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Inflammation-induced hyperalgesia: Effects of timing, dosage, and negative affect on somatic pain sensitivity in human experimental endotoxemia

Alexander Wegner [a], Sigrid Elsenbruch [b], Janina Maluck [b], Jan-Sebastian Grigoleit [b], Harald Engler [b], Marcus Jäger [a], Ingo Spreitzer [c], Manfred Schedlowski [b], Sven Benson [b,*]

[a] Clinic for Trauma Surgery, University Hospital Essen, University of Duisburg-Essen, Germany
[b] Institute of Medical Psychology & Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Germany
[c] Paul Ehrlich Institute, Federal Agency for Sera and Vaccines, Langen, Germany

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ABSTRACT

Background: Inflammation-induced pain amplification and hypersensitivity play a role in the pathophysiology of numerous clinical conditions. Experimental endotoxemia has recently been implemented as model to analyze immune-mediated processes in human pain. In this study, we aimed to analyze dose- and time-dependent effects of lipopolysaccharide (LPS) on clinically-relevant pain models for musculoskeletal and neuropathic pain as well as the interaction among LPS-induced changes in inflammatory markers, pain sensitivity and negative affect.

Methods: In this randomized, double-blind, placebo-controlled study, healthy male subjects received an intravenous injection of either a moderate dose of LPS (0.8 ng/kg *Escherichia coli*), low-dose LPS (0.4 ng/kg), or saline (placebo control group). Pressure pain thresholds (PPT), mechanical pain sensitivity (MPS), and cold pain sensitivity (CP) were assessed before and 1, 3, and 6 h post injection to assess time-dependent LPS effects on pain sensitivity. Plasma cytokines (TNF-α, IL-6, IL-8, IL-10) and state anxiety were repeatedly measured before, and 1, 2, 3, 4, and 6 h after injection of LPS or placebo.

Results: LPS administration induced a systemic immune activation, reflected by significant increases in cytokine levels, body temperature, and negative mood with pronounced effects to the higher LPS dose. Significant decreases of PPTs were observed only 3 h after injection of the moderate dose of LPS (0.8 ng/kg). MPS and CP were not affected by LPS-induced immune activation. Correlation analyses revealed that decreased PPTs were associated with peak IL-6 increases and negative mood.

Conclusions: Our results revealed widespread increases in musculoskeletal pain sensitivity in response to a moderate dose of LPS (0.8 ng/kg), which correlate both with changes in IL-6 and negative mood. These data extend and refine existing knowledge about immune mechanisms mediating hyperalgesia with implications for the pathophysiology of chronic pain and neuropsychiatric conditions.

1. Introduction

Experimental endotoxemia in humans constitutes an innovative translational model to elucidate the mechanisms and clinical relevance of afferent immune-to-brain communication. By signaling the brain via humoral and neural pathways, pro-inflammatory cytokines released following endotoxin application result in a number of behavioral changes collectively termed sickness behavior (Dantzer et al., 2008; Goehler et al., 2007; Schedlowski et al., 2014). Pain facilitation has been viewed as an important component of sickness behavior (Watkins and Maier, 2005). Increased pain sensitivity due to inflammation was conceptualized as an evolutionary-driven, adaptive response aimed at protecting the organism by promoting recuperative behaviors such as reduced physical activity (Watkins and Maier, 2005). Animal data document acute inflammation-induced changes in the response to painful (i.e., hyperalgesia) as well as normally non-painful stimulation (i.e., allodynia). However, the translation of findings in experimental animals to humans remains a major challenge (Schedlowski et al., 2014), especially in the context of both acute

* Corresponding author. Address: Inst. of Medical Psychology & Behavioral Immunobiology, University Hospital Essen, Hufelandstr. 55, 45122 Essen, Germany. Tel.: +49 201 723 4516; fax: +49 201 723 5948.
E-mail address: sven.benson@uk-essen.de (S. Benson).

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and chronic pain (Benson et al., 2012a). One increasingly recognized approach to delineate the effects of a systemic immune activation on pain is the experimental administration of bacterial endotoxin (i.e., lipopolysaccharide, LPS). LPS, the major component of the outer membrane of Gram-negative bacteria, is a prototypic pathogen-associated molecular pattern that stimulates via Toll-like receptor (TLR) 4-dependent pathways the synthesis and release of pro-inflammatory cytokines (Bahador and Cross, 2007; Miller et al., 2009; Schedlowski et al., 2014). These pro-inflammatory mediators control local and systemic immune responses to pathogens like LPS, and – more importantly in the context of pain – also have effects on the central nervous system, evoking behavioral, neuroendocrine, and metabolic changes (Bahador and Cross, 2007; Benson et al., 2012a; Dantzer et al., 2008; Schedlowski et al., 2014). Experiments in healthy humans, employing administration of low-dose LPS (0.4 ng/kg) (Benson et al., 2012b; Hutchinson et al., 2013) or higher LPS doses (2 ng/kg) (de Goeij et al., 2013) reportedly increased sensitivity to visceral (Benson et al., 2012b) and somatic pain (de Goeij et al., 2013; Hutchinson et al., 2013). These findings indicated that LPS-induced immune activation not only constitutes a model to assess afferent immune-to-brain signaling in basic pain research, but also to analyze effects of immune-targeted pain medications in clinical studies (de Goeij et al., 2013; Hutchinson et al., 2013). Indeed, the putative clinical relevance of inflammation-induced pain facilitation is supported by evidence that inflammatory mediators play a role in the pathophysiology of many chronic pain conditions, including neuropathic (Schafers and Sorkin, 2008; Üceyler et al., 2007), cancer-related (Wang et al., 2012), and post-operative pain (Geiss et al., 1997), as well as chronic inflammatory and functional pain conditions (Elsenbruch, 2011; Koch et al., 2007; Parkitny et al., 2013; Stürmer et al., 2004; Üceyler et al., 2011). However, it is difficult to establish cause-effect relationships in these complex clinical conditions, which calls for preclinical studies in healthy humans.

