Brain, Behavior, and Immunity 46 (2015) 35–43

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Modality and sex differences in pain sensitivity during human endotoxemia

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ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 24 November 2014
Accepted 25 November 2014
Available online 5 December 2014

Keywords:
Endotoxin
Lipopolysaccharide
Pain
Sickness behavior
Inflammation
Conditioned pain modulation
Cytokines
Pressure point thresholds
Cold pressor

ABSTRACT

Systemic inflammation can induce pain hypersensitivity in animal and human experimental models, and has been proposed to be central in clinical pain conditions. Women are overrepresented in many chronic pain conditions, but experimental studies on sex differences in pain regulation during systemic inflammation are still scarce. In two randomized and double blind placebo controlled experiments, we used low doses of lipopolysaccharide (LPS) as an experimental model of systemic inflammation. The first study employed 0.8 ng/kg LPS in a within-subject design of 8 individuals (1 woman), and the second study 0.6 ng/kg LPS in a between-subject design of 52 participants (29 women). We investigated the effect on (a) pressure, heat, and cold pain thresholds, (b) suprathreshold noxious heat and cold sensitivity, and (c) conditioned pain modulation (CPM), and differences between men and women. LPS induced significantly lower pressure pain thresholds as compared to placebo (mean change with the 0.8 ng/kg dose being −64 ± 30 kPa $P = .04$; with the 0.6 ng/kg dose −58 ± 55 kPa, $P < .01$, compared to before injection), whereas heat and cold pain thresholds remained unaffected (P's > .70). Suprathreshold noxious pain was not affected by LPS in men (P's ⩾ .15). However, LPS made women rated suprathreshold noxious heat stimuli as more painful ($P = .01$), and showed a tendency to rate noxious cold pain as more painful ($P = .06$) as compared to placebo. Furthermore, LPS impaired conditioned pain modulation, a measure of endogenous pain inhibition, but this effect was also restricted to women ($P < .01$, for men $P = .27$). Pain sensitivity correlated positively with plasma IL-6 and IL-8 levels. The results show that inflammation more strongly affects deep pain, rather than cutaneous pain, and suggest that women's pain perception and modulation is more sensitive to immune activation than men's.

1. Introduction

Chronic pain is one of the greatest challenges in modern health care, as satisfactory treatment and pain relief are still lacking for many painful disorders. Peripheral and central neurological changes have been demonstrated in chronic patients (Henry et al., 2011), and inflammation, both peripherally and centrally, has emerged as a potential mechanism driving pain development (de Oliveira et al., 2011a; Loram et al., 2012; Walker et al., 2014). Furthermore, the systemic inflammation that accompanies viral or bacterial infections in the sickness response also affects pain sensitivity in animal models (Watkins and Maier, 2000), and recently similar results have been shown in humans (Benson et al., 2012b; de Goeij et al., 2013; Hutchinson et al., 2013; Wegner et al., 2014). If unabated, it is believed that systemic inflammation may lead to chronic pathological pain (Ren and Dubner, 2010).

A sickness response is believed to represent an evolved generalized cytokine-driven response to immune challenge (Watkins and Maier, 2000). Pro-inflammatory cytokines, e.g. interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, that are released peripherally influence the central nervous system (CNS), causing

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http://dx.doi.org/10.1016/j.bbi.2014.11.014
0889-1591/

disruptions in mood and memory in humans (Reichenberg et al., 2001), as well as decreased social interaction, anorexia, anhedonia, sleepiness, fatigue and increased pain sensitivity (Dantzer, 2001). Animal studies show that several routes serve to transmit such information. These include cytokine-dependent signals through parts of the blood–brain barrier with greater permeability, receptor-mediated activation and signal transmission via the vagus nerve and cytokine receptor-dependent interactions with brain microvessels releasing prostaglandins (Rivest, 2010). Animal findings show that systemic inflammation affects pain regulation in several ways. Injections with lipopolysaccharide (LPS) increase the permeability of the blood–brain barrier (Lu et al., 2009) and activate glial cells in the CNS (Ren and Dubner, 2010). In animals, increased transport of inflammatory mediators and monocytes/macrophages over the blood-spinal cord barrier plays a role in the development of neuropathic pain (Echeverry et al., 2011), and activated glia in the CNS augment pain sensitivity during peripheral inflammation (Watkins and Maier, 2000). Glial activation is also implicated as pivotal in development of chronic pain (Watkins et al., 2007). This was indirectly supported by findings of high cytokine concentrations in the cerebrospinal fluid (CSF) of patients suffering from nociceptive pain, as well as widespread pain of unknown origin (Kadetoff et al., 2012; Lampa et al., 2012; Lundborg et al., 2010). Clinically, women are overrepresented in many chronic pain and inflammatory disorders (Bartley and Fillingim, 2013; Manson, 2010), but experimental studies on sex differences in pain sensitivity during systemic inflammation are scarce. In a recent study, deep pain sensitivity increased during low-dose endotoxemia in male subjects, but no effect on mechanical pain or suprathreshold pain was reported (Wegner et al., 2014). Previous studies have shown modulatory effects of systemic inflammation on both visceral (Benson et al., 2012a) and electrical pain thresholds as well as suprathreshold pain (de Goeij et al., 2013) in male subjects.

