INFLAMMATORY MARKERS AS A MEASURE OF TRANSFERRED STRESS IN RATS LIVING WITH TRAUMATIZED CAGE-MATE.

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ABSTRACT

The link between psychological stress and immune response has been developed recently. A focus has been placed on inflammatory agents as causative influences in animal models of depression [1, 2]. Stress has been determined to exacerbate inflammatory conditions [3], and to alter expression of inflammatory cytokines [4]. In this experiment, using an experimental model for chronic stress in rats, we consider the immunological effects of transferred stress, through level of TNF expression in brain tissue from animals housed with a experiencing chronic mild trauma. Female rats were exposed to stress conditioning by electric tail shock. These experimental animals were caged with a non-stressed partner. Brains were obtained from both stressed and non-stressed cage-mates upon sacrifice, and analyzed for relative levels of transcription of the inflammatory gene TNF. The shocked rats showed significantly higher levels of TNF expression as compared to their non-shocked cage-mates, both showed higher levels than control animals. These data suggest that stress, as indicated by higher levels of TNF expression, is not transferred to a large extent in rats housed with chronically stressed partners; however, it does offer further evidence that stress can create or exacerbate inflammatory pathways.

INTRODUCTION

Recently, chronic stress has increasingly been linked to greater susceptibility for various conditions: heart disease, cancer, inflammatory conditions, and so on. Previous reviews have shown a link between such stress and exacerbation of chronic respiratory inflammatory disease [5]. The biophysiological relationships, however, are yet unclear. This project contributes further analysis to a study which strives to form a preliminary translational model of the psychophysiological effects of chronic stress. This model aims to provide a framework for using animals to evaluate stressful conditions, including chronic and transferred stress.

In the initial phase of this prior experiment, adolescent female Sprague-Dawley rats were housed together in pairs. After an acclimation period, one rat from each partner group was exposed to chronic stress conditioning by electric tail shock, while the other rat received no chronic stress. After four weeks of this protocol, both rats were subjected to mild, varied stressors, such as foreign intruders, sham-shock, isolation, and food or water deprivation. After this final period, which also lasted four weeks, both rats were sacrificed and tissue samples were obtained. Home-cage rats were maintained during the experiment; they were housed in similar conditions, but were not exposed to chronic stress, mild stressors, or a stressed cage-mate (Figure 1). As stated above, while inflammatory markers are indicated in many psychological conditions, including depression, it is unclear whether they are a cause for disease progression or an immune response to such [1]. Therefore, we analyzed the presence of TNF expression in the stressed and non-stressed cage-mate pairings. TNF is an inflammatory cytokine. Made by macrophages in the innate immune system, TNF binds receptors that are linked to downstream cascades which activate NFκB, a pro-inflammatory transcription factor. TNF is also regulated by stress and transcription factor NFκB in many tissues and cells. Therefore, it is unclear how inflammation and stress interact in the brain.

RESULTS

Results of the Real Time PCR are given as Curve Threshold (Ct) values. (Figure 2) This is the point at which fluorescence in that sample reached a threshold level. Lower numbers correlate to higher levels of nucleic acid present in each sample, as determined by cycle of PCR at which fluorescence reached a threshold value. The relationship between the Ct values and the amount of nucleic acid present in each sample is determined by a standard curve. The standard curve is generated by reverse transcription PCR of RNA isolated from experimental and control animals after the third phase of the experimental protocol. The mean expression level for each population group was compared in order to determine a ratio: {[(Ct Value Experimental Samples) / (Ct Value Control Samples)]} offer insight into the relative levels of gene expression in those two populations.

CONCLUSIONS

This study aimed to determine further the psychophysiological aspects of chronic stress. One aspect of this is the level of stress transfer that can be measured in one experiencing secondary stress through a traumatized partner. Based on these results, level of stress as indicated by levels of TNF expression is not as high in the living with the stressed partner as in those stressed animals themselves. While both animals had higher inflammatory cytokine expression than those in the home-cage, they did not appear to be experiencing the same magnitude of biological response to the stress experienced. It is significant, however, that a higher level of TNF expression was induced by both chronic and transferred stress. This may appear to downplay the possible gravity of transferred stress, further studies into other pathways involved in psychophysiological responses to stress are necessary to further understand how different types of stress affect the brain.

REFERENCES


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Figure 1: Experimental Paradigm

Figure 2: Mean Curve Threshold (Ct) Values

Figure 3a: TNF cytokine expression in total brain homogenates

Figure 3b: TNF cytokine expression in total brain homogenates