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Research Report

Effects of peripheral inflammation on activation of p38 mitogen-activated protein kinase in the rostral ventromedial medulla

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ABSTRACT

In the present study, the activation of p38 mitogen-activated protein kinase (p38 MAPK) in the rostral ventromedial medulla (RVM) following the injection of complete Freund's adjuvant (CFA) into the rat hindpaw was examined in order to clarify the mechanisms underlying the dynamic changes in the descending pain modulatory system after peripheral inflammation. Phospho-p38 MAPK-immunoreactive (p-p38 MAPK-IR) neurons were observed in the nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis pars alpha (GiA). Inflammation induced the activation of p38 MAPK in the RVM, with a peak at 30 min after the injection of CFA into the hindpaw, which lasted for 1 h. In the RVM, the number of p-p38 MAPK-IR neurons per section in rats killed at 30 min after CFA injection (19.4±2.0) was significantly higher than that in the naive group (8.4±2.4) [$p<0.05$]. At 30 min after CFA injection, about 40% of p-p38 MAPK-IR neurons in the RVM were serotonergic neurons (tryptophan hydroxylase, TPH, positive) and about 70% of TPH-IR neurons in the RVM were p-p38 MAPK positive. The number of p-p38 MAPK- and TPH-double-positive RVM neurons in the rats with inflammation was significantly higher than that in naive rats [$p<0.05$]. These findings suggest that inflammation-induced activation of p38 MAPK in the RVM may be involved in the plasticity in the descending pain modulatory system following inflammation.

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Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; ERK, extracellular signal-regulated kinase; GFAP, glial fibrillary acidic protein; Gi, nucleus reticularis gigantocellularis; GiA, nucleus reticularis gigantocellularis pars alpha; JNK, c-Jun N-terminal kinase; LPGi, nucleus lateralis paragigantocellularis; LTD, long-term depression; MAPK, mitogen-activated protein kinase; MKP, MAPK phosphatase; NeuN, neuronal nuclei; NMDA, *N*-methyl-D-aspartate; NRM, nucleus raphe magnus; OX42, CD11b-Rat macrophages/microglial cells; PAG, periaqueductal gray matter; PB, phosphate buffer; PFA, paraformaldehyde; RT, room temperature; RVM, rostral ventromedial medulla; Sp5, spinal trigeminal nucleus; TBS, Tris-buffered saline; TBST, TBS containing 0.1% Triton X-100; TPH, tryptophan hydroxylase; TRPV1, vanilloid receptor type 1 of the transient receptor potential channel family; 7, facial nucleus

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1. Introduction

Transmission of input from nociceptors through the spinal dorsal horn is subject to both descending inhibition and facilitation from supraspinal structures. The rostral ventromedial medulla (RVM) including the nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis pars alpha (GiA) and the ventral nucleus reticularis gigantocellularis (Gi) receives projections from the periaqueductal gray matter (PAG) and sends projections to the spinal dorsal horn largely along the dorsolateral funiculus. The PAG and the RVM with its spinal projections constitute the efferent channel of the "descending pain modulatory system" (Fields and Basbaum, 1999; Millan, 2002; Ren and Dubner, 2002; Vanegas, 2004; Wei et al., 1999). The NRM is a major source of the descending serotonergic pathways that participate in spinal nociceptive modulation (Basbaum and Fields, 1984), and the GiA and Gi seem to modulate the activity of the NRM (Gilbert and Franklin, 2001; Terayama et al., 2002). These descending serotonergic pathways exert bi-directional control of nociception (Ren and Dubner, 2002; Suzuki et al., 2002; Wei et al., 1999).

Descending modulation exhibits dynamic changes in response to persistent noxious input following inflammation. Peripheral inflammation leads to an initial decrease in RVM excitability at 3–5 h and net descending inhibition followed by an increase in RVM excitability up to 24 h post-inflammation and net descending inhibition. The time course of the changes in RVM excitability and descending inhibition following peripheral inflammation is dependent, in part, on the activation of *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors (Guan et al., 2003; Terayama et al., 2000, 2002).

The p38 mitogen-activated protein kinase (p38 MAPK) is a member of the serine/threonine protein kinases implicated in the transduction of neurotrophic and cytokine signals from the cell surface to the nucleus (Obata et al., 2000; Shi and Gaestel, 2002). Mitogen-activated protein kinase (MAPK) not only regulates cell proliferation, differentiation and survival but also plays important roles in synaptic plasticity and memory formation (Alonso et al., 2003; Zhu et al., 2002).

