THE EFFECT OF REPEATED SOCIAL DEFEAT ON HIGH MOBILITY GROUP BOX-1 MRNA EXPRESSION IN THE DORSAL HIPPOCAMPUS

by

Ryan P. Madigan

Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Neuroscience

Texas Christian University

Fort Worth, Texas

May 2, 2016

THE EFFECT OF REPEATED SOCIAL DEFEAT ON HIGH MOBILITY GROUP BOX-1 MRNA EXPRESSION IN THE DORSAL HIPPOCAMPUS

Project Approved: 9/26/15

Supervising Professor: Gary Boehm

Department of Psychology

Brenton Cooper

Department of Psychology

Michael Chumley

Department of Biology

ABSTRACT

Repeated Social Defeat (RSD) is a paradigm that induces stress on mice. By introducing an intruder mouse into the home cage of several other mice, we introduce a stressful situation. Past studies have shown that this stress can cause inflammation in the brains of these mice. High Mobility Group Box Protein One (HMGB1) is the protein capable of priming the immune system to respond to a challenge. Therefore, if the stress paradigm, RSD, increases the expression of HMGB1, then it would support the hypothesis that stress can influence the immune system by increasing the expression of HMGB1. This study purposed measuring the amount of HMGB1 between control groups and groups that received RSD. We witnessed an increase in HMGB1 in mice that had undergone RSD when compared to home cage control animals that did not receive any treatment.

Human mobility group box 1 protein (HMGB1) is a DNA binding protein that loosely binds to DNA and stabilizes nucleosome formation (Javaherian, 1979). HMGB1 acts similarly to a transcription factor like protein. Of great importance in this paper is HMGB1's role in inflammatory responses. Macrophages, dendritic cells, and natural killer cells secrete HMGB1 in response to injury, infection, or other inflammatory stimulus (Semino, 2005). HMGB1 then acts as a cytokine molecule by signaling throughout the body in order to upregulate the inflammatory response. To do this, HMGB1 binds to the receptor for advanced glycation endproducts (RAGE) and Toll-like Receptor 2 (TLR-2) receptors as the first step in the inflammation pathway. Signaling then commences through these two receptors, activating the NFkB pathway (Park, 2004). In addition, HMGB1 activates the erk and p38 pathways. All three pathways promote pro-inflammatory cytokine production throughout the body using the myd88 pathway.

Research shows that repeated social defeat (RSD) as a paradigm, increases several cytokines, such as IL-1β (Woleb, 2011). RSD recreates a stress paradigm for the CD57/BL mice in their home cage. Several adhesion molecules in the brain, particularly in the hippocampus, increase after experiencing RSD (Sawicki, 2015). RSD introduces an aggressor mouse into the home cage of three CD57/BL mice.

Diffuse deposits of HMGB1 present in mice models of Alzheimer's disease, delaying the clearance of Amyloid-β (Takata, 2004). Amyloid-β forms plaques that hinder brain function and lead to neuronal cell death. Amyloid-β plaques are a leading model of Alzheimer's disease today. Using this model leads us to believe that RSD will increase the inflammatory response and expression of HMGB1 mRNA. Using an experimental design utilizing RSD and control animals, we look to show that there is an increase in HMGB1 as well as IL-1β in the hippocampus after RSD.

Method

Mice used. Mice used were bred in the Texas Christian University vivarium, from The Jackson Laboratory (Bar Harbor, ME) stock. Experiments utilized 4–6 month-old, male, C57BL6/J mice and 12 month-old, male CD-1. Animals were housed in groups of three in standard polycarbonate cages, with food and water available *ad libitum.* The animals were maintained on a 12-h light/dark schedule, with daylight hours occurring between 0700 and 1900 h. All animals were housed and cared for in accordance with NIH standards, and all protocols were approved by the IACUC of Texas Christian University.

