IL-10 Stimulates Microglia and Neurons to Produce Endogenous Opioid Peptides and Reduce Neuro-Inflammatory Chronic Pain

Miao Zhang
The High School Affiliated to Renmin University of China, Beijing, China
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Abstract: Chronic pain, persisting beyond three months, affects millions worldwide with no effective treatments. While some anti-inflammatory cytokines like IL-4 can trigger macrophages to produce pain-relieving opioid peptides, the role of IL-10 in this process remains unexplored. This study investigates IL-10's potential to stimulate opioid peptide production in neurons and glial cells and reduce pain. Using immunofluorescent staining, enzyme immunoassays, and the Von Frey test, we examined cells treated with LPS and cytokines, and assessed pain reduction in mouse models. Our findings suggest that IL-10 can trigger neurons and microglia to produce opioid peptides, albeit less efficiently than IL-4. These results indicate a potential new target for chronic pain treatment, combining IL-4 and IL-10 therapies, offering a promising alternative to current pain management strategies.
***Keywords*:** microglia, neuroinflammation, anti-inflammation, cytokine, endogenous opioid peptide, IL-4, IL-10

1. Introduction

Chronic pain, persisting beyond three months, is a growing global health concern. In 2021, 20.9% of American adults reported chronic pain, with 6.9% experiencing high-impact chronic pain that significantly limits daily activities (Rikard et al., 2019-2021). This condition disrupts normal life and negatively impacts various aspects of health, including mental well-being (Smith et al., 2001).

Despite its prevalence, effective treatments remain elusive. Current management strategies, including opioid therapy, often prove inadequate and can lead to complications such as addiction (Chou et al., 2020). In the United States, over 3 million people have experienced opioid use disorder (Azadfard et al., 2023), underscoring the urgent need for alternative approaches.

Existing tools merely manage chronic pain to an acceptable level (Hylands-White et al., 2016), with 67% of cancer patients with chronic pain reporting daily analgesic use (Glajchen, 2001). This paper explores a potential new treatment focusing on anti-inflammatory substances to reduce neuroinflammation in chronic pain. The objective of our research is to contribute to the development of more effective and safer chronic pain management strategies. To this end, we review relevant basic research, plan experiments to test our hypothesis, and discuss the likely outcomes.

2. Literature Review

2.1 Chronic pain

Chronic pain, lasting over three months, affects approximately 30% of adults worldwide (Ji et al., 2014). It can be neuropathic, nociceptive, or nociplastic, significantly impacting physical health, daily activities, emotions, work, and finances (Smith et al., 2001; Vergne-Salle & Bertin, 2021). Current treatments focus on pain management rather than relief, including painkillers, physical treatments, and cognitive behavior therapy (Hylands-White et al., 2016). Traditional painkillers may be ineffective for chronic pain and carry risks, leading to opioid use despite addiction potential. Some patients benefit from deep brain stimulation, while psychological treatments like commitment therapy help cope with chronic pain (Hylands-White et al., 2016).

2.2 Opioid Treatment for chronic pain

Opioid peptides, which are known for their analgesic properties, have been demonstrated to inhibit the pro-inflammatory factors that trigger hyperalgesia (Busch-Dienstfertig & González-Rodríguez, 2013). Dynorphin A 1-17, met-enkephalin and β-endorphin act as neurotransmitters and neuromodulators, exerting analgesic effects (Holden et al., 2005; Celik et al., 2020). These peptides act on μ-, δ-, and κ-receptors, thereby reducing neuronal excitability. This is achieved by blocking voltage-gated Ca^{2+} channels and opening K^{+} channels (Holden et al., 2005). The effects of opioids are similar and they are

used for various types of pain (Chau et al., 2008; Pasternak, 1993). Nevertheless, the use of opiates for the treatment of chronic pain is limited due to the occurrence of adverse effects and potential ineffectiveness.

2.3 Neuroinflammation (Inflammatory pain)

Neuroinflammation is an important factor in the development of chronic pain following injury, infection or stress (Ji et al., 2014). It involves the infiltration of immune cells, the activation of glial cells and the release of inflammatory substances in the nervous system. This response, which is coordinated by neurons, macroglia and microglia, involves cytokines, chemokines and the complement system (Isik et al., 2023). The diagnosis of neuroinflammation in the CNS is more difficult, but this form of inflammation has been shown to be more effective in the development and maintenance of pain (Ji et al., 2014). Inflammatory pain, the most prevalent form, occurs in both cancer and neuropathic pain (Busch-Dienstfertig & González-Rodríguez, 2013). The release of inflammatory mediators in the periphery leads to a reduction in the activation thresholds of the nociceptors, which enables an increased response of the pain receptors to weaker stimuli (Ji et al., 2014; Vergne-Salle & Bertin, 2021).

