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Full-length Article

Myocardial ischemia/reperfusion impairs neurogenesis and hippocampal-dependent learning and memory

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ABSTRACT

The incidence of cognitive impairment in cardiovascular disease (CVD) patients has increased, adversely impacting quality of life and imposing a significant economic burden. Brain imaging of CVD patients has detected changes in the hippocampus, a brain region critical for normal learning and memory. However, it is not clear whether adverse cardiac events or other associated co-morbidities impair cognition. Here, using a murine model of acute myocardial ischemia/reperfusion (I/R), where the coronary artery was occluded for 30 min followed by reperfusion, we tested the hypothesis that acute myocardial infarction triggers impairment in cognitive function. Two months following cardiac I/R, behavioral assessments specific for hippocampal cognitive function were performed. Mice subjected to cardiac I/R performed worse in the fear-conditioning paradigm as well as the object location memory behavioral test compared to sham-operated mice. Reactive gliosis was apparent in the hippocampal subregions CA1, CA3, and dentate gyrus 72 h post-cardiac I/R as compared with sham, which was sustained two months post-cardiac I/R. Consistent with the inflammatory response, the abundance of doublecortin positive newborn neurons was decreased in the dentate gyrus 72 h and 2 months post-cardiac I/R as compared with sham. Therefore, we conclude that following acute myocardial infarction, rapid inflammatory responses negatively affect neurogenesis, which may underlie long-term changes in learning and memory.

1. Introduction

Cognitive deficits in patients with cardiovascular disease have become increasingly common in the Western population (Vogels et al., 2007a; Nunes et al., 2003; Jefferson et al., 2011). Although technological advances have improved survival rates following adverse cardiac events, cognitive deficits remain and impose a significant, clinically measurable impact on memory. These outcomes interfere with quality of life and employment status, imposing a large financial burden on patients and their families. Ischemic heart disease is a primary cause of heart failure – a condition in which the heart is unable to efficiently supply blood to meet the needs of the body. In order to therapeutically address cognitive decline in this patient population, a basic understanding of the mechanisms that result in cognitive decline are required.

Recent studies suggest that patients with heart failure have damage to the hippocampus (Woo et al., 2009; Fujioka et al., 2000; Rauramaa et al., 2011), a brain region critical for normal learning and memory, and reduced volume in the parahippocampal gyrus (Woo et al., 2003) which projects to the hippocampus. The importance of these clinical assessments are supported by basic science research in rodents where selective lesioning of the hippocampus results in cognitive decline. Although experimental animal studies of cerebral vascular ischemia and stroke show pathological changes in the brain, the impact of myocardial ischemia-reperfusion (I/R), *i.e.*, reperfused myocardial infarction, in the brain has not been explored.

Ischemic brain injury such as stroke results from cerebrovascular occlusion causing oxidative stress and inflammation to the surrounding brain parenchyma. Myocardial infarction, one of the most common forms of cardiovascular disease, results from an occlusion of the coronary arteries within the myocardium, causing oxidative stress and inflammation to the infarct and surrounding areas. Treatments with thrombolytic medications and/or angioplasty restore blood flow through the artery – a process termed reperfusion.

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Reperfusion of ischemic tissues is often associated with oxidative stress, microvascular injury, and inflammation, causing greater damage to the myocardium. However, whether myocardial I/R induces damage to extra-cardiac tissues is currently unknown.

The aim of the present study was to determine if acute cardiac I/R in the mouse (produced by occluding the coronary artery for 30 min followed by reperfusion) could recapitulate the cognitive deficits observed clinically. Our results demonstrate a specific impairment in the hippocampal-dependent object location memory task and contextual fear conditioning paradigm two months post-cardiac I/R. Recent studies have demonstrated that a decrease in neurogenesis can underlie spatial learning and memory deficits (Saxe et al., 2006). Therefore, we examined doublecortin positive neurons in the dentate gyrus and found a decrease at 72 h post-cardiac I/R that was sustained at two months. Consistent with other studies demonstrating that inflammation can impair neurogenesis, we also observed reactive microgliosis and astrocytosis in the dentate gyrus as well as the CA1 and CA3 sub-regions of the hippocampus. These findings suggest that reperfused myocardial infarction can induce rapid and sustained hippocampal neuroinflammation and long-term impairments in cognition.

2. Methods

2.1. Animals

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23) and was approved by the Institutional Animal Care and Use Committees at the University of Alabama at Birmingham. Male C57BL/6 mice (20 weeks old) were housed at the UAB Animal Research Program under controlled conditions (23 ± 1 °C; 12-h light/12-h dark cycle) and received standard laboratory chow and water ad libitum.