Therefore, our goal was to complement and refine existing knowledge from human LPS studies to specifically address the following aspects of inflammation-induced hyperalgesia in healthy volunteers: First, we tested effects of LPS on various aspects of somatic pain sensitivity using different clinically-relevant pain models for musculoskeletal and neuropathic pain. Given previous evidence suggesting effects of the time point of pain assessment in relation to LPS administration in one pain model (Hutchinson et al., 2013), we herein carefully assessed time-dependent LPS effects. Since LPS effects on immune measures, mood, and neurocognitive functions are reportedly dose-dependent (Grigoleit et al., 2011; Mullington et al., 2000), we compared effects of a low-dose LPS (0.4 ng/kg body weight) with effects of a moderate-dose of LPS (0.8 ng/kg body weight) on measures of pain sensitivity. Finally, we assessed the interrelationship between LPS-induced changes in inflammatory markers, pain sensitivity and negative affect. Negative mood, especially anxiety, is closely related to hyperalgesia (Elsenbruch, 2011; Walker et al., 2014; Wiech and Tracey, 2009), and therefore, it is imperative to consider putative effects of LPS-induced negative affectivity on the response to pain.

2. Methods

2.1. Study participants

Fifty-nine healthy males (mean age: 27.6 ± 0.5 years; mean BMI: 24.0 ± 0.4 kg/m^2) participated in this study. Mean Hospital Anxiety and Depression Scale (HADS, see below) anxiety (2.9 ± 0.3) and depression scores (1.7 ± 0.2) were low and inside normal range. Groups did not differ significantly in any sociodemographic or psychological variable (data not shown). Two subjects were excluded from all following analyses due to altered baseline cytokine profiles (i.e., >3 standard deviations above total samples' mean baseline IL-6 and TNF-α levels), resulting in following group sizes: medium-dose (0.8 ng/kg) LPS, $N = 19$, low-dose (0.4 ng/kg) LPS, $N = 20$, placebo control group, $N = 18$.

The recruitment and screening process as well as safety measures have previously been described in detail (Benson et al., 2012b; Grigoleit et al., 2010). Briefly, healthy males aged 18–45 years participated in a detailed screening process including physical examination and a personal interview conducted by a physician (A.W.), and repeated laboratory analyses of blood samples (i.e., complete blood cell count, liver enzymes, renal parameters, electrolytes, coagulation factors, C-reactive protein) before and up to 1 week after completion of the study. A medical or psychological history, body mass index <18 or ⩾29 kg/m^2, current medications, smoking, or regular high alcohol use (>4 drinks per week) were exclusion criterions. To rule out clinically relevant symptoms of anxiety and/or depression, participants completed the Hospital Anxiety and Depression Scale (HADS; Herrmann-Lingen et al., 2005) during the screening process. Participants were told to refrain from strenuous exercise 48 h prior the study. Additional safety measures included a physical examination and normal blood cell counts 6 h after injection as a precondition for subjects being allowed to leave the laboratory. Further, participants were not allowed to drive a vehicle on the study day, and they underwent follow-up examinations including laboratory analysis of C-reactive protein levels 24 h after each session and 7 days after the final session. The study protocol was approved by the local ethics committee (permit No. 09-4271). All subjects provided written informed consent and were paid for their participation.

2.2. Study protocol

This randomized, double-blind, placebo-controlled study consisted of three study groups (see Fig. 1): subjects received an intravenous injection of either 0.8 ng LPS (medium-dose LPS group), 0.4 ng LPS (low-dose LPS group) per kilogram body weight dissolved in sterile water, or the same volume of saline (placebo control group). Endotoxin (reference standard endotoxin, lot G3E069; United States Pharmacopeia, Rockville, MD) had been subjected to a microbial safety testing routine approved by the German Federal Agency for Sera and Vaccines (Paul Ehrlich Institute, Langen, Germany), and was prepared for human use as previously described (Benson et al., 2012b; Grigoleit et al., 2010, 2012). Subjects and the investigator involved in pain assessments (J.M.) were blinded to the study condition (i.e., LPS or saline).

On the study day, an intravenous catheter was inserted in an antecubital forearm vein for repeated blood collection and endotoxin/placebo application. Subjects were injected between 9 am and 11 am. Pain sensitivity to different stimuli was assessed at baseline, 1, 3, and 6 h post injection (p.i.), as described below in greater detail. Blood samples were drawn in EDTA treated tubes at baseline (prior to baseline pain assessments), and 1, 2, 3, 4, and 6 h after injection, immediately centrifuged, and stored at −80 °C until analysis. Following blood collection, vital parameters (i.e., body temperature, blood pressure, heart rate) were measured with an aurical thermometer and a blood pressure cuff, respectively. Then, subjects completed validated questionnaires assessing state anxiety (state version of the State-Trait-Anxiety-Inventory (STAI) (Spielberger et al., 1970), and mood (Multidimensional Mood Questionnaire; MDBF) (Steyer et al., 1997) (see below). Subjects were discharged 6 h after injection if white blood cell counts returned to normal range. All subjects received a medical assessment 24 h and 1 week after the experimental sessions.

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