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The role of the AMPA receptor and 5-HT_3 receptor on aggressive behavior and depressive-like symptoms in chronic social isolation-reared mice

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HIGHLIGHTS

- The hypothalamic 5-HT_3 receptor was down–regulated by chronic social isolation (SI).
- The amygdalar AMPA receptor subunits were up-regulated by chronic SI.
- Intra-amygdalar infusion of (S)-AMPA exhibited the despair-like behavior.
- A systemic 5-HT_3 receptor agonist abolished the aggression in chronic SI mice.
- A systemic 5-HT_3 receptor agonist enhanced the aggression in short-term SI mice.

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ABSTRACT

Chronic social isolation (SI)-reared mice exhibit aggressive and depressive-like behaviors. However, the pathophysiological changes caused by chronic SI remain unclear. The hypothalamus and amygdala have been suggested to be associated with the stress of SI. In addition to serotonin 3 (5-HT_3) receptors, AMPA receptors have also been suggested to be involved in aggressive behavior and depressive-like symptoms in animals. Therefore, we examined whether chronic SI affects AMPA and 5-HT_3 receptor expression levels in these regions. A Western blot analysis revealed that after four weeks of SI, mice exhibited up-regulated AMPA receptor subunit (GluR1, GluR2) protein levels in the amygdala and down-regulated hypothalamic 5-HT_3 receptor protein levels. The AMPA/kainate receptor antagonist NBQX (10 mg/kg; i.p.) attenuated SI-induced depressive-like symptoms but not aggressive behavior. Intra-amygdalar infusions of the selective AMPA receptor agonist (S)-AMPA (10 μM) induced despair-like behavior, but not sucrose preference or aggressive behavior, in mice not reared in SI (naïve mice). Alternatively, treatment with the 5-HT_3 receptor agonist SR57227A (3.0 mg/kg; i.p.) decreased aggression levels. In addition, intra-hypothalamic infusions of the 5-HT_3 receptor antagonist ondansetron (3 μM) did not trigger aggressive behavior in naïve mice; however, the administration of ondansetron (0.3 mg/kg; i.p.) increased aggression levels in two-week SI mice, which rarely exhibited the aggressive behavior. Moreover, ondansetron did not affect the depressive-like symptoms of the SI mice. These results suggest that SI-induced up-regulation of GluR1 and GluR2 subunits protein levels in the amygdalar region and down-regulation of 5-HT_3 receptor proteins level in the hypothalamic region are associated with the effect of AMPA receptor agonist and 5-HT_3 receptor antagonist -induced aggressive behavior and depressive-like symptoms.

1. Introduction

Traits of aggression and anger are often observed in patients with major depressive disorder [1,2]. Excessive aggression likely develops as a consequence of generally disturbed emotional regulation, such as abnormal levels of anxiety [3]. The co-occurrence of depression and anxiety has been observed in certain types of patients [4–6]. Therefore, it is possible that aggressive behavior is a consequence of a depressive disorder combined with an abnormal anxiety level. Many of the symptoms caused by chronic social isolation (SI) have been observed in depression and anxiety disorders [7,8]. SI produces anhedonia, psychomotor retardation, neophobia and aggression [9,10]. Therefore, male mice

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate receptor; SDS, sodium dodecyl sulfate; PVDF, polyvinylidene difluoride; BSA, bovine serum albumin; ECL, enhanced chemiluminescence; GABA, gamma-aminobutyric acid; BNST, bed nucleus of the stria terminalis; DMN, dorsal medial nucleus; PVN, hypothalamic paraventricular nucleus; HPA, hypothalamic-pituitary-adrenocortical; 5-HT, serotonin; SI, social isolation; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione; SSRI, a selective 5-HT reuptake inhibitor; GluR, glutamate receptor; TST, tail suspension test; FST, forced swimming test; PBS, phosphate-buffered saline; SP, sucrose preference.

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exposed to long-term SI have been widely used to study the mechanisms of aggressive behavior with depressive-like symptoms [11–15].

Stress-linked receptors are present in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala [16,17]. The immunoreactivity for c-Fos in brain mapping studies of SI rats revealed that the activation of the medial and basolateral amygdala, hypothalamic attack area and the hypothalamic paraventricular nucleus (PVN) were involved in aggressive behavior toward an intruder [18]. Increased behavioral anxiety is associated with stress-induced activation of hypothalamic dorsomedial nucleus (DMN) neurons [19]. Hypothalamic DMN activity can elicit either excitatory or inhibitory responses in the PVN [20]. Clinical research has linked abnormal amygdalar function with social behavior deficits and depressive-like behaviors later in life [21]. This change was due to neurohumoral alterations in the hypothalamic-pituitary-adrenal (HPA) axis [22]. Moreover, it has been suggested that inappropriate SI-induced forms of aggression are associated with high or low HPA axis activity [23]. Chronic glucocorticoid deficiency induces abnormal aggression in rats [24]. However, the responsible neurochemical alterations in the amygdala and hypothalamic region which produce this aggressive and depressive-like behavior remain unclear. Therefore, understanding the roles of the amygdala and hypothalamic region on the aggressive and depressive-like behavior in chronic social SI-mice is important.

