INTRODUCTION

Inflammation is a fundamental reaction in the brain (neuroinflammation) that has been associated with a number of neurodegenerative diseases, including Alzheimer’s disease (AD) (Solitio et al., 2012). Within the brain, inflammation is defined broadly as prolonged activation of the brain’s immune cells, known as glial cells. Excessive activation of glial cells within the brains of AD patients is a hallmark of the disease, however the mechanism by which this contributes to disease pathology is relatively unclear (Jo et al., 2014). Recently, studies have shown that glial cells, known as astrocytes, are able to synthesize and release the inhibitory neurotransmitter GABA (Chen et al., 2000). Further, microglia, the primary immunocompetent cells of the brain, have been shown to be GABA(positive) cells, which express GABA(B) type receptors (Kuhn et al., 2004). Early characterizations of AD first described alterations in astrocyte location and activation in the disease and interestingly, differences in the total abundance of GABA within the brains of AD patients have recently been reported. Combined, these data provide support for the hypothesis that astrocytes regulate microglia activity through the release of GABA acting at GABA(B) type receptors. The activation of GABA(B) receptors may serve to reduce the activation status of these microglia, thereby reducing the number of pro-inflammatory cytokines present within the brain (Siné et al., 2012). In the present study, we examined the effects of the GABA(B) antagonist baclofen on chronic inflammation in relevant in vitro preparations (Silva et al., 2013). LPS, a bacterial endotoxin derived from the cell wall of gram-negative bacteria, is capable of mounting an immune response through the activation of toll-like receptor 4 (TLR4). Our data indicate that the administration of baclofen initially attenuated the pyrogenic effects of LPS administration, though this effect was lost after six weeks of injections. The administration of baclofen also rescued deficits in spatial learning memory seen in animals chronically administered LPS. Furthermore, a significant increase in the total abundance of B oligomers, believed to play a role in the pathology of AD, was seen in the brains of animals chronically administered LPS. Together, these data provide evidence that the modulation of GABA(B) receptors function altered the immune response evoked by activation of TLR4. These data also provide support for a potential role of GABA(B) in modulating alternate immune activity seen in AD patients.

EXPERIMENTAL PROCEDURE

Subjects: Subjects consisted of 30 male Sprague-Dawley rats (n=30) were used in this experiment. Subjects were divided into three groups (n=10/group) and randomly assigned to one of three treatment schedules: Saline-Saline (control), LPS-Saline (LPS), or LPS-Baclofen (LPS-Bac). All animals were individually housed in a standard animal facility with a 12:12 hour light, dark cycle with food and water available ad libitum throughout the course of the experiment. All procedures were performed in accordance with the institutional Animal Care and Use Committee and NHG guidelines.

Drug Administration: As part of a larger experiment, stereotaxic surgeries were performed on all animals under aspecic conditions and ketamine/xylazine/xylazine anesthesia. 8ml, of artificial cerebrospinal fluid (ACSF) was slowly infused into each lateral ventricle using the stereotaxic coordinates 0.7mm lateral to Bregma, 1.4mm ventral to the surface of the skull. Following a recovery period of one week after surgery, animals received intraperitoneal (i.p) injections of LPS 0.5mg/kg/h) or saline (1ml/kg) twice a week for seven weeks for a total of 14 injections. Four hours following LPS or saline injections, animals received i.p. injections of baclofen (1mg/kg) or saline (1ml/kg) resulting in three treatment groups: Saline-Saline (control), LPS-Saline (LPS), and LPS-Baclofen (LPS-Bac).

Temperatures: To ensure an immune response was evoked by the administration of LPS, rectal temperatures were tracked prior to drug administration and at 1, 2, and 3-day intervals post injection throughout the course of the experiment. Following the last injection, animals were given three weeks for temperatures to return to baseline prior to behavioral testing.

Morris Water Task: The Morris water task was conducted in a white circular tank, 1.8 m in diameter, 75 cm in height, and 4.7 mm in thickness. In the maze, the hidden platform, the morning water was made opaque with the addition of white non-toxic paint. For each subject, a 10cm x 10cm square platform was placed in the center of one of the four quadrants of the tank, 1.5 cm below the water. At the start of each trial, rats were placed into the maze and given sixty seconds to find the hidden platform located below the water. If after sixty seconds the animal was unsuccessful in locating the hidden platform, a trained experimenter guided the animal to the hidden platform. Once reaching the platform, animals were given twenty seconds to orient themselves to the distal spatial cues. Each animal performed four trials for each day of experimental testing. Twenty-four hours after the control group reached an average latency of ten seconds, a single probe trial was conducted in which the platform was removed from the maze and selective search behavior was recorded for each animal. Following the probe trial, two days of visible training in which the hidden platform was replaced with a visible platform was conducted in order to detect any visual or motor deficits.

SIDS Page/Western Blot: Tissue was prepared for western blotting as previously described (Solitio et al., 2013). For each sample, 20 microliters of protein were loaded at 10% SIDS-PAGE gels and proteins were separated by molecular weight through electrophoresis. Proteins were then transferred to nitrocellulose membranes and blocked in a solution of 5% milk to prevent non-specific antibody binding. Following blocking, membranes were incubated overnight in primary antibody (rabbit IgG 1:1000, Cell Signaling; mouse β-actin, 1:20000, ProteinTech) at 4°C. Following overnight incubation, membranes were washed and placed into secondary antibody solution (anti-rabbit 800, 1:5000, Li-Cor; IR dye anti-mouse 680, 1:5000, Li-Cor) for 2 hours. Membranes were imaged using the Odyssey CLX Infrared Imaging System and band intensity was obtained for each target of interest. Each sample was run in duplicate with β-actin to normalize protein levels.

RESULTS

Administration of baclofen attenuated the pyrogenic effects of chronic LPS.

In the Morris water task probe trial, all groups spent significantly more time in the target quadrant. Only controls showed a significant increase in the number of annulus crossings.

Administration of baclofen rescued LPS-induced deficits in learning and memory in the Morris water task. No differences were seen in visible performance, speed or percent thigmotaxis between animals.

CONCLUSION

☒ Baclofen attenuated the pyrogenic response evoked through the administration of LPS.

☒ Baclofen rescued learning and memory deficits seen in animals administered LPS.

☒ LPS and LPS-Bac animals show a significant increase in total Aβ oligomeric protein levels.

☒ Combined, these data provide support for the role of the GABA(B) receptor in modulating chronic neuroinflammation.

REFERENCES


