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ORIGINAL RESEARCH

Sufentanil enhances M1/M2 phenotypic polarization transition and alleviates LPS-triggered neuroinflammation in BV2 cells

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Abstract

To investigate the regulatory effects of Sufentanil (Suf) in BV2 cells stimulated with lipopolysaccharide (LPS). Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay, apoptosis was evaluated *via* flow cytometry, levels of inflammation factors were quantified using Enzyme-Linked Immunosorbent Assay (ELISA), proportions of cluster of differentiation (CD)206 and CD16/32 were determined through flow cytometry, and mRNA and protein expressions were analyzed using Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) and western blot. Our findings demonstrate that Suf (10 μM and 20 μM) exerted no cytotoxic effects, and LPS stimulation of BV2 cells mimicked a neuropathic pain cell model. In addition, treatment using Suf enhanced cell viability and inhibited apoptosis in LPS-triggered BV2 cells, mitigated LPS-induced inflammation and promoted M1/M2 phenotypic polarization transition in BV2 cells, and attenuated the activation of the nuclear factor kappa-B (NF-κB) pathway induced by LPS treatment. In conclusion, Suf can enhance M1/M2 phenotypic polarization transition and attenuate LPS-mediated neuroinflammation in BV2 cells.

Keywords

Sufentanil; M1/M2 phenotypic polarization; Neuroinflammation; Neuropathic pain

1. Introduction

Neuropathic pain, characterized by hyperalgesia and allodynia, arises from damage to the somatosensory nervous system [1], significantly impacts patients' quality of life and imposes a substantial economic burden. Approximately 5–10% of the general population is affected by neuropathic pain [2]. Despite its high prevalence, the precise mechanisms driving neuropathic pain remain incompletely understood. Thus, there is an urgent need to identify effective therapeutic agents and elucidate the underlying regulatory mechanisms.

Current neuropathic pain treatment is primarily based on anesthetic drugs to alleviate discomfort. Sufentanil (Suf), a lipophilic opioid agonist with selectivity for μ-opioid receptors, possesses analgesic and sedative properties. Also, being a derivative of fentanyl, Suf exhibits a significant affinity for μ-receptors and is widely utilized for cardiovascular response control [3]. It has been shown that the intrathecal administration of Suf can induce considerable analgesia in rats with neuropathic pain while markedly suppressing the expression of N-methyl-D-aspartic acid receptor (NMDAR) and calcitonin gene-related peptide (CGRP) [4]. Additionally, Suf effectively reduces the expression of inflammatory cytokines in rats with middle cerebral artery occlusion (MCAO) and improves neurological functions [5]. Moreover, Suf modulates the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, thereby alleviating endothelial barrier dysfunction induced by oxygen-glucose deprivation/reoxygenation [6]. Despite these findings, further investigations are warranted to elucidate the regulatory mechanisms of Suf.

The pathogenesis of neuropathic pain involves metabolic damage, central sensitization, excessive microglial activation, and stimulation of inflammatory mediators. Numerous studies have highlighted the critical role of excessive microglial activation in neuropathic pain [7]. However, the regulatory effects of Suf on M1/M2 polarization in neuropathic pain have yet to be elucidated.

In this study, we explore the regulatory effects of Suf on M1/M2 polarization in LPS-stimulated BV2 cells.

2. Materials and methods

2.1 Cell culture and treatment

The BV2 cells were purchased from the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) medium (Gbico, 11995065, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, 10099-141, Gbico, Grand Island, NY, USA) at 37 °C with 5% CO_2. To simulate a neuropathic pain cell model,

BV2 cells were treated with LPS (100 ng/mg) for 24 hours [8, 9]. Subsequently, BV2 cells were treated with varying concentrations of Suf (0 μM, 10 μM, 20 μM and 40 μM) for 24 hours.

2.2 CCK-8 assay

BV2 cells were seeded in a 96-well plate at a density of 1 $\times$ 10^4 cells per well. After 24 hours of incubation, 10 μL of CCK-8 solution (5 mg/mL, CA1210, Solarbio, Shanghai, China) was added to each well, following which cell viability was assessed using a spectrophotometer (ND-ONE-W, Thermo Fisher Scientific, Waltham, MA, USA) at an absorbance rate of 450 nm.