The p38 MAPK is activated in the dorsal root ganglion and spinal dorsal horn after peripheral noxious stimulation, nerve injury and inflammation and is involved in induction and/or maintenance of peripheral and central sensitizations (Ji et al., 2002a, 2003; Jin et al., 2003; Obata et al., 2004; Svensson et al., 2003; Tsuda et al., 2004). We have previously shown that extracellular signal-regulated kinase (ERK), another member of MAPK, is activated in the RVM of rats with thermal hyperalgesia following chronic restraint stress (Imbe et al., 2004). Recently, we have also demonstrated that inflammation induced the activation of ERK in the RVM, with a peak at 7 h after the injection of CFA into the hindpaw and a duration of 24 h (Imbe et al., 2005). Although several studies have reported that the RVM plays an important role in nociceptive transmission, no study has examined the time course of the activation of p38 MAPK in the RVM following peripheral inflammation.

In the present study, the activation of p38 MAPK in the RVM following the injection of CFA into the rat hindpaw was examined to clarify the mechanisms underlying the dynamic changes in the descending pain modulatory system after peripheral inflammation. The result showed that hindpaw inflammation increased phospho-p38 MAPK-immunoreactive (p-p38 MAPK-IR) neurons in the RVM.

2. Results

2.1. *Activation of p38 MAPK in the RVM following the injection of CFA into the hindpaw*

In the RVM of naive rats and rats with inflammation, almost all of the p-p38 MAPK-IR neurons were distributed in the NRM and GiA (Figs. 1, 2). These p-p38 MAPK-positive neurons showed strong staining of cell nuclei and weak staining of cell bodies. To confirm whether p-p38 MAPK-IR cells in the RVM were neurons or not, we examined the immunohistochemical colocalization of p-p38 MAPK and NeuN, OX42 or GFAP. All the p-p38 MAPK-IR cells with neuronal profile also expressed NeuN-IR (Figs. 3A–C). Although few small p-p38 MAPK-IR cells expressed OX42-IR (Figs. 3D–F) or GFAP-IR (Figs. 3G–I), we could easily distinguish neurons from microglia and astrocytes by size and shape. In the rostral and caudal RVM, although there were several p-p38 MAPK-IR neurons in the NRM and GiA in naive rats (Figs. 1B, 2B), hindpaw inflammation induced a robust increase in p-p38 MAPK-IR neurons (Figs. 1C, 2C) at 30 min after the injection of CFA into the hindpaw. A rapid and obvious increase in the number of p-p38 MAPK-IR neurons in the RVM (131.7% increase vs. naive group) was observed at 30 min after CFA injection (Fig. 4C). The numbers of p-p38 MAPK-IR neurons per section in the RVM in the inflammation groups (CFA 30-min group: 19.4±2.0, CFA-1 h group: 17.8±4.6) were significantly higher than that in naive rats (8.4±2.4) [$p<0.05$] (Fig. 4C). The percentage of p-p38 MAPK-IR neurons in the NRM was significantly higher than that in the GiA among such neurons in the RVM in CFA 30-min group (61.9±1.5% vs. 38.1±1.5%) and CFA-1 h group (66.7±6.1% vs. 33.3±6.1) [$p<0.05$] (Fig. 4). In the GiA, the numbers of p-p38 MAPK-IR neurons in the CFA-3 h and CFA-5 h groups were significantly higher than that in the naive group ($p<0.05$, Fig. 4B). There were no significant differences in the number of p-p38 MAPK-IR neurons per section in the RVM between the naive group (8.4±2.4) and saline groups (saline-30 min, 12.8±0.9; saline-1 h, 4.5±1.6; saline-3 h, 7.8±2.5; saline-5 h, 7.2±0.6; saline-7 h, 8.3±0.7) (Fig. 5). The numbers of p-p38 MAPK-IR neurons in the RVM in the CFA-30 min and CFA-1 h groups were significantly higher than those in saline-30 min and saline-1 h groups, respectively [$p<0.05$]. However, there were no significant differences in the number of p-p38 MAPK-IR neurons per section in the RVM between the naive and inflammation groups from 1 day to 2 weeks after CFA injection. In addition to the RVM, there were many p-p38 MAPK-IR neurons in other areas of the medulla, such as the locus coeruleus and medial vestibular nucleus, as well as a few p-p38 MAPK-IR neurons in the pontine and medullary reticular formations and dorsal paragigantocellular nucleus. No p-p38 MAPK-IR neurons were found in the spinal trigeminal nucleus or facial nucleus.