An Acute Inflammatory Response in a Diabetic Alzheimer's Disease Model

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INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia, accounting for 50 to 80 percent of all dementia cases. This neurodegenerative disease leads to neuronal death and tissue loss in the brain, resulting in the slow deterioration of memory, thinking, and eventually even the ability to perform daily tasks. While it is not a normal part of aging, AD is mostly diagnosed in people over the age of 65; thus, the main risk factor for Alzheimer’s disease is age itself, though it is likely other additional factors also contribute (Hessee & Akutsu, 2006). Neuropathological hallmarks of AD include neurofibrillary tangles (NFTs) formed by the aggregation of hyperphosphorylated tau protein, and amyloid plaques formed by the accumulation of β-amyloid (Aβ) proteins (Cvetko-Divoz et al., 2001). To date, the etiology of AD remains unknown but several models involving multiple pathways have been used to better understand the disease. In this experiment, two specific AD risk factors, Type 2 diabetes mellitus (DM) and inflammation of the brain, are of recent interest. Recent evidence has indicated a connection between Alzheimer’s disease and Type 2 DM. Using the DM model to investigate AD represents a useful tool to examine AD pathogenesis. In Type 2 DM, cells fail to use insulin properly, resulting in insulin resistance. This resistance consequently affects the metabolism of tau and Aβ proteins, potentially leading to formation or accumulation of Aβ and amyloid plaques characteristically seen in AD (Gasparini et al., 2002).

Chronic brain inflammation, commonly referred to as neuroinflammation, induces a neurotoxic effect by inducing Aβ proteins, senile plaques, and neuronal damage, leading to exacerbated symptoms and a more rapid disease progression. Alternatively, acute inflammatory responses have been suggested to serve a protective role in degenerative disorders (Frank-Cannon et al., 2009). A critical question in AD research is what aspects of chronic neuroinflammation exacerbate pathological features, as well as if an acute inflammatory response may play a neuroprotective role in reducing the aggregation of Aβ, and thus lowering the likelihood of cell loss. The purpose of this study was to investigate the role that acute inflammation plays in a model of AD. To accomplish this, we investigated an immune response induced by lipopolysaccharides (LPS) would alter AD-related behavioral or pathological features using the stepdown (STD) diabetic model of AD.

EXPERIMENTAL PROCEDURE

ANIMALS

Fifty-four male Sprague-Dawley (~9 weeks old) were used throughout the studies. The animals were single-housed in a standard animal facility with a 12–12 hr light-dark cycle, with food and water available ad libitum. All procedures were performed during the light phase and in accordance with the University of Nevada, Las Vegas Animal Care and Use Committee and NIH guidelines for ethical treatment of research subjects.

DRUG ADMINISTRATION

Rats were randomly distributed into four groups. To induce DM, streptozotocin (STZ) was injected intraperitoneally at 65 mg/kg. In two of the groups, bilateral injections of streptozotocin (STZ, 25 mg/ml; 0.1 ml) were administered on each site using stereotaxic coordinates: 0.7 mm posterior, 1.4 mm lateral to Bregma, and 3.5 mm ventral to the surface of the skull (Paxinos & Watson, 2009). The remaining two groups were infused with artificial cerebrospinal fluid (ACSF) to act as a control. A week after the surgeries, a single intraperitoneal injection of either LPS (1.0 mg/kg; 0.1 ml/kg of body weight) was then administered, resulting in the four groups: ACSF/Saline, STZ/ACSF, ACSF/LPS, and STZ/LPS.

TEMPERATURE AND WEIGHT RECORD

To ensure LPS induced an immune response, temperatures and weights were recorded after LPS injections. Racial temperatures were recorded for the first 72 hours and then one week later. Body weights were recorded throughout the course of the experiment.

MORRIS WATER MAZE

Spatial learning and memory was tested using the Morris Water Maze spatial learning task two weeks after LPS or saline injection. The water maze was conducted using a white, circular polyethylene tank (1.5 m in diameter, 76 cm in height, 4.77 mm thick). Food with LPS or saline water was placed on the platform, and water was divided into four quadrants for analysis. Four different large, colorful shapes and posters on the walls were used as spatial cues. Path length, swim speed, perimeter time (triphalomat), latency, and quadrant location were recorded.

In the experimental trials, rats were allowed to swim for 60 seconds to find the hidden platform (a 10 cm x 10 cm square clear plastic located 10 cm below the water’s surface) located in one of the four quadrants. If an animal failed to locate the platform after 60 seconds, they were guided to the platform. Once on the platform the animal was given 20 seconds to orient and familiarize itself to the spatial cues. The trial was repeated in identical fashion three more times, with a 30 second interval between trials. Four consecutive trials were performed each day for each animal. A tracking system was used to record all trials. After five days of training, a probe trial was done where the hidden platform was taken out and animals were allowed to search the maze for 60 seconds. The tracking system recorded the amount of time subjects spent in all four quadrants, as well as the number of times the animals path crossed over the former platform location versus analogous locations in other quadrants. Versatile platform training was then conducted wherein the platform was raised above water level to ensure motoric and visual function was equivalent between groups.

TISSUE COLLECTION

Rats were individually euthanized via CO2 asphyxiation, decapitated, and hippocampi and cortices were immediately dissected out and frozen. Hippocampi and cortices were homogenized using RIPA buffer (Bolton et al., 2012). Homogenization was performed using a handheld Polytron tissue homogenizer (Kinematica Inc). Lysates were centrifuged at 1,000 g for 15 minutes at 4°C, the supernatant was then collected and protein content was determined using the bicinchoninic acid assay (BCA; Pierce). Samples were frozen and stored at -80°C. Tissue was collected four weeks after LPS and saline injections.

SDS PAGE/WESTERN BLOT

Samples (20 μg) from the hippocampal tissue were separated on 10% SDS-PAGE gels. Proteins were electro-transferred to nitrocellulose membranes and blocked for 2 hours. Membranes were then incubated overnight in primary antibody (1:1,000; 11C, Cell Signaling) anti-β-actin (1:500, Cell Signaling), rabbit-anti-β-actin (1:1,000; Cell Signaling), rabbit-anti-NMDAR1, 1:1,000, Cell Signaling; rabbit-anti-NMDAR2A, 1:1,000, Cell Signaling; rabbit-anti-GluR1, 1:1,000, Cell Signaling; rabbit-anti-GluR2, 1:1,000, Cell Signaling). Following incubation, membranes were washed three times with phosphate-buffered saline and TWEEN® (PBST) for 5 minutes each, and then placed in secondary antibody (1:1,000) for 1 hour. A Bio-Rad F Film was used to visualize the bands. The bands were scanned in a GelDoc system and analyzed using ImageJ software. The densities of the bands were normalized to β-actin, the housekeeping protein.