Chronic Endotoxin Exposure Decreases Choline and Increases Lactate as Measured by Ex Vivo ^{1}HMRS

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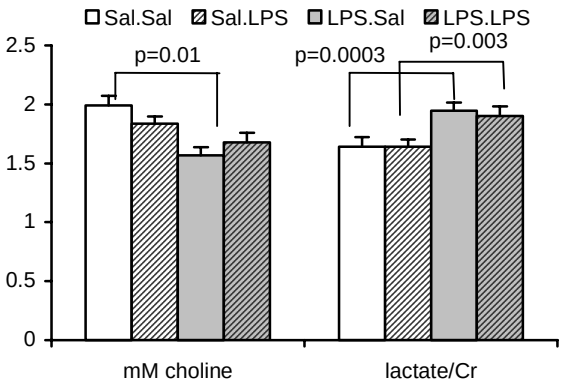
Introduction: Evidence suggests that inflammation in the brain is associated with the pathogenesis of several degenerative neurologic disorders, including Parkinson's, Alzheimer's, multiple sclerosis, and AIDS dementia.[1] During infection, bacterial products, such as the endotoxin lipopolysaccharide (LPS), cause the release of cytokines from immune cells. Inflammation in the brain results from the activation of glial cells, especially microglia, that produce a variety of proinflammatory and neurotoxic factors, including cytokines. Endotoxins are also considered systemic stressors activating the hypothalamo-pituitary-adrenal (HPA) axis. Studies have shown evidence of LPS tolerance developing after chronic treatment with LPS. During repeated endotoxemia, tolerance of both immune and HPA function develops.[2] Patients with chronic infections and/or degenerative neurologic disorders, which can affect immune and HPA responses, often have altered brain metabolites, however the underlying mechanism for these changes is not known.

The aim of this study was to evaluate the effects of chronic and acute LPS on brain metabolites as a model of potential immune-mediated brain injury.

Methods: Forty adult male Sprague-Dawley rats were divided into 4 groups (chronic/acute): 1. saline/saline 2. saline/LPS 3. LPS/saline 4. LPS/LPS. Each rat in groups 3 and 4 received i.p. LPS injections for a 10 day period according to an escalating schedule starting at 250 ug/kg on days 1-2 and increasing to 16 mg/kg on day 10. Rats in groups 1 and 2 received equivelent i.p. injections of saline. Injections were administered in the morning and evening, 9 hours apart. At day 11, groups 2 and 4 received an acute dose of 32 mg/kg LPS. Groups 1 and 3 received a saline injection. Rats were decapitated 2 hours after the final injection. The brains were quickly dissected over ice to isolate several regions of interest and frozen with liquid nitrogen. Individual brain regions were stored at -80ºC for later extraction. For extraction, individual brain regions were weighed and homogenized in five volumes (based on tissue wet weight) of 0.04 M $HClO_4$. Samples were centrifuged at 4°C. Supernatant was collected. Samples were re-homogenized and centrifuged, as above, and supernatants combined. TSP was added to a final concentration of 2.5 mM. Each 0.4 mL sample was analyzed on a Bruker 400 MHz NMR (9T) with a 5 mm QNP probe at room temperature. Acquisition parameters were: 30° pulse, 6 µs 4100 Hz spectral width, 128 averages, 4 s repetition time. After Fourier transformation, phasing, and baseline correction, metabolites were identified by ^{1}H chemical shift position, and signal areas of peaks corresponding to N-acetyl aspartate, glutamate, gamma-aminobutyric acid, total choline, total creatine(Cr), taurine, lactate, alanine, and myo-inositol were integrated and the concentration of each determined by referencing to TSP.

Results: Two-way ANOVA revealed chronic LPS resulted in a significant decrease in choline (p=0.004), and trends for an increase in lactate (p=0.1) and decrease in taurine (p=0.07). Acute LPS challenge alone did not have any significant effects on metabolite concentrations; however, it did have significant chronic by acute LPS interactions for alanine (p=0.04) and glutamate (p=0.03). To reduce sample variability, metabolite ratios to total creatine were also analized and showed similar changes; increased lactate/Cr (p<0.001), decreased choline/Cr (p=0.04), and a trend for taurine/Cr to decrease (p=0.19) with chronic LPS, and chronic by acute LPS interactions for glutamate/Cr (p=0.01) and taurine/Cr (p=0.07).

Discussion: Understanding the inflammatory processes within the brain has important implications for several degenerative neurologic disorders as well as understanding how systemic infections can affect the brain. We observed increases in lactate and decreases in choline within the hypothalamus, suggesting alterations in brain metabolism and cell membrane structure. This work demonstrates that, like studies done in other inflamatory and/or degenerative brain disorders, metabolite abnormalities can be measured using ^{1}HMRS. The hypothalamus was studied due to LPS's strong HPA axis activation and increased FOS immunostaining,[3,4] however further work is required to determine if similar changes are observed in other brain regions affected by degenerative disorders, such as the striatum and cortex.



References & Acknowledgements:

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