Brain, Behavior, and Immunity 44 (2015) 213–220

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Acute increases in intramuscular inflammatory cytokines are necessary for the development of mechanical hypersensitivity in a mouse model of musculoskeletal sensitization

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ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form 16 October 2014
Accepted 16 October 2014
Available online 22 October 2014

Keywords:
Muscle pain
Inflammation
TNF
IL-6
IL-1

ABSTRACT

Musculoskeletal pain is a widespread health problem in the United States. Back pain, neck pain, and facial pain are three of the most prevalent types of chronic pain, and each is characterized as musculoskeletal in origin. Despite its prevalence, preclinical research investigating musculoskeletal pain is limited. Musculoskeletal sensitization is a preclinical model of muscle pain that produces mechanical hypersensitivity. In a rodent model of musculoskeletal sensitization, mechanical hypersensitivity develops at the hind paws after injection of acidified saline (pH 4.0) into the gastrocnemius muscle. Inflammatory cytokines contribute to pain during a variety of pathologies, and in this study we investigate the role of local, intramuscular cytokines in the development of mechanical hypersensitivity after musculoskeletal sensitization in mice. Local intramuscular concentrations of interleukin-1β (IL-1), IL-6 and tumor necrosis factor-α (TNF) were quantified following injection of normal (pH 7.2) or acidified saline into the gastrocnemius muscle. A cell-permeable inhibitor was used to determine the impact on mechanical hypersensitivity of inhibiting nuclear translocation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) prior to musculoskeletal sensitization. The role of individual cytokines in mechanical hypersensitivity following musculoskeletal sensitization was assessed using knockout mice lacking components of the IL-1, IL-6 or TNF systems. Collectively, our data demonstrate that acidified saline injection increases intramuscular IL-1 and IL-6, but not TNF; that intramuscular pre-treatment with an NF-κB inhibitor blocks mechanical hypersensitivity; and that genetic manipulation of the IL-1 and IL-6, but not TNF systems, prevents mechanical hypersensitivity following musculoskeletal sensitization. These data establish that actions of IL-1 and IL-6 in local muscle tissue play an acute regulatory role in the development of mechanical hypersensitivity following musculoskeletal sensitization.

1. Introduction

Chronic pain is a significant health problem in the United States and is associated with high personal and economic costs. Total annual costs associated with chronic pain are estimated upwards of $635 billion in the United States alone, including treatment costs and lost work productivity (National Research Council, 2011). Pain is one of the leading causes of absenteeism from work and presents physical, social and psychological barriers to working (Wynne-Jones and Main, 2011). Chronic musculoskeletal pain conditions are particularly burdensome; low back pain, neck, and facial pain are musculoskeletal in origin and constitute three of the most prevalent chronic pain conditions in the United States (National Center for Health Statistics, 2006). Pain control for patients with musculoskeletal pain is poor and drug dependency can occur from opioid treatment (Panagiotou and Mystakidou, 2012). These demographic and epidemiologic data underscore the need for understanding mechanisms by which chronic musculoskeletal pain develops. Such knowledge may lead to new or more effective treatment interventions and thus improve quality of life for patients and reduce overall costs associated with this public health burden.

Data demonstrate that inflammatory cytokines are mediators and modulators of many pain conditions (de Oliveira et al., 2011;

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http://dx.doi.org/10.1016/j.bbi.2014.10.009
0889-1591/

Marchand et al., 2005; Okuse, 2007; Weiseler-Frank et al., 2005). The inflammatory cytokines interleukin-1β (IL-1), IL-6, and tumor necrosis factor-α (TNF) are all involved in muscle pain (Hoheisel et al., 2005; Schafers et al., 2003a). Elevated inflammatory cytokines are detected in plasma and tissue biopsies of patients with chronic pathologies characterized by muscle pain (Milligan et al., 2003; Stemkowski and Smith, 2012; Uceyler and Sommer, 2008). Targeting inflammatory cytokines for inhibition is effective in relieving pain of patients with rheumatoid arthritis (Miossec, 2013) and ankylosing spondylitis, a form of chronic inflammatory arthritis (Dougados et al., 2012). Inhibition of cytokines also relieves pain in pre-clinical models of neuropathic pain (Sommer et al., 2001), spinal nerve ligation pain (Schafers et al., 2003b), and experimental arthritis (Schaible et al., 2010; Segond von Banchet et al., 2009). Mice lacking components of the IL-1, IL-6, or TNF systems through genetic knockout do not develop the same intensity of pain as genetically intact mice in models of gouty arthritis, chronic inflammation, and nerve transection (Torres et al., 2009; Westlund et al., 2012; Xu et al., 1997).

Reduced tissue pH is a characteristic of painful conditions induced by inflammation, muscle spasm, exhausting exercise, cancer, and ischemia, for example (Hood et al., 1988; Issberner et al., 1996; Reeh and Steen, 1996; Shah et al., 2005). Intramuscular injection of acidified saline reduces local tissue pH by increasing extracellular hydrogen ion (H^+) concentrations, and injection of acidified saline into the gastrocnemius muscle of rodents is used to study chronic muscle pain (Sluka et al., 2001). In this rodent model, musculoskeletal sensitization by injection of acidified saline produces long-lasting bilateral secondary mechanical hypersensitivity at the hind paws (Sharma et al., 2009; Sluka et al., 2002; Sutton and Opp, 2014a,b). This rodent model of musculoskeletal sensitization is clinically relevant, in part, because intramuscular injection of acidified saline into human volunteers produces muscle hyperalgesia and referred pain (Frey Law et al., 2008).

