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Protein biomarkers of susceptibility and resilience to stress in a rat model of depression

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ABSTRACT

The molecular etiologies of psychological stress and major depressive disorder (MDD) are highly complex and many brain regions are involved. The prefrontal cortex (PFC) has gained attention in depression research due to its role in cognition including working memory and decision-making, which are impaired in MDD. The aim of the present study was to identify differentially regulated synaptosomal proteins from PFC in stress-exposed animals. The well-established chronic mild stress (CMS) rodent model was applied and three groups of rats were studied: unstressed controls, stress-susceptible and stress resilient. Large-scale proteomics based on relative iTRAQ quantification was applied on three synaptosomal Percoll gradient fractions and 27 proteins were found to undergo significant differential regulation. Gradient fraction two (F2) contained the highest amounts of synaptosomal proteins and is therefore recommended to be included in proteomic studies onwards, in addition to the traditionally used fractions F3 and F4.

The regulated proteins corroborate previous studies on depression regulated proteins; including GFAP, HOMER1 and glutamatergic transmission (vesicular transporter 1, VGLUT1). However, additional functionalities were represented – especially in stress-resilient rats – such as oxidative stress protection (peroxiredoxins PRDX1 and PRDX2), Na/K-transporter ATP1A2 and respiratory chain subunits (UQCRC1 and UQCRFS1), which illustrate the biochemical complexity behind the stress phenotypes, but may also aid in the development of novel treatment strategies.

1. Introduction

Novel molecular markers of depression are important for obtaining in-depth understanding of the biology behind depression and aid the development of improved treatments of depression. Although depression is a highly complex and heterogeneous disorder it can be modeled in rodent models, such as the chronic mild stress (CMS) rat model. The CMS model is a highly validated model of depression and therefore is a suitable model for studying this disease (Willner, 1997; Christensen et al., 2013; Wiborg, 2013; Henningsen et al., 2012a). The CMS rodent model has the advantages, as compared to clinical studies, that it allows full control of experimental settings and subjects investigated, and provides unrestrained access to tissue samples. The model is based on a variable chronic stress regime, which induces a behavioral and physiological condition that mimics a clinical depression. Chronic exposure to stress always causes a response, but some individuals appear to be less prone to the debilitating effects of stress (Hjemdal et al., 2010). By using the CMS model we have consistently reported that CMS exposure causes a non-uniform effect on sucrose intake in rats (Henningsen et al., 2012a; Bergstrom et al., 2008; Christensen et al., 2011) and based on this behavioral measure, the CMS-exposed rats can be divided into subgroups. Rats decreasing their sucrose intake are defined as anhedonic-like, and rats not decreasing their sucrose intake are defined as resilient. Thus, the CMS model can be used to study the biological underpinning of stress-susceptibility.

The prefrontal cortex (PFC) has long been implicated in depression research due to its role in cognition. Dysfunction of the PFC, and associated circuitries, is assumed to partly underlie the impairment of cognitive function and executive processes, including working memory and decision-making, which constitute common symptoms of major depressive disorder (MDD) (Lewicka, 1997; Pelosi et al., 2000).

A malfunction in PFC-related cognitive abilities can be mediated through altered neurotransmitter release in the PFC, and changes in

Abbreviations: ATP1A2, sodium/potassium-transporting ATPase subunit alpha-2; CMS, chronic mild stress; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GFAP, glial fibrillary acidic protein; HOMER1, homer protein homolog 1; iTRAQ, isobaric tags for relative and absolute quantitation; MDD, major depressive disorder; PFC, prefrontal cortex; PKC, protein kinase C; PRDX1, peroxiredoxin 1; PRDX2, peroxiredoxin 2; PRKCG, protein kinase C gamma type; UQCRC1, cytochrome *b*-c1 complex subunit 1, mitochondrial; UQCRFS1, cytochrome *b*-c1 complex subunit Rieske, mitochondrial; VGLUT1, vesicular glutamate transporter 1.

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neurotransmitter release in turn involve changes in neurotransmitter turnover processes, such as exocytosis and endocytosis. To study the biology of neurotransmitter release, one can use synaptosomes, which are artificial structures obtained by homogenization and gradient centrifugation of neuronal tissue (Dunkley et al., 2008). Synaptosomes contain the complete presynaptic terminal, mitochondria, and parts of the postsynaptic terminal (Dunkley et al., 1986, 2008). Synaptosomes are therefore suitable for studying changes in synaptic protein expression levels.

Although the molecular biology of depression has been an area of scientific interest for decades, a detailed molecular fingerprint of depression remains to be elucidated (Krishnan and Nestler, 2008). Given this situation, a proteomic screening approach was chosen for the present study.

In large-scale discovery proteomics it is often beneficial to fractionate the proteome to get increased analytical depth. In the present study, synaptosome fractionation was used to enrich the sample for proteins with neural function. A discontinuous Percoll gradient is commonly used to prepare synaptosomes from brain tissue synapses, and five relatively distinct bands (F1, F2, F3, F4 and F5) can be obtained for further analysis (Fig. 1) (Dunkley et al., 2008). Synaptosome fractionation is mostly used for obtaining viable synaptosomes to study transmitter release. Activity studies have shown that viable synaptosomes are predominantly located in fraction F3 and F4 and only to a lesser extent in fraction F2 (Dunkley et al., 2008). However, the proteomes of these fractions remains to be thoroughly characterized.

