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CHRONIC SOCIAL DEFEAT STRESS INCREASES DOPAMINE D2 RECEPTOR DIMERIZATION IN THE PREFRONTAL CORTEX OF ADULT MICE

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Abstract—The present study aimed to examine the effects of chronic social defeat stress on the dopamine receptors and proteins involved in post-endocytic trafficking pathways. Adult mice were divided into susceptible and unsusceptible groups after 10 days of social defeat stress. Western blot analysis was used to measure the protein expression levels of dopamine D2 receptors (D2Rs), a short (D2S) and a long form (D2L) and, D2R monomers and dimers, dopamine D1 receptors (D1Rs), neuronal calcium sensor-1 (NCS-1) and G protein-coupled receptor-associated sorting protein-1 (GASP-1), and reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the mRNA expression levels of D2S, D2L, D2R monomers and dimers, and D1Rs in different brain areas. We observed increased expression of D2S, D2L and D2Rs dimers in the prefrontal cortex (PFC) of susceptible and/or unsusceptible mice compared with controls. The only significant findings with regard to mRNA expression levels were lower expression of D2S mRNA in the amygdala (AMYG) of susceptible and unsusceptible mice compared with controls. The present study demonstrated that chronic social defeat stress induced increased expression of D2S, D2L, and D2R dimers in the PFC of susceptible and/or unsusceptible mice.

Key words: dopamine receptor isoforms, dopamine D_2 receptor dimers, G protein-coupled receptor-associated sorting protein-1, neuronal calcium sensor-1, social defeat.

INTRODUCTION

Social defeat refers to losing a confrontation with conspecific animals, including humans, in either a dyadic or group–individual context. Social defeat stress is an ethologically salient stressor that provides a relevant model for investigating the etiology of stress-related disorders in humans (Koolhaas et al., 1999). Specifically, the social defeat stress paradigm has been widely used as an animal model for depression, anxiety disorders, and drug abuse (Martinez et al., 1998; Blanchard et al., 2001).

Few studies have investigated the effects of social defeat stress on changes in dopamine receptors: three studies focused on changes in the sensitivity or binding of dopamine D1 receptors (D1Rs) (Kudryavtseva et al., 2008; Avgustinovich and Alekseyenko, 2010; Novick et al., 2011), and one focused on dopamine D2 receptors (D2Rs) (Burke et al., 2011). The two isoforms of D2Rs, a long (D2L) and a short form (D2S), have been identified (Dal Toso et al., 1989; Giros et al., 1989). The two isoforms, generated by alternative splicing from the same gene, show differential distributions (McVittie et al., 1991) and functions (Usiello et al., 2000; Xu et al., 2002). In a recent study of postmortem brains, Kaalund et al., (2013) reported an increased D2S/D2L ratio (increased D2S mRNA and decreased D2L mRNA) in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia compared with controls. Similarly, increases in both D2S and D2L mRNA levels were reported in the frontal cortex of patients with schizophrenia compared with controls (Tallerico et al., 2001). Following the recognition that G protein-coupled receptors (GPCRs), including dopamine receptors, can form dimers or oligomers, efforts to identify the physiological relevance of this phenomenon have increased. Of particular interest is that significantly enhanced expression of D2R dimers and decreased expression of D2R monomers were reported in the post-mortem striatal tissues of schizophrenia

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Abbreviations: AFB, animal-free blocker; AISS, amphetamine-induced sensitized-state; AMYG, amygdala; D1Rs, D1 receptors; D2Rs, D2 receptors; DLPFC, dorsolateral prefrontal cortex; EDTA, ethylenediaminetetra acetic acid; EGTA, ethylene glycol tetraacetic acid; GASP-1, G protein-coupled receptor-associated sorting protein-1; GPCRs, G protein-coupled receptors; GPK, G protein-coupled receptor kinase; HIP, hippocampus; HRP, horseradish peroxidase; NCS-1, neuronal calcium sensor-1; PBS, phosphate- buffered saline; PFC, prefrontal cortex; RT-PCR, reverse transcription-polymerase chain reaction; ST, striatum.

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patients, and that amphetamine facilitated D2R dimerization in both the striatum (ST) of amphetamine-induced sensitized-state (AISS) rats and in rat striatal neurons (Wang et al., 2010). Additionally, D2Rs have been reported to form dimers in a variety of neurological diseases such as Alzheimer's, Parkinson's, and Huntington's (Fuxe et al., 2008; Franco, 2009). Taken together, these observations point to the possible role of altered splicing and dimerization of D2Rs in the pathophysiological mechanisms of schizophrenia. To date, no studies have explored the effects of social defeat stress on altered D2S and D2L expression and D2R dimerization in animal brains.

