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Effect of acute lipopolysaccharide-induced inflammation in intracerebroventricular-streptozotocin injected rats

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ABSTRACT

Lipopolysaccharide (LPS) is often used to investigate the exacerbatory effects of an immune-related challenge in transgenic models of various neurodegenerative diseases. However, the effects of this inflammatory challenge in an insulin resistant brain state, as seen in diabetes mellitus, a major risk factor for both vascular dementia (VaD) and Alzheimer's disease (AD), is not as well characterized. We investigated the effects of an LPS-induced inflammatory challenge on behavioral and biological parameters following intracerebroventricular (ICV) injection of streptozotocin (STZ) in male Sprague–Dawley rats. Subjects received a one-time bilateral ICV infusion of STZ (25 mg/mL, 8 μL per ventricle) or ACSF. One week following ICV infusions, LPS (1 mg/mL, i.p.) or saline was administered to activate the immune system. Behavioral testing began on the 22nd day following STZ-ICV infusion, utilizing the open field and Morris water maze (MWM) tasks. Proteins related to immune function, learning and memory, synaptic plasticity, and key histopathological markers observed in VaD and AD were evaluated. The addition of an LPS-induced immune challenge partially attenuated spatial learning and memory deficits in the MWM in STZ-ICV injected animals. Additionally, LPS administration to STZ-treated animals partially mitigated alterations observed in several protein levels in STZ-ICV alone, including NR2A, $GABA_{B1}$, and β-amyloid oligomers. These results suggest that an acute LPS-inflammatory response has a modest protective effect against some of the spatial learning and memory deficits and protein alterations associated with STZ-ICV induction of an insulin resistant brain state.

1. Introduction

Diabetes mellitus is a complex metabolic disorder generally characterized by excessive levels of glucose in the blood. The observed hyperglycemia is generally caused either by a reduction of insulin production by the pancreatic β-cells or when tissues become unresponsive to the effects of insulin. Type 2 diabetes mellitus (T2DM) is the form that arises in the context of insulin resistance and is usually accompanied by other vascular risk factors, including hypertension, dyslipidemia, and obesity (Reaven, 1998). Unlike its typical mechanism of action in the peripheral body, insulin has a limited effect on glucose metabolism in the central nervous system. Within the brain, insulin plays an important role in neurotransmitter release and reuptake, synaptic plasticity, and learning and memory (Belgardt and Bruning, 2010). Disruption to insulin signaling within the brain leads to an impairment in neuronal function, deficits in learning and memory, accumulation of amyloid proteins, and is closely associated with dementia (Craft et al., 1998; Zhao and Alkon, 2001).

Longitudinal studies have identified that patients with T2DM have nearly double the risk for developing both Alzheimer's disease (AD) and vascular dementia (VaD; Arvanitakis et al., 2004; Leibson et al., 1997; Ott et al., 1999). Dementia, as a general syndrome, is characterized by a decline in mental ability and cognitive functions, often severe enough to disrupt daily activities (Jellinger, 2013). The most common form of dementia, Alzheimer's disease (AD), is a progressive neurodegenerative disorder marked by cognitive impairments and pathological hallmarks that include beta-amyloid (Aβ) plaques, neurofibrillary tangles, and neuronal loss (Selkoe, 2000). Vascular dementia, the second leading cause of dementia, is marked by impaired executive function, memory loss, arises from a cerebrovascular origin, and is usually associated with brain hemorrhage or cerebral brain infarction (Schneck, 2008).

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While various aspects and risk factors associated with T2DM appear to potentially increase the risk of dementia, numerous studies indicate that insulin resistance within the brain confers the greatest risk for AD and VaD (Craft et al., 1998; Frölich et al., 1998; Kuusisto et al., 1997; Matsuzaki et al., 2010). Mounting evidence suggests that there is considerable overlap of many key features between T2DM and the two leading types of dementia, including the aggregation of Aβ proteins, increased tau phosphorylation, altered glycogen synthase kinase-3β activity (GSK-3β), increased oxidative stress, altered insulin signaling, and vascular abnormalities (Li and Holscher, 2007).

The vast majority of transgenic AD models are based on a number of mutations that exist in three genes, the *APP* (amyloid precursor protein), *presenilin-1* and *presenilin-2*, that lead to early-onset (<60 years) AD, however, these mutations only account for 1% of AD cases (Ridha et al., 2006). The vast majority of AD cases are sporadic in origin (sporadic AD) and are less clearly influenced by a single mutation but rather some combination of many potential genetic and environmental risk factors (Pedersen et al., 2004), which includes T2DM and insulin perturbations. Additionally, while a number of potential models of VaD exist, the majority of these models rely on hypoperfusion, hypertension, and ischemia to induce limited aspects of VaD (Jiwa et al., 2010), without considering insulin perturbations within the brain as a causal risk factor.

