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Substance P receptor mediated maintenance of chronic inflammation in EAE

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Abstract

Substance P (SP) is a modulatory, pro-inflammatory neuropeptide. We investigated the role of the SP receptor, neurokinin-1 (NK-1), in EAE. Our data show that in the chronic phase, mice lacking NK-1 have improved mobility and decreased numbers of LFA-1 high CD4+ T cells and MOG-specific, IFN-γ producing CD4+ T cells. SR140333, an NK-1 antagonist, administered alone during the chronic phase of EAE was not sufficient to ameliorate symptoms. These results indicate that SP, through NK-1, contributes to maintenance of CNS inflammation, and combining NK-1 antagonists with conventional anti-inflammatory treatments may enhance the success of treatments for diseases like multiple sclerosis.

Keywords: Substance P; Neurokinin; Neuropeptide; EAE; SR140333; MS

1. Introduction

The discovery of Substance P (SP) immunoreactive astrocytes in myelin plaques from multiple sclerosis (MS) patients led to the hypothesis that SP, a known participant in inflammation and pain, may play a role in MS (Barker and Larner, 1992; Kostyk et al., 1989). Studies of cerebral spinal fluid taken from MS patients and controls, however, showed either no difference or a trend toward lower SP levels in the patients (Qureshi et al., 2000; Rosler et al., 1990). Although the lack of increased SP in cerebral spinal fluid does not necessarily indicate an absence of SP in the pathology, there has been no further in depth investigation into the role of SP in MS.

The importance of SP and neurokinin-1 (NK-1), the highest affinity SP receptor, has been investigated in other autoimmune and inflammatory responses. In CNS trauma, NK-1 and/or SP have increased expression on glia, neurons, and endothelium after experimental CNS injuries (Lin, 1995; Mantyh et al., 1989; Stumm et al., 2001), and treatment with NK-1 antagonist SR140333 minimizes infarct size in a rat model of ischemia (Yu et al., 1997). This indicates a general role for SP and NK-1 in response to CNS damage. SP and NK-1 are also found in affected tissue in multiple autoimmune diseases, such as Crohn's disease, rheumatoid arthritis, and diabetes (Goode et al., 2000; Mantyh et al., 1995, 1994; Menkes et al., 1993; Persson-Sjogren et al., 2005;

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Sakai et al., 1998). In addition to the injured tissue, SP and NK-1 are expressed on infiltrating leukocytes in these diseases, indicating that leukocyte expression of these molecules may also be critical for pathology (Lambert et al., 1998; Mantyh et al., 1988, 1994; Persson-Sjogren et al., 2005). In the context of infectious disease, infiltrating cells associated with the inflamed tissue, especially CD4+ T cells, express NK-1 (Blum et al., 2001; Cook et al., 1994; Tripp et al., 2002). Macrophage from inflammatory lesions have been shown to respond to SP by increased release of inflammatory cytokines and to respond to inflammatory cytokines by secreting SP (Arsenescu et al., 2005; Castagliuolo et al., 1997). In the absence of NK-1, or under the inhibition of NK-1 by a chemical antagonist, the inflammatory response to infectious agents is inhibited (Blum et al., 1993, 2003; Elsawa et al., 2003; Sturiale et al., 1999). This phenomenon was demonstrated in the brain using a mouse model of trypanosome-induced meningitis (Kennedy et al., 2003, 1997). SP and NK-1 are clearly very important in brain injury and in both autoimmune and infectious pro-inflammatory immune responses. It is therefore very plausible that SP and NK-1 play a role in CNS autoimmunity.

