Biomarkers of mood disorders in blood

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Introduction

Chronic stress is known to exert long lasting effects in the brain including genomic modification, physiological alterations and dysregulation of the HPA axis1. This type of stress has been implicated as a trigger for depression as well as other mood disorders. Rodent models have provided some insight into the genomic changes that occur following chronic subordination stress. Nestler has shown that chronic subordination in mice produced long lasting down regulation of brain derived neurotrophic factor (BDNF) in the brain2, yet little is known about gene expression changes detected in the blood following chronic stress. Blood biomarkers may offer an unexpected informative window into brain functioning and disease state. To determine the effect of chronic subordination stress on blood gene expression, whole blood samples were collected from aggressive and subordinate animals prior to and following a ten day chronic subordination paradigm.

Materials and Methods

Animals and Treatment

CF-1 male mice were housed in groups of 3  in 12:12 hour light:dark cycle with food and water available ad libitum. Aggressive animals were established by isolation for 6 weeks prior to use in the test paradigms. On Day 1 of the chronic social subordination procedure, group housed animals were exposed to an aggressive animal for a five minute attack. A partition was inserted between the animals to prevent physical but not sensory isolation from the aggressor. Each subordinated animal was exposed to a new aggressor for 10 days.

Repeat Sampling

Before subordination, blood was collected from all animals via the saphenous vein. Rapid sampling from the saphenous vein was used because it causes minimal discomfort or stress to the animal. The blood was stored in RNAlater at -70 °C until use. After the second round of behavior testing, animals were sacrificed and blood was collected via truncation into RNAlater and frozen at -70°C until use.

Animal Testing and Selection

Animals (n=4) were selected for use based on behavioral data from light:dark testing. Previous experiments showed no learning with sensory isolation from the aggressor. Each subordinated animal was exposed to a new aggressor for 10 days.

RNA Isolation and Analysis

RNA was isolated using Mouse RiboPuro™ RNA Isolation Kit (Ambion®). RNA isolation was completed according to the manufacturer directions. RNA isolates were analyzed by spectrophotometry (Nanodrop, Beckman). All samples contained high amounts of RNA with the most concentrated sample containing 125 ng/µl. The 260/280 ratio was used to determine the quality of RNA present, samples with a value under 1.7 were not used. Total RNA (400ng) was reverse transcribed (First Strand Kit, SABiosciences) into cDNA and quantified by qRT-PCR.3 qRT-PCR was performed using a custom PCR array plate designed to detect genes of interest researched based on findings from other studies on genetics and depression, anxiety and aggression4,5 (SABiosciences). For each plate SABio software available to users on their website (www.SABiosciences.com/pr) was used to analyze data to determine Ct, fold change and regulation.

Results and Conclusions

• PCR array analysis of subordinated mice resulted in four down regulated genes (>2.5 fold change): Androgen Receptor, two GABA receptor subunits and inositol triphosphate 1a receptor.

• PCR array analysis of 48 genes in aggressive CF-1 male mice produced pronounced changes in BDNF (upregulation) and Avpr1b (down- regulation), vasopressin receptor 1b.

• Aggressive and subordinated mice showed different patterns in gene expression after ten days of chronic subordination stress.

• The stress paradigm produced changes in gene expression in blood that are consistent with findings in brain.

Future Research

• Determine the pathways of regulated genes to find relationships linked to stress, depression, and mood disorders

• Analyze brain regions of interest to compare and contrast gene regulation with blood sample data

Gene Symbol  Gene  Relation to Mood Disorders
Ar  Androgen Receptor  Androgen Receptor isotype is a marker of vulnerability to developing depression
Apvr1  Vasopressin Receptor 1A  An increase in vasopressin receptors in the brain is found in depressive animals6
Dsrl  Brain derived neurotrophic factor  Stress decreases Bdnf in animals6
Drd1a  Dopamine receptor D1A  Increased dopamine associated with aggression5
Gabra1  GABA A Receptor Alpha 1  GABA, receptors regulate rapid changes in anxiety and the acute stress response
Gabra3  GABA A Receptor Alpha 3  
Gabrg2  GABA A Receptor Gamma 2  
Htra1  Insoluble 1, 4,5-triphosphate receptor
Htra2  Neuregulin U receptor 2  Expression of Nmur2 in the PVN suggests a role in mediating stress response6
Hspa1a  Somatostatin Receptor 1a  Reduced hypothalamic somatostatin receptor expression is seen in aggressive animals
Hspa1b  Somatostatin Receptor 4  

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References