RSD. Mice were subjected to RSD. An aggressive intruder male CD-1 mouse (retired breeder) was introduced into cages of established male cohorts (three per cage) of C57BL/6 mice for 6 consecutive nights between 4:00 and 6:00 P.M. (2 h). We observed, during each cycle, submissive behavior including upright posture, fleeing, and crouching to ensure that the resident mice showed subordinate behavior. If the intruder did not initiate a defeat within 5–10 min or was defeated by any of the resident mice, then a new intruder was introduced. At the end of the 2 h period, the intruder was removed and the residents were left undisturbed until the following day when the paradigm was repeated. Different intruders were used on consecutive nights. The health status of the mice was carefully examined throughout the paradigm; Mice that were injured or moribund were removed from the study. Control mice were left undisturbed in their home cages until euthanized.

Hippocampus removals and RT-PCR. Dorsal hippocampus samples were collected from two separate batches of animals. All tissue was collected under RNase-free conditions and stored in RNAlater (Austin, TX) at −20 ◦C until processing to isolate the RNA (RNeasy Micro kits, Qiagen,Valencia, CA). RNAyields were quantified using a NanoDrop ND-1000

spectrophotometer (Thermo Scientific, NanoDrop Products, Wilmington, DE) before being diluted to a uniform concentration for qRT-PCR procedures. We utilized a BioRad CFX Connect RealTime PCR Thermal Cycling System (Applied Biosystems, Foster City, CA) for the RT and PCR steps, and samples were amplified using BioRad Primers and Probes (Foster City, CA). Target genes were normalized to β-actin prior to analysis, using the BioRad CFX Manager 3.1 procedure for quantification of gene expression and statistical analysis.

Results

HMGB1 expression. In order to test the hypothesis that RSD can increase the level of HMGB1, we used BioRad Software statistical analysis to analyze the levels of HMGB1 in both HCC and animals that underwent RSD (Figure 1.) We saw a significant increase in those animals that underwent RSD with a p=0.030232. These results support our hypothesis.

Discussion

We saw an increase with RSD compared to HCC. Our hypothesis stated that RSD most likely proceeds through an HMGB1 pathway, and based on the data we support the hypothesis.

In future projects, a higher number of subjects can be selected in order to reduce the size of error bars. However, in the past this lab has had success with n values of this size. The results are in accord with previous literature suggesting that RSD in combination with LPS will increase inflammatory signals in the brain.

Possible targets after this study include the receptor for advanced glycation end products (RAGE) and toll-like receptor 2 (TLR2). Both of these signal downstream of HMGB1 and would be expected to be activated as well. This can then be used to further pinpoint an exact pathway in RSD creates inflammation and stress in these animals. Further, a study in which HMGB1 is

blocked by an antagonist would be insightful into HMGB1's importance in other inflammatory processes through RSD.

Works Cite

Dinarello, Charles. *Blood* 87.6 (1996): 2095-147. Print.

Javaherian, Kashayar, Mohamad Sadeghi, and Leroy F. Liu. "Nonhistone Proteins HMG 1 and HMG 2 Unwind DNA Double Helix." *Nucl Acids Res Nucleic Acids Research* 6.11 (1979): 3569-580. Web.

Lee, Sanghee, Youngpyo Nam, Ja Young Koo, Donghyun Lim, Jongmin Park, Jiyeon Ock, Jaehong Kim, Kyoungho Suk, and Seung Bum Park. "A Small Molecule Binding HMGB1 and HMGB2 Inhibits Microglia-mediated Neuroinflammation." *Nature Chemical Biology Nat Chem Biol* 10.12 (2014): 1055-060. Web.

Lopez-Castejon, Gloria, and David Brough. "Understanding the Mechanism of IL-1β Secretion." *Cytokine & Growth Factor Reviews* 22.4 (2011): 189-95. Web.

Park, J. S., D. Svetkauskaite, Q. He, J.-Y. Kim, D. Strassheim, A. Ishizaka, and E. Abraham. "Involvement of Toll-like Receptors 2 and 4 in Cellular Activation by High Mobility Group Box 1 Protein." *Journal of Biological Chemistry* 279.9 (2003): 7370-377. Web.

