Available online at www.sciencedirect.com
ScienceDirect
Brain, Behavior, and Immunity 21 (2007) 68–78
BRAIN, BEHAVIOR, and IMMUNITY
www.elsevier.com/locate/ybrbi

Effects of carrageenan and morphine on acute inflammation and pain in Lewis and Fischer rats

Karamarie Fecho [a,b,c,*], Elizabeth L. Manning [b], William Maixner [b,c], Charles P. Schmitt [d]

[a] Department of Anesthesiology, Division of Pain Medicine, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[b] Curriculum in Neurobiology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[c] Center for Neurosensory Disorders, School of Dentistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[d] Copperline Solutions, LLC, Chapel Hill, NC 27516, USA

Received 19 December 2005; received in revised form 15 February 2006; accepted 16 February 2006
Available online 17 April 2006

Abstract

The present study used inbred, histocompatible Fischer 344 (FIS) and Lewis (LEW) rats to begin to explore the role of the hypothalamic–pituitary–adrenal (HPA) axis in the immune processes and pain behavior associated with the carrageenan model of acute hindpaw inflammation. Because the HPA axis contributes in part to morphine's analgesic and immunomodulatory properties, the present study also assessed the effects of morphine in carrageenan-inflamed LEW and FIS rats. The results showed that carrageenan-induced hindpaw swelling and pain behavior were greater in FIS than in LEW rats. The enhanced hindpaw swelling in FIS rats correlated with an increase in myeloperoxidase (MPO; a measure of neutrophils) in the inflamed hindpaw. FIS rats showed lower circulating levels of TNFα, higher IL-6 levels, and similar IL-1β and nitric oxide levels, when compared to LEW rats. Morphine produced a significant decrease in carrageenan-induced hindpaw swelling and MPO in both strains, but morphine did not significantly alter circulating cytokine/mediator levels. Morphine's analgesic effects were greater in the inflamed than the noninflamed hindpaw, and they did not correlate with morphine's anti-inflammatory effects. In fact, low doses of morphine produced a mechanical allodynia and hyperalgesia in the noninflamed hindpaw of FIS, but not LEW, rats. These results suggest a positive relationship between HPA axis activity and acute inflammation and inflammatory pain. In contrast, little evidence is provided for HPA axis involvement in morphine's anti-inflammatory or analgesic effects.

Keywords: Lewis; Fischer 344; HPA axis; Pain; Allodynia; Hyperalgesia; Carrageenan; Inflammation; Morphine; Analgesia

1. Introduction

Studies on inbred, histocompatible Lewis (LEW) and Fischer 344 (FIS) rats have identified a negative relationship between the responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis and susceptibility to autoimmune and chronic inflammatory disorders. LEW rats are susceptible to the development of a variety of autoimmune and chronic inflammatory disorders, whereas FIS rats are resistant to the same disorders. This disparity is believed to reflect a difference in HPA axis function. The HPA axis response to behavioral stressors or proinflammatory mediators is blunted in LEW rats, leading to reduced synthesis and secretion of corticosterone; the opposite is true for FIS rats (Dhabhar et al., 1993; Ferrick et al., 1991; Happ et al., 1988; Peers et al., 1993; Perretti et al., 1993; Rivest and Rivier, 1994; Sternberg et al., 1989a,b, 1992; Wei and Sternberg, 2004; Wei et al., 2002, 2003; Wilder et al., 1982). In general, corticosteroids produce immunosuppressive effects and suppress many aspects of inflammation (see, Riad et al., 2002 for review). Thus, FIS rats are believed to be inflammation-resistant due to heightened HPA axis activity and elevated corticosteroid levels. LEW rats, in contrast, are believed to be inflammation-susceptible due to blunted HPA axis

* Corresponding author. Fax: +1 919 966 4873.
E-mail address: kfecho@aims.unc.edu (K. Fecho).

0889-1591/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2006.02.003

activity and reduced corticosteroid levels. In agreement with this hypothesis are studies showing that interruption of the HPA axis by pharmacological blockade of glucocorticoid receptors (Sternberg et al., 1989a) or adrenalectomy (Mason et al., 1990) can cause an inflammation-resistant host to become susceptible to chronic inflammation. Conversely, reconstitution of the HPA axis via transplantation of fetal hypothalamic tissue from resistant FIS rats into susceptible LEW rats restores the blunted HPA axis response in LEW rats and decreases susceptibility to chronic inflammatory disorders (Misiewicz et al., 1997).

The relationship between HPA axis activity and acute inflammation is less understood. While corticosteroids are known to produce many immunosuppressive effects, recent evidence suggests that corticosteroids can *enhance* the acute inflammatory response. For example, corticosteroids can enhance neutrophil activity and prolong the neutrophil lifespan (Cox, 1995). Other studies show that acute stressors can act through the HPA axis to cause a redistribution of neutrophils and other leukocytes from the blood to peripheral sites of inflammation, thereby enhancing peripheral inflammatory responses (see Dhabhar and McEwen, 1997, 1999; Dhabhar et al., 1995, 1996).