2.4 Microglia

Microglia, the main innate immune cell of the central nervous system (Rossum and Hanisch, 2003), is a cerebral macrophage of myeloma origin (Eggen et al., 2013). It plays a crucial role in inflammatory neurodegenerative disorders and brain inflammation (Shabab et al., 2016).Microglia comprise about 10% of all cells in the central nervous system (Colonna & Butovky, 2017). Their primary function is tissue defense and protection in immune system mechanisms (Rossum & Hanisch, 2003). Microglia emit trophic substances promoting neuronal circuit growth and survival (Nayak et al., 2014). They also sculpt neuronal synapses, regulate synaptic transmission intensity, and can induce cell death in poorly differentiated neurons (Colonna & Butovsky, 2017; Nayak et al., 2014).

2.5 Microglia phenotype

Microglia can alter their physiological state in response to changes in the neural microenvironment, leading to activation (Dubbelaar et al., 2018). The M1 phenotype has cytotoxic effects, while the M2 phenotype has neuroprotective effects, influencing neuroinflammation and pain regulation (Tang & Le, 2015). M2 microglia promote debris clearance, wound healing and tissue homeostasis by producing anti-inflammatory cytokines (IL-4 and IL-13) (Dubbelaar et al., 2018). In the event of a confrontation with pathology and damage, microglia undergo a phenotypic switch to the M2 type in order to carry out tissue repair (Dubbelaar et al., 2018). This phenotypic plasticity allows microglia to adapt to a variety of environmental signals and to fulfill a wide range of functions in the CNS.

2.6 IL-4

IL-4 is a versatile anti-inflammatory cytokine produced mainly by activated T cells (Busch-Dienstfertig & González-Rodríguez, 2013). It plays multiple roles in immune regulation, including promoting T-cell proliferation, stimulating B cells, activating macrophages, and aiding in chronic inflammation and wound healing. In animal studies, IL-4 reduced pain sensitivity and lowered levels of inflammatory cytokines (Busch-Dienstfertig & González-Rodríguez, 2013). IL-4 activates the JAK-STAT pathway, promoting pain relief by activating opioid receptors and promoting pro-opiomelanocortin (POMC) production. It also shifts microglia to an M2 phenotype when activated by LPS, contributing to pain management (Fenn et al., 2012).

2.7 IL-10

Interleukin 10 (IL-10) is a major anti-inflammatory cytokine generated by activated immune cells, essential for regulating immune responses (Ip et al., 2017). It suppresses excessive inflammatory responses by targeting various leukocytes (Ouyang et al., 2010). IL-10 regulates inflammatory responses through the Stat3 pathway, activating Jak1 and Tyk2, leading to Stat3 phosphorylation (Sanjabi et al., 2009). It also inhibits inflammation by regulating metabolic pathways like mTOR signaling, preventing mTORC1 activation via STAT3, affecting the switch from OXPHOS to glycolysis, and reducing inflammatory responses after TLR stimulation (Ip et al., 2017). These mechanisms contribute to IL-10's potent anti-inflammatory effects and potential role in pain modulation.

3. Hypothesis

This study investigates IL-10's influence on opioid peptide secretion by neurons and microglia and its impact on chronic pain. We hypothesize that IL-10 stimulates these cells to produce endogenous opioid peptides, reducing neuroinflammation-induced chronic pain. To test this, we will:

(1) Compare immunofluorescent images of control, LPS, LPS+IL-4, and LPS+IL-10 groups.

(2) Detect intracellular opioid peptide content in primary microglia groups.

(3) Measure opioid peptide content in mixed primary microglia and neuron cultures.

(4) Test mechanical hypersensitivity in a chronic pain mouse model.

The null hypothesis states that IL-10 cannot reduce chronic pain. Through these experiments, we aim to elucidate IL-10's potential role in pain management and opioid peptide production.

4. Methods

4.1 Cell Experiments

Primary microglia (Shanghai Anwei Biotechnology Co., Ltd.) were cultured in DMEM with 10% FBS, antibiotics, and antifungals at 37°C, 5% CO2. Cells were divided into four groups: control, LPS, LPS+IL-4, and LPS+IL-10. LPS (100 ng/ml) was added to non-control groups for 2 hours, followed by IL-4 (200 ng/ml) or IL-10 (100 ng/ml) overnight incubation.For immunofluorescent staining, cells were fixed with 4% PFA, stained with Actin-Tracker Green-488 and DAPI, then mounted and imaged using Echo Revolve Microscope.Enzyme immunoassays were performed on primary microglia and mixed microglia-neuron cultures (50% each). Cells were lysed, and supernatants were incubated with antibodies against ENK, END, or DYN. After adding streptavidin-HRP and tetramethylbenzidine, absorbance was measured at 450 nm. Duplicate measurements were taken for each sample.These experiments aim to visualize cellular responses and quantify opioid peptide production under different conditions, providing insights into IL-10's effects on microglia and neurons.