2.3 Reverse transcription-quantitative PCR (RT-qPCR)

RNA extraction from BV2 cells was performed using TRIzol reagent (Invitrogen, 15596018, Carlsbad, CA, USA). Subsequently, RNA was reverse transcribed into cDNA using the PrimeScript® RT reagent kit (Takara, RR037B, Kusatsu, Japan). Quantitative PCR (qPCR) was conducted using the SYBR® Premix Ex Taq™ II kit (Takara, RR820A, Dalian, China), and the relative mRNA expression was determined using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were as follows:

Interleukin-1 beta (IL-1β):
F: 5′-CGACAAAATACCTGTGGCCT-3′,
R: 5′-TTCTTTGGGTATTGCTTGGG-3′;
IL-6:
F: 5′-GAAACCGCTATGAAGTTCCTCTCTG-3′,
R: 5′-TGTTGGGAGTGGTATCCTCTGTGA-3′;
Tumor Necrosis Factor-α (TNF-α):
F: 5′-CATCTTCTCAAAATTCGAGTGACAA-3′,
R: 5′-TGGGAGTAGACAAGGTACAACCC-3′;
IL-10:
F: 5′-GCTCTTACTGACTGGCATGAG-3′,
R: 5′-CGCAGCTCTAGGAGCATGTG-3′;
Glyceraldehyde-3-phosphate dehydrogenase (GADPH):
F: 5′-AGGTCGGTGTGAACGGATTTG-3′,
R: 5′-GGGGTCGTTGATGGCAACA-3′.

2.4 ELISA

Commercial ELISA kits were utilized to quantify the levels of tumor necrosis factor-α (TNF-α, ab208348, Abcam, Shanghai, China), interleukin-1β (IL-1β, ab197742), IL-6 (ab222503), and IL-10 (ab255729).

2.5 Flow cytometry

For cell apoptosis assessment, the fluoresceine isothiocyanate (FITC) Annexin V apoptosis detection Kit (556547, BD Biosciences, San Jose, CA, USA) was used. Briefly, BV2 cells were stained with FITC Annexin V (5 μL) and propidium iodide (5 μL) in the dark, following which cell apoptosis was analyzed using flow cytometry (BD FACSCalibur, BD Biosciences, San Jose, CA, USA).

Regarding changes in macrophage subsets, BV2 cells were incubated with CD16/32 antibody (553141, 1:100, BD Biosciences, San Jose, CA, USA) or anti-CD206 antibody (MAB25351, 1:100, R&D Systems, Inc., Minneapolis, MN, USA) in the dark. The proportions of M1-type macrophages (CD16/32) and M2-type macrophages (CD206) were then determined using flow cytometry.

2.6 Western blot

The proteins were isolated from BV2 cells using radio immunoprecipitation assay (RIPA) lysis buffer (P0013B, Beyotime, Shanghai, China) supplemented with protease and phosphatase inhibitors (Roche, 4906837001, Beijing, China). Protein separation was achieved through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto polyvinylidene difluoride (PVDF) membranes (Sigma, St Louis, USA). Primary antibodies were incubated with the membranes for 12 hours, followed by a secondary antibody (1/5000; ab6721) for 2 hours. The protein blots were visualized using the enhanced chemiluminescence system (Thermo Fisher Scientific, USA).

The primary antibodies used in these experiments included p-p65 (1/1000; ab76302), p65 (0.5 μg/mL; ab16502), p-inhibitor of NF-κB alpha (IκBa, 1/10,000; ab133462), IκBa (1/1000; ab32518), and β-actin (1 μg/mL; ab8226).

2.7 Statistical analysis

The data are expressed as mean $\pm$ standard deviation (SD), and statistical analyses were conducted using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Each experiment was performed at least three times. Comparisons between two groups were assessed using Student's *t*-test, and comparisons among multiple groups were evaluated using One-way analysis of variance (ANOVA). A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1 Sufentanil facilitated cell viability and suppressed cell apoptosis in LPS-stimulated BV2 cells

Considering that our initial experiments indicated that cell viability remained unchanged following treatment with 10 μM and 20 μM Suf but was reduced after exposure to 40 μM Suf (Fig. 1A), 10 μM and 20 μM Suf were selected for subsequent investigations. Additionally, while LPS induction led to a decrease in cell viability, this effect was reversed following treatment with Suf (10 μM and 20 μM) (Fig. 1B). Furthermore, it was observed that LPS treatment increased cell apoptosis, which could be mitigated by Suf treatment (10 μM and 20 μM) (Fig. 1C,D). Taken together, these findings indicate that Suf promotes cell viability and suppresses cell apoptosis in LPS-stimulated BV2 cells.

