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Mechanical allodynia corresponds to Oprm1 downregulation within the descending pain network of male and female rats exposed to neonatal immune challenge

Siyang Yan, Amanda C. Kentner *

School of Arts & Sciences, Health Psychology Program, MCPHS University (formerly Massachusetts College of Pharmacy & Health Sciences), Boston, MA 02115, United States

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ABSTRACT

Exposure to painful procedures and/or stressors during the early neonatal period can reprogram the underlying neurocircuitry involved in nociception and neuropathic pain perception. The reprogramming of these systems can result in an enduring elevation in sensitivity towards mechanical and thermal stimuli. Recent evidence suggests that exposure to mild inflammatory mediators during the neonatal period can induce similar pain responses in both adolescent and adult rats. Therefore, we sought to profile changes in the expression of several genes across brain areas involved in the active modulation of nociception and neuropathic pain using a well-recognized model of neonatal inflammation. In the present study male and female Sprague-Dawley rats were administered either the inflammatory endotoxin lipopolysaccharide (LPS; 0.05 mg/kg, i.p.) or saline (equivolume) on postnatal days (PND) 3 and 5. During adolescence, hind paw mechanical withdrawal thresholds were evaluated using an electronic von Frey anesthesiometer. Animals challenged neonatally with LPS (nLPS) had increased pain sensitivity on this measure which was associated with decreased Oprm1 expression in the prefrontal cortex (PFC) and periaqueductal gray (PAG) of both male and female rats. Although a 'second hit' with LPS in adolescence (aLPS) did not confer protection or reveal additional vulnerabilities, aLPS given to animals treated neonatally with saline was associated with increased pain sensitivity, but only in females. Interestingly, adolescent inflammatory challenge decreased Hcrt2 mRNA in the PAG and elevated Trpv1 in the PAG and PFC of both sexes. There was no effect of inflammatory treatment on either anxiety or depressive-like behavior suggesting that affective functioning did not account for differences in mechanical pain sensitivity. Finally, a preliminary investigation demonstrated that administration of a broad spectrum antibiotic cocktail attenuated the mechanical sensitivity that followed nLPS. Together, these data extend upon evidence that inflammation imparts long term changes in quality of life and pain responses via interference within the descending pain network. Moreover, they highlight a potential window of opportunity to target the microbiota-gut-brain axis and reverse pain processing disturbances following perinatal inflammation.

1. Introduction

Complications arising from perinatal infections include cerebral palsy and cognitive delay, (Stoll et al., 2004; Wheater and Rennie, 2000) in addition to an elevated risk for neurosensory impairments including hearing and vision loss (Bassler et al., 2009; Stoll et al., 2004). Furthermore, a recent accumulation of evidence is suggestive of altered nociceptive neuronal circuits after perinatal peripheral inflammation, at least in animal models (Campbell et al., 2015; Boissé et al., 2005; Ruda et al., 2000; Zouikr et al., 2014a, 2014b, 2015). Specifically, neonatal exposure to the endotoxin lipopolysaccharide (nLPS) has led to hyperalgesia and allodynia in rats later in life (Boissé et al., 2005; Campbell et al., 2015; Zouikr et al., 2014a, 2014b, 2015). The mechanisms underlying this long-term reprogramming of pain sensitivity likely involves both peripheral and central systems given the widespread influence that inflammation has across the hierarchy of structures involved in pain processing (see Grace et al., 2014).

The anatomical and physiological organization of the periaqueductal gray (PAG) and its indirect projections to the dorsal spinal cord via the rostral ventromedial medulla (RVM) is a well-recognized descending pain pathway with the capacity to both facilitate and inhibit nociceptive signals (reviewed by Lau and

* Corresponding author.
E-mail address: amanda.kentner@mcphs.edu (A.C. Kentner).

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Vaughan, 2014; Vanegas, 2004). A classic understanding of the supraspinal influence of the PAG in antinociception has focused on the GABA disinhibition hypothesis of analgesia to explain opioid action. This view has recently evolved to include cannabinoids, orexin (hypocretin), and transient receptor vanilloid type-1 (TRPV1). Indeed, the mechanisms of pain processing appear to be more elaborate than originally hypothesized; u-opioid receptor, cannabinoid receptor 1 (CB)1, hypocretin receptor 1 and 2, and TRPV1 each have integral actions throughout the descending pain pathway (Lau and Vaughan, 2014; Mobarakeh et al., 2005; Palazzo et al., 2008) and are likely candidates underlying the reported disruptions in pain processing following perinatal inflammation. Specifically, decreased transmission of one, or a combination, of these systems within the descending pain pathway may account for the reduced Fos activation reported in the PAG following neonatal inflammation (Zouikr et al., 2016). This network is further complicated by findings that the structure and activation of the PAG, in response to inflammatory-evoked pain, is sexually dimorphic (see Loyd and Murphy, 2009) highlighting the importance of evaluating both male and female animals.

