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Research Report

Chemical stimulation of the intracranial dura activates NALP3 inflammasome in trigeminal ganglia neurons

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ABSTRACT

Inflammasomes are molecular platforms that upon activation by cellular infection or stress trigger the maturation of proinflammatory cytokines such as interleukin (IL)-1β to engage innate immune defenses. Increased production of IL-1β in pain and inflammation such as headache is well documented. However, limited evidence addresses the participation of inflammasomes in inflammatory pain. The present study used rat inflammatory dural stimulation-induced model of intracranial pain to assess whether headache-related pain can induce the activation of NACHT, LRR, and PYD-containing protein (NALP)-3 inflammasome pathway in the trigeminal ganglia (TG) and which cells express NALP3 inflammasome proteins and IL-1β. Chemical stimulation of the intracranial dura caused a total drug dose- and time-dependent induction of activated caspase-1 and mature IL-1β proteins. Application of a selective caspase-1 inhibitor diminished these effects. Immunohistochemistry revealed that both NALP3 inflammasome and IL-1β immunoreactivity were existed mainly in small to medium-sized C-type neurons and increased over time, with intense cytoplasmic staining after 3 days of dural inflammation. Overall, the present observation indicated that dural inflammation promoted assembly of the multiprotein NALP3 complex, activated caspase-1, and induced processing of IL-1β, which provides an indirect evidence of the participation of NALP3 inflammasome in the cascade of events involved in the genesis of headaches by promoting IL-1β maturation in the TG. This may contribute to strategies for headache control.

Abbreviations: CGRP, calcitonin gene-related peptide; DMSO, dimethyl sulfoxide; GFAP, glia fibrillary acidic protein; IS, inflammatory soup; IL-1β, interleukin (IL)-1β; NALP3, NACHT, LRR and PYD-containing protein (NALP)-3; PAN, primary afferent neuron; SGC, satellite glial cell; TG, trigeminal ganglia

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1. Introduction

The intracranial dura receives nociceptive fibers sensory innervation from the trigeminal ganglion(TG) that is thought to be involved in some types of headaches, including migraine (Feindel et al., 1960; Keller et al., 1985; O'Connor and van der Kooy, 1986). Pain is the only sensation that can be evoked by stimulation of the intracranial meninges. Activation and sensitization of primary afferent neurons (PANs) innervating the dura mater is the crucial step in the pathogenesis of a migraine attack (Goadsby et al., 2009). Elucidation of the expression pattern of chemo-sensing molecules in the PANs of the headache circuit will add to our understanding of headache pathophysiology and could facilitate the development of new therapeutics.

The cytokine cascade involved in pain and inflammation is a tremendously complex system encompassing glial, immune, and neuronal cell interactions. Increased production of cytokines of the interleukin (IL)-1 family, such as IL-1β, in such cascades is well documented, indicating a pivotal role of this cytokine in various pain-producing states (Ren and Torres, 2009). IL-1β has been shown to play a central role in the generation of mechanical hyperalgesia (Ferreira et al., 1988), and inflammatory hyperalgesia can be prevented experimentally by administration of an endogenous IL-1 receptor antagonist (Cunha et al., 2000). Furthermore, a clinical investigation showed significant differences in the genotypic distribution of the IL-1beta+3953C/T polymorphism between migraineurs and controls ($p=0.004$) and a significantly greater frequency of the IL-1beta+3953 T allele in patients with migraine without aura (MwoA) than in healthy controls ($p=0.004$) (Yilmaz et al., 2010). IL-1β was also among the cytokines whose levels were elevated in jugular vein blood during migraine attacks (Perini et al., 2005).

