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Adipocytokine signaling is altered in flinders sensitive line rats, and adiponectin correlates in humans with some symptoms of depression

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ABSTRACT

Major depression is a complex multi-factorial disorder with a lifetime diagnosis of nearly 1 out of 6. We used the Flinders Sensitive Line (FSL) of rats, a model of depression, and the parent Sprague–Dawley (SD) rats to identify genes, gene ontology categories and pathways associated with depression. Depression-like behavior was verified in the FSL line by forced swim testing, with FSL animals exhibiting greater immobility compared to SD rats. RNA samples from the hippocampus were isolated from a group of experimentally naïve FSL and SD rats for microarray analysis. Microarray analysis yielded a total of 361 genes that were differentially regulated between FSL and SD rats, with catechol-O-methyltransferase (COMT) being the most up-regulated. The genes that were differentially regulated between FSL and SD rats were subjected to bioinformatic analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID), which yielded several gene ontology categories that were overrepresented. Subsequent pathway analysis indicated dysregulation of the adipocytokine signaling pathway. To test the translational impact of this pathway, metabolic factors and psychiatric symptoms were evaluated in a sample of human research participants. Results from our human subjects indicated that anxiety and a subset of depressive symptoms were correlated with adiponectin levels (but not leptin levels). Our results and those of others suggest that disruption of the adipocytokine signaling pathway may be a critical component of the depressive-like behaviors observed in the FSL rats and may also be an important indicator of depressive and anxiety symptoms in humans.

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1. Introduction

Major depressive disorder (MDD) is a serious illness characterized by deep sadness, feelings of hopelessness and despair, and increased somatic symptoms (e.g., disturbed sleep, appetite changes, and pain). By the year 2020, depression will be the 2nd most disabling health problem in the world. Up to 15% of individuals with severe MDD die by suicide (American Psychiatric Association, 2000). Current medications to treat depression exhibit limited efficacy (e.g. Fountoulakis and Möller, 2011). Residual depressive symptoms are common, increase the risk for relapse, and may result in a more chronic course of illness (reviewed in Kurian et al., 2009). Thus, new treatments for major depression are needed to address the high rates of resistance to current interventions and the chronic residual symptoms in many patients treated for depression. A better understanding of the pathophysiological mechanisms associated with depression will contribute to this need for improved therapeutic strategies.

The heterogeneity of MDD suggests that multiple neurocircuits and neurochemicals are involved in its pathogenesis. The most widely accepted neurochemical theory of depression is the monoamine hypothesis which postulates that depression is a pathology caused by alterations in brain serotonergic and noradrenergic systems. However, this theory is incomplete (Lee et al., 2010), and the role of the immune system in depression is increasingly appreciated and supported (reviewed in Loftis et al., 2010; Raedler, 2011; Leonard and Maes, 2012).

To identify genes that contribute to depression and the biological mechanisms through which they act, we used the Sprague Dawley (SD) derived Flinders Sensitive Line (FSL) rats to perform genome-wide expression profiling. The FSL rats, a well-validated genetic model for depression, were previously generated by selective breeding of out-bred SD rats for differences in the effects of the anticholinesterase agent diisopropylfluorophosphate (DFP) (Overstreet and Russell, 1982; Overstreet, 1986). FSL rats are more sensitive to DFP and cholinergic agonists, a feature shared by depressed humans (Janowsky et al., 1994). Subsequent evaluations of the FSL rats revealed that, in addition to their cholinergic hypersensitivity, they

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express behavioral (e.g., reduced locomotor activity, increased immobility, and cognitive deficits (Overstreet, 1993)) and physiological (e.g., psychomotor retardation, lower body weight, and reduced appetite (Overstreet et al., 2005)) features similar to those found in MDD. Recent proteomic analyses found that a number of analytes previously associated with MDD were similarly altered in hippocampus and prefrontal cortex of FSL rats (Carboni et al., 2010; Piubelli et al., 2011), including alterations in proteins associated with energy metabolism, cellular localization and transport, cytoskeleton organization, and apoptosis.

The hippocampus is increasingly thought to be involved in the pathophysiological mechanisms of depression (Duman, 2002; Santarelli et al., 2003; Campbell et al., 2004; Videbach and Ravnkilde, 2004), and therefore was selected for gene expression profiling. Repeated intrahippocampal administration of prednisolone (a commonly prescribed glucocorticoid) increased anxiety and depression-like behavior in mice, and altered expression of genes associated with cell death and inflammation (Kajiyama et al., 2010). Imaging studies show that hippocampal volumes are reduced in some patients with MDD (Bremner et al., 2000; MacQueen et al., 2003; Sheline et al., 1996), possibly due to reduced neuropil sizes (Rosoklija et al., 2000). Early life stress may underlie the reduced hippocampal volumes observed in some patients with MDD (see Frodl and O'Keane, in press for review). Efficacious antidepressant treatments function in part, by normalizing disturbed neuroplasticity (Michael-Titus et al., 2008) and facilitating axonal and dendritic sprouting (Vaidya et al., 1999) — processes that can help restore synaptic connections within the neuropil. Although the role of hippocampal neurogenesis in the development and persistence of depression is not completely understood, its requirement for antidepressant efficacy is well accepted (Lewitus et al., 2009; Santarelli et al., 2003; Malberg et al., 2000).