3.2 Sufentanil attenuated inflammation in LPS-induced BV2 cells

Assessment of inflammation factors revealed that the mRNA expressions of IL-1β, IL-6 and TNF-α were upregulated, while IL-10 was downregulated following LPS induction. However,

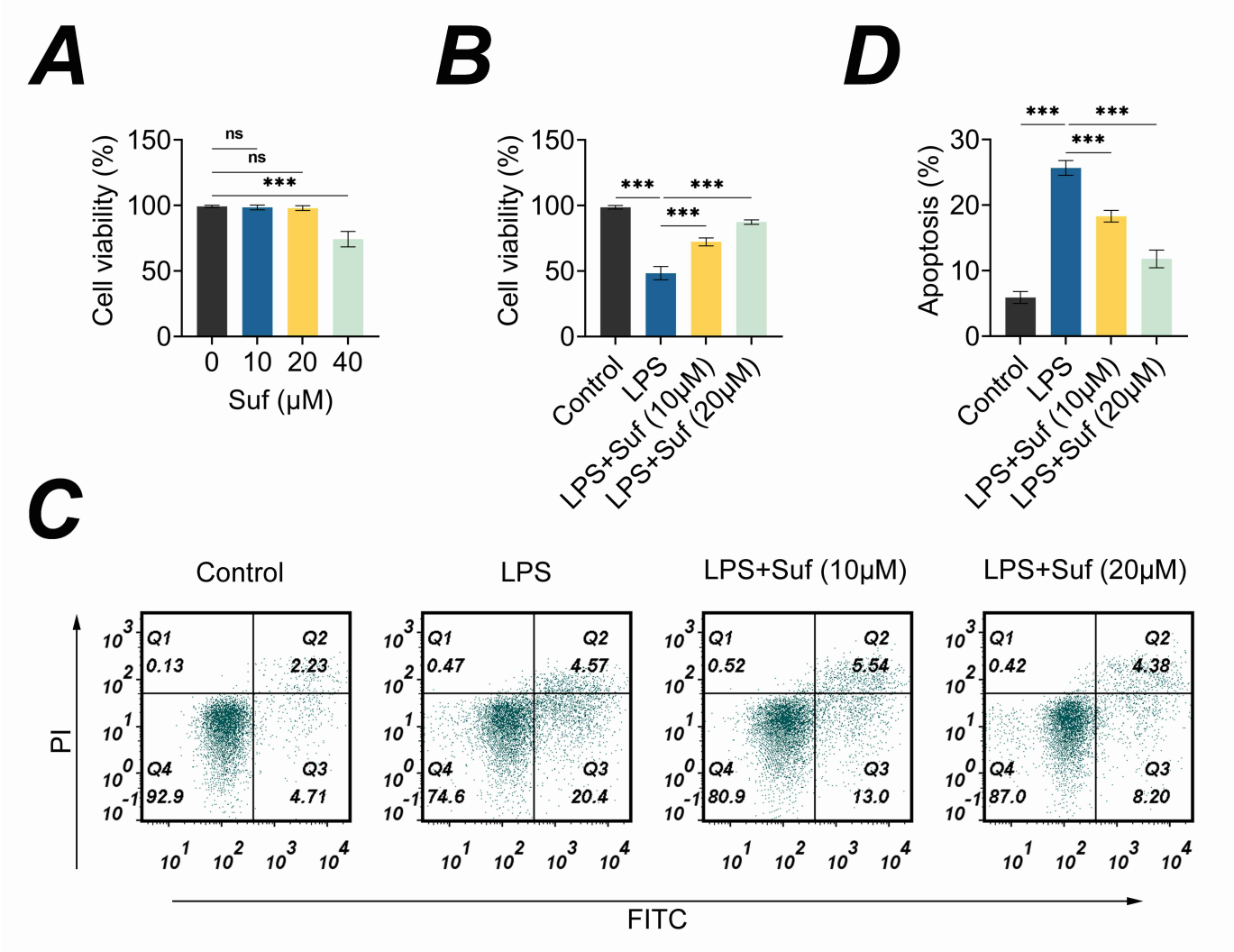

FIGURE 1. Sufentanil enhances cell viability and suppresses cell apoptosis in LPS-triggered BV2 cells. (A) Cell viability assessed using the CCK-8 assay with varying concentrations of Sufentanil (Suf) (0 μM, 10 μM, 20 μM and 40 μM). (B) Confirmation of cell viability in Control, LPS, LPS + Suf (10 μM), and LPS + Suf (20 μM) groups using the CCK-8 assay. (C,D) Measurement of cell apoptosis in Control, LPS, LPS + Suf (10 μM), and LPS + Suf (20 μM) groups through flow cytometry. ***$p < 0.001$. Suf: Sufentanil; LPS: lipopolysaccharide; ns: no significance; PI: propidium iodide; FITC: fluoresceine isothiocyanate.

these effects could be effectively counteracted following treatment with Suf (10 μM and 20 μM) (Fig. 2A). Consistently, ELISA results showed similar alterations in the levels of IL-1β, IL-6, TNF-α and IL-10 (Fig. 2B). Overall, these findings support that Suf can attenuate inflammation in LPS-induced BV2 cells.

3.3 Sufentanil enhanced M1/M2 phenotypic polarization transition in LPS-stimulated BV2 cells

