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Locus Coeruleus and Hippocampal Tyrosine Hydroxylase Levels in a Pressure-Overload Model
of Heart Disease

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ABSTRACT

Studies have indicated that approximately 30% of people with heart disease experience major depressive disorder (MDD). Despite strong clinical evidence of a link between the two diseases, the neurobiological processes involved in the relationship are poorly understood. A growing number of studies are revealing similar neuroanatomical and neurochemical abnormalities resulting from both depression and heart disease. The locus coeruleus (LC) is a group of neurons in the pons that synthesize and release norepinephrine, and that is known to play a significant role in depression pathobiology. For example, there is evidence that tyrosine hydroxylase (TH) is elevated in the LC in depression. In addition, there is evidence that the LC plays a role in cardiovascular autonomic regulation. The hippocampus is another region that exhibits abnormalities in both depression and heart disease. In this study, the levels of TH in the hippocampus and LC were examined in the guinea pig pressure-overload model of heart disease. TH levels were also measured in the pressure-overload model treated with vagal nerve stimulation, a new investigational therapeutic intervention in heart disease. This study found that there were no changes in TH levels in the LC or the hippocampus of the pressure-overload model or in the pressure-overload model treated with vagal nerve stimulation.

1. INTRODUCTION

Cardiovascular disease (CVD) and major depressive disorder (MDD) are two of the most prominent diseases in our healthcare system today. CVD is the leading cause of death in the United States, killing an estimated 610,000 people annually (CDC, NCHS, 2015). The National Institute of Health reports that 6.7% of Americans suffer from depression (Kessler et al., 2005). While the two diseases can appear as separate entities, they are known to be clinically linked. Approximately 30% of people with heart disease develop symptoms of depression (Lesperance and Frasere-Smith, 2000). Also, people with depression are more likely to develop CVD (Hare, Toukhsati, Johansson, and Jaarsma, 2014). Unfortunately, depressed patients with heart disease typically experience more adverse events, such as an increase in symptoms, poorer responses to medication, a slower rehabilitation, and ultimately a poorer quality of life. Depression also increases the probability of additional cardiac complications, as well as increases the mortality rate (Nair, Farmer, Gongora, and Dehmer, 2012; Parissis et al., 2007). There is also evidence of a correlational relationship, with the mortality rate and number of cardiac complications increasing with the severity of the depression (Hare et al., 2014). Despite the many associations between the two diseases, the underlying biological relationship is very poorly understood.

The locus coeruleus (LC), a group of neurons located in the pons, is an area of interest in the neurobiological link between CVD and MDD. It is the primary source of the neurotransmitter norepinephrine in the brain and plays a role in many physiological responses, including numerous behaviors. Tyrosine hydroxylase (TH), an enzyme found in the LC, is the rate-limiting enzyme in the biosynthesis of norepinephrine. Studies have shown the relevancy of the LC in the pathophysiology of depression. The expression level of TH in the LC in brain tissue from depressed humans was shown to be significantly higher than that of age-matched

sudden-death control cases (Ordway, Smith, and Haycock, 1994; Zhu, Klimek, Dilley, Haycock, Stockmeier, Overholser, Meltzer, and Ordway, 1999). Research has also found that the LC plays a role in central control of the cardiovascular system by being connected to many cardiovascular regulatory centers. For example, nitrergic stimulation in the LC was shown to modulate heart rate and blood pressure (Yao, Finkelstein, and Lawrence, 1999).

The hippocampus, known to play a vital role in learning and memory, is an additional brain area known to present pathology in depression, while likewise being impacted pathologically by heart disease. In regards to CVD, research has shown that the release of proinflammatory cytokines that occurs in CVD deleteriously affects the limbic system, particularly of the hippocampus (Liu et al., 2013). Apoptosis has been shown to occur in the hippocampus following experimentally-induced myocardial infarction in rats (Wann et al., 2007; Kaloustian et al. 2008). In the pathology of depression, atrophy in the hippocampus is associated with MDD, with recurrent depression being associated with a greater loss of hippocampal volume (Sapolsky, 2001).

Another interesting similarity between CVD and MDD involves a treatment known as vagal nerve stimulation (VNS). VNS is administered through a pulse generating device that is surgically implanted into the chest and connected to the left vagus nerve. Initially used for epilepsy, VNS is now approved for use in patients with treatment-resistant depression. Research has shown marked improvement in patients with treatment-resistant MDD treated with VNS, despite the mechanism not being fully understood (Schlaepfer et al., 2008). More recently, VNS has gained great interest as a potential treatment to alleviate cardiac pathology following a myocardial infarction. VNS was shown to significantly increase the long-term survival after chronic heart failure in a rat model (Li et al, 2004). More recently, VNS was evaluated in

patients with chronic heart failure, and showed some promising results (Premchand, 2014).

While many of the underlying mechanisms of VNS are unknown, its role as a potentially effective treatment in both MDD and CVD make it a point of interest.