IL-1β is synthesized as an inactive cytoplasmic precursor that is proteolytically processed into its biologically active form by caspase-1, a cysteine protease, in response to proinflammatory stimuli (Burns et al., 2003; Martinon and Tschopp, 2004). The processing of pro-IL-1β involves the activation of an intracellular multiprotein caspase-1-activating complex termed the inflammasome, which plays an important role in innate immunity and is an active player in inflammatory disorders (Martinon et al., 2002; Ogura et al., 2006). Inflammasomes contain NOD-like receptor (NLR) proteins and are named according to which NLR protein is present. The NALP3 (also known as NLRP3 or CIAS1) inflammasome is probably the best studied. Activation of the inflammasome results in processing and secretion of proinflammatory IL-1β and IL-18, consequently triggering the inflammatory response.

Although the promotion of IL-1β maturation by the NALP3 inflammasome is an important step in the inflammatory cascade, few studies have explored its association with pain (Martinon et al., 2009). The present study investigated the involvement of inflammasome pathway in the rat inflammatory dural stimulation-induced model of intracranial pain. We focused mainly on the peripheral part of pain transmission in which the inflammasome activation was caused by this form of inflammatory pain. Our results indicate that a molecular platform (the NALP3 inflammasome) consisting of NALP3 and caspase-1 is present mainly in C-type neurons of the TG in rats suffering from dural inflammation. Inflammatory dural stimulation induced rapid processing of IL-1β, activated caspase-1, and promoted assembly of the NALP3 inflammasome. Moreover, inhibition of caspase-1 selectively decreased the cleavage of inactive IL-1β and thus blocked its activation.

2. Results

2.1. *Dural stimulation induces processing of IL-1β*

To determine whether dural inflammation induced processing of the proinflammatory cytokine IL-1β, we analyzed TG lysates from naïve and dural IS-stimulated animals for IL-1β protein (Fig. 1). The level of mature IL-1β in the TG lysates had increased 3 h after dural stimulation, decreased slightly between 3 h and 6 h after stimulation, and then continued to rise over the next 1–3 d under repeated inflammatory dural stimulation. Therefore, dural inflammation induces processing of the IL-1β precursor into the mature inflammatory cytokine, and this may be important in the onset of headache.

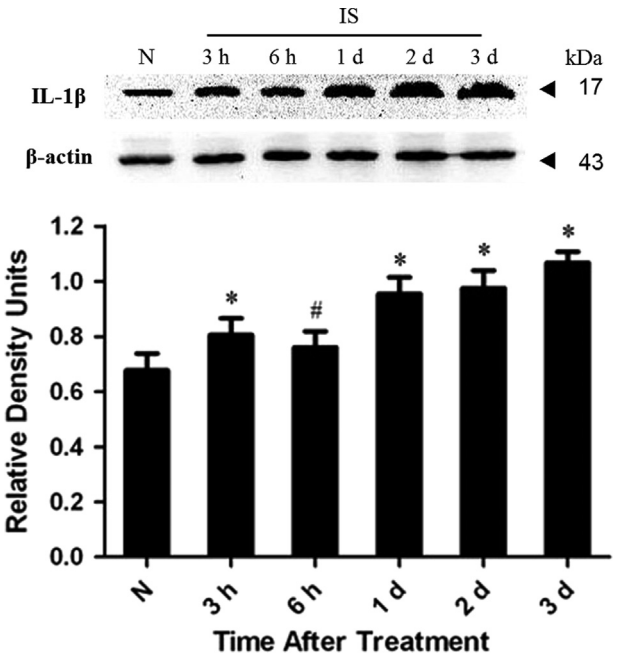

Fig. 1 – Dural inflammation induces processing of interleukin(IL)-1β. Representative immunoblot analysis of left trigeminal ganglion lysates from treatment-naive animals (N) and treated rat trigeminal ganglia after 3 h, 6 h, 1 d, 2 d, and 3 d of inflammatory stimulation. Trigeminal ganglion lysates were immunoblotted with antibodies against IL-1β. β-actin was used as internal standard and protein loading control. The data are presented as the mean ± standard error of the mean (SEM). $^{*}p<0.0001$, $^{\#}p<0.01$ in comparison with the treatment-naïve group. $N=5$ per group.