Recently, Schubert et al. (2012) proposed that abnormalities in clathrin-mediated endocytosis and protein trafficking are core pathophysiological processes in schizophrenia and bipolar disorders. Among the various proteins involved in the internalization and trafficking of dopamine receptors, we were interested in the neuronal calcium sensor-1 (NCS-1) and G protein-coupled receptor-associated sorting protein-1 (GASP-1). NCS-1 is the mammalian ortholog of frequenin, a calcium-binding protein implicated in mediating several aspects of neurotransmission (Weiss et al., 2000) and neurotransmitter release (Pan et al., 2002; Scalettar et al., 2002). It has been reported that NCS-1 is involved in modulating G protein-coupled receptor kinase (GRK)-mediated desensitization of activated D2Rs (Kabbani et al., 2002). Moreover, significantly elevated levels of NCS-1 have been reported in the DLPFC of schizophrenic and bipolar patients (Koh et al., 2003). GASP-1 is a recently discovered sorting protein for GPCRs that seems to be involved in directing internalized GPCRs to lysosomes for degradation (Whistler et al., 2002; Moser et al., 2010). GASP mediates the degradation of internalized D2Rs in response to dopamine treatment (Bartlett et al., 2005).

We hypothesized that social defeat stress would alter the expression of D2S, D2L, D2R dimers, NCS-1, and GASP-1 and that the degree of such changes may differ between susceptible and unsusceptible mice. The present study aimed to investigate the effects of chronic social defeat stress on the expression of D2S, D2L, D2R monomers and dimers, D1Rs, NCS-1, and GASP-1 in several key brain regions of mice.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J mice and male CD1 mice (Central Lab Inc., Japan), aged 7 and 14 weeks and weighing 18–23 and 40–44 g, respectively, at the time of delivery were used throughout the study. They were housed in groups in a temperature-controlled room under a 12-h light/dark cycle (lights on 07:00) with food and water available *ad libitum* before the social defeat procedure. All possible efforts were made to minimize animal suffering, and the number of animals used was in accordance with the Guidelines for Animal Experiments, Chonbuk National University Medical School. The study was approved by the Institutional Animal Care and Use Committee (Certification No. 2013-1-0159).

Chronic social defeat stress

The procedure for inducing social defeat stress was performed as reported previously (Berton et al., 2006; Tsankova et al., 2006). Male CD1 mice were screened for aggressiveness level by measuring the latency period prior to attack of a naive C57BL/6J mouse; only mice that attacked in less than 30 s on 3 consecutive days were used, yielding a sample population consisting of ~85% of the aggressors screened originally. C57BL/6J mice were introduced into the home cage of an unfamiliar CD1 aggressor mouse and allowed to interact for 10 min. During this exposure, all subject mice were defeated and showed signs of subordination (i.e., lying on their backs, freezing or upright submissive postures). If the aggressor did not attack the intruder, the aggressor was removed and replaced by a new aggressor mouse. After 10 min of full interaction, the defeated mouse was separated from the aggressive resident by placing in the other half of the cage, which was separated by a perforated Plexiglas divider to allow sensory contact for the rest of the day. The next day, the C57BL/6J mouse was randomly exposed to a new resident CD1 aggressor mouse to prevent habituation. The social defeat procedure lasted 10 consecutive days and severely wounded mice were excluded from the experiment. The C57BL/6J control mice were housed by pair in equivalent cages with members of the same strain, one on each side of a perforated plexiglass partition, which changed daily with another C57BL/6J mice.

Based on the results of the social avoidance test, animals were divided into susceptible and unsusceptible subgroups on day 11. This was accomplished by placing mice in an interaction test box (42 × 42 cm) with an empty wire mesh cage (10 × 4.5 cm) located at one end. Their movement was tracked for 2.5 min, followed by 2.5 min in the presence of an unfamiliar aggressor confined within the wire mesh cage. The duration of the subject's presence in the "interaction zone" (defined as the 8-cm-wide area surrounding the wire mesh cage) was obtained using the automated video tracking system based on the spontaneous motor activity recording tracking (SMART) software (Panlab, Barcelona, Spain). The interaction ratio was calculated as 100 × (time spent in the interaction zone with an aggressor)/(time spent in the interaction zone without an aggressor). Based on previous work[37], an interaction ratio of 100 was set as the cut-off value: mice with scores <100 were defined as "susceptible," and mice with scores $\geqslant 100$ were defined as "unsusceptible." The overall timeline of the experimental procedures is depicted in Fig. 1.

Preparation of brain tissue

After the social avoidance test (day 12) (Fig.1), mice were sacrificed by cervical dislocation. Serial coronal sections (10–15 μm thick) were made in a pre-cooled cryostat (Richard-Allan Scientific, USA). When appropriate sections guided by Allen reference atlas: A digital color brain atlas of the C57BL/6J atlas appeared, the prefrontal cortex (PFC, bregma +1.84 mm), corresponding to the

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