The proportions of CD206 (M2 phenotype marker) and CD16/32 (M1 phenotype marker) were assessed to confirm macrophage subtype changes. Following LPS induction, CD206 expression decreased while CD16/32 expression increased. However, these alterations were reversed after treatment with Suf (10 μM and 20 μM) (Fig. 3). Collectively, these findings indicate that Suf enhances the transition from M1 to M2 phenotypic polarization in LPS-stimulated BV2 cells.

3.4 Sufentanil retarded the NF-κB pathway in LPS-mediated BV2 cells

Following LPS induction, the results showed that the expressions of p-p65 and p-IκBa were both increased, while IκBa protein expression was reduced. In addition, these effects could be reversed after treatment with Suf (10 μM and 20 μM) (Fig. 4), suggesting that Suf inhibits the NF-κB pathway in LPS-mediated BV2 cells.

4. Discussion

Suf has been investigated for its potential therapeutic benefits in various diseases. However, its regulatory effects in the progression of neuropathic pain require further exploration. In this study, we first confirmed that Suf (10 μM and 20 μM) did not exhibit cytotoxicity. Subsequently, LPS induction in BV2 cells was employed to mimic a neuropathic pain cell model [8, 9]. Our findings demonstrated that Suf treatment enhanced cell viability and suppressed cell apoptosis in LPS-triggered

