INTRODUCTION

Among industrialized nations, the United States has the worst incidence of childhood maltreatment with 3 million cases per year (U.S. Department of Health and Human Services, 2012). Studies have shown that individuals who were maltreated when they were younger are more susceptible to drug abuse and addiction (e.g., alcohol, nicotine, and marijuana; Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007). The maternal separation stress paradigm is used to model the effects of early life stress (ELS) on the central nervous system. The maternal separation (MS) paradigm involves separating the mother from her pups for a specific period of time (typically 3-4 h) starting on postnatal day 7. Following separation, the pups are maintained in the home cage. While maternal separation does not involve the complete removal of the mother (e.g., motherless pups), its effects mimic those of maternal deprivation, where the mother is removed for a prolonged period (typically 24-48 h) starting on postnatal day 1. Maternal separation results in changes in brain development, including alterations in gene expression (e.g., brain-derived neurotrophic factor, BDNF) and changes in the hypothalamic-pituitary-adrenal (HPA) axis (Lippmann et al., 2007).

METHODS

Subjects: Four males and four females were exposed to the MS paradigm (n = 8 per group). The animals were housed in standard conditions (12:12 h light-dark cycle, controlled temperature, and ad libitum access to food and water) and were 2-4 months old at the beginning of the experiment. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of our institution.

RESULTS

Figure 1: MS = Maternally Separated; C = Control; Ex = Exercise; Sed = Sedentary; Co = Cocaine; Sal = Saline

CONCLUSIONS

Under standard conditions, both β-actin and BDNF showed weak signals which indicated the need to develop a western blot protocol appropriate with the target protein’s quantification. The first experiment used a low percentage of polyacrylamide gel. Since the protein of interest has a very small molecular weight, BDNF transfers very fast and goes through the pores of the gel very easily. Furthermore, the low percentage of the polyacrylamide gel attributed to the scattered appearance of the bands. Thus, a higher percentage of polyacrylamide gel was needed. The subsequent experiments used 14% polyacrylamide gels and was proven to better accommodate the molecular weight of BDNF based on the following experiments’ results. During the second experiment, aside from the increased percentage of the polyacrylamide gels, the dilutions for both primary antibodies were changed. The experiment found that 1:5000 dilution of β-actin is better than 1:3000 while 1:500 dilution of BDNF is better than 1:300 based on the result. For the third and the fourth experiment, we found that 5% milk in Tris-buffered saline with Tween 20 (TBST) as the blocking buffer resulted in stronger signals for both β-actin and BDNF than 5% milk in Phosphate Buffered Saline (PBS). Furthermore, it is important to note that the BDNF band from experiment 4 (20-25 kDa) is likely not BDNF. Previous studies have observed a band of this size with other anti-BDNF antibodies, and the band is also present in brain lysate from BDNF knockout mice (Nemeroff et al., 2009). Moreover, BDNF band from experiment 5 could be either a mature BDNF homodimer (28 kD) or pro-BDNF (32 kD) based on its size. Lastly, we did not see any BDNF bands on the second membrane in experiment 5 suggesting that mature BDNF (14 kD) is not “blowing through”.

The results of the present study opened other new possibilities for improving the western blot protocol, which will be used to quantify hippocampal BDNF content. One of the possibilities is using a gradient gel to resolve low molecular weight proteins like mature BDNF.

Finally, it will be important to test the anti-BDNF antibody for specificity on peripheral tissue samples lacking BDNF to determine whether or not the bands seen in experiment 4 (20-25 kDa) are truly BDNF. Finally, the study could also probe blots with an anti-pro-BDNF antibody to determine whether anti-BDNF antibody is recognizing pro-BDNF, as seems to be the case in experiment 5.

SELECTED REFERENCES


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