In the present study we identified associations with several genes and found dysregulation of the adipocytokine signaling pathway in the FSL rat model of depression. We chose to conduct the microarray investigation under resting conditions (i.e., using experimentally naïve rats) to conform to studies involving depressed patients (e.g., Shelton et al., 2011) and to determine whether differences in gene expression would be evident in the absence of stress or pharmacological manipulation. We found significant gene expression differences at all levels of analysis, including at the single gene level, at the biological process level (gene ontology) and at the pathway level. We followed up these preclinical findings with a study in humans to determine whether disrupted adipocytokine signaling was similarly associated with symptoms of depression or anxiety. Our translational findings highlight important new directions for depression research and diagnosis and further support the utility and relevance of the FSL rat model.

2. Materials and methods

2.1. Animals

Male FSL rats (302.9 ± 23.4 g; Dr. Amir H. Rezvani, Duke University) and male SD rats (294.9 ± 17.6 g; Harlan Laboratories) were housed in the same room for 3 months prior to the start of the experiments. Rats were pair-housed under conditions of constant temperature (20–22 °C) and humidity (30–45%) with free access to food and water. The room was maintained on a 12:12 h light:dark cycle with lights off at 1800 h. All animal studies were approved by the Institutional Animal Care and Use Committee at the Portland VA Medical Center and were performed in accordance with the guidelines of the National Institutes of Health.

2.2. Forced swim test

To evaluate depressive-like behavior in FSL and SD rats, forced swim testing (FST) was performed to assess behavioral despair (immobility). Rats participating in the behavioral testing were not used for the microarray experiment because the stress associated with the swim test could alter gene expression (e.g. Drossopoulou et al., 2004). The FST was performed as previously described (Loftis et al., 2006; Wilhelm et al., 2011). Briefly, rats were placed in a clear acrylic cylinder (40 cm height; 18 cm diameter) filled to 30 cm high with 25 °C (±2 °C) water. The water was sufficiently deep that the rats would swim or float in the water without limbs or tail touching the floor of the container. Rats were exposed to a 15-min practice swim (training session), followed 24 h later by a 5-min test swim. Both the training and test sessions were video recorded, and the test sessions were scored by an independent observer. Three behaviors were measured: swimming, climbing and immobility. These behaviors were defined by Cryan et al. (2002):

- Immobility — floating in the water without struggling and using only small movements to keep the head above water
- Swimming — moving limbs in an active manner (more than required to keep head above water) causing movement around the cylinder
- Climbing — making active movements with the forepaws in and out of the water, usually directed against the wall.

The predominant behavior during 5-s intervals of the 5-min test swim was assessed. The main dependent variables for this task were time spent immobile and latency to start floating. The total time spent immobile was calculated and subjected to a *t*-test (two-tailed, unpaired).

2.3. Flow cytometry

Animals (n = 7 from each group) were euthanized via CO_2 asphyxiation and whole brains were rapidly removed. A single-cell suspension of mononuclear cells was prepared using a 100 μm cell strainer (BD Biosciences, San Jose, CA, USA). Recovered cells were washed in RPMI 1640, resuspended in 8 ml of 40% Percoll (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) underlaid with 3 ml of 80% Percoll to form a discontinuous gradient in a 15-ml centrifuge tube. The gradient was centrifuged at 500 ×*g* for 30 min, and the cells at the 40% to 80% interface harvested. Cells were washed, stained for flow cyotmetric analysis (antibodies for CD11b/c-APC, CD45-PE, CD3-FITC, and CD161a-PE), and acquired on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

2.4. RNA isolation, amplification and microarray analysis

Animals were euthanized by rapid decapitation and hippocampal sections were removed on dry ice, as previously described (Loftis and Janowsky, 2002). Hippocampi were placed in RNAlater (Qiagen, Inc., Valencia, CA, USA) and stored at −80 °C until processed for microarray analysis. RNA was isolated from tissue samples using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) as described in the product manual.

Microarray experiments were conducted in the Affymetrix Microarray Core of the Oregon Health & Science University Gene Microarray Shared Resource. Messenger RNA was amplified and labeled from 200 ng of total RNA in two steps using the MessageAMP Premier RNA Amplification kit (Ambion, Inc., Austin, TX, USA) as described in the product manual. Target yield was measured by UV_{260} absorbance and quality was assessed by examining yield and size distribution of the in vitro synthesis reaction products using a 2100 Bioanalyzer and RNA 6000 LabChip (Agilent Technologies, Santa Clara, CA, USA). Labeled target was fragmented at 95 °C in the presence of high magnesium concentration to produce a uniform distribution of short cRNAs. Ten micrograms of the fragmented material was combined with biotinylated hybridization control oligomer and biotinylated control cRNAs for BioB, BioC, BioD and CreX (Affymetrix, Santa Clara, CA, USA) in hybridization buffer and hybridized on the