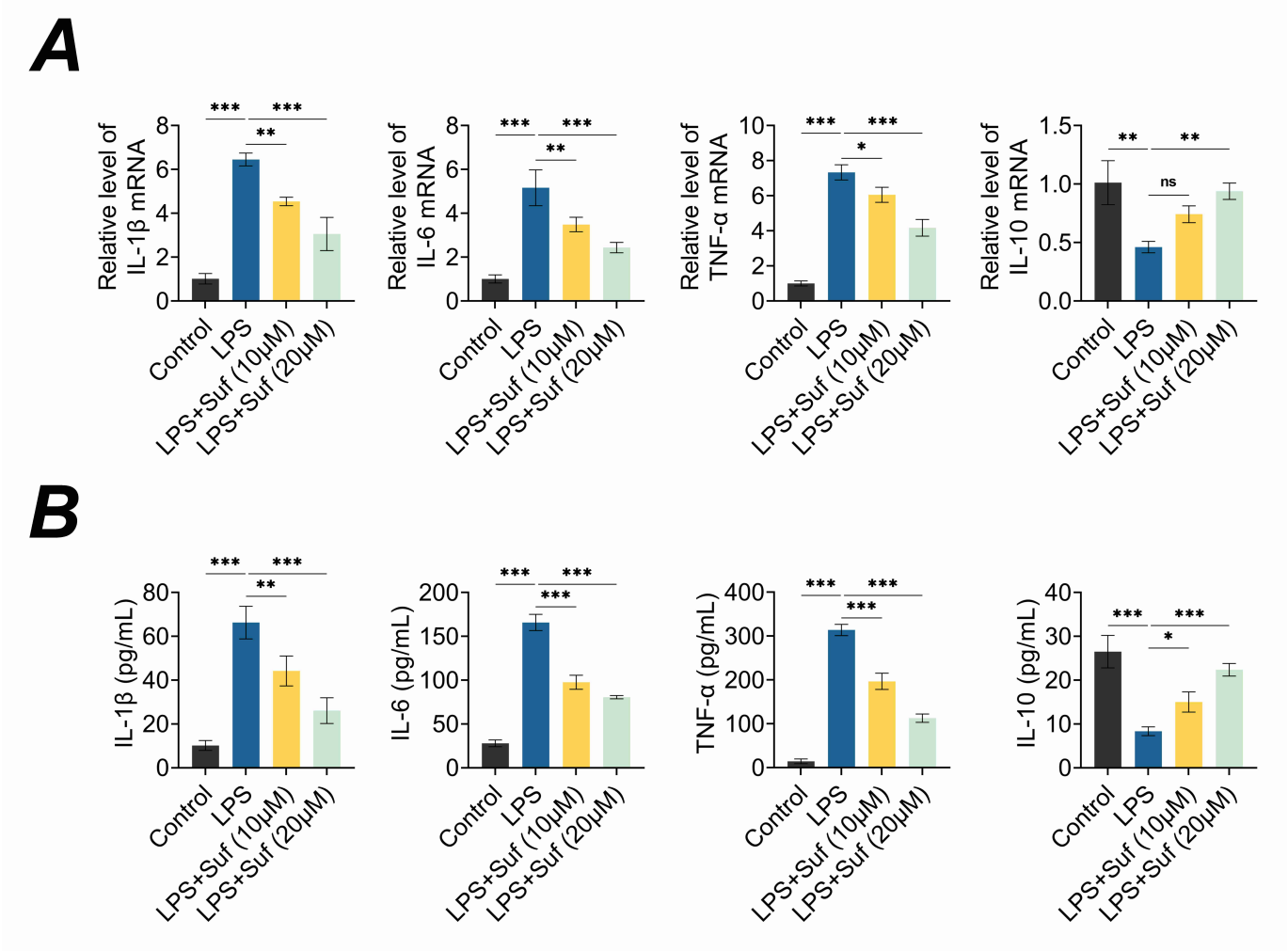

FIGURE 2. Sufentanil attenuates inflammation in LPS-induced BV2 cells. (A) Assessment of mRNA expressions of IL-1β, IL-6, TNF-α and IL-10 in Control, LPS, LPS + Suf (10 μM), and LPS + Suf (20 μM) groups *via* RT-qPCR. (B) Analysis of IL-1β, IL-6, TNF-α and IL-10 levels in Control, LPS, LPS + Suf (10 μM), and LPS + Suf (20 μM) groups using ELISA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Suf: Sufentanil; LPS: lipopolysaccharide; IL-1β: interleukin-1β; TNF-α: tumor necrosis factor-α; ns: no significance.

BV2 cells.

Inflammation plays a critical role in various diseases [10], including neuropathic pain and other chronic pain conditions, and in these contexts, proinflammatory and anti-inflammatory factors are known to exert important functions. For instance, hydroxytyrosol has been shown to alleviate oxidative stress and inflammation, thereby improving neuropathic pain [11]. Conversely, Fascin-1 has been implicated in exacerbating neuropathic pain in rats by promoting inflammation [12]. Additionally, enhancer of zeste homolog 2 (EZH2) methyltransferase has been reported to attenuate neuroinflammation associated with neuropathic pain [13]. In our study, we observed heightened inflammation following LPS treatment, which was mitigated by Suf treatment (10 μM and 20 μM).

Microglia, one of the most important immune cells in the nervous system, undergo polarization into M1 and M2 phenotypes within 24 hours of a nerve injury. M1 polarization promotes a proinflammatory phenotype associated with neuropathic pain, while M2 polarization exhibits an anti-inflammatory phenotype that counteracts M1 polarization [14]. Activated microglia trigger proinflammatory responses and release proinflammatory factors, leading to neuronal damage and the development of neuropathic pain [15]. Several studies have investigated M1/M2 polarization in neuropathic pain. For instance, Paeonol was found to modulate microglial M1 and M2 polarization, thereby alleviating neuropathic pain [16]. Similarly, stigmasterol has been shown to regulate microglial M1/M2 polarization and mitigate neuropathic pain [17]. Moreover, electroacupuncture has been found to regulate programmed cell death 1 ligand 1 (PD-L1) expression, promoting M2 microglia polarization and ameliorating neuropathic pain [18]. In this study, we observed that Suf enhanced the transition from M1 to M2 phenotypic polarization in LPS-stimulated BV2 cells.

