European Review for Medical and Pharmacological Sciences 2021; 25: 5268-5274

Effects of remifentanil on human C20 microglial pro-inflammatory activation

N. CAPPOLI[1], P. ACETO[2,3], E. TABOLACCI[4,5], D. MEZZOGORI[6], L. SOLLAZZI[2,3], P. NAVARRA[1,5], C. DELLO RUSSO[1,5]

[1]Università Cattolica del Sacro Cuore, Dipartimento di Sicurezza e Bioetica, Sezione di Farmacologia, Rome, Italy
[2]Fondazione Policlinico Universitario A. Gemelli IRCCS, Dipartimento di Scienze dell'Emergenza, Anestesiologiche e della Rianimazione, Rome, Italy
[3]Università Cattolica del Sacro Cuore, Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Rome, Italy
[4]Università Cattolica del Sacro Cuore, Dipartimento di Scienze della Vita e Sanità Pubblica, Sezione di Medicina Genomica, Rome, Italy
[5]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[6]Università Cattolica del Sacro Cuore, Dipartimento di Neuroscienze, Sezione di Fisiologia, Rome Italy

Abstract. – OBJECTIVE: Remifentanil (RF) is a potent short-acting μ-opioid receptor agonist. Although preferred for its unique pharmacokinetics, the clinical use may be limited by hyperalgesia. Preclinical studies have shown a potential role of microglia on the development of hyperalgesia, with limited and conflicting evidence on RF. Considering the role of microglia in the initiation and maintenance of brain inflammation and their different responses among species, we aimed at characterizing RF effects on human adult microglia *in vitro*.

MATERIALS AND METHODS: RF was tested at clinically relevant concentrations on the human microglial C20 cell line. Expression and release of interleukin-6 (IL-6) and brain derived neurotrophic factor (BDNF) were assessed under basal and inflammatory conditions.

RESULTS: The expression and secretion of IL-6 significantly increased in C20 cells in response to pro-inflammatory cytokines. RF did not modify this response neither under basal nor under inflammatory conditions. No toxicity due to RF was detected. The drug displayed a modest stimulatory effect on the production of BDNF.

CONCLUSIONS: Although RF does not exert direct pro-inflammatory actions on human adult microglia, its effects on BDNF, a crucial mediator of pain transmission, suggest a possible role on neuroinflammation and pain perception.

Key Words:
Human microglia, Remifentanil, Opioid-induced hyperalgesia, Brain derived neurotrophic factor, Interleukin-6, Molecular biomarkers, Pharmaco-therapy.

Introduction

Remifentanil (RF) is a potent μ-opioid receptor agonist whose main advantages are a short action and a lack of cumulative effects[1]. Like for other opioids, both analgesic and main adverse effects are mediated by the activation of μ-opioid receptors (MORs)[2], encoded by the *OPRM1* gene in humans[3]. Both chronic and acute opioid use have been associated with the development of opioid-induced hyperalgesia (OIH), with a prevalence of 14-28% in different clinical settings[4]. In the perioperative one, OIH is characterized by an abnormal increase of pain intensity[5]. It is the main cause of prolonged hospitalization, increased opioid consumption and side effects[1]. OIH is more often associated with the use of RF, particularly when used at high doses and after rapid withdrawn[1].

The pathogenesis of OIH is not completely elucidated[1]. Interestingly, OIH is apparently associated with the activation of the brain derived neurotrophic factor (BDNF) pathway[6]. In particular, morphine-induced hyperalgesia (MIH) in rats is mediated by increased microglial BDNF[7]. Consistently, the inhibition of microglial activation is associated with reduced MIH[8]. On the other hand, the role of microglia in the development of remifentanil-induced hyperalgesia (RIH) is poorly investigated. In one study, minocycline, a known inhibitor of microglial inflammatory response, did not exert any modu-

Corresponding Author: Paola Aceto, MD, Ph.D; e-mail: paola.aceto@policlinicogemelli.it

latory effect on RIH in rats[9]. In contrast, a more recent study showed a protective action of minocycline on the long-term potentiation at C-fiber synapses induced by RF withdrawal. The latter is a known mechanism underlying synaptic plasticity and hyperalgesia. In this setting, minocycline reduced microglial activation and tumor necrosis factor-α (TNF-α) release induced by RF withdrawal[10].

