]. Moreover, when patients with some other disease suffer from MDD, the disease often gets worsened (i.e., diabetes is often accompanied by depression) [3]. Thus, MDD is rapidly becoming the greatest contributor to the global burden of disease. There are a plethora of discussions regarding the cause of depression, but it has not yet been completely resolved.

Recently, some studies have suggested that depression is related to the immune system and inflammation [4–6]. These studies suggest that indoleamine 2,3-dioxygenase 1 (IDO1), which is activated by several proinflammatory cytokines, such as interferons, tumor necrosis factor α (TNFα) and interleukin-6, is related to depression. IDO1 is the initial and rate-limiting enzyme that converts tryptophan (TRP) to kynurenine (KYN). When IDO1 activity is increased by cytokines, it is possible that increased KYN and abnormal TRP metabolites cause depressive-like behavior.

Similarly, decreased TRP could be the cause of depressive-like behavior, and consequently, serotonin is not fully synthesized [7]. Thus, there is increasing interest in the role of TRP metabolites and the kynurenine pathway (KP) as well as the serotonin pathway in MDD [8–14]. There are multiple studies regarding the relationship between IDO1 and MDD [15,16], but there are few reports on the role of kynurenine 3-monooxygenase (KMO). KMO is a pivotal enzyme in KP and normally oxidizes KYN to 3-hydroxykynurenine (3-HK) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen. KMO localizes to the outer membrane of mitochondria and is primarily expressed in the peripheral tissues and phagocytes, such as macrophages. In the brain, KMO is expressed in astrocytes, it is also associated with several neurologic disorders, such as ischemia, bipolar disorder, schizophrenia and cognitive impairment [17–19].

The purpose of this study was to clarify the relationships between KMO and MDD. In this study, we investigated whether KMO knockout (KO) mice show depressive-like behaviors and change of the KP metabolites in the serum and hippocampus and the monoamines in the hippocampus by using high performance liquid chromatography (HPLC). We also investigated whether the administration of antidepressants, sertraline and imipramine improved depression-like behaviors in the KMO KO mice.

2. Materials and methods

2.1. Mice

All mice were mature males aged 10 weeks. KMO gene-deficient mice, i.e., KMO knockout (KMO K.O.) mice, of a C57BL/6J background were obtained from Knockout Mouse project (KOMP). Animals were housed in Kyoto University School of Medicine animal facilities under specific pathogen-free conditions and were maintained on 12-h light/dark cycle (lights on at 8:00 a.m.) at 25 °C. They were maintained under free access to food and water. The protocol for all animal experiments was approved by Animal Experimentation Committee of the Graduate School of Medicine, Kyoto University. The procedures involving mice and their care were conducted in conformity with the international guidelines, Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985).

2.2. Drug

2.2.1. Antidepressants

Sertraline was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). It was suspended in saline containing 0.3% (w/v) carboxymethyl cellulose sodium. Imipramine, purchased from Sigma-Aldrich Co., LLC. (St. Louis, MO, USA), was dissolved in 0.9% NaCl solution. Both compounds were chronically (28 days) injected intraperitoneally (*i.p.*) once a day. The injection volumes were 10 ml/kg for mice. Behavioral tests were performed 30 min after the last injection.

2.3. Behavioral analysis

All behavioral analysis was performed between 10:00 a.m. and 6:00 p.m. To investigate the behavior of KMO KO mice, the behavioral tests were carried out sequentially with sucrose preference test (1st–3rd day), Y-maze test (4th day), open field test (5th day), and forced swimming test (6th and 7th day). To reduce the influence of previous experiment, sequence of behavioral tests was in order of stress strength from low (e.g. sucrose preference test) to high (e.g. forced swimming test). Behavioral experiments were carried out in a sound-attenuated and air-regulated experimental room, to which mice were habituated for at least 1 h.

2.3.1. Open field test

Open field test was performed according to the method described in the previous report [20]. A light (50 W) was positioned 100 cm above the center of the floor of the apparatus. To measure locomotor activity in a novel environment, a mouse was placed in a transparent acrylic cage with a black frosting Plexiglas floor ($45 \times 45 \times 30$ cm), and locomotion, rearing, and time spent in the center zone (15×15 cm) were measured every 1 min for 10 min using digital counters with infrared sensors (SCANET MV-40 OF; MELQUEST Co., Ltd., Toyama, Japan).

2.3.2. Sucrose preference test

Sucrose preference test was conducted according to the method outlined in the previous report [21]. In the sucrose preference test, mice were tested for a decreased sensitivity to reward, a core aspect of depression. The entire procedure was performed over 3 days, during which mice were given the choice to drink a liquid from one of two bottles placed side-by-side into their home cages. The animals were not deprived of food or water before the experiment. During the first day of the procedure, mice were habituated to the two-bottle configuration of their home cages. Thus, on the first day, each cage was supplied with two identical bottles, each containing 200 ml of regular tap water. On the second day (24 h later), water was replaced in one of the bottles with a 1% sucrose solution. On the third day (24 h later), all bottles were weighed in order to estimate the amount of liquid consumed by mice.

2.4. Forced swimming test

Forced swimming test, a standardized test of depressive-like behavior for which depression is inferred from increased duration of immobility, was conducted as described previously [22], with slight modifications. Each mouse was placed in a transparent glass cylinder (20 cm high, 8 cm in diameter), that contained water at 22 ± 1 °C to a depth of 13.5 cm, and was forced to swim for 15 min. After that, they were removed and dried with a towel. They were again forced to swim in a similar environment for 6 min 24 h later. The duration of swimming was measured by using a SCANET MV-40 AQ apparatus (MELQUEST Co., Ltd., Toyama, Japan). The duration