Previous research has shown that Suf inhibits the activation of the NF-κB pathway [19]. Notably, in BV2 cells, suppression of the NF-κB pathway has been demonstrated to promote microglial M2 polarization, thus mitigating neuroinflammation [20]. Consistent with these findings, our study revealed that LPS treatment activated the NF-κB pathway and that this

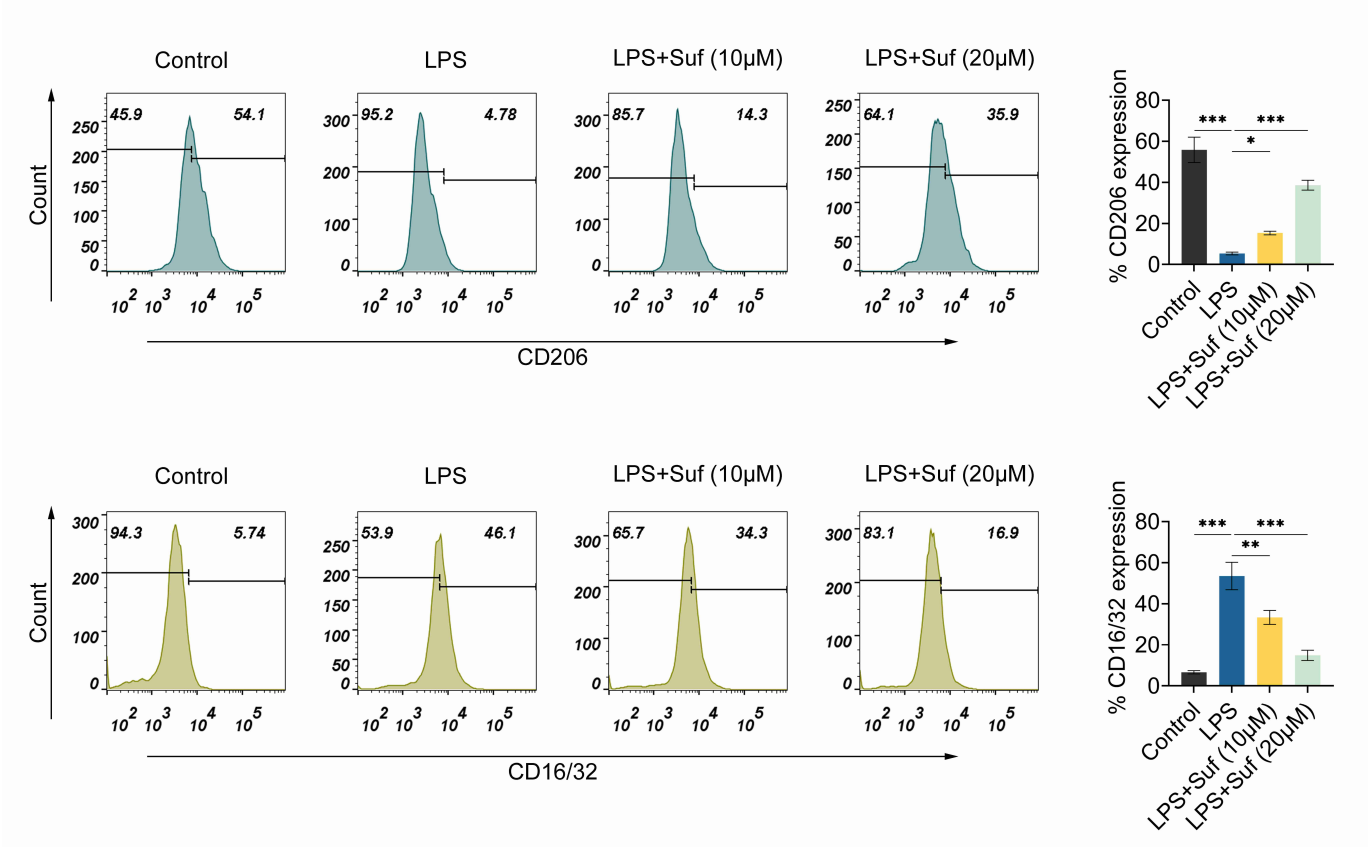

FIGURE 3. Sufentanil enhances M1/M2 phenotypic polarization transition in LPS-stimulated BV2 cells. Flow cytometry analysis of CD206 and CD16/32 proportions in Control, LPS, LPS + Suf (10 μM), and LPS + Suf (20 μM) groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Suf: Sufentanil; LPS: lipopolysaccharide; CD: cluster of differentiation.

