Available online at www.sciencedirect.com

ScienceDirect

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

Prior stress exposure increases pain behaviors in a rat model of full thickness thermal injury

Jennifer E. Nyland [a,*], Samuel A. McLean [b], Dayna L. Averitt [c]

[a] Pain Management Research Area, United States Army Institute of Surgical Research, Fort Sam Houston, TX, United States

[b] Departments of Anesthesiology and Emergency Medicine, University of North Carolina at Chapel Hill, NC, United States

[c] Department of Biology, Texas Woman's University, Denton, TX, United States

ARTICLE INFO

Article history:
Accepted 1 September 2015

Keywords:
Burn pain
Thermal injury
Hyperalgesia
Allodynia
Stress

ABSTRACT

Thermal burns among individuals working in highly stressful environments, such as firefighters and military Service Members, are common. Evidence suggests that pre-injury stress may exaggerate pain following thermal injury; however current animal models of burn have not evaluated the potential influence of pre-burn stress. This sham-controlled study evaluated the influence of prior stress exposure on post-burn thermal and mechanical sensitivity in male Sprague-Dawley rats. Rats were exposed to 20 min of inescapable swim stress or sham stress once per day for three days. Exposure to inescapable swim stress (1) increased the intensity and duration of thermal hyperalgesia after subsequent burn and (2) accelerated the onset of thermal hyperalgesia and mechanical allodynia after subsequent burn. This stress-induced exacerbation of pain sensitivity was reversed by pretreatment and concurrent treatment with the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine. These data suggest a better understanding of mechanisms by which prior stress augments pain after thermal burn may lead to improved pain treatments for burn survivors.

1. Introduction

Each year, more than 700,000 civilians seek care for burns in the United States and more than 50,000 undergo hospitalization [1]. Such injuries are common among individuals working in highly stressful environments, such as firefighters, disaster relief workers, and military Service Members [2,3]. Even with currently available medications, moderate or severe pain after burn is common and remains a substantial clinical challenge [4,5]. In order to reduce patient suffering and enhance recovery, new understanding of the mechanisms that mediate pain after burn is urgently needed [6].

One important area of research that may advance understanding and that has received little study is the potential influence of pre-burn stress exposure on post-burn pain. More than 30 years ago, Dr. Patrick Wall first proposed that stress systems may enhance pain after injury [7]. Subsequent decades of research have supported Dr. Wall's hypothesis and demonstrated links between neurobiological stress systems and variation in pain outcomes after trauma exposure [8–11]. However, to date, the potential influence of

* *Corresponding author at*: Pain Management Research Area, US Army Institute of Surgical Research, 3698 Chambers Pass, JBSA Fort Sam Houston, TX 78234, United States. Tel.: +1 210 539 5015; fax: +1 210 539 1460.
E-mail address: jen.e.nyland@gmail.com (J.E. Nyland).

http://dx.doi.org/10.1016/j.burns.2015.09.007

pre-injury stress has not been evaluated in animal models of thermal burn. This is an important issue, because if pre-injury stress commonly exerts a significant influence on outcomes after burn in humans, but is not included in animal models of burn pain, then their utility is diminished.

In this study we evaluated the influence of pre-burn stress exposure on the intensity and duration of post-injury mechanical allodynia and thermal hyperalgesia in a rat model of thermal burn. We chose a well-known animal model of stress, forced swim, as our pre-burn stressor because this exposure incorporates both physical and psychological stress [12–14], and prior physical and psychological stress are common among civilians and military personnel experiencing burn. We hypothesized that pre-burn stress exposure would enhance burn-evoked pain behaviors consistent with both increased intensity and duration of pain. Further, we anticipated that stress-related effects could be prevented by pretreatment with the serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine. SNRIs such as duloxetine are efficacious in a variety of clinical pain syndromes [15] and have been shown to prevent stress-induced hyperalgesia in a non-injury animal model [16].

2. Methods

2.1. Subjects

Adult (275–350 g) male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in these experiments. Rats were pair housed in a 12:12 h light: dark cycle with *ad libitum* access to food and water. All studies were approved by the U.S. Army Institute of Surgical Research Institutional Animal Care and Use Committee and conform to federal guidelines and guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. This study was also conducted in compliance with the Animal Welfare Act, Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used in these experiments and to minimize potential suffering.

2.2. Drugs

Duloxetine (pharmaceutical grade) was obtained from Sigma Aldrich under license from Eli Lilly. Duloxetine was dissolved in 5% glucose solution and administered subcutaneously [17]. Control subjects received an equivalent volume of 5% glucose.

2.3. Behavioral testing paradigms

Rats were first acclimated to the testing room for 2 h and to the testing apparatuses for 20 min on a non-testing day. Following acclimation, baseline measurements for mechanical and thermal sensitivity were recorded; a rest period of 2 h between mechanical and thermal testing was used to prevent behavioral sensitization. Pain behaviors were recorded at seven time points: a baseline measurement was taken 24 h prior to the first stress exposure and 24 h following the last stress exposure prior to injury. Additional measurements were taken at 24 and 96 h after thermal injury and then once a week up to three weeks. Throughout the study, testing with the mechanical stimulus always preceded testing with the thermal stimulus, as the thermal stimulus was considered noxious. Observers were blinded to the experimental condition.

2.4. Assessment of mechanical sensitivity

The force (in grams) required to elicit voluntary withdrawal of the hindpaw from a mechanical stimulus was determined using a Dynamic Plantar Anesthesiometer (Ugo Basile; Collegeville, PA) as previously described [18]. Briefly, rats were individually placed in a non-restrictive Plexiglas box on an elevated grid platform. After 20 min acclimation to the testing apparatus, a blunt mechanical stimulus was applied to the plantar surface of the hindpaw with slowly increasing force until the rat voluntarily withdrew the paw. The force of the mechanical stimulus was increased with a ramp of 3 g/s over 10 s with a cutoff of 30 g to avoid lifting of the paw by the device. Recordings were collected in triplicate and displayed as the average of all three recordings for each rat.

2.5. Assessment of thermal sensitivity

Rats were allowed 2 h rest following mechanical testing to avoid behavioral sensitization. After the resting period, sensitivity to a thermal stimulus was determined using the Paw Thermal Stimulator (Univ. California, San Diego, CA) as previously described [19]. Rats were individually placed in a non-restrictive clear Plexiglas box resting on an elevated glass plate maintained at 30 °C. Following 20 min acclimation to the apparatus, a radiant beam of light was positioned under the plantar surface of the hindpaw and the latency (in seconds) for the rat to remove the paw from the thermal stimulus was recorded. The intensity of the beam was set to produce baseline paw withdrawal latencies (PWL) of approximately 10–12 s. A maximal latency of 20 s was used to prevent excessive tissue damage due to repeated application of the thermal stimulus. As with the mechanical testing, PWLs were recorded in triplicate and averaged for each rat.

2.6. Forced swim stress

Forced swim sessions began approximately 24 h following baseline mechanical and thermal sensitivity measurements. To induce stress, rats were individually placed in cylindrical polyethylene containers measuring 30-cm in diameter and 50-cm in height and containing 20-cm of room temperature water. This was sufficient to force the rats to swim, as they were unable to reach the bottom of the cylinder. The sessions lasted for 20 min once daily. In order to assess the effectiveness of forced swim to induce stress, the activity during each swim session was recorded on video and later analyzed to determine the percentage of time spent immobile, as increased immobility is a characterized measure of learned helplessness [14]. The videos were scored in a blinded manner with the order of each subject/recording randomly assigned. Immobility was observed as making only the bodily