2.2. Animal model and surgery

Mice were anesthetized with 1.5–2.0% isoflurane, intubated, and ventilated on a Harvard ventilator. In the open-chest cardiac I/R group, a lateral thoracotomy was performed followed by immediate ligation of the left anterior descending artery (LAD), and the ligation was removed 30 min later as previously published by the authors (Durgan et al., 2010). Studies for stereological assessment of hippocampal reactive gliosis also included closed-chest cardiac I/R mice. In this group, the occluding device was implanted subsequent to thoracotomy, followed by chest closure as previously described by the authors (Durgan et al., 2010). One week later, the closed-chest I/R mice were subjected to a 30-min occlusion followed by release. Sham mice for both groups were subjected to the same procedure respectively but without ligation. Open-chest and closed-chest cardiac I/R mice had similar inflammatory cell numbers in the CA1, CA3, and dentate gyrus subregions of the hippocampus at 72 h post-surgery, therefore we opted to use open-chest cardiac I/R mice and their corresponding sham controls for the remaining experiments.

2.3. Echocardiography

Mouse echocardiography was performed under anesthesia with tribromoethanol (0.25 mg/g IP), and isoflurane (≈1%) as needed, using a VisualSonics Vevo 770 High-Resolution System with a RMV707B scan head as previously described (Ismahil et al., 2014). Mice were imaged on a heated, bench-mounted adjustable rail system (Vevo Imaging Station) that allowed steerable and hands-free manipulation of the ultrasound transducer.

2.4. Behavioral procedures

2.4.1. Habituation

All animals were trained and tested during the same lighting and time of day conditions. Animals were given 30 min to habituate to the behavioral testing room before training or testing. All behavioral chambers were cleaned with 70% ethanol cleaning solution before and after mouse use.

2.4.2. Open field

Mice were placed in the center of an open-field chamber (42 × 42 × 30 cm). Movements were tracked with Ethovision (Noldus) for 5 min. Horizontal motor (distance traveled), movement speed, and central activity (distance traveled in central area/total distance traveled) were evaluated. Mean value and SEM were calculated in each group.

2.4.3. Zero maze

The zero maze consisted of a round track (65 cm diameter) divided into four zones of equal area by two sets of walls along the track, separated by 180 degrees around the track. The animals were put in the center of the arena and observed for 4 min with a camera-driven tracker system, *i.e.*, Ethovision (Noldus, The Netherlands). The system recorded the position of the animal in the arena at 8 frames/s, and the data was analyzed for time spent in each open and closed zone, speed of locomotion, and frequency of entries into a zone.

2.4.4. Contextual fear conditioning

Two months post-surgery animals were placed in a fear-conditioning chamber and given a 2 min interval of habituation followed by a 2 s, 0.6 mA mild electrical shock. This 2 min interval was then repeated followed by an additional 2 s, 0.6 mA shock. Animals were then returned to their home cage. Twenty-four hours after training, testing was performed by placing the animals back in the same conditioning chamber in which no foot shock was delivered. The freezing behavior was recorded for 5 min. FreezeFrame 4 (ActiMetrics) was used to analyze the freezing behavior in which the cutoff for freezing was while the animal was immobile excluding breathing.

2.4.5. Object location memory task

During the acquisition phase, the animals were exposed to two identical Lego™ monkey toys in mirrored positions in the left and right corners of an arena (42 × 42 × 30 cm). The animals were allowed to explore both objects during a 10 min. training period, and interaction was measured by Ethovision (Noldus). After a delay of 24 h, one toy was moved to the opposite corner of the arena. The other toy was placed in its original location. The toy that was moved to the novel position was randomly switched in half of the test trials for each group to control for potential bias towards one object. Exploratory behavior was monitored for 5 min, and tracking was recorded with Ethovision (Noldus). The percentage of time the mouse spent with the object in the novel location as a percentage of total time spent with both the new and old object was reported.

2.5. Immunohistochemistry and stereology

Mice from each group (closed-chest sham, closed-chest I/R, open-chest sham, open-chest I/R) were anesthetized with 5% isoflurane before transcardiac perfusion with PBS (1.5 mM potassium dihydrophosphate, 2.7 mM sodium phosphate, and 150 mM sodium chloride, pH, 7.4) followed by 4% paraformaldehyde. Brains were post-fixed in 4% paraformaldehyde for 24 h, cryoprotected in 30% sucrose, embedded in OCT, and cut (30 μm) coronally on a