The major aim of the present study was to look for changes in protein expression profiles in synaptosomes extracted from the PFC of rats exposed to CMS, comparing anhedonic-like rats, resilient rats and non-stressed controls.

A secondary aim of the present study was to conduct a proteomic comparison of fraction F2, F3, and *F*4, to clarify whether the fractions that are commonly used to study viable synaptosomes also hold the highest level of synaptic proteins.

We report a list of 27 proteins found to undergo significant differential regulation. Of specific interest, the glial fibrillary acidic protein, was found to be downregulated in the anhedonic-like group, confirming earlier studies and suggesting stress susceptibility to be associated with a glial deficit.

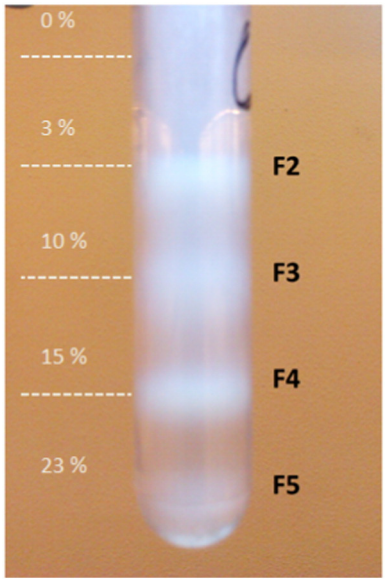

Fig. 1. Discontinuous Percoll gradient consisting of four different concentrations of Percoll (3, 10, 15 and 23%) layered on top of each other. The homogenised prefrontal cortices were layered on top of the gradient and centrifuged at 20,000 ×*g* for 5 min to produce four fractions. The four fractions, annotated F2, F3, F4 and F5, are located in the interfaces of the specific Percoll gradients.

2. Materials and methods

2.1. Animals

5–6 weeks old male Wistar rats were purchased from Taconic M&B, Denmark. The animals were singly housed, food and water was available ad libitum, and the animals were kept on a standard 12-h light/dark cycle except when one of these parameters was changed according to the stress regime. All procedures involving animals were accepted by the Danish National Committee for Ethics in Animal Experimentation (2008/561-447).

2.2. Sucrose consumption test

Animals were adapted for five weeks to consume a palatable sucrose solution (1.5%). In this period, the sucrose test was performed twice a week during the first three weeks and once a week during the last two weeks. The animals were food and water deprived 14 h prior to the sucrose test. The test consisted of a one-hour period of free access to a bottle of the sucrose solution. The sucrose consumption was measured by weighing the bottles pre- and post-testing. Baseline sucrose consumption was calculated for each animal and is defined as the mean sucrose consumption from the last three sucrose tests prior to stress start. During the stress period, the sucrose consumption test was performed once a week.

2.3. Chronic mild stress

Based on the baseline sucrose consumption animals were divided into two matched groups (Equal mean baseline ± 0.5 g), control and CMS groups, and placed in separate rooms. The CMS group was exposed to eight weeks of chronic mild stressors. Briefly, this procedure consisted of seven different stressors and lasted from 10 to 14 h. The mild stressors consisted of interchanging periods of intermittent illumination, stroboscopic light, grouping, food and/or water deprivation, soiled cage, 45° cage tilting and no stressors according to the week schedule illustrated below (Table 1). The control group was left unchallenged except for 14 hour food and water deprivation before sucrose consumption test.

During grouping, animals were housed with different partners, the individual rat alternatively being a resident or an intruder. Following exposure to stress for four weeks, rats were categorised as anhedonic-like (defined as a > 30% within-subject decrease in sucrose intake) or stress-resilient (defined as a < 10% within-subject decrease in sucrose intake). Rats with sucrose consumption not corresponding to either category were excluded from the experiment.

2.4. Tissue preparation

A preliminary study was conducted to examine the efficiency of the synaptosome fractionation protocol as a tool to fractionate and enrich a sample for a subtype of proteins. Three rats were decapitated and brains were removed and PFC immediately dissected. The PFCs were pooled and processed as described for the treatment study below.

For the CMS study, three groups of animals; control ($n = 9$), anhedonic-like ($n = 9$) and stress-resilient ($n = 9$), were decapitated and brains were removed and PFC immediately dissected. This was performed by removing the olfactory bulb and making a coronal section of the brain to isolate the PFC according to Paxinos and Watson, (1998). Immediately hereafter the tissue was snap-frozen on dry ice and stored at −80 °C until further processing. Within each group three PFCs were pooled resulting in three subgroups; control ($n = 3$), anhedonic-like ($n = 3$) and stress-resilient ($n = 3$), to ensure sufficient amounts of tissue sample for synaptosomal fractionation.