The aim of the present study was to use LEW and FIS rats to begin to decipher the role of the HPA axis in the immunological processes and pain behavior associated with a model of acute inflammation; namely, carrageenan-induced hindpaw inflammation. Carrageenan-induced hindpaw inflammation is a neutrophil-mediated acute inflammatory response that produces hindpaw swelling, erythema, and localized hyperthermia, with clinical symptoms peaking at $1\frac{1}{2}$ to 3 h after the intraplantar injection of carrageenan (Cunha et al., 1991, 1992, 1999, 2005; Di Rosa et al., 1971; Ferreira et al., 1988, 1993; Handy and Moore, 1998; Leung et al., 2001; Poole et al., 1999; Ribeiro et al., 2000; Tsuji et al., 2003; Vinegar et al., 1969; Wei et al., 1995; Winter et al., 1962). The carrageenan-inflamed hindpaw also is painful, and carrageenan-induced hindpaw pain can be reliably measured using established behavioral pain assays, such as the Hargreaves radiant heat test and the von Frey monofilament test (e.g., Fecho et al., 2005; Ferreira et al., 1988). Because morphine is known to modulate both pain and immunological processes (see Vallejo et al., 2004 for review), in part through effects on the HPA axis (see Mellon and Bayer, 1998; Kosten and Ambrosio, 2002; Kiefer and Wiedemann, 2004 for reviews), the present study also included an assessment of morphine's effects in carrageenan-inflamed LEW and FIS rats.

2. Methods

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of North Carolina. Adult male LEW and FIS rats, weighing 175–225 g at 55–60 days old, were purchased from Charles-River Laboratories (Raleigh, NC). The rats were housed two per cage in plastic cages in a temperature- and humidity-controlled colony room under a 12 h day–night cycle. Food and water were provided ad libitum. The rats received a 1 week habituation period before the experimental procedures were initiated, during which time they were handled daily by the investigators, exposed to the behavioral testing room, and given one training session in each behavioral pain assay.

2.2. Morphine administration

Morphine sulfate (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile 0.9% saline and prepared to concentrations of 0 (saline vehicle alone), 2.5, and 5.0 mg/ml. Morphine was administered 1 h after intraplantar injection of carrageenan in a 1 ml/kg volume by subcutaneous (sc) injection into the left lower abdomen, to achieve a final dose of 0 (control), 2.5 or 5.0 mg/kg. The behavioral pain assays were intiated $\frac{1}{2}$ h after the morphine injection, in the order described below.

2.3. Carrageenan-elicited hindpaw inflammation

On the test day, the width of the left and right hindpaw of each rat, defined as the distance from the plantar to the dorsal surface of the center of the hindpaw, was measured using manual calipers. Rats then received an intraplantar (ipl) injection of 100 μl of 3.5% (w/v) carrageenan in sterile 0.9% saline into the right hindpaw, using a 1-cc syringe and a 27-gauge needle. To control for the influence of the injection itself, 100 μl of sterile 0.9% saline was injected into the intraplantar region of the left hindpaw of each rat. Hindpaw width was measured again 4 h after the carrageenan injection, after which time the rats were euthanized. The data were presented as the change in hindpaw width (in mm) from pre-injection to 4 h post-injection.

2.4. Behavioral pain assays

The behavioral pain assays were conducted in the order described below on the baseline and test days, with approximately 30 min of home cage rest between each assay. Baseline assessments of nociceptive sensitivity were taken for all rats 24 h before the test day. On the test day, the tail-flick test was conducted at $1\frac{1}{2}$ h after the carrageenan injection (or $\frac{1}{2}$ h after morphine), the Hargreaves test was initiated at 2 h after the carrageenan injection (or 1 h after morphine), and the von Frey monofilament test was initiated at $2\frac{1}{2}$ h after the carrageenan injection (or $1\frac{1}{2}$ h after morphine). All behavioral pain assays were conducted during the peak of the acute phase of the inflammatory response (Vinegar et al., 1969; Winter et al., 1962). Pilot studies using untreated rats confirmed that the order in which we ran the behavioral assays and the rest intervals that we included between assays resulted in stable responding that did not differ from baseline values (data not shown).

2.4.1. Hargreaves test for thermal hyperalgesia

The Hargreaves radiant heat method (Hargreaves et al., 1988) was used to measure hindpaw sensitivity to a noxious thermal stimulus, applied via an IITC Model 336 Paw/Tail Stimulator Analgesia Meter (Woodland Hills, CA). Rats were placed in individual plexiglass cages on a clear glass platform and given 15 min to acclimate to the testing environment. The stimulator source was used to present a focused beam of radiant light onto the midplantar region of the hindpaw. The idle intensity of the light was set at 2% of the maximum intensity to permit accurate placement of the beam of light to the appropriate region of the hindpaw; the active intensity of the light was set at 50% maximum. The light stimulus was turned off manually when the rat withdrew the hindpaw from the noxious beam of light, or automatically if the 20 s cut-off time was reached. Each rat received 3 trials/hindpaw, with a period of 5–10 min separating each trial, and the results from the 3 trials were averaged for analysis. Data were expressed in terms of the latency (s) to hindpaw withdrawal and the percent maximum possible effect (MPE; defined as $[(\text{Latency}_{\text{test}} - \text{Latency}_{\text{baseline}})/(20 - \text{Latency}_{\text{baseline}})] \times 100$).