Considering that OIH comprises reciprocal signaling between neurons and microglia and given the significant differences in the immune response among species[11], it is relevant to characterize the effects of RF on human microglia. In this study, we used a novel *in vitro* experimental model of adult human microglial cells[12] to explore the hypothesis that RF, at clinically relevant concentrations[13], may modulate microglial activation, thus contributing to the development of neuroinflammation and OIH.

Materials and Methods

Materials

BrainPhys medium and N2 supplement were from StemCell Tecnologies (Vancouver, Canada). Fetal Bovine Serum, L-glutamine and penicillin/streptomycin antibiotics were purchased by Biochrom AG (Berlin, Germany). Normocin was from Invivogen (San Diego, CA, USA). Human recombinant TNFα and interleukin 1β (IL-1β) were produced in yeast (Thermo Fisher Scientific, Waltham, MA, USA). Human interferon γ (IFNγ, Sigma-Aldrich, Saint Louis, MO, USA) was produced in *Esherichia Coli* and certified as containing <0.1 EU/μg endotoxin. Cytokines were reconstituted in certified endotoxin free water (G-Biosciences, St. Louis, MO, USA) and further diluted, as previously detailed[14]. A mixture of all cytokines (TII = TNFα, IL-1β and IFNγ) was prepared and tested at different concentrations. Each cytokine was used at the same concentration in the mixture, *e.g.* 1 pg/ml TII is equal to 1 pg/ml TNFα + 1 pg/ml IL-1β + 1 pg/ml IFNγ.

Cell Cultures and RF Treatment

The human microglial C20 cell line was developed through SV40/hTERT (Simian Virus 40/human Telomerase Reverse Transcriptase) immortalization of primary cultures of human microglia derived from cortical tissue of adult subjects, kindly provided by Dr Álvarez-Carbonell (Case Western Reserve University, Cleveland, OH, USA)[12]. The cells were seeded at 20,000 cells/cm^2 and cultured in complete medium (CM), i.e. BrainPhys medium supplemented with 1% N2, 1% FBS, 2.5 mM L-glutamine and antibiotics (100 IU/ml penicillin, 100 μg/mL streptomycin and 100 μg/mL normocin). The cells were passed at 90-95% confluency. For functional studies, the cells were plated at 60,000 cells/cm^2 and treated the day after plating in CM. RF (ULTIVA, Aspen Pharma Trading Ltd, Dublin, Ireland) was dissolved in saline buffer at 50 μg/ml and further diluted in CM.

IL-6 and BDNF Measurements

IL-6 levels were quantified in the incubation media by DuoSet ELISA (R&D Systems, Minneapolis, MN, USA)[14]. The intracellular levels of mature BDNF were determined by a specific ELISA kit (Biosensis, Thebarton, South Australia), according to the manufacturer's instructions.

mRNA Analysis in Real Time PCR (qPCR)

Expression of inflammatory genes was quantified through qPCR[15]. Total RNA was extracted using TRIZOL (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and treated with DNase-free™ Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA). 1 μg RNA was converted into cDNA through ImProm II Reverse Transcriptase (Promega, Madison, WI, USA) using random hexamers. Amplification was performed in a final volume of 20 μl using Brilliant SYBR Green QPCR Master Mix (Agilent, Santa Clara, CA, USA) in a MX3000P real time PCR machine (Stratagene, Santa Clara, CA, USA), as it follows: 35 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. Microglial surface markers were assessed as previously described[16]. Previously validated primers were used for the assessment of *IL-6* mRNA (NM_000600.4), F394 (CCTTCCAAAGATGGCTGAAA) and R524 (TGGCTTGTTCCTCACTACT); and β-actin (*ACTB)* (NM_001101.4) F427 (TGGGACGACATGGAGAAA) and R573 (GAAGGTCTCAAACATGATCTGG)[14]. *BDNF* gene expression was assessed through a validated primer set: F1705 (AGTGCCGAACTACCCAGTCGTA) and R1780 (CTTATGAATCGCCAGCCAATTC) (http://www.rtprimerdb.org/assay_report.php?assay_id=352), yielding a 76 bp amplicon. Primers for *OPRM1* mRNA (NM_001008504.3)