2. METHODS

2.1 Pressure-Overload and Vagal Nerve Stimulation

Guinea pigs were utilized in this study (n=18). The guinea pigs were randomly assigned to one of three treatment groups: 1) sham control group (CO), 2) pressure-overload group (PO), and 3) pressure-overload in combination with vagal nerve stimulation group (PO + VNS). The pressure-overload in the PO and PO + VNS group was produced by a 15-20% chronic aortic constriction. For the PO + VNS group, a vagal nerve stimulation device was implanted in the guinea pigs two weeks prior to the surgery to produce pressure-overload. The device was attached to the right cervical vagus nerve and delivered stimulation (20 Hz, 0.1 mA) in a continuous cycle of 14 seconds on and 48 seconds off. The guinea pigs were maintained for 60 days post-PO. The PO + VNS group received stimulation during the 60 days. The sham controls were also maintained for the same length of time. The animals were then terminated, and the target brain tissues were dissected and stored at 80°C. This protocol was approved by East Tennessee State University's IACUC.

2.2 Collection of Locus Coeruleus and Hippocampal Tissue

Tissue from the LC was collected from the brainstem, using a micro-punch (2-mm diameter). Hippocampal tissue was collected from a 50 um thick section of the temporal lobe, using a micro-punch (3.5-mm diameter). The collected tissue was stored at 80°C.

2.3 Measuring Tyrosine Hydroxylase Immunoreactivity

Protein from the micropunched LC and hippocampal tissue was isolated, solubilized, and separated by 4-10% polyacrylamide gel electrophoresis. The separated proteins were transferred to PVDF membranes and treated with polyclonal primary antibody generated against rabbit TH. The membrane was then exposed to horseradish peroxidase linked secondary antibody. The TH-immunoreactivity (ir) was then quantified and normalized to actin-ir by chemiluminescence using a G-box imaging system.

2.4 Statistical Analysis

TH-ir levels were analyzed for each group (CO, PO, PO+VNS) for the LC and hippocampal tissue. Statistical analysis was completed using a one-way analysis of variance (ANOVA) with a p-value set at 0.05 to determine statistical significance.

3. RESULTS

3.1 Locus Coeruleus

The LC is the primary site for the synthesis of norepinephrine in the brain. This synthesis is dependent upon the levels of TH; the rate-limiting enzyme. Levels of TH in the LC tissue were measured to determine whether there were any changes induced in the PO group or in the PO group receiving VNS. No significant changes were found between the TH levels of the sham controls and the PO group. In addition, TH levels from the PO group that received VNS did not exhibit a significant difference from the sham controls or from the PO group. These results are expressed in **Figure 1**.

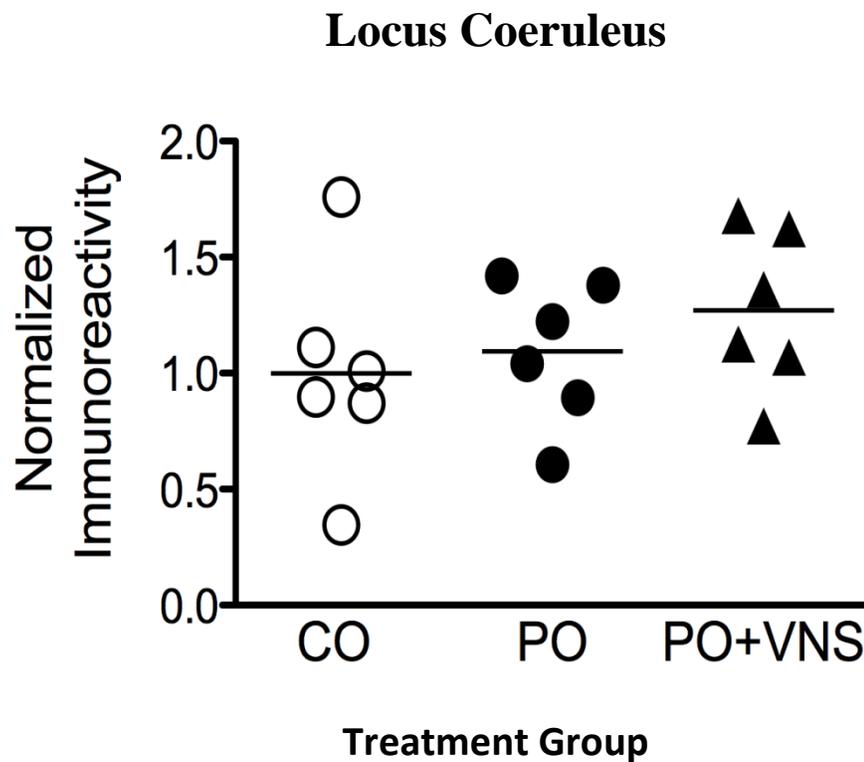


Figure 1: Measurement of Tyrosine Hydroxylase Immunoreactivity in the LC. TH-ir normalized by actin-ir was examined in control (CO; open circles), pressure-overload (PO; closed circles), and pressure-overload with VNS (PO+VNS; closed triangles) groups in micropunched tissue samples collected from the LC and hippocampus.