There are several drugs which block SP interactions with NK-1 now in clinical trials aimed at treating chemotherapy-associated nausea (NCT00285272, NCT00104403), depression related to fibromyalgia (NCT00264628), post-traumatic stress disorder (NCT00211861), and overactive bladder (NCT00174798). In this paper, we investigated the efficacy of SR140333, a NK-1 antagonist currently being tested as a treatment for human inflammatory bowel disease (NCT 00232258) and used in multiple rodent models of inflammation (Pinter et al., 2002; Santos et al., 2004; Schuiling et al., 1999; Weinstock et al., 2003), in the autoimmune MOG-induced experimental autoimmune encephalomyelitis (EAE) model in mice.

To investigate the role of SP and NK-1 in CNS autoimmunity, we examined the course of EAE in NK-1 deficient animals. We found that the absence of SP receptors did not interfere with the induction of EAE, but there was a significant difference in the effector phase of EAE as characterized by less severe clinical symptoms and inflammation in the NK-1 deficient mice. Despite this, treatment of mice during the effector phase of the disease with SR140333 had no significant effect on the development of clinical symptoms, suggesting that a role for NK-1 blocking would be a part of an integrated therapy that includes additional anti-inflammatory components.

2. Materials and methods

2.1. Mice

Mice used were 10–13-week old females. NK-1 −/− mice backcrossed on the C57Bl/6 background (9 generations) (Blum et al., 2003) were bred at the University of Wisconsin-Madison or University of Iowa animal facility. C57Bl/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) or bred at the University of Wisconsin-Madison School of Medicine and Public Health animal facility. All experimental protocols were approved by the University of Wisconsin-Madison School of Medicine and Public Health institutional animal care and use committee.

2.2. NK-1 immunofluorescence microscopy

Spinal cord was isolated from spinal column 16 days after EAE induction. Tissue was embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA), frozen on dry ice, and kept at −80 °C until use. 10 μm sections were fixed in 1% formalin, and 3% goat serum and 0.1% saponin in TBS were used to permeabilize, block, and to dilute antibodies. Antibodies and reagents used included: antibody #19 raised in chicken against neurokinin-1 provided by CURE/Digestive Diseases Research Center, Antibody/RIA Core, NIH grant #DK41301 (Los Angeles, CA), anti-Mac1 (M1/70) prepared by conventional methods and labeled with Alexa 488 (Molecular Probes, Invitrogen, Carlsbad, CA), anti-CD4 (GK1.5) prepared by conventional methods and biotinylated, goat anti-chicken antibody labeled with Alexa 647 (Molecular Probes), and streptavidin labeled with Alexa 568 (Molecular Probes). Images were acquired with a Nikon C1 Laser Scanning Confocal (Nikon, Melville, NY) with upright microscope at 100× and 400× magnification. Nikon EZ-C1 software (Nikon) tif images were exported to Adobe Photoshop 8 (Adobe Systems Inc., San Jose, CA) for adjustment. Arrowheads and asterisks were added using Adobe Illustrator (Adobe Systems, Inc).

2.3. EAE induction and evaluation

Myelin oligodendrocyte glycoprotein peptide (MOG 35–55) synthesized by CyberSyn (Lenni, PA) or by the University of Wisconsin-Madison Biotechnology Center (Madison, WI) was dissolved at 2 mg/ml in sterile PBS and mixed by sonication in equal volumes with complete Freund's adjuvant (CFA), supplemented with *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI) to 5 mg/ml. 100 μg of the resultant MOG/CFA slurry was injected subcutaneously between the shoulder blades of the mouse. 200 ng of Pertussis toxin (List Biological Laboratories, Inc. Campbell, CA) was injected intraperitoneally at the time of EAE induction and two days following induction. Clinical scores of the animals were monitored daily beginning 7 days after EAE induction. Clinical scoring was as follows: 0, no motor defect; 1, tail weakness; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, complete fore limb and hind limb paralysis; 5, moribund or dead. For all experiments, animals that did not show clinical score of 1 or above by/on day 20 after EAE induction were excluded from analysis as having insufficient induction; this was the case for approximately 18% of C57Bl/6 and 22% of NK-1 −/− animals. Statistical comparison of disease severity by clinical score