were F1031 (CTCTTCAGCCATTGGTCTTC) and R1209 (GTCCATAGCACACGGTAATG), amplifying a region of 179 bp and validated *via* sequencing analysis. The relative quantification of target transcripts *vs. ACTB* endogenous level was calculated as previously detailed[15]. qPCR efficiency ranged between 96% and 107.9%. After qPCR, amplicons were resolved and correctly sized using 2% agarose gels with 0.1 μg/ml ethidium bromide (EB).

Sequencing Analysis

To verify the exact sequence of *OPMR1* qPCR products, cDNA was amplified by PCR using KAPA Taq ReadyMix PCR kit (KAPA Biosystems, Saint Louis, MO, USA) and products were resolved on 1.5% agarose gel with EB. PCR products were purified by Illustra™ ExoProStar™ (GE Healthcare, Rome, Italy), according to the manufacturer's protocol. They were sequenced in both strands with primers designed for qPCR through BigDye Terminator v3.1 Cycle Sequencing Kit. Sequencing products were purified with XTerminator™ Solution Buffer before separation on a 3130 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA).

Cell Morphology and Scanning Electron Microscopy (SEM) Analysis

C20 cell morphology was analyzed by labeling cytoskeletal F-actin filaments with phalloidin (ActinGreen™ 488 ReadyProbes™, Thermo Fisher Scientific, Waltham, MA, USA). The cells were seeded at 7,900 cells/cm^2 in 24-well plates with 12 mm glass coverslips (Glaswarenfabrik Karl Hecht Gmbh & CO KG, Sondheim vor der Rhön, Germany). After 24 hours of incubation in CM, the cells were washed with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} (PBSw/o) and fixed with 4% (w/v) paraformaldehyde for 15 min. They were then permeabilized with 0.25% of Triton X100 for 5 min. After 30 min of ActinGreen™ 488 probe incubation, the cells were washed twice with PBSw/o and 4',6-Diamidino-2-Phenylindole Dihydrochloride (DAPI, diluted 1/2000) was added for 10 min. Coverslips were mounted with ProLong mountant (Thermo Fisher Scientific, Waltham, MA, USA) and images captured using confocal microscopy (Olympus BX63, Segrate, MI, Italy).

For SEM analysis, after overnight incubation in CM, the cells were washed with 0.2 M PBS and treated with 1% Glutaraldehyde for 2-3 min. They were then sequentially dehydrated with 30%, 60%, 80% ethanol for 5 min each and twice with 100% ethanol for 5 min. Coverslips were dried in vacuum-assisted desiccator for 1-2 hours and stored at room temperature. Coverslips were then sprayed on the surface with an electrically conductive 40 nm thick layer of gold-palladium (60-40). SEM images were recorded with a scanning electron microscope (Supra™ 25, Zeiss, Milan, Italy).

Data Analysis

Data were analyzed from at least 3 different experiments on cells from different thaws/batches. Data were pooled to analyse IL-6 and BDNF expression and release/production by one-way ANOVA with Bonferroni's post hoc test, using GraphPad Prism 5 software (San Diego, CA, USA).

