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Early single Aspirin-triggered Lipoxin blocked morphine anti-nociception tolerance through inhibiting NALP1 inflammasome: Involvement of PI3k/Akt signaling pathway

Yu Tian [a], Ming Liu [a], Qi-Liang Mao-Ying [a], Huan Liu [a], Zhi-Fu Wang [a], Meng-Ting Zhang [a], Jun Wang [a], Qian Li [a], Shen-Bin Liu [a], Wen-Li Mi [a], Hong-Jian Ma [a], Gen-Cheng Wu [a,b], Yan-Qing Wang [a,b,*]

[a] Department of Integrative Medicine and Neurobiology, School of Basic Medical Sciences, Shanghai Medical College, Fudan University, Shanghai 200032, China
[b] Institutes of Brain Science, Brain Science Collaborative Innovation Center, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China

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ABSTRACT

Clinical usage of opioids in pain relief is dampened by analgesic tolerance after chronic exposure, which is related to opioid-associated neuroinflammation. In the current study, which is based on a chronic morphine tolerance rat model and sustained morphine treatment on primary neuron culture, it was observed that Akt phosphorylation, cleaved-Caspase-1-dependent NALP1 inflammasome activation and IL-1β maturation in spinal cord neurons were significantly enhanced by morphine. Moreover, treatment with LY294002, a specific inhibitor of PI3k/Akt signaling, significantly reduced Caspase-1 cleavage, NALP1 inflammasome activation and attenuated morphine tolerance. Tail-flick tests demonstrated that pharmacological inhibition on Caspase-1 activation or antagonizing IL-1β dramatically blocked the development of morphine tolerance. The administration of an exogenous analogue of lipoxin, Aspirin-triggered Lipoxin (ATL), caused a decline in Caspase-1 cleavage, inflammasome activation and mature IL-1β production and thus attenuated the development of morphine tolerance by inhibiting upstream Akt phosphorylation. Additionally, treatment with DAMGO, a selective μ-opioid receptor peptide, significantly induced Akt phosphorylation, Caspase-1 cleavage and anti-nociception tolerance, all of which were attenuated by ATL treatment. Taken together, the present study revealed the involvement of spinal NALP1 inflammasome activation in the development of morphine tolerance and the role of the μ-receptor/PI3k–Akt signaling/NALP1 inflammasome cascade in this process. By inhibiting this signaling cascade, ATL blocked the development of morphine tolerance.

1. Introduction

Morphine anti-nociception tolerance, characterized as progressive loss of analgesic potency after continuous morphine exposure that necessitates dose escalation to achieve equal pain relief, is one of the major problems associated with long-term usage of morphine in clinical pain management. Understanding mechanisms of morphine tolerance and identification of solutions are of clinical significance.

Among the extensive studies regarding the mechanism underlying morphine tolerance, the contribution of opioid-induced neuroinflammation has been well documented (Wang et al., 2012). IL-1β, one of the major pro-inflammatory cytokines, was reported to be highly up-regulated by chronic morphine treatments (Johnston et al., 2004; Raghavendra et al., 2002; Tai et al., 2006). Either blocking IL-1R or neutralizing IL-1β was shown to be effective in blocking the development of tolerance (Chen et al., 2012b; Johnston et al., 2004; Shavit et al., 2005). IL-1β possesses biological activity only after proteolysis (Denes et al., 2012), however, the modulation on IL-1β maturation after chronic morphine treatments remains unclear. It was reported that chronic morphine tolerance could be completely blocked by continuous intrathecal infusion of Aspirin-triggered Lipoxin (ATL), an exogenous analogue of lipoxin (Jin et al., 2012). As the first identified endogenous anti-inflammatory and pro-resolving lipid mediator derived from arachidonic acid (Chiang et al., 2000), lipoxin was worthy of noticing. It was shown to decrease pro-inflammatory cytokine production and to promote the resolution of acute inflammation (Freire and Van Dyke, 2013). In addition to blocking the development of

* Corresponding author at: Department of Integrative Medicine and Neurobiology, Shanghai Medical College, Fudan University, Mail Box 291, 138 Yi-Xue-Yuan Road, Shanghai 200032, China.
E-mail address: wangyanqing@shmu.edu.cn (Y.-Q. Wang).

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morphine tolerance, ATL was reported to be effective in alleviating inflammation-, neuropathy- and cancer-associated pain (Hu et al., 2012; Li et al., 2013; Svensson et al., 2007). Preliminary data demonstrated that development of morphine tolerance was significantly blocked at early stages by single ATL treatment only when delivered at certain time points. However, the intriguing underlying mechanism occurred at this particular time point has yet to be determined.

