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Myocardial hypersensitivity to ischemic injury is not reversed by clonidine or propranolol in a predator-based rat model of posttraumatic stress disorder

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ABSTRACT

- Individuals with posttraumatic stress disorder (PTSD) are at increased risk for cardiovascular disease. We previously reported that a predator-based model of PTSD increases myocardial sensitivity to ischemic injury. Heightened sympathetic signaling has a well-established role in the formation of anxiety associated with PTSD and may also contribute to worsening of myocardial injury in the ischemic heart. Thus, we examined whether suppression of sympathetic tone protects the ischemic heart in rats subjected to this model of PTSD. Rats were treated with saline or clonidine throughout the 31-day stress paradigm. Behavior on the elevated plus maze (EPM) was assessed on Day 32, and hearts were subjected to an ischemic insult on day 33. Stressed rats exhibited increased anxiety on the EPM and significantly larger myocardial infarcts following ischemia. Clonidine reversed the anxiety-like behavior but had no impact on infarct size. In a subsequent experiment, rats were treated with propranolol in their drinking water throughout the stress paradigm. Propranolol had no effect on either anxiety or myocardial sensitivity to ischemic injury. These findings suggest that the myocardial hypersensitivity to ischemic injury observed in this model is not caused by increased sympathetic tone or chronic β-adrenergic receptor signaling.

1. Introduction

Individuals who are exposed to life-threatening trauma such as wartime combat, assault, rape, or motor vehicle accidents are at risk of developing posttraumatic stress disorder (PTSD). People with PTSD experience extreme psychological distress by repeatedly reliving their trauma through intrusive flashback memories (Ehlers et al., 2004; Ehlers et al., 2002; Reynolds and Brewin, 1999). These memories are accompanied by other debilitating symptoms, including emotional numbing, avoidance of stimuli associated with the trauma, and a persistent state of increased arousal and hypervigilance (Nemeroff et al., 2006; Pitman et al., 2012; Zoladz and Diamond, 2013).

In addition to psychological manifestations, PTSD is also associated with cardiovascular abnormalities including increased heart rate and blood pressure, increased risk of cardiac arrhythmias, increased atherosclerosis, and increased risk of myocardial infarction (Ahmadi et al., 2011; Beristianos et al., 2016; Khazaie et al., 2013; Vaccarino et al., 2013). Despite evidence that PTSD increases the likelihood of experiencing a myocardial infarction, the impact of PTSD on the ischemic heart has not been well studied. Such investigations are difficult to conduct in human patients because PTSD is associated with increased rates of smoking, sedentary lifestyle, diabetes, depression, and other factors that influence cardiovascular function (Dedert et al., 2010; Zen et al., 2012) and potentially confound efforts to address the direct impact of posttraumatic stress on the heart.

Animal models provide a tool to examine the impact of posttraumatic stress on the myocardial response to ischemic injury. Predator-based animal models of PTSD have been the subject of multiple recent reviews (Deslauriers et al., 2018; Schoner et al., 2017; Zoladz and Diamond, 2016). Predator exposure is an appealing stressor to use in animal models of PTSD because of its high ecological validity and the instinctual fear response it elicits. Previous work demonstrated that rats exposed to a predator-based model of PTSD developed a number of physiological and behavioral abnormalities that are remarkably similar

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to those observed in people with PTSD, including a robust fear-conditioned memory of the traumatic experience, increased anxiety-related behavior, reduced baseline levels of corticosterone, and an exaggerated startle response (Zoladz et al., 2008). These animals also exhibited impaired memory for new information, greater cardiovascular and hormonal reactivity to an acute stressor, exaggerated physiological and behavioral responses to an α_2-adrenergic receptor antagonist (yohimbine), reduced growth rate, reduced thymus weight, greater adrenal gland weight, enhanced negative feedback of the HPA axis, and increased DNA methylation in the hippocampus (Roth et al., 2011; Zoladz et al., 2008; Zoladz et al., 2012, 2013). Decreased brain serotonin levels, increased brain norepinephrine levels, increased production of reactive oxygen species, increased production of proinflammatory markers, and decreased production of anti-inflammatory markers have also been reported in rats exposed to this model (Wilson et al., 2014a; Wilson et al., 2014b; Wilson et al., 2014c; Wilson et al., 2013). Importantly, many of these effects can be blocked by clinically used drugs including clonidine, amitriptyline, sertraline, and valproic acid (Wilson et al., 2014b; Wilson et al., 2014c; Zoladz et al., 2013).

Rats subjected to the predator-based model of PTSD have been previously shown to exhibit increased sympathetic activity and to be hypersensitive to myocardial ischemic injury (Rorabaugh et al., 2015). Some of the behavioral and cardiovascular effects of this model can also be blocked by suppressing sympathetic tone with clonidine (Zoladz et al., 2013). Furthermore, prior studies have demonstrated that chronic β-adrenergic receptor stimulation worsens myocardial injury in the ischemic heart and that this can be blocked by propranolol (Hu et al., 2006; Rorabaugh et al., 2017a). Thus, the goal of the present study was to determine whether the myocardial hypersensitivity to ischemic injury that develops in animals exposed to this form of stress can be blocked by suppressing sympathetic tone or by blocking β-adrenergic receptors.