4.2 Mice experiments

The experimental group consisted of male C57BL/6 mice that were seven to eight weeks old at the time of the experiment. The animals were from a standard FMMU breeding facility. The experimental group was divided into four subgroups: The experimental groups were divided into four groups: control group, CFA, CFA+IL-4 and CFA+IL-10. In the non-control groups, chronic inflammatory pain was induced by CFA injection (50% CFA, 10 μl). One week after the CFA injection, the mice were administered IL-4 (200 ng) or IL-10 (100 ng) intraperitoneally daily for a period of eight to ten days.

Mechanical sensitivity was determined using the Von Frey test according to the Dixon up-and-down paradigm. The animals were placed in plastic boxes with metal grid floors for a period of 30 minutes before the test began. Various bending forces (0.008–2 g) were applied to the hind paw. Licking, biting or sudden withdrawal were observed as positive reactions. The tests were performed before the cytokine injections, 5–60 minutes after them and 24 hours later. A 50% withdrawal threshold was defined as the pain threshold.

The aim of this study is to analyze the efficacy of IL-10 in reducing mechanical hypersensitivity in a model of chronic inflammatory pain and to compare it with IL-4 and control conditions.

5. Anticipated Results

5.1 IL-10 can reduce inflammatory response caused by LPS

LPS was used to excite primary rat microglia, which were then exposed to either IL-4 or IL-10 2 hours later. After 24 hours, functional alterations and transcription profiles were assessed. Based on observations from Lively and Schlichter's experiment (2018), we analyzed the results as our experimental protocols were similar.

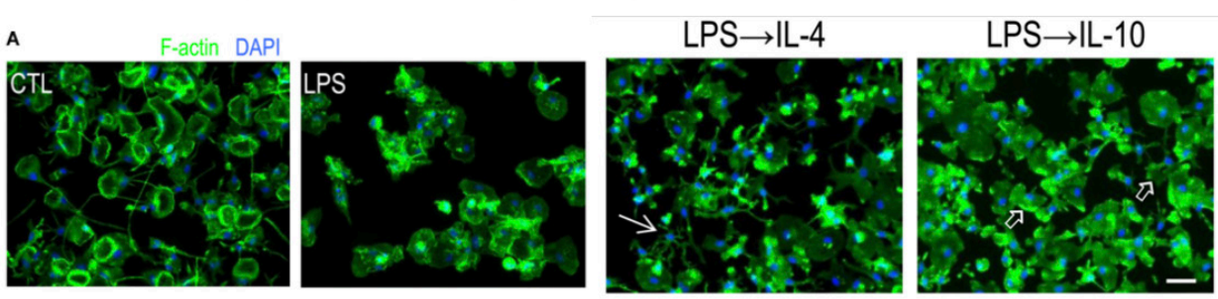

Figure 1. The fluorescence images

Figure 1 shows primary rat microglia that were treated with lipopolysaccharide (LPS) over a period of 24 hours. In addition, the cells were treated with interleukin-4 (IL-4) or interleukin-10 (IL-10). (adapted from Lively & Schlichter, 2018)

5.2 IL-10 increased the expression of opioid peptides

Figure 2 shows intracellular opioid peptide (ENK, END, DYN) content in primary microglia. The LPS group shows a slight, non-significant increase in all peptides compared to control. LPS+IL-4 demonstrates a significant increase in all peptides (Celik et al., 2020). Notably, LPS+IL-10 also shows a substantial increase, though lower than LPS+IL-4. DYN consistently shows the lowest concentration across all groups. These results suggest IL-10 can stimulate microglia to produce opioid peptides endogenously, albeit less efficiently than IL-4. DYN appears less responsive to cytokine stimulation in microglia.

