ELSEVIER

Journal of Neuroimmunology 181 (2006) 100 – 105

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

Neurokinin 1 receptor signaling mediates sex differences in μ and κ opioid-induced enhancement of contact hypersensitivity

Jay C. Elliott *, Alison F. Wagner, Donald T. Lysle

Department of Psychology, CB # 3270, Davie Hall, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3270, USA

Received 4 July 2006; received in revised form 23 August 2006; accepted 30 August 2006

Abstract

Contact hypersensitivity (CHS) is a type of cutaneous inflammation that is exacerbated by neurogenic factors. Both μ- and κ-opioids enhance CHS to a greater extent in females than males. It was hypothesized that potentiated neurokinin 1 (NK1) receptor signaling following opioid treatment accounts for sex differences in the magnitude of CHS. Following morphine or spiradoline treatment the NK1 receptor antagonist SR140,333 significantly attenuated the magnitude of CHS in females but not males. By contrast, the NK2 antagonist SR48968 had no effect on morphine modulation of CHS. Taken together, these data indicate that NK1 receptor signaling is a key mediator of sex differences in opioid-induced enhancement of CHS.

Keywords: Contact hypersensitivity; Opioid; Sex difference; Substance P

1. Introduction

Contact hypersensitivity (CHS) is an antigen-specific type of cutaneous inflammation induced by repeated topical application of sensitizing chemicals. It is classified as a form of delayed-type hypersensitivity (DTH) and is a prototypical *in vivo* memory T-lymphocyte mediated immune response (Enk and Katz, 1995). Delayed-type hypersensitivity is an important immunological process involved in host defense and tissue damage produced by bacterial infections such as tuberculosis (Dannenberg, 1994) and chlamydia (Yang, 2001). Our laboratory has shown that course of CHS in rats can be significantly exacerbated by acute treatment with μ- or κ-opioids and that the magnitude of this exacerbation is significantly greater in females than males (Elliott et al., 2006, 2003; Nelson et al., 1999). We have also demonstrated that both μ- and κ-opioids act via CNS opioid receptors to alter CHS in females and males (Elliott et al., 2003). Therefore, it follows that there must be activation of physiological systems influenced by central opioid systems that regulate cutaneous immune responses following antigen exposure. Activation or sensitivity to activation of these systems by μ- or κ-opioids should be greater in females to account for sex differences in CHS inflammation following opioid administration. Neurogenic inflammation is a physiological process that fits these criteria and may be a proximate interface between the central nervous and cutaneous immune systems.

Neurogenic inflammation involves the antidromic release of tachykinins such as substance P and neurokinin A, as well as other neuropeptides by C and Aδ sensory neurons into peripheral tissues following injury or antigen exposure (Holzer, 1998). Under normal conditions, release of these peptides stimulates immunological and vasoactive processes that promote tissue repair and containment of infection (Steinhoff et al., 2003), however neurogenic processes also contribute to the pathophysiology of a variety of inflammatory conditions including CHS. For instance, selective ablation of substance P containing cutaneous sensory neurons prior to elicitation of contact hypersensitivity significantly attenuates inflammation (Beresford et al., 2004), whereas treatment with substance P or a selective neurokinin 1 (NK1) agonist prior to elicitation enhances CHS inflammation (Gutwald et al., 1991). Similarly, increasing substance P

* Corresponding author. Tel.: +1 843 792 6333; fax: +1 843 792 4423.
E-mail address: elliojc@musc.edu (J.C. Elliott).

0165-5728/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2006.08.011

activity by pharmacological inhibition or gene knockout of its catalytic enzymes significantly enhances the magnitude of CHS (Scholzen et al., 2001; Scholzen et al., 2003). Such increases were blocked by pretreatment with the NK1 antagonist SR140,333, indicating a key role for this tachykinin receptor subtype in CHS enhancement by substance P. Collectively, these data provide strong evidence that substance P is an important modulator of CHS and other types of cutaneous inflammation through its action on NK1 receptors in the skin.

