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Superoxide dismutase overexpression protects against glucocorticoid-induced depressive-like behavioral phenotypes in mice

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ABSTRACT

In the stress response, activation of the hypothalamic–pituitary–adrenal axis, and particularly the release of glucocorticoids, plays a critical role. However, dysregulation of this system and sustained high plasma levels of glucocorticoids can result in depression. Recent studies have suggested the involvement of reactive oxygen species (ROS), such as superoxide anion, in depression. However, direct evidence for a role of ROS in the pathogenesis of this disorder is lacking. In this study, using transgenic mice expressing human Cu/Zn-superoxide dismutase (SOD1), an enzyme that catalyzes the dismutation of superoxide anions, we examined the effect of SOD1 overexpression on depressive-like behavioral phenotypes in mice. Depressive-like behaviors were induced by daily subcutaneous administration of the glucocorticoid corticosterone for 4 weeks, and was monitored with the social interaction test, the sucrose preference test and the forced swim test. These tests revealed that transgenic mice overexpressing SOD1 are more resistant to glucocorticoid-induced depressive-like behavioral disorders than wild-type animals. Furthermore, compared with wild-type mice, transgenic mice showed a reduction in the number of 8-hydroxy-2′-deoxyguanosine (a marker of oxidative stress)-positive cells in the hippocampal CA3 region following corticosterone administration. These results suggest that overexpression of SOD1 protects mice against glucocorticoid-induced depressive-like behaviors by decreasing cellular ROS levels.

1. Introduction

Chronic and uncontrollable psychological stress causes psychiatric diseases, including major depression [1]. WHO has estimated that depression affects more than 121 million people worldwide, and the continuous increase in patients with depression is a major medical and social problem, with an economic burden estimated at approximately 83 billion dollars in the United States alone [2].

Decreased monoaminergic neurotransmission appears to play a major role in the pathogenesis of depression, based on observations that brain levels of serotonin and noradrenaline are reduced in patients with major depression [3]. Drugs that stimulate monoaminergic neurotransmission by inhibiting monoamine reuptake (such as selective serotonin reuptake inhibitors and serotonin and noradrenaline reuptake inhibitors) or by inhibiting monoamine degradation (such as tranylcypromine) are widely used for patients with major depression [3]. However, these drugs have several drawbacks, such as low remission rates, delayed response times and adverse side effects [4]. Thus, the development of better antidepressants is of major clinical importance.

It was previously thought that depression involved only a dysfunction of synaptic (monoaminergic) neurotransmission. However, new studies indicate that structural changes also play a major role in the pathogenesis of depression [5]. Indeed, atrophy of brain regions involved in mood and cognition has been observed in patients with major depression, and decreased neural synaptogenesis and adult neurogenesis in the hippocampus has been found in animals subjected to chronic psychological stress [6–9]. Furthermore, it was recently reported that the inhibition or enhancement of adult neurogenesis in the hippocampus by transgenic techniques induces or suppresses, respectively, depressive-like phenotypes in mice [10].

Abbreviations: ANOVA, analysis of variance; EC-SOD, extracellular SOD; HPA axis, hypothalamic–pituitary–adrenal axis; PC-SOD, lecithinized SOD; ROS, reactive oxygen species; SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

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In the systemic response to psychological stress, activation of the hypothalamic-pituitary-adrenal (HPA) axis, especially the release of glucocorticoids, plays a critical role [11]. The acute response to stress mediated by the HPA axis has various homeostatic functions, including reducing energy consumption and controlling glucocorticoid levels through negative feedback regulation [11]. However, excessively high or long-lived psychological stress impairs negative feedback regulation, resulting in sustained high plasma glucocorticoid levels [12]. This hypercortisolemia is thought to play an important role in depression, because chronic glucocorticoid administration to animals causes depressive-like behavioral disorders, neural synaptic loss and inhibition of adult neurogenesis [13–15]. Furthermore, high plasma glucocorticoid levels are observed in patients with depression [16]. Thus, animals given glucocorticoid (such as corticosterone) administration are good models of depression [17].

To protect against reactive oxygen species (ROS), the body has a well-developed antioxidant system, with catalase, superoxide dismutase (SOD) and glutathione peroxidase the main antioxidant enzymes. SOD, which has three isoforms (Cu/Zn-SOD (SOD1), manganese-SOD and extracellular (EC)-SOD), catalyzes the dismutation of the superoxide anion to hydrogen peroxide [18]. We previously reported that PC-SOD, a lecithinized derivative of SOD1 with a higher stability in plasma and a greater affinity for tissue, has therapeutic effects in animal models of various diseases, such as idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, ulcerative colitis, focal cerebral ischemic injury and spinal cord injury-induced motor dysfunction [19–23].