Recently, the regulation on a multi-protein complex platform responsible for IL-1 family member maturation, called inflammasomes, was reported. The inflammasome is a Caspase-1-containing and activity-dependent multi-protein complex involved in innate immune response and responsible for IL-1β maturation (Martinon et al., 2002). The components of inflammasomes include the NAcht leucine-rich-repeat proteins (NALP) protein family member, Caspase-1 and adaptor protein apoptosis-associated speck-like protein (ASC), which contains a Caspase-activating recruitment domain that connects the NALPs with Caspase-1 (Lamkanfi, 2011; Martinon et al., 2002). The composing NALP family member, recognized as the inflammasome subtype-defining protein, differs in regard to the particular type of tissue and specific evoking stimuli (Karatas et al., 2013; Lamkanfi et al., 2011; Martinon et al., 2002). NALP1-, NALP3- and IPAF-containing inflammasomes are among the most researched inflammasome subtypes. NALP1/NLRP1 inflammasome was a subtype of inflammasomes that mostly resided in neurons of central nervous system so far as known. Neuronal NALP1 inflammasomes existed as pre-assembled state and were activated in acute phase after the onset of injuries in central nervous system such as ischemic stroke and brain injury (Abulafia et al., 2009; de Rivero Vaccari et al., 2009, 2008), as well as retinal ganglion cell death as complication of acute glaucoma (Chi et al., 2014). The NALP1 inflammasome-mediated neuronal IL-1β release caused the post-injury inflammatory cascade thus determined the outcome of injury (Dietrich and Bramlett, 2010). Fluctuation in potassium concentration was the only reported mechanism in mediating NALP1 inflammasome activation by far (Pelegrin and Surprenant, 2006; Silverman et al., 2009). The involving signaling pathway in inflammasome activation was still unknown. Results from our previous studies on neuropathic pain research based on rodent model demonstrated that alleviated neuropathic pain after ATL treatment was attributed to reduced IL-1β production and maturation resulting from spinal inflammasome inhibition (Li et al., 2013). Considering that morphine tolerance and neuropathic pain shared common in certain mechanisms (Mayer et al., 1999), we hypothesized that inhibited inflammasome activation might be involved in attenuated morphine tolerance after ATL administration at particular time points. If this was the case, the exact inflammasome subtype and the underlying signaling pathway needed to be determined.

The present study demonstrated the involvement of the cascade of spinal μ-receptor/PI3k–Akt signaling/NALP1 inflammasome in the development of morphine anti-nociception tolerance; The blocking effect of single ATL administration on the development of morphine tolerance was attributed to the attenuated spinal NALP1 inflammasome activation. Our study provides a new perspective in understanding the underlying mechanism of morphine tolerance and suggests a promising therapy in clinical pain management.

2. Material and method

2.1. Subjects

Pathogen-free male Sprague-Dawley rats weighing 180–200 g and 8-week-old C57BL/6 mice from The Animal Center of Shanghai Institute of Biological Science, The Chinese Academy, were used in all the experiments. The animals were group-housed in temperature-controlled (23 ± 1 °C) room of 12-h light-dark cycle with access to standard chow and water *ad libitum*.

All procedures described here strictly followed the rules issued by National Institutes of Health guide for care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All the experiments were performed with the approval of Animal Care and Use Committee in Fudan University, and adhered to guidelines of Committee for Research and Ethical Issues of IASP. All efforts were made to reduce the number of animals used and to minimize their sufferings.

2.2. Chronic morphine tolerance induction and tail-flick test

Morphine hydrochloride was purchased from No. 1 Pharmaceuticals of Shenyang, China. Chronic morphine tolerance was induced by a 5-day course of subcutaneous morphine administration. Animals were given morphine injections twice daily with 12-h interval at a dose of 10 mg/kg body weight for five consecutive days. To examine the anti-nociceptive effect of morphine, tail-flick test was conducted after each morning injection. For value measurement, one third of tail-length from distal was immersed into hot water (52 ± 0.5 °C for rat; 48 ± 0.5 °C for mice), the time latency until a brisk tail flick was recorded before and 30 min after morphine injection for baseline and experimental value respectively. A 12-s cut-off was set to prevent permanent tissue damage from the heat. Quadruple measurements were done for each value. The maximum potential efficiency (MPE %) was calculated as the followings formulation:

$$\text{MPE } \% = (\text{Experimental Value} - \text{Baseline Value})/(12\ s\text{-Baseline Value}) \times 100\%$$

2.3. Drug delivery and lumbar puncture

Aspirin-triggered-Lipoxin (ATL, from Calbiochem, Merck) and IL-1β neutralizing antibody (R&D) were dissolved in normal saline; Caspase-1 inhibitor YVAD (Calbiochem, Merck), PI3k/Akt pathway inhibitor LY294002 (Cell Signaling) and Boc-2 (Phoenix Pharmaceuticals) were dissolved in DMSO, and diluted into working solution using normal saline.

Drugs were delivered into the spinal space via lumbar puncture with a 30-gauge needle between L5 and L6 vertebrae as described previously (Xu et al., 2006). Animals were anesthetized with inhaled mixture of 1.5% isoflurane/air using gas delivery system (Baxter). Once the tip of the needle was in, a brisk tail twitch was considered as indication of the exact intrathecal spot. A volume of 20 μl drug-containing fluid was introduced into spinal space. Boc-2 was administrated an hour before ATL delivery; ATL and other drugs were intrathecally delivered 2 h before 4th morphine injection if no specific notification.

2.4. Primary culture

Primary spinal neuron and astrocyte culture were prepared as previous described with brief alteration. Without notification all of the culture medium and buffer were from GIBCO. Spinal cords were harvested from newly born SD rats (within 24 h) and gathered in 10 cm petri-dish with chilled HBSS. The meninges-free spinal cords were subjected to trypsin digestion and triturated into single cell suspension. The cell pellets after centrifugation were re-suspended with complete culture medium, plated onto poly-L-Lysine (Millipore) coated culture plates and maintained in 5% CO_2, 37 °C incubator. For neuronal cultures, after 4 h of attachment, culture medium was replaced with Neurobasal culture

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