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Review

Prenatal stress-induced increases in hippocampal von Willebrand factor expression are prevented by concurrent prenatal escitalopram

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HIGHLIGHTS

- Prenatal stress augmented expression of von Willebrand factor.
- Effects of prenatal stress on vWF were prevented by *in utero* escitalopram treatment.
- Chronic stress in adulthood decreased blood vessel length.
- Escitalopram reduced reactive oxygen in the hippocampus.
- Prenatal experience causes complex changes in adult cerebrovascular endpoints.

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ABSTRACT

Prenatal stress has been linked to deficits in neurological function including deficient social behavior, alterations in learning and memory, impaired stress regulation, and susceptibility to adult disease. In addition, prenatal environment is known to alter cardiovascular health; however, limited information is available regarding the cerebrovascular consequences of prenatal stress exposure. Vascular disturbances late in life may lead to cerebral hypoperfusion which is linked to a variety of neurodegenerative and psychiatric diseases. The known impact of cerebrovascular compromise on neuronal function and behavior highlights the importance of characterizing the impact of stress on not just neurons and glia, but also cerebrovasculature. Von Willebrand factor has previously been shown to be impacted by prenatal stress and is predictive of cerebrovascular health. Here we assess the impact of prenatal stress on von Willebrand factor and related angiogenic factors. Furthermore, we assess the potential protective effects of concurrent anti-depressant treatment during *in utero* stress exposure on the assessed cerebrovascular endpoints. Prenatal stress augmented expression of von Willebrand factor which was prevented by concurrent *in utero* escitalopram treatment. The functional implications of this increase in von Willebrand factor remain elusive, but the presented data demonstrate that although prenatal stress did not independently impact total vascularization, exposure to chronic stress in adulthood decreased blood vessel length. In addition, the current study demonstrates that production of reactive oxygen species in the hippocampus is decreased by prenatal exposure to escitalopram. Collectively, these findings demonstrate that the prenatal experience can cause complex changes in adult cerebral vascular structure and function.

Contents

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1. Introduction

Stress experienced *in utero* is linked to deficits in social behavior, learning deficits, alterations in stress regulation, and susceptibility to adult disease in both humans and rodent models [42,46,47]. Despite the profound importance of vascular integrity in cerebral function, less has been established regarding developmental stress and the cardiovascular system. Vascular disturbances later in life may lead to cerebral hypoperfusion which has been implicated in a variety of neurodegenerative and psychiatric diseases including depression and Alzheimer's disease [10,11]. Indeed, studies have shown that rodents born to stress-treated dams exhibit altered sensitivity of the cardiovascular system in adulthood [23] and show altered adult resilience to subsequent events [30,32].

Although our collective understanding of the impact of prenatal stress on the adult cardiovascular system is growing, little is known about the potential protective effects of *in utero* antidepressant treatment in the presence of chronic stress, a common animal model of the clinical state of maternal depression [5,28,34]. For this study we used the selective-serotonin reuptake inhibitor (SSRI) escitalopram which has been purported to have superior efficacy and fewer discontinuations compared to other second-generation antidepressants [7]. In addition, this research was conducted as part of a NIH-funded Translational Research Center in Behavioral Sciences in which human investigation was also undertaken. Escitalopram was the most commonly prescribed SSRI to pregnant women at the Emory Women's Mental Health Program and in order to foster experimental continuity, is was also selected as the SSRI for the rodent research. Particular care was taken during these experiments to ensure that the prenatal escitalopram (Escit) dosing was maintained within a clinically relevant range of serum drug concentrations throughout gestation in order to produce a state as close to the clinical condition as possible in the context of a rodent model [5]. Using this model, the current set of experiments were designed to determine the impact of *in utero* experience on the expression of noted markers of vascular health, including von Willebrand factor (vWF), an important marker of vascularization and vascular compromise which has previously been shown to be altered by adverse prenatal environment [32].

vWF was first noted as a clotting factor, but has since been established to have multiple vascular functions [12]. vWF impacts thrombosis [45], inflammatory processes ([37]; Petri et al., 2010), and has been associated with functional outcome [3] and mortality following stroke [6]. Furthermore, polymorphisms in vWF increase the risk of cardiovascular disease [44] and stroke [9]. Due to the diverse roles of vWF and the potential functional implications of altered *vWF* expression, gene expression findings are expanded upon to include examination of vascularization (vessel length) and vascular compromise (oxidative stress) under the combined conditions of prenatal stress and/or Escit with and without an adult stress challenge.

2. Methods

2.1. Animals

Prenatal stress or Escit-exposed offspring were kept on a 12:12 light:dark cycle (lights on at 7:00 AM) in a humidity (60%) and temperature (20 °C–23 °C) controlled facility. Rodent Diet 5001 (Purina Mills, Richmond, IN) chow and water were available *ad libitum* throughout the study. Three days after birth, rat pups were sexed and litters were culled. Animals were weaned into same-sex pairs on post-natal day (PND) 21. Only male offspring were used in the current study. No more than two pups were used from each litter in order to prevent litter effects [21]. Each group was assigned between 8 and 12 pups. Experimental groups included non-stress/saline (Control), non-stress/Escit, stress/saline (Stress), and Stress + Escit. All experiments were performed in accordance with the Institutional Animal Care and Use Committee of Emory University and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All groups and sample sizes are assembled in Supplemental Table 1 and described in the text below.

2.2. Prenatal stress exposure

Rats used in this experiment were bred in house from male Sprague-Dawley experienced breeders and nulliparous females weighing 200–225 g purchased from Charles River Laboratories (Charles River, Wilmington, MA). Both the stress paradigm and drug administration paradigms have been previously characterized [5,13]. In addition to the experimental design considerations in selection of Escit that are discussed in the Introduction, Escit was also advantageous because it is soluble in saline. The solubility in saline allows for consistent minipump release without clogging and without irritation to the rat's dermal layers which sometimes occurs with drugs that have to be dissolved in more noxious vehicle solutions. Nulliparous female rats were implanted with Alzet 28-day osmotic minipumps (model 2ML4, Alzet, Cupertino, CA) delivering either 0.9% saline or 12.2 mg/kg/day Escit oxalate in 0.9% NaCl based upon the weight of the pregnant dam on gestational day (G) 21 [5,13]. The estimated expected weight was based on assessment of G21 weights from 4 previous studies ($n = 36$). For these studies, the actual dose on G21 was 12.2 mg/kg/day. This results in steady-state serum drug concentrations that are always within the clinically observed range even though the dose is slightly higher early in the experiment prior to the weight gain associated with pregnancy [5]. This dosing strategy has been shown to mimic clinically relevant serum drug concentrations throughout gestation [5]. Escit serum drug concentrations were verified in each pregnant rat by a small blood sample on G15. Three days after minipump implantation, females were bred with retired breeder males. On G9, the chronic unpredictable mild stress model of depression began and consisted of restraint, cage tilt, damp bedding, cage changes, noise, and overnight illumination [5]. Prenatal

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