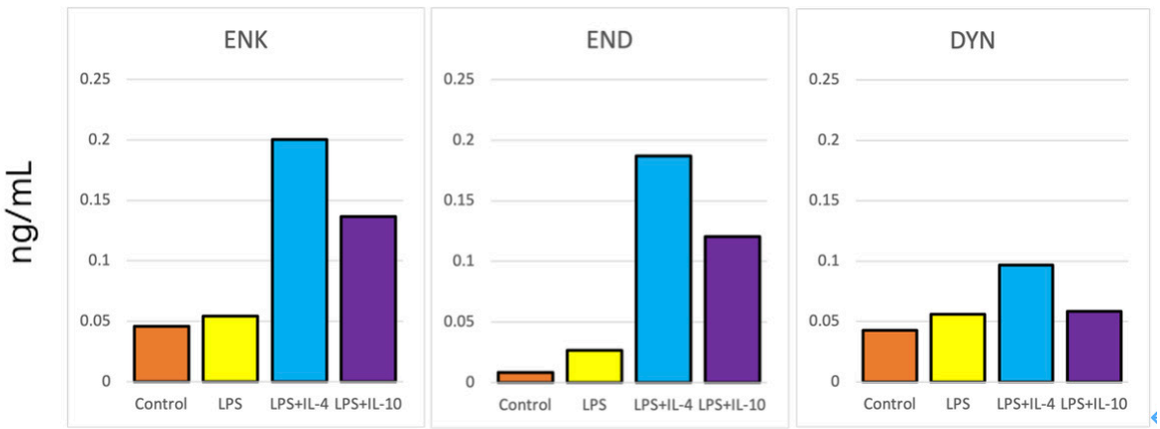

Figure 2. The intracellular content of the opioid peptides enkephalin (ENK), endorphin (END) and dynorphin (DYN) in primary microglial cells measured by enzyme-linked immunosorbent assay (ELISA), with the results plotted with idealised data

5.3 IL-10 can stimulate neurons to produce opioid peptides

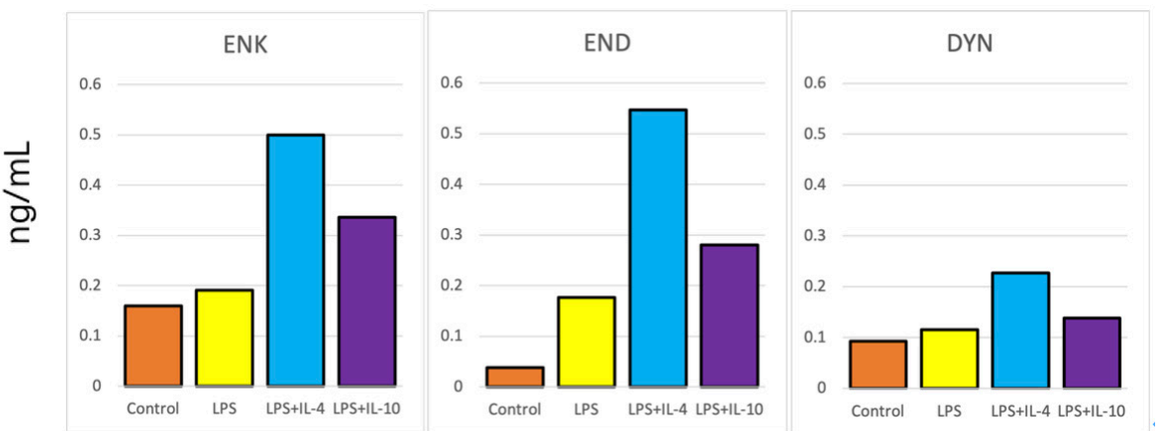

Figure 3. Opioid peptides ENK, END, and DYN intracellular content in the mixture of primary microglia and neurons (50% each) measured by enzyme immunoassays (drawn with idealized data)

Figure 3 illustrates the intracellular content of opioid peptides (ENK, END, and DYN) in a mixture of primary microglia and neurons (50% each), as measured by enzyme immunoassays.

The LPS group shows a trend of increased ENK and DYN compared to the control, with a significant increase in END. The LPS+IL-4 group demonstrates a significant growth in all opioid peptides compared to both control and LPS groups. The LPS+IL-10 group shows a significant increase in ENK and END, and a modest increase in DYN compared to control and LPS groups.

Notably, DYN levels are consistently lower across all groups. The LPS+IL-10 group shows lower peptide levels compared to LPS+IL-4, but higher than control and LPS groups. Overall, intracellular content of all peptides is higher in this neuron-microglia mixture compared to microglia alone (Figure 2).

These results indicate that IL-10 can stimulate neurons to produce opioid peptides, albeit with lower efficiency than IL-4. This neuron-specific response contributes to the overall increase in opioid peptide production observed in the mixed culture.