2. Methods

2.1. Animals

Male Sprague-Dawley rats were obtained from a breeding colony at Ohio Northern University. The colony was initially established using Sprague Dawley rats obtained from Charles River Laboratories (Wilmington, MA). The rats were housed on a 12-h light/dark cycle (lights on 0700–1900 h) in standard polycarbonate cages with free access to food and water. This investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Ohio Northern University. Previous work has demonstrated that females do not develop myocardial hypersensitivity to ischemic injury following exposure to this predator-based model of PTSD (Rorabaugh et al., 2015). Thus, only males were included in the present study.

2.2. Predator exposure and psychosocial stress

Rats were randomly assigned to “psychosocial stress” or “no stress” groups. On Day 1, rats in the psychosocial stress groups were immobilized in plastic DecapiCones (Braintree Scientific; Braintree, MA) and placed in a perforated wedge shaped Plexiglas enclosure (Braintree Scientific; Braintree, MA; 20x20x8 cm). This enclosure was then taken to a cat housing room and placed in a metal cage (61x53x51 cm) with an adult female cat for 1 h. The Plexiglas enclosure prevented any contact between the cat and rats, but the rats were still exposed to all non-tactile sensory stimuli associated with the cat. Canned cat food was smeared on top of the enclosure to direct the cat's attention toward the rats. An hour later, the rats were returned to their home cages. Rats in the psychosocial stress group were given two acute stress sessions which were separated by 10 days. The first stress session took place during the light cycle (between 0800 and 1300 h), and the second stress session occurred during the dark cycle (between 1900 and 2100 h). The stress sessions took place during different times of the day to add an element of unpredictability as to when the rats might re-experience the predator exposure (Roth et al., 2011; Zoladz et al., 2008; Zoladz et al., 2012, 2013; Zoladz et al., 2015). Rats in the stressed group were also subjected to unstable housing conditions throughout the 31-day stress paradigm. The animals were pair housed, and their cage mates were changed daily such that no rats in the psychosocial stress group had the same cage mate on two consecutive days during the 31-day stress period.

Animals in the “no stress” control group were also pair housed. These animals were not exposed to the cat and maintained the same cage mates throughout the duration of the study. Rats in this group were handled daily to control for potential handling effects on stressed animals.

2.3. Clonidine treatments

Rats received daily intraperitoneal injections of clonidine (0.05 mg/kg) (obtained from Sigma Aldrich, St. Louis, MO) or saline starting 24 h after the first cat exposure and continuing throughout the stress paradigm. The last injection was administered 30 min prior to behavioral testing on the elevated plus maze on day 32. Hearts were isolated on day 33. The injections were always administered in the morning (between 0900 and 1200 h). The clonidine dose (0.05 mg/kg) was chosen based on previous findings that this dose ameliorates anxiety-like behavior on the elevated plus maze and also blocks increased heart rate and blood pressure in rats exposed to this stress paradigm (Zoladz et al., 2013).

2.4. Propranolol treatments

Propranolol-treated rats were administered propranolol in their drinking water (0.5 g/l) starting on day 2 and continuing until day 33 when hearts were isolated and subjected to an ischemic insult. Water bottles were changed every other day. Control animals were given drinking water without propranolol. Prior work demonstrated that chronic treatment of rats with this propranolol dose decreases blood pressure (Asahi et al., 2001) and preserves cardiac function in a rat model of ischemia-induced cardiomyopathy (Warner et al., 1992). More recently, work in our lab demonstrated that chronic treatment with this dose of propranolol also protects the heart from ischemic injury in a transgenic mouse model that exhibits increased sympathetic tone (Rorabaugh et al., 2017a).

2.5. Elevated plus maze (EPM)

Anxiety-related behavior was measured on day 32 on the EPM as previously described (Rorabaugh et al., 2015; Zoladz et al., 2008). Rats were placed on the maze for 5 min, and their behavior was recorded by a video camera located above the maze. Behavior on the maze was evaluated offline by two separate investigators who were blind to the experimental conditions of the animals. Time spent in the open arms was used to assess anxiety. The number of closed arm entries was also measured to assess potential differences in overall locomotor activity.

2.6. Langendorff isolated heart preparation and measurement of infarct size

Rats were anesthetized with sodium pentobarbital (100 mg/kg) on day 33. Hearts were quickly removed and mounted on a Langendorff isolated heart apparatus. Contractile function of the left ventricle was measured as previously described (Rorabaugh et al., 2015). Hearts were equilibrated for 25 min prior to 20 min of global ischemia and 2 h of reperfusion as previously described (Rorabaugh et al., 2017a; Rorabaugh et al., 2015; Rorabaugh et al., 2016; Rorabaugh et al., 2017b). Preischemic contractile function was assessed immediately