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Potential role of CXCL10/CXCR3 signaling in the development of morphine tolerance in periaqueductal gray

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ABSTRACT

Tolerance to morphine antinociception hinders its long-term use in clinical practice. Interaction between neuron and microglia has been proved to play critical role in the mechanism of morphine tolerance, while CXCL10/CXCR3 signaling has been implicated in neuron-glia signaling and morphine analgesia. This study aims to investigate whether CXCL10/CXCR3 signaling in periaqueductal gray (PAG) contributes to the development of morphine tolerance by modulating neuron-microglia interaction. The results showed that the expressions of CXCR3 and CXCL10 were gradually increased in parallel with repeated morphine administration and activation of microglia. CXCR3 was co-localized with neuronal marker NeuN, while CXCL10 was derived from microglia. Microglia inhibitor minocycline significantly attenuated the expression of CXCL10, besides, both minocycline and CXCR3 inhibitor alleviated the development of morphine tolerance. Taken together, our study provided the evidence that CXCL10/CXCR3 signaling in PAG is involved in the development of morphine analgesic tolerance via neuron-microglia interaction.

1. Introduction

Tolerance to morphine-induced antinociceptive effect hinders its prolonged usage in the clinic. The roles of neuronal intracellular cascades including desensitization of opioid receptors, endocytosis of opioid receptor and functional changes of glutamate receptors in the mechanisms of morphine tolerance have been well investigated (Martini and Whistler, 2007; Tai et al., 2007; Williams et al., 2013). Accumulating evidences suggest that microglia may play an essential role in the development of morphine tolerance (Horvath et al., 2010; Wang et al., 2010b; Eidson and Murphy, 2013a). Microglia could be activated in response to morphine-induced neuronal changes, while microglia-derived proinflammatory factors including cytokines and chemokines, in turn, promote the neuronal sensitization (Horvath et al., 2010; Wang et al., 2010b). These studies indicate the importance of the interaction between neuron and microglia in the mechanism of morphine tolerance.

Periaqueductal gray (PAG) and its descending projections to rostral ventromedial medulla and spinal cord comprise an essential neural circuit for opioid-mediated analgesia (Basbaum and Fields, 1978). Recent studies demonstrated that opioid tolerance is accompanied by activation of microglia in PAG (Eidson and Murphy, 2013b), and inhibition of microglia activities could attenuate the development of morphine tolerance (Eidson and Murphy, 2013a). Although the contribution of microglia activation in PAG to morphine tolerance has been reported, little is known about the underlying mechanism of neuron-microglia interaction (Cui et al., 2006).

Chemokines, a family of small cytokines, could directly induce chemotaxis of responsive cells. Several studies indicate that chemokine receptors, such as CXCR2, are co-expressed by opioid-containing

Abbreviations: CaMKII, Ca^{2+}/calmodulin dependent protein kinase II; CREB, cAMP response element binding protein; CXCL9, C-X-C motif chemokine 9; CXCL10, C-X-C motif chemokine 10; CXCL11, C-X-C motif chemokine 11; CXCR3, C-X-C motif chemokine receptor 3; rmCXCL10, recombinant mouse CXCL10 protein; GABA, gamma-aminobutyric acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding adapter molecule 1; IL-1β, interleukin-1 beta; NeuN, neuronal nuclei; NFκB, nuclear factor kappa B; PAG, periaqueductal gray; TNFα, tumor necrosis factor alpha

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leukocytes. Inhibiting some chemokines (CXCL1 and CXCL2/3) could substantially result in the decreased number of opioid-containing immune cells in inflammatory tissue and in consequence abolish the endogenous peripheral opioid analgesia (Brack et al., 2004; Machelska, 2007), whereas some chemokines (e.g. CCL5, CXCL12, and CX3CL1) are described to be able to induce pain or decrease central analgesic effects of opioid receptor agonists in animals without inflammation (Chen et al., 2007; Oh et al., 2001). Chemokines could also emerge as potential modulators of neuron-microglia interaction in opioid tolerance-related neuroinflammation and chronic neuropathic pain (Biber et al., 2008; Old and Malcangio, 2012). The chemokine receptor CXCR3 could be activated by its ligands including CXCL9, CXCL10 and CXCL11 (Loetscher et al., 1998). Activation of CXCR3 is involved in NMDA-induced hippocampal cell death (van Weering et al., 2011), entorhinal cortex lesion (Rappert et al., 2004) and brain ischemia (Biber et al., 2001) by inducing neuron-microglia interaction. Previous study has reported that CXCL10 was upregulated in nervous system with neuroinflammatory pain (Müller et al., 2010). Recently, our studies have shown that single morphine administration promoted CXCL10 expression in spinal neurons, while blocking the function of CXCL10 could enhance the effect of morphine analgesia in cancer pain animal (Ye et al., 2014; Bu et al., 2014). In addition, CXCR3 was co-localized with neuron, astrocyte and microglia in bone cancer pain model (Guan et al., 2015), suggesting CXCL10/CXCR3-related neuron-microglia interaction may play a critical role in the formation of bone cancer pain and morphine analgesic effect. Activation of microglia could be responsible to neuronal changes and aggravate the development of morphine tolerance by releasing pro-nociceptive factors such as chemokines (Horvath et al., 2010; Wang et al., 2010b). However, the role of CXCL10/CXCR3 in the morphine tolerance in PAG remains unclear. Thus, in the present study, we hypothesized that activation of CXCL10/CXCR3 signaling might participate in the mechanism of morphine tolerance by modulating neuron-microglia interaction.

