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Programming of formalin-induced nociception by neonatal LPS exposure: Maintenance by peripheral and central neuroimmune activity

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ABSTRACT

The immune and nociceptive systems are shaped during the neonatal period where they undergo fine-tuning and maturation. Painful experiences during this sensitive period of development are known to produce long-lasting effects on the immune and nociceptive responses. It is less clear, however, whether inflammatory pain responses are primed by neonatal exposure to mild immunological stimuli, such as with lipopolysaccharide (LPS). Here, we examine the impact of neonatal LPS exposure on inflammatory pain responses, peripheral and hippocampal interleukin-1β (IL-1β), as well as mast cell number and degranulation in preadolescent and adult rats. Wistar rats were injected with LPS (0.05 mg/kg IP, Salmonella enteritidis) or saline on postnatal days (PNDs) 3 and 5 and later subjected to the formalin test at PNDs 22 and 80–97. At both time-points, and one-hour after formalin injection, blood and hippocampus were collected for measuring circulating and central IL-1β levels using ELISA and Western blot, respectively. Paw tissue was also isolated to assess mast cell number and degree of degranulation using Toluidine Blue staining. Behavioural analyses indicate that at PND 22, LPS-challenged rats displayed enhanced flinching ($p < .01$) and licking ($p < .01$) in response to formalin injection. At PNDs 80–97, LPS-challenged rats exhibited increased flinching ($p < .05$), an effect observed in males only. Furthermore, neonatal LPS exposure enhanced circulating IL-1β and mast cell degranulation in preadolescent but not adult rats following formalin injection. Hippocampal IL-1β levels were increased in LPS-treated adult but not preadolescent rats in response to formalin injection. These data suggest neonatal LPS exposure produces developmentally regulated changes in formalin-induced behavioural responses, peripheral and central IL-1β levels, as well as mast cell degranulation following noxious stimulation later in life. These findings highlight the importance of immune activation during the neonatal period in shaping immune response and pain sensitivity later in life. This is of clinical relevance given the high prevalence of bacterial infection during the neonatal period, particularly in the vulnerable population of preterm infants admitted to neonatal intensive care units.

1. Introduction

The neonatal period represents a unique developmental phase during which both the nociceptive and immune systems are highly plastic. The maturation and fine-tuning of pain processing and immune responses are dependent on environmental, immunological and sensory stimuli encountered during this vulnerable period of development (Adkins et al., 2004; Fitzgerald, 1995, 2005; Whitelaw and Parkin, 1988). Previous studies have demonstrated that exposure to the bacterial mimetic, lipopolysaccharide (LPS) during the neonatal period produces long-term alterations in the immunological and neuroendocrine responses later in life (Ellis et al., 2005; Hodgson et al., 2001; Shanks et al., 1995, 2000; Spencer et al., 2006; Walker et al., 2009a, 2004). There is now good evidence that pain behaviour can also be altered by early pain experience (Beggs et al., 2012; Ren et al., 2004; Walker et al., 2009b; Wang et al., 2004). Of particular interest, experimental

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administration of LPS in rodents and humans has been reported to produce transient immune activation and pain facilitation including thermal hyperalgesia, mechanical allodynia and hyperalgesia associated with an upregulation of IL-1β in the circulation as well as in the inflammed tissue (Hutchinson et al., 2013; Mason, 1993; Watkins and Maier, 2000; Watkins et al., 1995; Wegner et al., 2014). These studies assessed pain responses following LPS administration in adulthood. However, less is known about the impact of neonatal LPS exposure on future inflammatory pain responses.

Immune system to brain communication modulates a variety of physiological responses including pain (Dantzer and Kelley, 2007; Dantzer et al., 2008; Maier, 2003; Marchand et al., 2005; Ren and Dubner, 2010). Pro-inflammatory cytokines, particularly IL-1β, released by immune cells in response to infection play a key role in this bidirectional communication (Besedovsky and del Rey, 2011; Maier, 2003; Watkins et al., 2007; Watkins and Maier, 2005). Central action of pro-inflammatory cytokines produces sickness behaviours such as loss of appetite, sleepiness, fever and fatigue (Dantzer, 2001; Kent et al., 1992). Importantly, hyperalgesia is also considered an integral part of sickness behaviour aimed at reducing activity (Watkins and Maier, 2000, 2005; Watkins et al., 1994a,b). IL-1β, among the many pro-inflammatory cytokines that have been implicated in peripherally and centrally-mediated hyperalgesia, is the most well characterized (Ferreira et al., 1988; Fukuoka et al., 1994; Oka et al., 1993; Watkins et al., 1994b). Intracerebroventricular (ICV) injection of recombinant IL-1β (rIL-1β) in rats significantly decreases paw lick latency to thermal noxious stimulus (Oka et al., 1993). Blocking the activation of IL-1β using an IL-1 receptor antagonist attenuated thermal hyperalgesia (Oka et al., 1993), and formalin-induced hyperalgesia (Watkins et al., 1997).