Intracerebroventricular (ICV) administration of the diabetogenic drug streptozotocin (STZ) leads to brain insulin resistance and several AD-related abnormalities, including a progressive deterioration of memory, the aggregation of Aβ peptides, and tau hyperphosphorylation (Deng et al., 2009; Grünblatt et al., 2007). STZ-ICV has been useful in understanding the role of an insulin resistant brain state due to its ability to prevent insulin receptor autophosphorylation and reduce the expression of insulin receptors, thus resulting in a brain specific disruption of insulin signaling (Lester-Coll et al., 2006; Sharma and Gupta, 2001; Shingo et al., 2012). STZ toxicity, resulting from DNA alkylation, is selective for insulin producing cells and insulin secreting cells, typically mediated by selective uptake through the glucose transporter, GLUT2, which is predominately found in pancreatic β-cells but is also found, to a lower extent, in the brain (Szkudelski, 2001; Arluison et al., 2004). Additionally, STZ at moderate doses has been demonstrated to decrease the ability of insulin receptors to autophosphorylate (Kadowki et al., 1984). While the exact mechanism of the effects of STZ within the central nervous system (CNS) is not well understood, it is assumed that the mechanisms are similar to the peripheral effects occurring within pancreatic β-cells due to the presence of GLUT2 and insulin receptors in the brain.

Additionally, neuroinflammation has been implicated in playing a fundamental role in the rapid progression of the neuropathological changes observed in dementia and neurodegenerative disorders (McGeer and Rogers, 1992; Zotova et al., 2010). The activation of microglia is thought to initially serve a protective role in the early stages of AD by aiding in Aβ clearance (Chung et al., 1999). However, this initial protective role can quickly become neurotoxic due to the ability of Aβ itself to act as a proinflammatory agent, thus resulting in chronic microglia activation (Matsuoka et al., 2001). The sustained and continual release of several proinflammatory cytokines by the activated microglia, including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα), facilitates the progression of both Aβ and tau pathologies (Akiyama et al., 2000; Bhaskar et al., 2014). A recent study indicates that priming of microglia results in an exaggerated response to subsequent inflammatory stimulation, which can arise from both systemic inflammation and neuroinflammation, and is capable of evoking the transition into and the acceleration of AD pathologies (Perry and Holmes, 2014).

Lipopolysaccharide (LPS), a component derived from the outer membrane of Gram-negative bacteria, is capable of inducing an immune response through the activation of Toll-like receptor 4 resulting in elevated levels of inflammatory cytokines (Block et al., 2007). Peripheral inflammatory events, such as LPS administration, can result in neuroinflammation by increasing the levels of several proinflammatory cytokines, including IL-6, IL-1β, and TNFα, directly within the CNS (Erickson and Banks, 2011). LPS-induced immune challenges are routinely utilized in transgenic models of AD, Parkinson's disease, and prion disease to evaluate the effects of neuroinflammation on deficits associated with these neurodegenerative disorders (Kitazawa, 2005; Sheng, 2003; Takeda et al., 2013).

Considerable research has been conducted to advance our understanding of the risk factors associated with sporadic AD and VaD. Despite the substantial research invested in the STZ-model (for a review see Salkovic-Petrisic et al., 2011), investigations utilizing an inflammatory challenge in STZ-ICV treated animals are not well characterized. The purpose of this study was to investigate the effects of a one-time, acute LPS-induced immune activation in STZ-ICV infused male Sprague–Dawley rats. We assessed anxiety-like behavior in the open field task and spatial learning and memory in the Morris water maze (MWM) task. After behavioral testing, we examined a number of proteins in the hippocampus related to inflammation, learning and memory, synaptic plasticity, and common core pathological features associated with T2DM, AD and VaD.

2. Materials and methods

2.1. Subjects

A total of forty-two adult male Sprague–Dawley rats (Taconic, Cambridge City, IN) were used in this experiment. Rats weighed approximately 250 g at the start of the experiment and were pair-housed until the time of surgery. Following surgery, subjects were individually housed. Rats were housed in a temperature (22 ± 1 °C) and humidity controlled vivarium with lights maintained on a 12:12 light–dark cycle. Standard rat chow and water were available *ad libitum*. All behavioral tests were conducted during the light phase of the daily cycle and began 3 h after lights were turned on. All procedures were approved by the University of Nevada, Las Vegas, Institutional Animal Care and Use Committee and were performed in accordance with NIH guidelines for the care and use of laboratory animals.

2.2. Drug treatments

The animals were randomly divided into four groups. Rats were administered either artificial cerebrospinal fluid (ACSF; Tocris Bioscience, Minneapolis, MN) or STZ (Sigma–Aldrich, St. Louis, MO) dissolved in ACSF at a concentration of 25 mg/mL, similar to previously described protocols (Sharma and Gupta, 2001). STZ was made fresh prior to each surgery. During surgeries, animals received a bilateral ICV infusion of 8 μL per ventricle of either ACSF or STZ, which was slowly infused at a rate of 1 μL every 10 s, followed by a 1 min rest period to ensure proper drug delivery. LPS was dissolved in physiological saline to a concentration of 1 mg/mL (*Escherichia coli*, serotype 0111:B4; Sigma–Aldrich). One week following surgeries, animals were injected intraperitonealy with either saline (1 mL/kg) or LPS (1 mg/mL/kg), resulting in four treatment groups: ACSF-ICV/saline-i.p (controls, $n = 10$), ACSF-ICV/LPS-i.p. (LPS, $n = 11$), STZ-ICV/saline-i.p. (STZ, $n = 10$), and STZ-ICV/LPS-i.p (STZ/LPS, $n = 11$). For an overview of the experimental protocol and timeline, see Fig. 1.