In this study we focus on inflammatory cytokines as mediators of musculoskeletal sensitization-induced mechanical hypersensitivity. Specifically, we hypothesize that IL-1, IL-6, and TNF are critical for the development of mechanical hypersensitivity following musculoskeletal sensitization. To test this hypothesis, we quantified cytokine concentrations in the mouse gastrocnemius muscle after intramuscular injection with normal or acidified saline; we targeted transcription of these three cytokines by inhibiting nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); and we used genetically modified mice lacking components of the IL-1, IL-6 or TNF systems to determine relative contributions of these cytokines to mechanical hypersensitivity after musculoskeletal sensitization. We now report that IL-1 and IL-6, but not TNF, increase in muscle after intramuscular injection of acidified saline. Local inhibition of nuclear translocation of NF-κB in the gastrocnemius muscle prior to musculoskeletal sensitization blocks the development of mechanical hypersensitivity; and genetic manipulation of the IL-1 and IL-6, but not TNF, systems prevents mechanical hypersensitivity following musculoskeletal sensitization. Collectively, our new data demonstrate that intramuscular IL-1 and IL-6 are local mediators of the development of mechanical hypersensitivity following musculoskeletal sensitization.

2. Methods

2.1. Animals

Adult male mice (8–12 weeks; 25 g) were used in this study. Mice were either purchased from the Jackson Laboratory (Bar Harbor, ME), or bred in-house as detailed later. All mice were maintained on a 12:12 h light:dark cycle at 27 °C with *ad libitum* access to food and water. Mice were group housed, and mice shipped from the Jackson Laboratory were allowed a minimum of one week to acclimate after arrival in our animal facility. All procedures using mice in these studies were approved in advance by the University of Washington Institutional Animal Care and Use Committee (IACUC), in accordance with the US Department of Agriculture Animal Welfare Act and the National Institutes of Health policy on Humane Care and the Use of Laboratory Animals.

2.2. Musculoskeletal sensitization

Acidified saline injections were used to produce long-lasting bilateral secondary mechanical hypersensitivity at the hind paws (Sharma et al., 2009; Sluka et al., 2001; Sutton and Opp, 2014a,b). Mice were lightly anesthetized using isoflurane and injected unilaterally into the gastrocnemius muscle with 20 μL of pyrogen-free saline using a 31 g insulin syringe. All mice were randomized into normal (pH 7.2) or acidified (pH 4.0) saline injection groups. Saline was adjusted pH 4.0 using 0.1 M HCl or NaOH. Five days later, mice were injected once again with the same pH saline as they received in the first injection. The leg into which injections were made was randomized among mice to prevent any lateralization bias, but injections were always given into the same muscle in each mouse. After each injection, animals were immediately returned to their home cage and observed by the investigator until fully ambulatory.

2.3. Mechanical hypersensitivity testing

The von Frey filament test is used to measure sensitivity to a non-noxious punctate pressure stimulus. All habituation and testing procedures took place at light onset and were completed during the first 2 h of the light period. Mice were habituated to a galvanized steel mesh testing platform for a minimum of 60 min during each of 3 daily sessions prior to baseline testing. During testing, mice were given a minimum of 30 min on the testing platform, or until they were quiet, before testing began. Calibrated filaments (0.07, 0.45, & 1.45 g) were presented in ascending order to the glabrous skin of the hind paw until the filament bowed slightly for 3 continuous seconds (Sharma et al., 2009; Smith et al., 2004; Sutton and Opp, 2014a,b). Testing continued until all 3 filaments were presented 5 times per hind paw. There was a minimum of a 1-min interval between each filament presentation, and presentations alternated between paws. As such, approximately 2-min lapsed between filament presentations to the same paw, and each mouse received a total of 30 filament presentations. Positive responses were recorded when the paw was retracted in response to the filament pressure. If mice became too active, testing was suspended until they were quiet. Mice were tested on 3 baseline days and on days 1, 3, 7, 14, and 21 after the second sensitization injection, as previously reported (Sutton and Opp, 2014a,b).

2.4. Experiment 1: Quantification of intramuscular cytokine concentrations after acidified saline injection

A total of 62 mice were used for this experiment. The gastrocnemius muscles ipsilateral and contralateral to the injection site were collected from all mice. Four mice that were not injected were sacrificed at light onset to serve as un-injected controls. Fifty-eight mice ($n = 29$ per injection group) were injected intramuscularly at light onset with either 20 μL normal (pH 7.2) or acidified (pH 4.0) saline. Twenty-four mice ($n = 4$ per time point per injection group) were sacrificed 60, 90, or 120 min after the first intramuscular injection (injection 1). The remaining 34 mice were housed for 5 days without manipulation. Five days after the first injection,