Sawicki, C.m., D.b. Mckim, E.s. Wohleb, B.l. Jarrett, B.f. Reader, D.m. Norden, J.p. Godbout, and J.f. Sheridan. "Social Defeat Promotes a Reactive Endothelium in a Brain Region-dependent Manner with Increased Expression of Key Adhesion Molecules, Selectins and Chemokines Associated with the Recruitment of Myeloid Cells to the Brain." *Neuroscience* 302 (2015): 151-64. Web.

Semino, C. "NK/iDC Interaction Results in IL-18 Secretion by DCs at the Synaptic Cleft Followed by NK Cell Activation and Release of the DC Maturation Factor HMGB1." *Blood* 106.2 (2005): 609-16. Web.

Takata, Kazuyuki, Yoshihisa Kitamura, Daiju Tsuchiya, Toshiyuki Kawasaki, Takashi Taniguchi, and Shun Shimohama. "High Mobility Group Box Protein-1 Inhibits Microglial A? Clearance and Enhances A? Neurotoxicity." *J. Neurosci. Res. Journal of Neuroscience Research* 78.6 (2004): 880-91. Web.

Wohleb, Eric S., Ashley M. Fenn, Ann M. Pacenta, Nicole D. Powell, John F. Sheridan, and Jonathan P. Godbout. "Peripheral Innate Immune Challenge Exaggerated Microglia Activation, Increased the Number of Inflammatory CNS Macrophages, and Prolonged Social Withdrawal in Socially Defeated Mice." *Psychoneuroendocrinology* 37.9 (2012): 1491-505. Web.

Yang, H., M. Ochani, J. Li, X. Qiang, M. Tanovic, H. E. Harris, S. M. Susarla, L. Ulloa, H. Wang, R. Diraimo, C. J. Czura, H. Wang, J. Roth, H. S. Warren, M. P. Fink, M. J. Fenton, U. Andersson, and K. J. Tracey. "Reversing Established Sepsis with Antagonists of Endogenous High-mobility Group Box 1." *Proceedings of the National Academy of Sciences* 101.1 (2003): 296-301. Web.

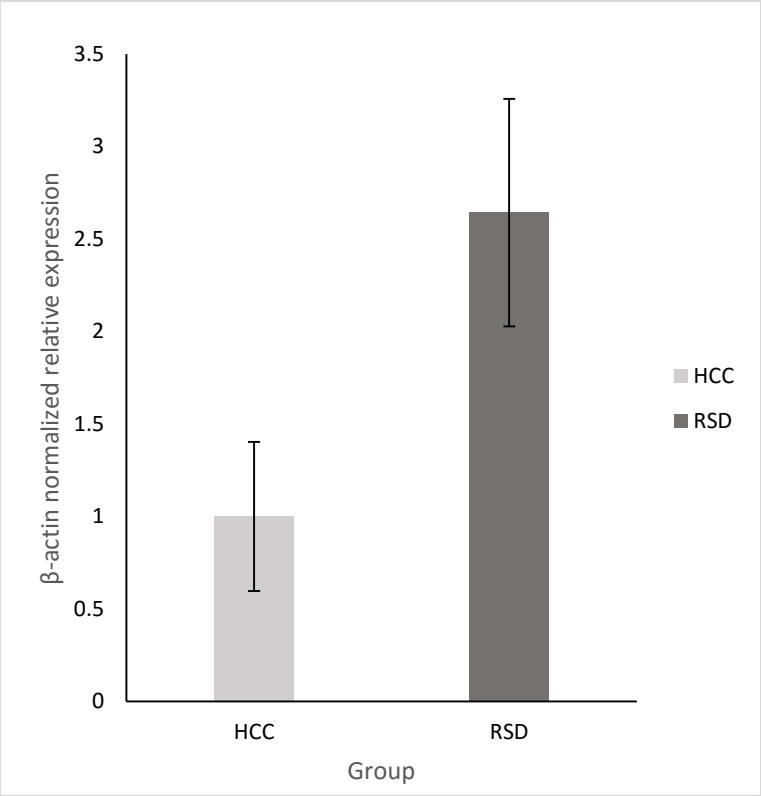

Figure 1. Results from HMGB1 expression in Home Cage Controls (HCC) and Repeated Social Defeat (RSD) mice. Results indicate a significant difference between HCC and animals that received RSD.