Sex differences in substance P signaling may contribute to differential inflammatory outcomes in males and females. For instance, estradiol has been shown to stimulate expression of NK1 mRNA and suppress neutral endopeptidase mRNA expression in rat uterine tissue (Pinto et al., 1999). Moreover, intestinal mast cell histamine release is greater in females than males following application of exogenous substance P and that this sex difference is dependent on progesterone (Bradesi et al., 2001). These data suggest that female gonadal hormones can stimulate substance P activity in some tissues by inhibiting substance P metabolism and/or increasing sensitivity to its effects. Since greater morphine-induced enhancement of CHS in females is dependent on the activational effects of female gonadal hormones (Elliott et al., 2003), it is possible that sex differences in cutaneous substance P/NK1 receptor signaling may account for the differential effect of morphine on CHS in males and females.

Neurogenic inflammation in the skin can be influenced by central nervous system activity. For instance, immobilization stress has been shown to cause degranulation of mast cells in the skin, and this effect is abolished by depleting sensory neurons of neuropeptides (Singh et al., 1999) or pretreatment with an NK1 eptor antagonist (Erin et al., 2004). Although there is a well-established connection between the opioid system and enhanced substance P signaling (Li et al., 2000), Nelson and Lysle (2001a) provided the first evidence for central opioid interactions with substance P in modulating cutaneous CHS. This paper demonstrated attenuation of morphine's enhancement of CHS following both systemic and local treatment with the NK1 antagonist WIN51,708 at the elicitation phase in male rats. These data support the hypothesized interaction between the opioid system and the peripheral substance P/NK1 system in the enhancement of CHS. However, this investigation did not test putative sex differences in the relative contribution of NK1 receptor signaling in opioid enhancement of CHS. Therefore, a central purpose of the present study was to evaluate the effects of a highly selective NK1 antagonist on μ-opioid modulation of CHS in males and females. It was hypothesized that NK1 receptor blockade with the NK1 antagonist SR140,333 would attenuate morphine enhancement of CHS to a greater extent in females than males. Since the data supported this initial hypothesis, additional experiments were conducted to determine whether SR140,333 also produces sex-selective effects on κ-opioid enhancement of CHS and to determine any NK2 receptor involvement in μ-opioid effects on CHS.

2. Materials and methods

2.1. Animals

Pathogen-free adult Fischer rats (3–4 months of age) obtained from Charles River (Raleigh, NC) were used in all experiments. All animals were gonadally intact and females were freely cycling. Animals were individually housed and kept on a 12:12 light cycle with lights off at 6 PM. Food and water were available *ad libitum* at all times. Animals were habituated to the colony room for a period of at least 1 week prior to the initiation of any experimental procedures.

2.2. Drugs

The μ-opioid agonist morphine sulfate was furnished by NIDA (Bethesda, MD) and the κ-opioid agonist spiradoline mesylate was purchased from Sigma (St. Louis, MO). The NK1 antagonist SR140,333 and NK2 antagonist SR48968 were generously provided by Dr. X. Emonds-Alt of Sanofi-Aventis (Bordeaux, France). SR140,333 was chosen since it is a highly potent and selective NK1 antagonist (Rupniak et al., 2003). It is also poorly brain-penetrant, so any effects of the antagonist are likely due to its effects on peripheral NK1 receptors. Morphine and spiradoline were dissolved to in endotoxin-free sterile water, whereas SR140,333 and SR48968 were initially dissolved in dimethyl sulfoxide (DMSO) and the mixture adjusted with saline such that the vehicle for the desired drug concentrations consisted of 50% DMSO. All drugs were delivered by subcutaneous injection to a site on the lateral lower abdomen in a final volume of 1 ml/kg.

2.3. Induction of contact hypersensitivity

Sensitization and elicitation of CHS with the antigen 2,4-dinitrofluorobenzene (DNFB, Sigma, St. Louis, MO) was performed as previously described (Nelson et al., 1999). Briefly, animals were sensitized with 100 μl of 1% DNFB (in a 4:1 mixture of acetone and olive oil) applied to the shaved abdomen on days 1 and 2 of the experiment. On day 6, 25 μl of a 0.5% DNFB solution was applied to the treatment ear (right pinna) to elicit the CHS response. As a control, an equivalent volume of vehicle alone was applied to the opposing ear (left pinna).

2.4. Inflammation measurement and data analysis

Swelling was quantified by measuring pinna thickness using a digimatic caliper (Mitutoyo, Kawasaki, Japan). At each time-point both the right (DNFB treated) and left (control) pinnae were measured twice and the means of each pinna thickness calculated for statistical analysis. If measurements differed by more than 0.05 mm, one additional measurement was taken and the mean calculated from the three measurements for use in the analyses. This additional