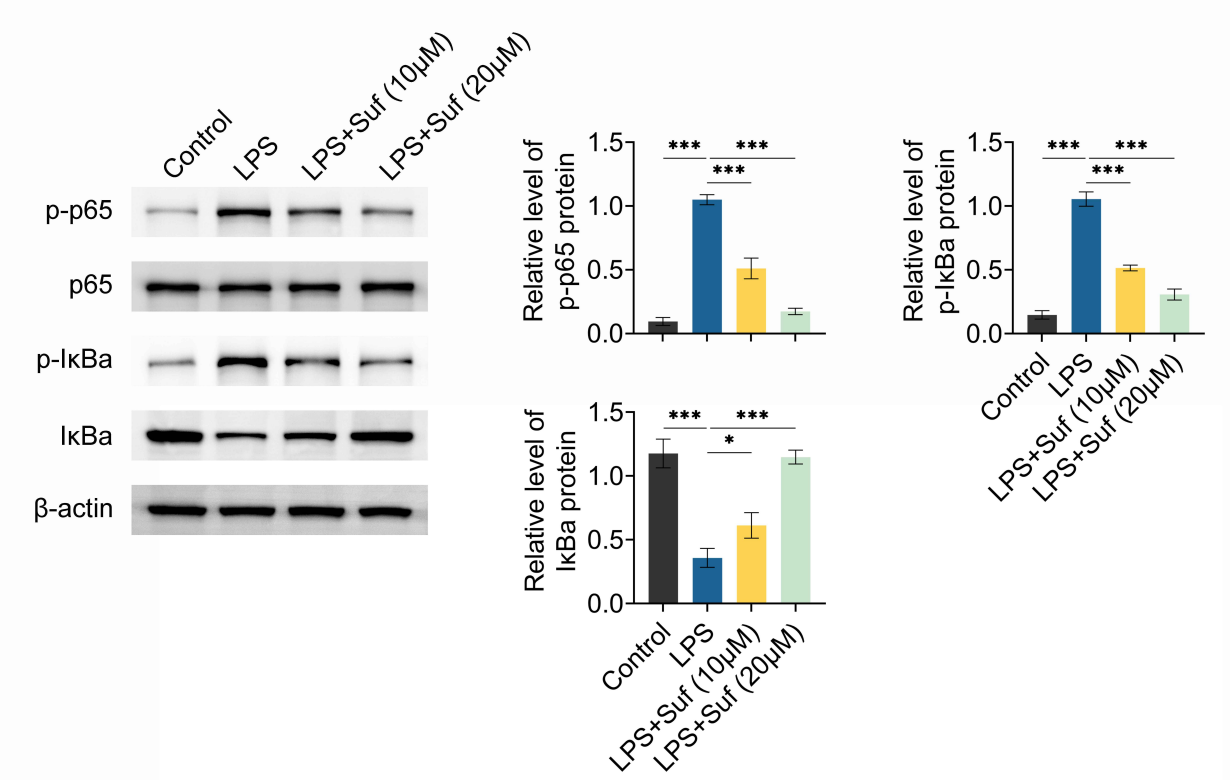

FIGURE 4. Sufentanil inhibits the NF-κB pathway in LPS-mediated BV2 cells. Western blot analysis of p-p65, p65, p-IκBa and IκBa protein expressions in Control, LPS, LPS + Suf (10 μM), and LPS + Suf (20 μM) groups. *$p < 0.05$, ***$p < 0.001$. Suf: Sufentanil; LPS: lipopolysaccharide; IκBa: inhibitor of NF-κB alpha.

effect could be effectively attenuated following treatment with Suf (10 μM and 20 μM).

5. Conclusions

This study represents the first demonstration that Suf enhances the transition from M1 to M2 phenotypic polarization and alleviates LPS-induced neuroinflammation in BV2 cells. Future investigations are needed to further elucidate additional regulatory effects of Suf in the progression of neuropathic pain.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

JRY and HL—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. JRY, HL, SLB and CSD—supervised the data collection, analyzed and interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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