Results

Morphological and Molecular Characterization of C20 Microglial Cells

The morphology of C20 cells at basal conditions is shown in Figure 1A. SEM analysis diplayed different cell morphologies, allowing for the visualization of fine protrusions and filaments (Figure 1B-C). The staining for the actin cytoskeletal protein confirmed the presence of distinct morphologies *in vitro* (Figure 1D-F). Most C20 cells presented an elongated-bipolar shape, whereas few cells exhibited a globular morphology or a round-small shape. The same was also shown for the embryonic microglial cell line HMC3[14]. The expression of human microglial markers and lack of the macrophage chemokine receptor, CCR2, confirmed the human microglial origin of C20 cells (Figure 2A), as previously demonstrated for the HMC3 cells[16]. The lineage markers were identified within a specific human microglial transcriptomic signature[17] and assessed through qPCR. The mean threshold cycles, fixing 0.150 as threshold, are the followings: 32.47 for ionized calcium-binding adaptor protein-1 (*IBA1*); 34.63 for C-X3-C Motif Chemokine Receptor 1 (*CX3CR1*); 30.94 for the purinergic receptor *P2RY12*; 34.99 for transmembrane protein 119 (*TMEM119*); 27.26 for colony stimulating factor 1 receptor (*CSF1-R)* and 15.30 for *ACTB*. To test the effects of RF on the C20 cells, we first confirmed *OPRM1* expression at basal conditions (Figure 2B-C).

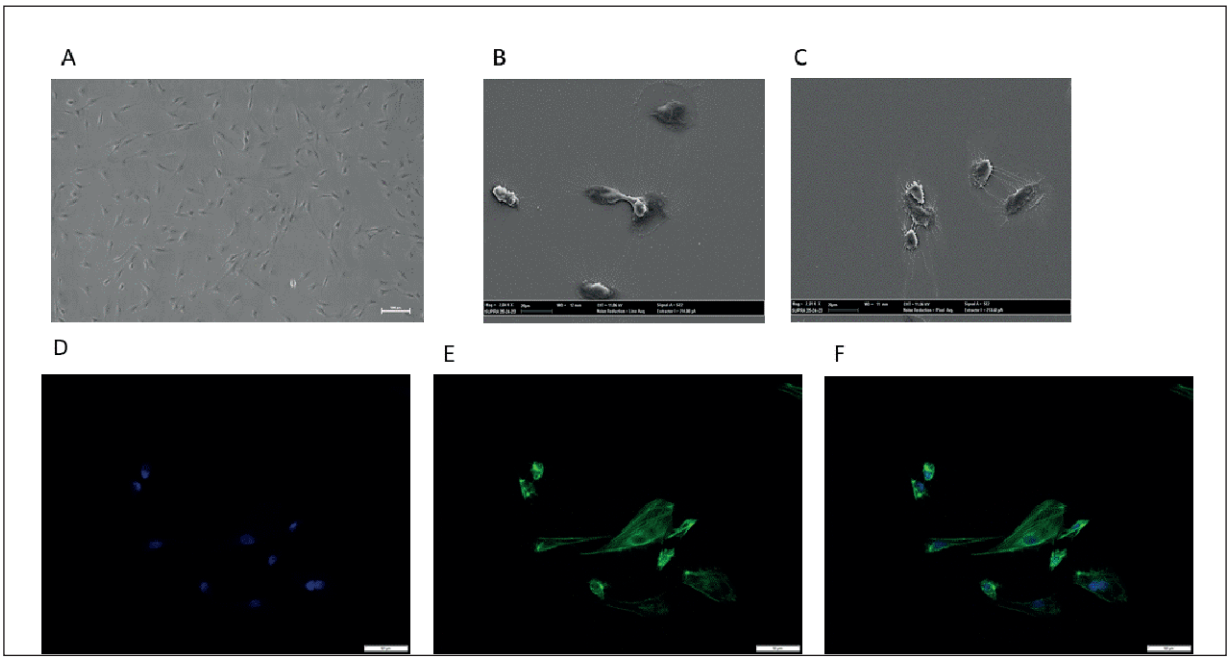

Figure 1. Morphology of C20 cells. **A,** Phase contrast microscopy of C20 cells at day 1 post plating. Scale bar 100 µm (10× magnification). **B-C,** Two representative SEM images. Scale bar 20 µm (2K-X magnification). **D-F,** Representative confocal images of DAPI nuclear staining *(blue)*, F-actin staining (*green*), and merge in (**F**) Scale bar 50 µm (20× magnification).

