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Neural circuitry mediating inflammation-induced central pain amplification in human experimental endotoxemia

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ABSTRACT

Background & aims: To elucidate the brain mechanisms underlying inflammation-induced visceral hyperalgesia in humans, in this functional magnetic resonance imaging (fMRI) study we tested if intravenous administration of lipopolysaccharide (LPS) involves altered central processing of visceral pain stimuli. *Methods:* In this randomized, double-blind, placebo-controlled fMRI study, 26 healthy male subjects received either an intravenous injection of low-dose LPS ($N = 14$, 0.4 ng/kg body weight) or placebo ($N = 12$, control group). Plasma cytokines (TNF-α, IL-6), body temperature, plasma cortisol and mood were assessed at baseline and up to 6 h post-injection. At baseline and 2 h post-injection (test), rectal pain thresholds and painful rectal distension-induced blood oxygen level-dependent (BOLD) responses in brain regions-of-interest were assessed. To address specificity for visceral pain, BOLD responses to non-painful rectal distensions and painful somatic stimuli (i.e., punctuate mechanical stimulation) were also analyzed as control stimuli. *Results:* Compared to the control group, LPS-treated subjects demonstrated significant and transient increases in TNF-α, IL-6, body temperature and cortisol, along with impaired mood. In response to LPS, rectal pain thresholds decreased in trend, along with enhanced up-regulation of rectal pain-induced BOLD responses within the posterior insula, dorsolateral prefrontal (DLPFC), anterior midcingulate (aMCC) and somatosensory cortices (all FWE-corrected $p < 0.05$). Within the LPS group, more pronounced cytokine responses correlated significantly with enhanced rectal pain-induced neural activation in DLPFC and aMCC. No significant LPS effects were observed on neural responses to non-painful rectal distensions or mechanical stimulation. *Conclusions:* These findings support that peripheral inflammatory processes affect visceral pain thresholds and the central processing of sensory-discriminative aspects of visceral pain.

1. Introduction

Recurrent abdominal pain constitutes one of the most common pain conditions worldwide, and is the hallmark symptom of functional gastrointestinal disorders such as the irritable bowel syndrome (IBS) (Longstreth et al., 2006). The pathophysiology of chronic abdominal pain and visceral hyperalgesia or hypersensitivity in IBS remains elusive and likely involves disturbances of the brain-gut axis mediated by both central and peripheral mechanisms (Elsenbruch, 2011; Wilder-Smith, 2011). On the one hand, the importance of central pain amplification is increasingly well-characterized owing to a growing number of brain imaging studies documenting altered pain-related neural responses in brain areas associated with emotional arousal and endogenous pain inhibition in patients with IBS (Berman et al., 2008; Tillisch and Labus, 2011). These findings complement and extend knowledge from the broader somatic pain field addressing the brain mechanisms involved in pain chronicity and hyperalgesia in other chronic pain conditions or experimental pain models (Bingel and Tracey, 2008; Tracey and Mantyh, 2007). On the other hand, and this may be unique to IBS as a condition involving the brain-gut axis, there is convincing evidence supporting a role of peripheral immune functions involving not only local but also systemic immune activation (Ohman and Simren, 2010), including increases in

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peripheral pro-inflammatory cytokine concentrations such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-8 (Dinan et al., 2008, 2006; Liebregts et al., 2007; Scully et al., 2010), which reportedly correlate with IBS symptom scores and disease severity (Dinan et al., 2008; Hughes et al., 2013). These clinical observations support the notion that visceral pain in IBS patients is associated with both local and low-grade systemic immune activation, leading to the hypothesis that inflammatory mediators may constitute relevant factors in the development and/or maintenance of visceral hypersensitivity, at least in a subset of IBS patients (Ohman and Simren, 2010; Wilder-Smith, 2011). However, whether peripheral pro-inflammatory mediators contribute to altered central pain processing via afferent immune-to-brain signaling remains unknown from existing patient data. This calls for preclinical studies addressing afferent neuro-immune interactions in human visceral pain while integrating brain imaging techniques with clinically-relevant models of inflammation.

Experimental endotoxemia constitutes an established translational model of systemic inflammation to elucidate immune-to-brain communication in humans (Benson et al., 2012a; Hutchinson, 2014; Schedlowski et al., 2014). Application of low-dose endotoxin (e.g., lipopolysaccharide, LPS) induces a transient inflammatory response, including the release of pro-inflammatory cytokines such as TNF-α and IL-6 (Andreasen et al., 2008). This model offers thus the unique opportunity to establish cause-effect relationships between inflammatory processes and pain (Benson et al., 2012a). In a proof-of-concept study involving low-dose intravenous LPS application in humans, we previously established visceral hypersensitivity in a rectal-distension model (Benson et al., 2012b). To elucidate the putative central mechanism(s) underlying these effects, we conducted the present brain imaging study in order to address if LPS-induced visceral hypersensitivity involves altered central processing of visceral pain stimuli.

