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iTRAQ-BASED QUANTITATIVE ANALYSIS OF HIPPOCAMPAL POSTSYNAPTIC DENSITY-ASSOCIATED PROTEINS IN A RAT CHRONIC MILD STRESS MODEL OF DEPRESSION

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Abstract—Major depressive disorder (MDD) is a prevalent psychiatric mood illness and a major cause of disability and suicide worldwide. However, the underlying pathophysiology of MDD remains poorly understood due to its heterogenic nature. Extensive pre-clinical research suggests that many molecular alterations associated with MDD preferentially localize to the postsynaptic density (PSD). Here, we used a rodent chronic mild stress (CMS) model to generate susceptible and unsusceptible subpopulations. Proteomic analysis using an isobaric tag for relative and absolute quantitation (iTRAQ) and tandem mass spectrometry was performed to identify differentially expressed proteins in enriched PSD preparations from the hippocampi of different groups. More than 1500 proteins were identified and quantified, and 74 membrane proteins were differentially expressed. Of these membrane proteins, 51 (69%) were identified by SynaptomeDB search as having a predicted PSD localization. The unbiased profiles identified several PSD candidate proteins that may be related to CMS vulnerability or insusceptibility, and these two CMS phenotypes displayed differences in the abundance of several types of proteins. A detailed protein functional analysis pointed to a role for PSD-associated proteins involved in signaling and regulatory functions. Within the PSD, the N-methyl-D-aspartate (NMDA) receptor subunit NR2A and its downstream targets contribute to CMS susceptibility. Further analysis of disease relevance indicated that the PSD contains a complex set of proteins of known relevance to mental illnesses including depression. In sum, these findings provide novel insights into the contribution of PSD-associated proteins to stress susceptibility and further advance our understanding of the role of hippocampal synaptic plasticity in MDD.

Key words: major depressive disorder, chronic mild stress, hippocampus, postsynaptic density, iTRAQ, proteomics.

INTRODUCTION

Major depressive disorder (MDD) is a common and debilitating mental illness affecting approximately 2.5% of the general population and is predicted to be the number two cause of illness worldwide by 2020 (Lecrubier, 2001). Symptoms of the illness include cognitive impairment and memory loss, implicating synaptic dysfunction in the pathophysiology of MDD (Levens and Gotlib, 2009; Barabassy et al., 2010; Boyle et al., 2010). This possibility is supported by animal studies demonstrating a reduction of dendritic spine numbers and hippocampal neuronal function (Radley et al., 2006; Liu and Aghajanian, 2008). Postmortem studies have suggested that somatodendritic, axonal, synaptic, and glial cell number changes are all involved in the inhibition of adult hippocampal neurogenesis (D'Sa and Duman, 2002; Sheline et al., 2003; Pittenger and Duman, 2008; Lorenzetti et al., 2009). This is also consistent with brain imaging studies that have reported a reduction in hippocampal volume in postmortem animal models of either stress or depression (D'Sa and Duman, 2002; Pittenger and Duman, 2008). In animal models, stress-induced hippocampal neuropathological changes may be summarized as follows: loss of dendritic spines, decrease in the number and length of dendrites, loss of synapses, loss of glia, and the impairment of neurogenesis (D'Sa and Duman, 2002; Sheline et al., 2003; Pittenger and Duman, 2008). To date, however, the protein substrates involved in synaptic plasticity in the hippocampus of

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Abbreviations: 2DE, two-dimensional gel-electrophoresis; ANOVA, analysis of variance; CMS, chronic mild stress; DAVID, database for annotation, visualization and integrated discovery; FASP, filter-aided sample preparation method; FDR, false discovery rate; FST, forced swim test; GO, gene ontology; GRAVY, grand average hydropathicity; HCD, higher energy collisional dissociation; iTRAQ, isobaric tag for relative and absolute quantitation; MDD, major depressive disorder; MW, molecular weight; NMDA, N-methyl-D-aspartate; Nrgn, neurogranin; p*I*, isoelectric point; PSD, postsynaptic density; SCX, strong cation exchange; SPT, sucrose preference test; TMDs, transmembrane domains; UniProt, universal protein resource.

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MDD have not been well-explored (Czeh and Lucassen, 2007).