In addition to its traditional role in memory formation and consolidation (Bird and Burgess, 2008), the hippocampus has recently been implicated in pain modulation *via* upregulation of IL-1β expression (del Rey et al., 2011) and is considered to be a critical supraspinal region involved in the transition from acute to chronic pain (Apkarian, 2008; Apkarian et al., 2011). Rats that were subjected to spare nerve injury exhibited mechanical hyperalgesia associated with significant increase in IL-1β mRNA levels in the hippocampus 10 days and 24 days following injury (del Rey et al., 2011). Neonatal LPS exposure is also associated with an elevation of IL-1β protein expression in the hippocampus following an emotional stress in adulthood (Walker et al., 2010). Since pain is an emotional aversive experience, we hypothesized that neonatal LPS exposure is likely to produce increased pain sensitivity in inflammatory pain models (i.e. formalin test) via upregulation of hippocampal IL-1β.

An increasing body of literature documents the important role of immune cells in the initiation and maintenance of chronic pain (Austin et al., 2012; Costigan et al., 2009; Grace et al., 2011). The majority of documented research in this area focuses mainly on astrocytes, microglia, and T cells. Significantly, very few studies have investigated the effects of mast cells in the pathogenesis of pain. This knowledge gap is particularly surprising given the well-characterized role of mast cells in mediating inflammatory cascades within tissue, which corresponds with the release of a number of hyperalgesic agents, and their close proximity to nociceptors in the peripheral nervous system and dura within the CNS (Kumar and Sharma, 2010; Silver and Curley, 2013). Upon degranulation (i.e. following physical or chemical injury), mast cells release algogenic substances such as histamine, bradykinin and substance P, that contribute directly to the hyper-sensitization of local nociceptor which results in hyperalgesia (Xanthos et al., 2011). Nerve-resident peripheral nerve mast cells are the first to be activated following tissue damage and contribute to the recruitment of neutrophils and macrophages (Zuo et al., 2003). More importantly, mast cells can release IL-1β following infection, toxic injury or trauma, subsequently contributing to neuropathic hyperalgesia (Sandler et al., 2007; Sommer et al., 1999).

The aim of the present study was to investigate the behavioural and immune changes in response to neonatal LPS exposure and subsequent formalin injection in preadolescent (i.e. PND 22) and adult (i.e. PNDs 80–97) rats, with particular focus on circulating and hippocampal IL-1β as well as on mast cell number and degranulation in tissue following formalin injection. Our hypothesis is that neonatal LPS exposure will produce both peripheral and central changes in mast cell and IL-1β responses that will accompany the formalin-induced behavioural hyperalgesia.

2. Materials and methods

2.1. Experimental procedures

23 experimentally naïve female Wistar rats were obtained from the University of Newcastle Animal House and allowed one week acclimatization prior to mating in a vivarium. Mating resulted in 184 offspring, 116 of which were used in this study; the remaining pups were allocated to other studies. After two weeks, the male was removed from the harem upon which dams were housed individually in custom designed polycarbonate-perspex home boxes (43.5 cm × 28 cm × 12.5 cm; Mascot Wire Works, Sydney, Australia) until delivery (PND 1). Pups were left undisturbed with their mother until PND 3 and 5 when they were briefly removed from their home boxes, weighed and injected (IP) with either LPS (*Salmonella enterica*, serotype *enteritidis*; Sigma-Aldrich Chemical Co., USA, dissolved in 20 μL sterile pyrogen-free saline, 50 μg/kg) or an equivolume of sterile pyrogen-free saline (Livingstone International, Australia). All pups from the same litter were treated identically.

Following neonatal drug administration, pups were left undisturbed with their mother until testing, when they were exposed to the formalin test as described below. Rats were randomly assigned testing days based on the two ages examined: PND 22 and PNDs 80–97, with a maximum of three pups per litter being assigned to each group. Following the formalin test, blood, paw, and brain tissue samples were collected and subsequently used for assessment of plasma IL-1β levels, histological analysis of mast cells infiltration and degranulation, and IL-1β protein levels in the hippocampus. Animals were distributed evenly from all litters used per treatment to avoid potential litter bias. Until their allocated testing day, rats were maintained in a temperature (21 ± 1 °C) and humidity (45%) controlled environment, under a 12 h/12 h light-dark cycle (light on 0600 h) with food and water available *ad libitum*.

All experiments were carried out in accordance with the 2013 National Health and Medical Research Council of Australian Code for the care and use of animals for scientific purposes. All procedures were reviewed and approved by the Ethics committee of the University of Newcastle (Ethics approval no. A-2010-127).

2.2. Formalin behavioural testing and analysis

Preadolescent rats (PND 22) were injected with 1.1% formalin into the plantar surface of the left hindpaw and adult rats (PND 80–97) were injected with 2.25% formalin into the plantar surface of the right hindpaw. Injections were made using a 31G needle at PND 22 and a 30 G needle at PNDs 80–97. The choice of formalin concentration, site of injection and injection volume (10 μL for PND 22 rats and 50 μL for PNDs 80–97 rats), testing apparatus as well as behavioural scoring techniques are all described in detail in our previous studies (Zouikr et al., 2014a,b; Zouikr et al.,