5.4 IL-10 reduces mechanical allodynia induced by CFA

The mechanical sensitivity was determined using a top-down Dixon model based on the response of the hind paw to the von Frey filament bending point (Wang et al., 2019). A comparison of the previously analysed results with the graph produced by Selleck and colleagues (2020), which depicts the outcomes of the von Frey test with IL-4 injection, suggests a high degree of similarity between the two sets of data. We concluded that IL-10 can stimulate microglia and neurons to produce opioid peptides but at a lower rate and a lower efficiency. The graph of IL-10 injection was anticipated to have the same trend as the graph of IL-4 but with a lower paw withdrawal threshold for all data checkpoints.

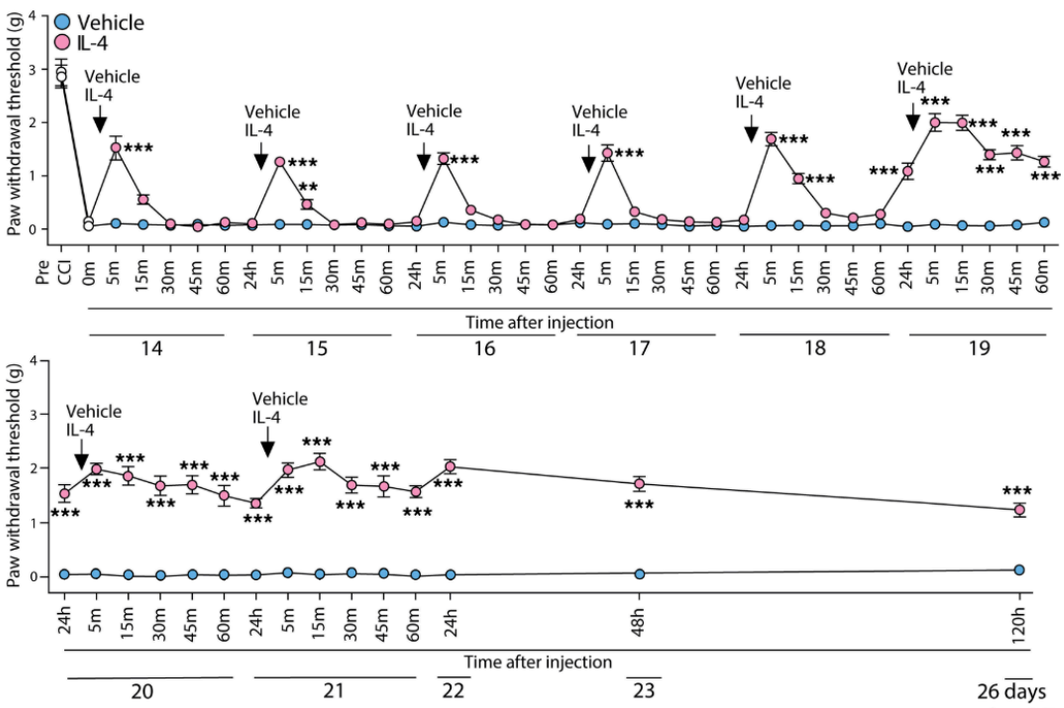

Figure 4. The time course of IL-4-induced analgesia

The time course of IL-4-induced analgesia was investigated by administering IL-4 on days 14-26 after chronic constriction injury. The analgesic effect was tested before, 5-60 minutes after, and 24 hours after injection of IL-4. The data were adapted from Celik et al. (2020).

6. Discussion

Our findings indicate that IL-10 can stimulate neurons and microglia to produce opioid peptides, potentially reducing mechanical hypersensitivity in neuroinflammation mouse models. However, IL-10 appears less efficient than IL-4 in inducing opioid peptide production, allowing us to reject the null hypothesis.

IL-10, a well-characterised anti-inflammatory cytokine, has been linked to pain modulation through the reduction of pro-inflammatory factors (Yanik et al., 2020). In contrast to IL-4, which directs microglia towards the M2 phenotype (Fenn et al., 2019), IL-10 has been observed to enhance opioid peptide production, potentially through alternative pathways.

Study limitations include the lack of physical testing, limited exploration of cytokine concentration effects, and incomplete understanding of IL-10-induced opioid peptide production mechanisms. Future research should address these gaps, including in-lab experiments, testing various cytokine concentrations, and investigating IL-10's specific pain reduction pathways.

Our findings suggest a potential new target for treating chronic pain, offering a safer and potentially more effective long-term alternative to opiate treatment. IL-10 injections could help chronic pain patients by reducing inflammation responses and associated pain.

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Author Bio

Miao Zhang (Dec 14, 2005) Female, Student (High school), The High School Affiliated to Renmin University of China, cellular biology/neuroscience.