Proinflammatory Activation of C20 Microglia

C20 cells were treated for 16 hours with TII, tested in the concentration range of 1 pg/ml to 1 ng/ml. IL-6 release was quantified and taken as microglial activation readout[14,16]. Optimal response was reached at 50 pg/mL TII, with maximal release of IL-6 obtained at 1 ng/ml TII (Figure 3A). Increased IL-6 release was paralleled with significant hyperexpression of *IL-6* mRNA (Figure 3B). Preliminary proteomic analysis (PPA) revealed increased release of other in-

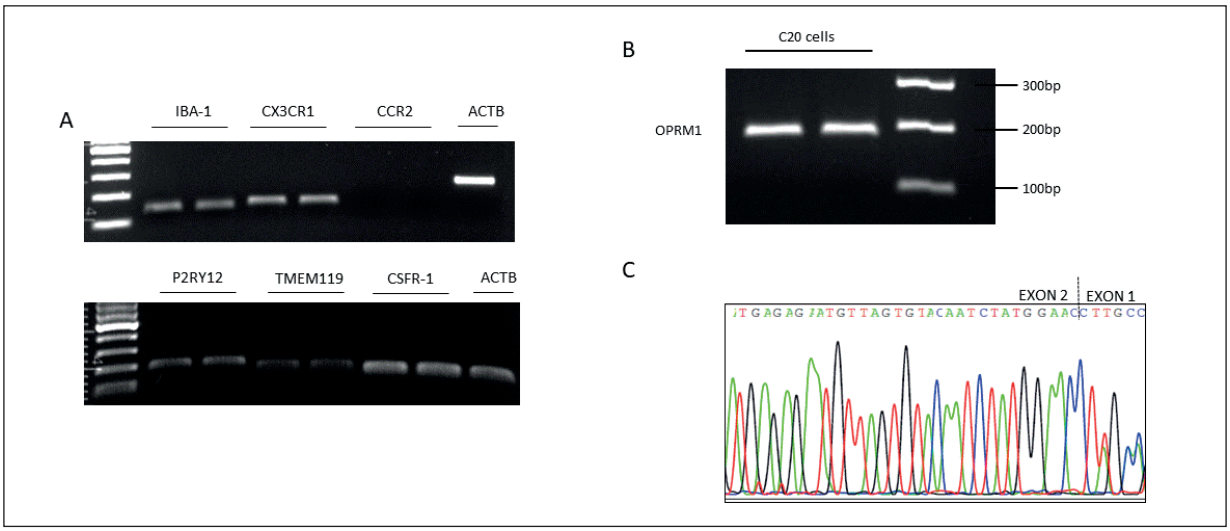

Figure 2. Expression of microglial lineage markers and *OPRM1*. Images show qPCR products resolved on 2% agarose gel. **A,** C20 cells express specific microglial lineage markers and lack the macrophage marker, CCR2. **B,** *OPRM1* expression in C20 cells. **C,** Electropherogram of *OPRM1* sequenced with reverse primer; dashed line delineates the boundary between exon 1 and 2.