2. Material and methods

2.1. Animals

Adult male Swiss Webster mice, weighing 20–25 g, were purchased from Animal Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Animals were housed under a 12-hour light/dark cycle at room temperature of 22 ± 1 °C and relative humidity 40–60% with food and water available ad libitum. The experimental protocols were reviewed and approved by Institutional Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science & Technology. All experimental protocols and animal handling procedures were carried out in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals.

2.2. Drug administration

Animals were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally). In order to facilitate injection, stainless cannula guides (0.60 mm external and 0.35 mm internal diameters) was implanted unilaterally into dorsal part of PAG (− 4.6 mm posterior to bregma, +/− 0 mm lateral to the midline and − 2 mm ventral to the dorsal surface of the skull) according to the previous study (Masse et al., 2008). A metallic cannula dummy was placed in the cannula guides after surgery to avoid blood clots. Animals were allowed a 7-day recovery period before the following experiments.

The following drugs were micro-injected into PAG 30 min before morphine administration, respectively: CXCR3 inhibitor AMG487 (10 or 20 μg, 0.25 μL, diluted in 20% 2-hydroxypropyl-β-cyclodextrin, once daily; Sigma, St. Louis, MO, USA), microglia inhibitor minocycline (10 pmol, 0.25 μL, diluted in saline, once daily; Sigma, St. Louis, MO, USA) (Wei et al., 2008; Eidson and Murphy, 2013a), recombinant mouse CXCL10 protein (rmCXCL10; 20 μg, 0.25 μL, diluted in saline, once daily; ProSpec-Tany TechnoGene, Rehovot, Israel).

2.3. Chronic morphine tolerance

To induce chronic morphine tolerance, mice were repeatedly administrated with morphine subcutaneously (10 mg/kg, twice daily with 12 h intervals), from day 1 (D1) to day 7 (D7) (Zhou et al., 2010; Ferrini et al., 2013).

2.4. Mechanical nociceptive tests

Nociceptive thresholds of mice were assessed by measuring paw withdrawal thresholds via von Frey filaments as described previously (Liu et al., 2012; Zhou et al., 2013). Briefly, behavioral tests were performed on day 0 (D0) and 30 min after morphine administration from day 1 to day 7. Mice were tested individually in a deep rectangular stainless-steel tank and allowed 15 min for habituation before tests. The region between foot pads in the plantar aspect of right hind paw was stimulated by a series of von Frey hairs with logarithmically incrementing forces (0.04, 0.07, 0.16, 0.4. 0.6, 1, 2, 4, 6, 8, 10, 15 and 26 g). Abrupt paw withdrawal, licking, or shaking was considered as positive responses. Once a withdrawal response was elicited, the test would be repeated starting with the next descending filament until no response occurred. An interval of 10 s was applied between the stimulations of filaments. The lowest amount of force that elicits a positive response was recorded. Three trials were performed on each animal with a time interval of 10 min, and the average value was considered as the paw withdrawal thresholds, represented in grams (g). All behavioral tests were conducted under blind conditions.

2.5. Real-time polymerase chain reaction

Total RNA was isolated from PAG with Trizol (Invitrogen, Carlsbad, CA). RNA was used to synthesize cDNA with SuperScript Reverse Transcriptase (Invitrogen, Carlsbad, CA). Real-time PCR reaction was performed with StepOne Real-time PCR System (Applied Biosystems) according to the manufacturer's instructions. Specific primers for mouse CXCR3 and endogenous control mouse GAPDH were obtained from PrimerDepot (Table 1). Relative quantification of mRNA was determined by using $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Data were presented as fold changes normalized to control group.

2.6. Western blot analysis

Animals were sacrificed and total proteins of PAG tissues were extracted immediately. The tissues were homogenized in a radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Wuhan, China) containing 1% Phenylmethanesulfonyl fluoride. The protein concentration was determined by BCA assay (Boster, Wuhan, China). After denatured by boiling in a sample buffer, 50 μg proteins from each sample were separated on SDS polyacrylamide gel and then transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA) by electrophoresis. The membranes were blocked with 5% non-fat milk

Table 1
Primers for real-time PCR.

Target genes	GeneBank accession no.	Primers
CXCR3	NM_009910.2	Forward: 5′-ACAGCACCTCTCCCTACGAT − 3′ Reverse: 5′-AATCTGGGAGGGCAAAGAGC − 3′
GAPDH	NM_008084.2	Forward: 5′-ATGAGAGAGGCCCAGCTACT-3′ Reverse: 5′-ACTGTGCCGTTGAATTTGCC-3′