Complex factors underlie the perception and response to pain, some of which include illness, shifted attention, motor disruptions, and emotional state (Ossipov et al., 2010). The prefrontal cortex (PFC) is one region that appears to regulate the affective component of pain and its intensity over time (Baliki et al., 2006; Lorenz et al., 2002; Porro et al., 2002). Along with the PAG, the PFC and anterior cingulate cortex (ACC) are part of the descending antinociception network in which the latter structures modulate cognitive and emotional information related to pain (Petrovic et al., 2002). Notably, this network can be disrupted by peripheral inflammatory challenges. In one recent study evaluating inflammatory nociception, systemic administration of LPS to men and women resulted in elevated pain sensitivity and reports of anxiety that coincided with reduced activity in both the ventrolateral PFC and the rostral ACC. Interestingly, the reactivity of the descending pain network was sex-specific, potentially accounting for sex differences in pain perception (Karshikoff et al., 2016). For these reasons we evaluated anxiety and depressive-like behaviors in male and female animals to determine if underlying affective disruptions may be associated with changes in pain sensitivity following nLPS.

Importantly, measureable changes in the magnitude of pain sensitivity and the types of pain behaviors displayed following nLPS appear to vary as a function of developmental age (Zouikr et al., 2015, 2014a). For example, inflammatory pain responses are not observable on postnatal day (P) 7 although increased licking and flinching are reported in rats at P13 and 22 respectively (Zouikr et al., 2014a). Moreover, formalin injection was associated with heightened licking and flinching and increased levels of circulating interlukin-1β in juvenile rats while adults only demonstrated increased flinching (Zouikr et al., 2015). Indeed, previous studies indicate that early life stressors can impart long term physiological and behavioral phenotypes that may either appear, or remit, depending on developmental stage (i.e. puberty) (Zuckerman et al., 2003; MacRae et al., 2015; Meyer et al., 2006). Given the focus of previous research on either the early neonatal/juvenile periods, or adulthood (Boissé et al., 2005; Campbell et al., 2015; Zouikr et al., 2014a, 2015) we chose to evaluate adolescent animals since very little is known about the effects of nLPS on pain sensitivity at this age.

Studies are now providing compelling evidence that the composition of the microbiota is important for the regulation of pain processing and other behaviors (see Mayer et al., 2014; Cryan and Dinan, 2012). Indeed, germ-free mice lacking detectable bacteria in the gut are hypoalgesic in response to inflammatory challenges, including LPS (Amaral et al., 2008). Additionally, prenatal LPS, used as a microbiome disruptor, has been implicated in subtle social impairments (Foley et al., 2014) and prebiotics have been reported to reverse LPS-induced anxiety like behavior in adult rats (Savignac et al., 2016). Since LPS is a natural byproduct of many enteric gut bacteria (Bested et al., 2013) and inflammation is associated with altered composition of the microbiota (see Cryan and Dinan, 2012; Grenham et al., 2011), there may be a mechanistic link between the gastrointestinal and immune systems in mediating pain sensitivity following nLPS. Therefore, the purpose of the current study was to investigate the influence of neonatal inflammatory challenge on a) the association between pain sensitivity and behavioral indicators of anxiety/depression, and b) the expression of several genes critical to the descending pain network in male and female adolescent rats. Finally, we conducted a preliminary investigation to determine the role of the microbiota on pain sensitivity by utilizing a broad spectrum antibiotic cocktail to reverse allodynia in our neonatal inflammatory model.

2. Experimental procedures

2.1. Animals and housing

Sixteen timed pregnant Sprague-Dawley rats were purchased from Charles River (Wilmington, MA) and housed at 20 °C on a 12 h light/dark cycle (0700-1900 light) with free access to food and water. All experimental procedures were approved by the MCPHS University Institutional Animal Care and Use Committee and were carried out in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). A flowchart of the study procedures is located in Fig. 1.

2.2. Neonatal and adolescent inflammatory treatments

Litters were left undisturbed from postnatal day (P)1, the day of birth, until P3 when each litter was adjusted to 12 pups. Litters were culled in order to create an equal proportion of males and females, wherever possible. A dual lipopolysaccharide (LPS; *Escherichia coli*, serotype 026:B6; L-3755, Sigma St. Louis MO) administration protocol was used on P3 and P5. Pups were injected i.p. with 50 μg/kg of LPS (nLPS) or an equivolume of pyrogen-free saline (nSaline), in a balanced manner within litters, as described previously (MacRae et al., 2015a). Body weights of pups (3 pups per sex and P3P5 treatment per litter) were recorded from a subset of dams (n = 8) between P3-6 in order to determine growth differences as a function of neonatal infection and sex. For all remaining measures reported, no more than one male and one female rat per litter were included in each group in order to avoid litter effects. Please see Table 1 for an outline of all group names and treatments. Weaning involved the removal of each dam (P22) and placing her offspring into a clean cage in same sex pairs. One group of adolescent male and female rats were tested for baseline mechanical paw withdrawal thresholds and then subdivided to receive a second i.p. injection of either LPS (aLPS; 50 μg/kg) or an equivalent volume of pyrogen-free saline (aSaline) on P40 (n = 7–8). Three hours later these animals underwent behavioral testing (i.e. locomotor activity/open field test, and mechanical allodynia) followed by tissue collection. Because sensitivity to pain is tied to affective status (Roeska et al., 2009; Shi et al., 2010; Tang and Gibson, 2005), a second set of neonatally treated rats was evaluated on the light-dark (L/D) test on P40 and for sucrose preference across P40-P42 (n = 7–8). Locomotor activity and behavior in the L/D and open field tests were tracked using automated behavioral monitoring software (Cleversys TopScan, Reston, VA).

Beginning 5 days prior to the second challenge on P40 (immediately after the first mechanical allodynia baseline), a subset of