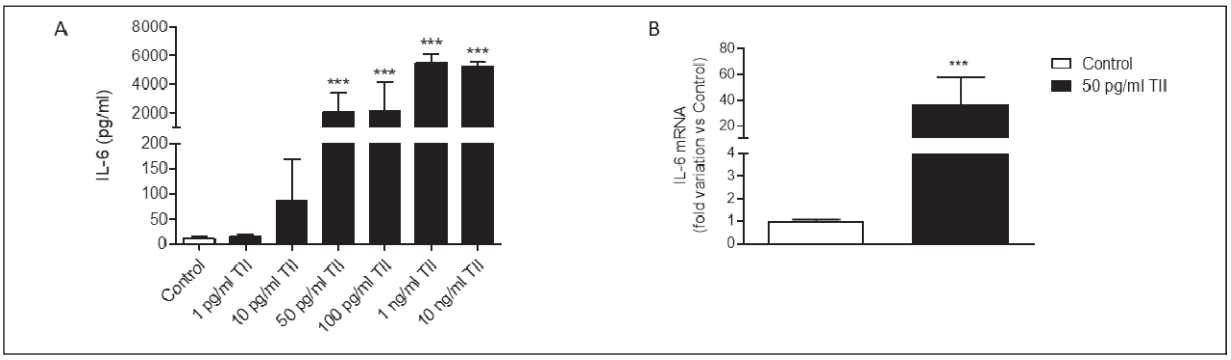

Figure 3. Proinflammatory activation of C20 cells and IL-6 release. **A,** C20 cells were stimulated with different concentrations of TNFα, IL-1β and IFNγ (TII) mixture for 16 hours. Data are shown as pg/ml, mean ± SD (n=11-27) of 6 independent experiments. ***, $p<0.001$ *vs.* Control. **B,** Quantification of IL-6 transcript by qPCR before and after TII stimulation. Data are expressed as fold variation *vs.* Control, means ± SD (n=12). ***, $p<0.001$ *vs.* Control.

flammatory chemokines, *i.e.,* the monocyte chemotactic protein 1 (MCP-1), IL-8 and the C-X-C Motif Chemokine Ligand (CXCL) 1 (CXCL1), in response to TII (data not shown).

Effects of RF on IL-6 and BDNF

When tested in a range of clinically relevant concentrations (1.25-20 ng/ml)[13], RF did not exert any toxic effect on C20 cells after 16 hours of incubation both under basal and inflammatory conditions. Cytotoxic effects of RF were excluded assessing cell viability[14] and lactate dehydrogenase activity in the incubation medium at the end of treatment (data not shown). When first tested in 6 hour-experiments, between 1.25 and 20 ng/ml, RF did not modify the release of IL-6 both at basal conditions and in cells activated with 50 pg/ml TII (data not shown). Similarly, when tested at 5 ng/ml in 16 hour-experiments, RF did not modulate neither the secretion (Figure 4A) nor the expression (Figure 4B) of IL-6 in C20 cells, at basal and inflammatory conditions. RF concentration of 5 ng/ml was selected according to the mean blood concentration detected during surgical anesthesia[13]. In PPA, 5 ng/ml RF increased the release of MCP1, IL-8 and CXCL1, whereas completely reduced the amount of CXCL12 (data not shown).

A significant increase of *BDNF* expression was observed after 16 hours of RF treatment at 5 ng/ml (Figure 4C). TII produced a modest increase of *BDNF* mRNA levels after 16 hour-treatment, although not modified by RF. Under basal condi-