The aim of the present study was thus to investigate how an experimental systemic inflammation affects different modalities of pain sensitivity in healthy men and women. In addition, we wanted to investigate if inflammation affects conditioned pain modulation (CPM) – i.e. "pain inhibits pain". This function is of great clinical interest, since chronic pain patients show impairments in this pain modulatory response (Jensen et al., 2009; Staud, 2009, 2012; Yarnitsky, 2010) and that CPM may predict the development of chronic pain (Yarnitsky et al., 2008). We chose to include the analysis of sex differences in our study based on previous knowledge that women are generally more sensitive than men to evoked pain stimulation and this is true for various modalities, including pressure (Fillingim et al., 2009). In addition, women have been reported to have increased temporal pain summation and a reduced function of exercise induced hypoalgesia as well as CPM compared to men (Fillingim et al., 2009).

LPS injections, extensively used in experimental models of inflammation, activate plasma pro-inflammatory cytokine levels (TNFα, IL-1β, and IL-6) in a highly dose dependent manner (Grigoleit et al., 2011; Suffredini et al., 1999), ranging from low-dose studies in humans, causing mild sickness, to sepsis-like effects in animal models (Inagaki et al., 2012; Reichenberg et al., 2001). We carried out two independent experiments, using low doses of LPS (0.8 ng/kg i.v. in a first study, and 0.6 ng/kg i.v. in a subsequent study) to experimentally approach the systemic cytokine production that accompanies chronic inflammatory situations in clinical settings. Plasma TNFα and IL-6 levels were used as measures of inflammation together with interleukin-8 (IL-8), a pro-inflammatory cytokine implicated in pain processing (Kadetoff et al., 2012). Our main hypotheses were that transient LPS induced activation of the inflammatory system in healthy subjects would be reflected as decreased pain thresholds, increased pain ratings of suprathreshold noxious stimuli, and impaired endogenous pain inhibition. In addition, exploratory analyses were made regarding sex differences.

2. Materials and methods

2.1. Design

Two double-blind experiments with different LPS doses (0.8 ng/kg and 0.6 ng/kg) were performed. The experiments were similar but varied in dose, design (within-subjects vs. between-subjects), and timing of tests. The first study, using the 0.8 ng/kg dose in a within-subjects design, was performed to validate the LPS effects, the cytokine profiles, and time contingency of pain sensitivity. With a slight change of timing of pain tests and blood samples, the second study also used a dose lowered to 0.6 ng/kg and a between-subject design to improve blinding and to facilitate scanning of subjects with MR for a separate experiment. The studies were approved by the Regional Ethical Review Board in Stockholm and participants provided written informed consent.

2.2. Participants and study outline

For inclusion, subjects had to be 18–50 years old, right-handed, medication free, non-smokers without history of drug abuse, inflammatory, psychiatric or sleep disorders, or chronic pain, with a normal body mass index. Participants were recruited by advertising and screened through questionnaires and a health examination by a physician. They were asked not to engage in strenuous physical activities, sleep regular hours and refrain from alcohol the day before the experiment. If the participants felt ill, e.g. coming down with a cold, they were instructed to call and were rescheduled for a later appointment. C-reactive protein (CRP) was assessed to exclude an ongoing infection on the experimental day. Baseline pain testing was conducted before any blood samples or injections. Fig. 1 summarizes the timing of the tests and questionnaires throughout the study day for both experiments.

Eight healthy participants (7 men and 1 woman, mean age 24 ± SD 3.7) were recruited to a randomized and balanced double blind cross-over design. They were injected two times, once with an i.v. injection of 0.8 ng/kg body weight LPS (*Escherichia Coli*, Lot nr G3E0609, United States Pharmacopeia Rockville, MD), and once with saline injection, 28 days apart. The female participant was studied during her follicular phase, but was excluded from all pain analyses due to equipment failure during the first experimental day.

One year later, 52 healthy subjects were recruited (29 women and 23 men, mean age 28.6 ± 7.1 years). 31 (18 women) subjects were injected with 0.6 ng/kg LPS (*E. Coli*, Lot nr G3E0609, United States Pharmacopeia Rockville, MD) intravenously (i.v.), and 21 (11 women) subjects were injected with saline in a double blind randomized order. Women were tested in the early follicular phase, except when employing contraceptives abrogating menses (7 subjects). 13 women did not use hormonal contraceptives and 16 used varying types of hormonal contraceptives. None had reached menopause. One female subject was excluded from all pain analyses as she chose to interrupt the pain tests. One female participant was excluded from all cytokine analyses because of difficulties with drawing blood. The LPS group was designed to be slightly larger to ensure enough statistical power for correlation analyses done only in this group.

2.3. Questionnaires

The screening questionnaires included the Swedish versions of the Hospital Anxiety and Depression Scale (HADS) (Zigmond and