It was reported that 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f) quinoxaline-2,3-dione (NBQX), an AMPA/kainate receptor antagonist, exerted an antidepressant effect in rodent models of depression [25]. In addition, the involvement of AMPA-type glutamate receptors in the regulation of social behavior has been suggested by experiments in mice deficient for the GluR1 subunit-containing AMPA receptors showing reduced intermale aggression [26]. By contrast, the enhancement of AMPA receptor function exerted an antidepressant effect in mice with chronic mild stress [27], and the antidepressant-like behavior induced by the NMDA receptor antagonist ketamine was exerted by enhancing AMPA receptor activation [28]. Moreover, long-term SI-induced depressive-like behavior was abolished by ketamine [29], and NBQX also attenuated the aggressive behavior of long-term SI mice by the up-regulation of prefrontal AMPA receptor expression [30]. Recently, however, it was suggested that anxiety- and depression-like behaviors were regulated by the activity of the AMPA receptor in the amygdala [31]. Therefore, there is a possibility that abnormal region-specific AMPA receptor expression may produce distinct behavioral results.

Serotonin (5-HT) has also been considered a central neuromodulator of the induction of depression and aggression. Social deprivation-induced inappropriate forms of aggression have also been associated with reduced functioning of the serotonin system in adulthood [23]. According to a study of the effects of fluoxetine, a selective 5-HT reuptake inhibitor (SSRI), in chronic SI mice [11], the serotonergic system regulated the chronic SI-induced aggressive behavior. Moreover, acute tryptophan depletion, which reduces 5-HT levels, induced depressive moods and increased aggression in humans [32]. 1-(m-chlorophenyl)-biguanide (mCPBG), a 5-HT_3 receptor agonist, blocked both aggressive and anxious behaviors in adolescent anabolic androgenic steroid-treated hamsters, indicating that 5-HT_3 receptor stimulation plays a critical role in the circuit modulating developmental anabolic androgenic steroid-induced changes in both aggressive and anxious behaviors [33]. In addition, the stimulation of 5-HT_3 receptors could produce antidepressant-like effects in behavioral tests of rodents [34]. These results indicated that the contribution of the 5-HT_3 receptor is important for inducing emotional changes, such as those associated with depressive moods and aggression. However, the effects of 5-HT_3 agonists on chronic SI-induced aggressive behavior remain unclear, although it has been suggested that 5-HT_3 antagonists are ineffective for SI-induced aggressive behavior [35,36].

Despite many results suggesting the contribution of AMPA and 5-HT_3 receptors to aggression and depressive mood, the contribution of these receptors in SI mice is unclear. Moreover, the relationships between AMPA and 5-HT3 receptors in mice exhibiting aggression and depressive mood are also unclear. Therefore, in the present study, we investigated the roles of the AMPA receptor and 5-HT_3 receptor in the SI mouse amygdalar and hypothalamic regions which have been suggested to be associated with aggressive behavior and depressive-like symptoms. by measuring the protein levels of AMPA receptor subunits (GluR1 and GluR2) and the 5-HT_3 receptor in the amygdalar and hypothalamic regions of SI mice. In addition, we also investigated whether agonists or antagonists of these receptors affected the aggressive and depressive behaviors of naïve and SI mice.

2. Materials and methods

2.1. Chemicals and antibodies

(S)-AMPA (013-25511) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). SR57227 (S1688) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ondansetron hydrochloride dehydrate (O5212) was purchased from LKT Laboratory (St. Paul, MN, USA). The rabbit monoclonal anti-NMDAR1 antibody (clone 1.17.2.6, 1:1000 for Western blot) was purchased from Merck Millipore (Billerica, MA, USA). The mouse monoclonal antibody against GAD65/67 (sc-365180 for [C-9], 1:100 for immunofluorescence) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). NBQX disodium salt (ab120046), the rabbit monoclonal anti-glutamate receptor 1 (GluR1) antibody (ab32436, 1:1000 for Western blot), the rabbit monoclonal anti-glutamate receptor 2 (GluR2) antibody (ab52932, 1:1000 for Western blot). The rabbit polyclonal antibody against the 5-HT_{3A} receptor (AV13046, 1:1000 for Western blot) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A rabbit monoclonal antibody against β-actin (D6A8) (#8457, 1:1000 for Western blot) was purchased from Cell Signaling Technology (Danvers, MA). TO-PRO-3 (T3605, 1:1000 for immunofluorescence) was purchased from Life Technologies (Carlsbad, CA, USA).

2.2. Ethics statement, animal care and drug administration

This study was approved by the Animal Care Committee of Ohu University (No. 2013-44). All animal procedures were performed in accordance with the guidelines of the Animal Care Committee of Ohu University. Special care was taken to reduce animal distress and to use the minimum number of animals needed for all studies. Adult CD1 mice (male; aged 3 weeks) were supplied by Charles River Laboratories Japan, Inc. (Yokohama, Japan). All mice were housed at 25 ± 2 °C on a 12 h–12 h light (08:00–20:00) - dark (20:00–08:00) cycle with access to food and water ad libitum. In the group of social isolation, mice were individually housed for two to four weeks in the cage, while the control grouped mice were housed in the cage under normal conditions (five mice per each cage). Mice were handled individually every day for the 7 days before the behavioral test. The mice that were to have a guide cannula implanted into the skull were handled individually once daily for the 3 days before surgery, and then twice daily during the 4 days after the recovery from anesthesia to the date of behavioral tests.

2.3. Behavioral tests

All behavioral tests were performed during the light phase (10:00–14:00) of the light–dark cycle. The resident-intruder test was performed as described by Pinna et al. [11]. Depressive-like behavior was performed as described previously [37]. Different mice were used for the resident-intruder test, tail suspension test (TST) and forced swimming test (FST), and the sucrose preference test in order to avoid the effect of previous test. Before these behavioral tests using SI mice, 4 weeks old of mouse had been housed individually for 2 or 4 weeks in our facility. Therefore the SI mice were used at the age of 6 (for 2 weeks of SI) or 8 (for 4 weeks of SI) weeks old when the behavioral