3.2 Hippocampus

Noradrenergic neurons of the LC project to the hippocampus where they synthesize and release norepinephrine. Levels of TH-ir were measured in hippocampal tissue from the three groups of animals to determine whether any changes were induced by PO or by PO plus VNS. No significant changes in TH-ir levels were observed between the sham controls and the PO group. In addition, no significant changes in the levels of TH-ir were observed in the PO group that also received vagal nerve stimulation. These results are expressed in **Figure 2**.

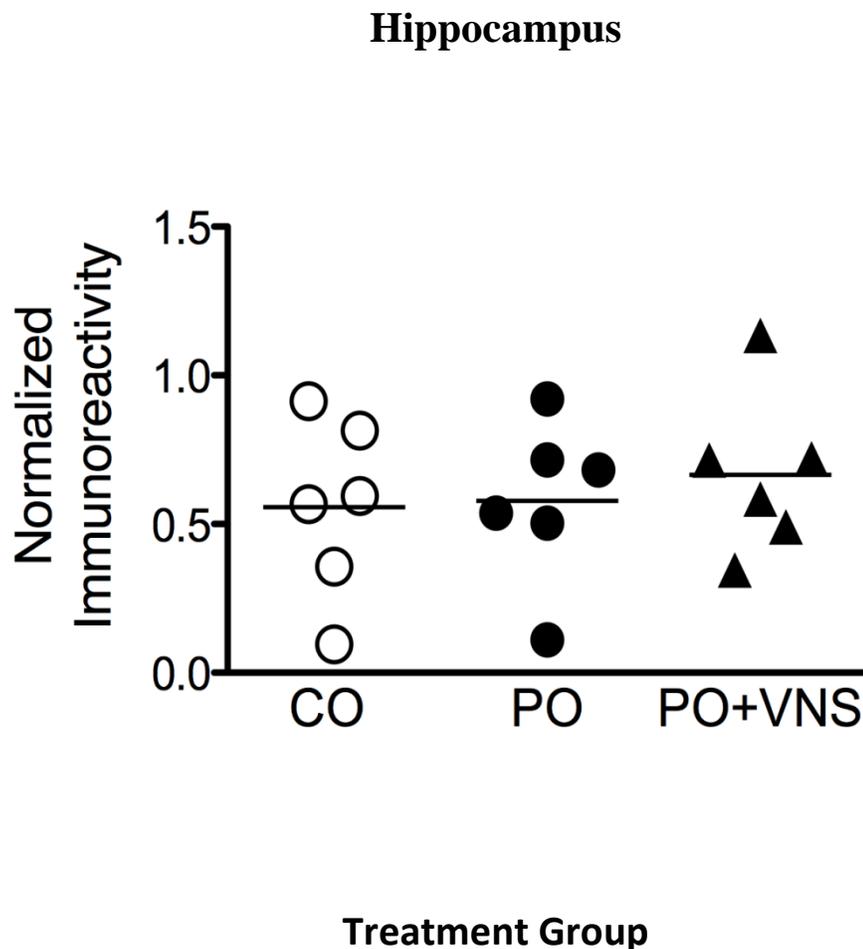


Figure 1: Measurement of Tyrosine Hydroxylase Immunoreactivity in the Hippocampus. TH-ir normalized by actin-ir was examined in control (CO; open circles), pressure-overload (PO; closed circles), and pressure-overload with VNS (PO+VNS; closed triangles) groups in micropunched tissue samples collected from the LC and hippocampus.

4. DISCUSSION

The results from this study suggest that the PO guinea pig model does not significantly impact LC or hippocampal biochemistry at the level of the enzyme TH. Additionally, the results from the PO model combined with VNS imply that this treatment does not significantly influence the levels of TH in the LC or hippocampus. Despite a lack of significant differences in TH-ir, a positive trend was observed between the controls and the PO model. A positive trend was also observed between the controls and the PO model receiving VNS. Due to the small sample size, it is possible that a significant difference went undetected. By increasing the sample size, it would have allowed for a better statistical analysis between the groups. It is noteworthy that elevation in the activity of the LC is typically paralleled by an increase in the expression of TH protein, since increased activity translates to increased norepinephrine release requiring greater synthetic capacity for norepinephrine (Zhu, Klimek, Dilley, Haycock, Stockmeier, Overholser, Meltzer, and Ordway, 1999). Therefore, the present findings suggest that the PO model used did not significantly increase the activity of the LC. Likewise, at least in PO animals, VNS did not appear to change the activity of the LC.

This study indicated that the PO produced no effect on TH-ir. However, the lack of significant effects may have resulted from the modesty of the PO model with respect to the severity of heart failure. It has been shown that damage to the hippocampus occurs in rats during myocardial infarctions (Kaloustian et al. 2008). In this study, the PO was created by a 15-20% chronic aortic constriction. A higher amount of chronic aortic constriction, simulating a more severe condition, may have influenced brain pathology.

People with CVD have an increased probability of developing MDD. Depression significantly increases the morbidity and mortality in individuals with CVD, making continued research in this area a significant need.

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