Recent studies suggest that ROS, such as superoxide anions, play an important role in the pathogenesis of depression by causing neural structural damage. Chronic glucocorticoid administration increases levels of oxidative stress markers and decreases levels of endogenous antioxidants in the brains of animals. Furthermore, increased levels of oxidative stress markers have been observed in the brains of depressive patients [24–26]. It has been reported that reducing ROS levels by genetic deletion of nicotinamide adenine dinucleotide phosphate oxidase suppresses depressive-like phenotypes in mice [27]. These observations suggest that antioxidant proteins such as SOD may be able to alleviate the symptoms of depression. However, there is currently no report providing experimental support for this concept.

In this study, we found that compared with wild-type mice, transgenic mice expressing human SOD1 showed resistance to depressive-like behavioral disorders induced by chronic glucocorticoid administration. Based on these findings, we propose that SODs are promising therapeutic candidates for depression.

2. Materials and methods

2.1. Materials

Sucrose was obtained from Nacalai Tesque (Kyoto, Japan). Peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulins was from Abcam (Cambridge, MA, USA). An antibody against 8-hydroxy-2′-deoxyguanosine (8-OHdG) was from the Japan Institute for the Control of Aging (Shizuoka, Japan). Corticosterone was obtained from Tokyo Chemical Industry (Tokyo, Japan). The assay kit for SOD was from Cayman Chemical (Ann Arbor, MI). Olive oil was from Wako Pure Chemical Industries (Osaka, Japan). The DV-Track Video Tracking System was from Muromachi Kikai (Tokyo, Japan). Malinol was from Muto Pure Chemicals (Tokyo, Japan). Mayer's hematoxylin was from Muto Pure Chemicals (Tokyo, Japan).

2.2. Animals and corticosterone administration

Transgenic mice expressing the human SOD1 transgene (C57BL/6, hSOD1$^{+/-}$) were gifts from Dr. T. Araki (Department of Peripheral Nervous System Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan). All experiments in this study were performed on 8–10-week-old male mice. C57BL/6 mice (wild-type) were from Japan Charles River (Yokohama, Japan). The experiments and procedures described here were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and were approved by the Animal Care Committee of Keio University.

To induce depressive-like behavior, corticosterone (0.5 mg in 200 μl olive oil) was subcutaneously injected daily for 4 weeks. The vehicle group was injected with olive oil only.

2.3. Social interaction, sucrose preference and forced swim tests

The social interaction test was performed as described previously with minor modification [28]. For habituation to the environment, the mouse was kept for 150 s in an open field chamber (50 cm × 50 cm) with an empty wire mesh circle cage (diameter, 8 cm) located along one of the walls (Fig. 1A). Then, the mouse was kept for 150 s in the same chamber, but with an unfamiliar C57BL/6 mouse enclosed in the mesh cage. An open field was divided into 5 zones, 1 interaction zone and 4 corner zones (Fig. 1A), and the time spent in the interaction zone and corner zones was measured by a computer based video-tracking system.

The sucrose preference test was performed as described previously with some minor modifications [29]. Mice were habituated to drink 1% sucrose solution for 4 h. The next day, after a 6-h water deprivation period, mice were placed with identical bottles of 1% sucrose and water for 4 h, and the drinking bottles were switched position every 2 h to avoid location preference. After the test, the consumption of each fluid was determined, and the sucrose preference index (%) was calculated as ([sucrose solution consumption]/[sucrose solution consumption + water consumption]).

The forced swim test was conducted as described previously [30]. Mice were subjected to forced swimming for 5 min, and the immobility time was calculated.

2.4. Measurement of SOD activity

SOD activity in the hippocampal homogenate was measured using an assay kit according to the manufacturer's instructions.

2.5. Immunohistochemical analysis

The brains were fixed in 4% buffered paraformaldehyde for 48 h and then embedded in paraffin before being cut into 6-μm-thick sections. Sections were treated for antigen retrieval using a microwave in 0.01 M citrate buffer for 15 min, and then incubated with 0.3% hydrogen peroxide in methanol for removal of endogenous peroxidase. Sections were blocked with 2.5% goat serum for 30 min and then incubated for 12 h with antibody against 8-OHdG (1:200 dilution) at 4 °C. After blocking with 3% BSA for 10 min, sections were incubated with peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulin for 1 h. After addition of 3,3′-diaminobenzidine, sections were incubated with Mayer's hematoxylin. Samples were mounted with malinol and analyzed with a NanoZoomer-XR digital slide scanner (Hamamatsu Photonics, Shizuoka, Japan). The number of 8-OHdG-positive cells was determined using Definiens Tissue Studio software (Definiens AG, Munich, Germany).