Within the brain, a large distributed network is involved in the processing of painful stimuli, comprised of multiple distinct but functionally connected areas involving cortical, midbrain and subcortical regions. Size and complexity of this brain network reflect the complex sensory-discriminative, affective-emotional and cognitive components comprising the experience of pain (Bingel and Tracey, 2008; Simons et al., 2014; Tracey and Mantyh, 2007). Activations within this network have previously been observed in response to both experimental visceral and somatic pain stimuli (Bingel and Tracey, 2008; Tillisch et al., 2011; Tracey and Mantyh, 2007), although some evidence exists to support differences between visceral and somatosensory signal processing both in the periphery and within the central nervous system (Aziz et al., 2000; Dunckley et al., 2007, 2005a,b; Eickhoff et al., 2006), including data indicating that attentional modulation of perception of pain intensity for visceral and somatic pain, respectively, is reflected in different brain regions (Dunckley et al., 2007). Together, this calls for studies addressing visceral and somatic pain stimuli in multiple regions of interest (ROIs). Using event-related functional magnetic resonance imaging (fMRI), we tested the hypothesis that experimental endotoxemia results in decreased rectal pain thresholds along with increased rectal distension-induced neural (i.e., blood oxygen level-dependent, BOLD) responses within brain regions mediating sensory-discriminatory aspects of pain (i.e., thalamus, posterior insula, and somatosensory cortices) as well as brain areas involved in affective-emotional and cognitive components of the pain response (i.e., cingulate cortex, prefrontal cortices, anterior insula, amygdala). As secondary ROIs, we additionally analyzed brain areas involved in descending pain inhibition (i.e., PAG, rostral ventromedial medulla). To address the specificity for visceral pain, we included non-painful visceral stimuli as well as painful somatic stimuli as control stimuli.

2. Materials and methods

2.1. Study sample

Twenty-six healthy males (mean age: 26.3 ± 0.7 years; mean BMI: 23.0 ± 0.5 kg/m^2) were randomly assigned to the LPS group (N = 14) or control group (N = 12). The recruitment and rigorous screening process as well as the safety measures have previously been described in detail (Benson et al., 2012b; Grigoleit et al., 2011, 2010; Kullmann et al., 2014; Wegner et al., 2014). Briefly, healthy male volunteers aged 18–45 years were recruited and subjected to an in-depth screening process consisting of a physical examination and a personal interview conducted by a physician, completion of standardized questionnaires, and repeated laboratory analyses of blood samples. All participants were evaluated digitally for anal tissue damage (e.g., painful hemorrhoids) that may interfere with balloon placement. Exclusion criteria included any previous or current medical or psychological conditions upon physical examination or self-report, body mass index <18 or ⩾29 kg/m^2, smoking, or any abnormality upon laboratory analyses of blood samples (i.e., complete blood cell count, liver enzymes, renal parameters, electrolytes, coagulation factors, C-reactive protein). Cut-off values for laboratory parameters were based on specifications of the Division of Laboratory Research of the University Hospital Essen (see Supplementary Table 1). The frequency and severity of gastrointestinal complaints during the past month was assessed with a standardized screening questionnaire (Lacourt et al., 2014), with sum scores ⩾10 resulting in exclusion (Lacourt et al., 2014). Presently increased scores (i.e., sum scores ⩾11) on the Hospital Anxiety and Depression Scale (HADS) (Herrmann-Lingen et al., 2005) were also exclusionary (for details on screening questionnaires, see Section 2.7). The study protocol was approved by the local ethics committee (permit No. 09-4271). All subjects gave written informed consent and were paid for their participation.

2.2. Study design

In this randomized, double-blind, placebo-controlled study, participants underwent standardized visceral and somatic pain assessments, described in detail below, in a baseline and a test phase (Fig. 1, study design). Following the baseline, subjects were randomly assigned to receive an intravenous injection of either saline (control group) or 0.4 ng LPS (Reference Standard Endotoxin, lot G3E069; United States Pharmacopeia, Rockville, MD) per kilogram body weight dissolved in sterile water (LPS group). A dose of 0.4 ng LPS was sufficient to induce visceral hyperalgesia in a previous study of our group (Benson et al., 2012b). The endotoxin used 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 (Grigoleit et al., 2010).

A total of four event-related fMRI scanning sessions were accomplished during which neural activation induced by visceral and somatic pain stimulation, respectively, were assessed at baseline (visceral baseline, somatic baseline) and test (visceral test, somatic test). Study day starting times were standardized (i.e., 09:00) to control for circadian effects, and the test phase was begun 120 min post-injection. Blood samples for analyses of plasma concentrations of TNF-α, IL-6, and cortisol were drawn at baseline (i.e., prior to baseline pain assessments), prior to injection of LPS or placebo (−0.25 h) and 1, 2, 3, 4, and 6 h after injection, along with assessments of vital (i.e., body temperature, blood pressure, heart rate) and mood parameters. The rectal balloon was removed after each distension session in order to avoid putative

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