Recent studies have used molecular profiling technologies to examine the brains of postmortem depressed patients and rodent models of depression. Several groups have used microarray approaches to demonstrate abnormalities in the expression of transcripts related to synaptic transmission, particularly glutamate and GABAergic signaling, in the brains of depressed patients with or without suicide, especially in the frontal and limbic regions (Choudary et al., 2005; Sequeira et al., 2007, 2009; Klempan et al., 2009). A number of proteomic studies investigating the overall level of hippocampal tissue from various stress systems have shown that stressful events alter the expression of proteins with roles in energy metabolism, neurogenesis, synaptic plasticity, and neurotransmission (Martins-de-Souza et al., 2010; Martins-de-Souza et al., 2012a,b). Although it is unclear whether altering these molecular dysregulations can affect overall hippocampal structure and function, there is evidence that significant changes occur at the subcellular level, particularly at neuronal synapses.

Because of the complexity of the central nervous system, the uses of fractionation enrichment techniques are usually necessary to achieve a reliable quantitation of low-abundance proteins. Within the synapse, the postsynaptic site contains a high concentration of proteins, including receptors and their intracellular signaling components that receive and transduce synaptic information. This postsynaptic electron-dense structure, termed the postsynaptic density (PSD), is a highly-organized structure attached to the postsynaptic neuronal terminal and is comprised of a complex network of cytoskeletal scaffolding and signaling proteins. These proteins facilitate the movement of receptor and signaling complexes. The PSD is critical to normal neurotransmission but is also critical to adaptive behaviors such as learning and memory. It has been strongly implicated in neuropsychiatric disorders (Jay et al., 2004), such as MDD, through its roles in synaptic plasticity and cognitive function (Duric et al., 2013). Although constituents of the PSD have been implicated in MDD using conventional detection techniques (Kinnunen et al., 2003; Duric et al., 2013), no study has yet focused on exploring global changes in the hippocampal PSD fraction through the use of a high-throughput proteomic screen.

In this study, using quantitative proteomics, we examined changes in the expression levels of hippocampal PSD-associated proteins in a chronic mild stress (CMS) rat model of depression. This model generates CMS-susceptible and unsusceptible subpopulations, reflecting the two hedonic responses to CMS. To measure the relative changes in protein expression, we used isobaric tag for relative and absolute quantitation (iTRAQ) followed by tandem mass spectrometric (LC-MS/MS) analysis. Our large-scale subcellular proteome profiles identified several differential PSD-associated proteins that may be specifically related to stress vulnerability. These data should provide a valuable resource for deciphering the molecular mechanism(s) underlying the abnormal synaptic plasticity observed in MDD.

EXPERIMENTAL PROCEDURES

Ethics statement

This study was approved by the Ethics Committee of the Chongqing Medical University (Chongqing, China), and all procedures of animal care and treatment were in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals). Special care was taken to minimize the number and suffering of animals.

Animals and CMS treatment

Eighty-one healthy adult male Sprague–Dawley rats, purchased from the animal facility at the Chongqing Medical University (Chongqing, China), were housed individually, given access to food and water *ad libitum*, and maintained on a 12-h light/dark cycle (light from 7:30 to 19:30) at a constant temperature of 21–22 °C and humidity of 55 ± 5% (unless otherwise noted). Animal weight was approximately 165 g when adaptation for sucrose consumption was initiated and approximately 250 g at the start of the stress regime.

The rats were allowed one week to acclimate to the environment and then 1% sucrose solution was given to them, as well as water for five weeks for sucrose habituation. During this period, sucrose preference of all rats was tested twice weekly during the first three weeks and once weekly during the last two weeks. According to the sucrose intakes of the three final baseline tests, the rats were divided randomly into two groups and placed in separate rooms. Stress groups were exposed to a four-week CMS procedure, whereas the control group was left undisturbed.

The CMS protocol was performed according to the procedure described in our previous studies (Hu et al., 2013; Yang et al., 2013). In short, rats were subjected to a variety of mild stress factors: paired housing, a 45° cage tilt along the vertical axis, a soiled cage (with 300 ml water spilled into bedding), exposure to an empty water bottle immediately following a period of acute water deprivation, stroboscopic illumination (300 flashes/min), continuous overnight illumination, and white noise. The procedure including the time and length of stressors are described in Appendix Table 1. At the end of every week, the sucrose preference of all of the rats was assessed, and the forced swim test (FST) was carried out in the final week.

Sucrose preference test (SPT)

The SPT was conducted as a measure of the anhedonic effect of CMS. A two-bottle preference test was used, where the rats had access to both water and a 1% sucrose solution for 24 h during a no-stress period. The positions of the two bottles (on the left and right sides of the cages) were randomly varied. All fluid consumption was recorded by weighing the two bottles before testing