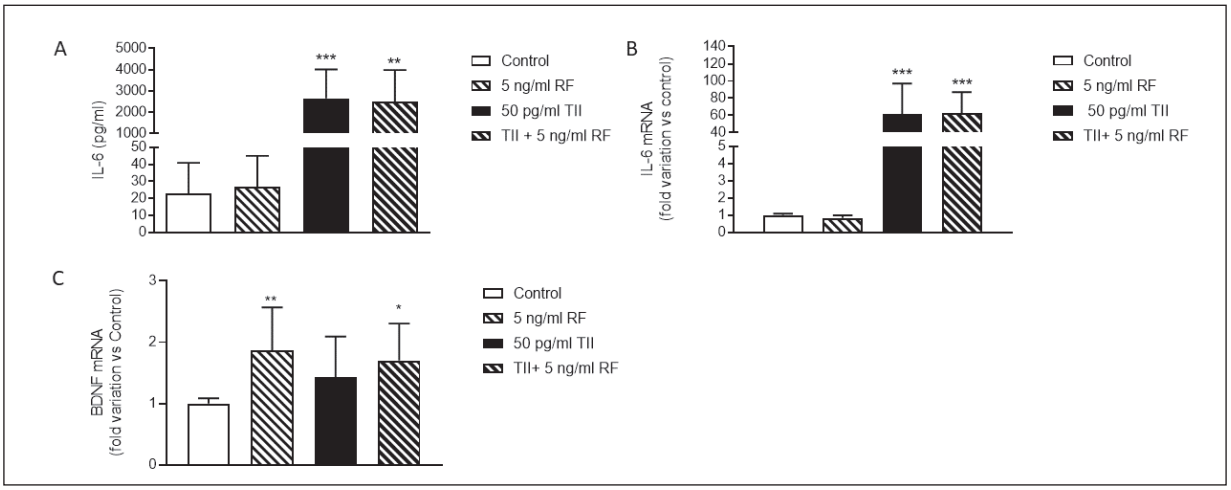

Figure 4. Modulatory effects of RF on IL-6. Cells were incubated in CM (Control) or activated with 50 pg/ml TII for 16 hours. RF at 5 ng/ml was tested under basal and inflammatory conditions. **A**, IL-6 levels are shown as pg/ml, mean ± SD of 4 different experiments (n=4-11). **, $p<0.01$ and ***, $p<0.001$ *vs.* Control. **B-C,** *IL-6* and *BDNF* mRNA levels were assessed by qPCR. Data are expressed as fold variation vs Control, means ± SD (n=9). *, $p<0.05$ and ***, $p<0.001$ *vs.* Control.

tions the level of mature BDNF in the incubation medium was below the detection limit of the assay. However, confluent C20 cell cultures produced sizable amount of mature BDNF, detected intracellularly at basal conditions. At the cell density used for functional experiments the intracellular levels of BDNF remained undetactable and were not modified by TII. However, 5 ng/ml RF increased the intracellular BDNF concentrations above the detection limit of the assay in 3 out of 5 replicates obtained from 3 different experiments (mean concentration 15.27 pg/ml ± 12.84 SD, n=5).

Discussion

In this study, we provide additional data on the inflammatory response of the human microglial C20 cell line[12]. When stimulated with TII, the C20 cells increase IL-6 expression and secretion, in line with previous observations[12,18,19]. RF did not modify IL-6 expression and release by C20 cells, neither at basal nor at inflammatory conditions, in contrast to what detected in peripheral immune cells[1]. Moreover, a PPA showed a marked release of pro-inflammatory chemokines in response to TII in C20 microglia. Thus, it is possible that, when exposed to inflammatory cytokines, microglial cells release factors relevant for the recruitment of peripheral immune cells and the maintenance of neuroinflammation. Interestingly, RF increased the release of MCP-1, IL-8, and CXCL1 and completely abolished CXCL12 secretion. Chemokines were studied by a PPA, evaluating the release of 36 pro- and anti-inflammatory molecules in a single sample per treatment. Thus, these data deserve future investigations to further analyze the effects of RF on other inflammatory pathways.

In this study, we observed a significant increase in the microglial expression of BDNF in response to RF treatment. However, RF did not significantly affect the expression of BDNF under inflammatory conditions in the C20 cells. The role of BDNF in OIH is not completely elucidated[20]. In a rat model, sensory neurons released BDNF in response to noxious signals, leading to the activation of astrocytes and microglia which sustained neuroinflammation and mechanical allodynia[21]. Conversely, exogenous BDNF displayed antinflammatory properties in the murine BV2 microglial cells exposed to the bacterial endotoxin, *via* upregulation of BDNF specific TrkB receptors and modulation of inflammatory transcription factors[22]. The regulation of BDNF signaling is complex, with increased activity associated to enhanced cleavage of pro-BDNF and production of mature BDNF. In our experimental conditions, mature BDNF was undetectable in the incubation media. This is in line with the rapid intracellular uptake of the neurotrophin observed *in vitro,* according to the datasheet of the BDNF ELISA kit. Interestingly, RF increased intracellular BDNF concentrations above the detection limit of the assay in 3 out of 5 replicates obtained in 3 different experiments, in agreement with the mRNA results.

Conclusions

We further characterized the inflammatory response of the human microglial C20 cell line, a novel experimental model suitable to study the biology of human adult microglia *in vitro*[12]. We studied for the first time the effects of RF on human microglia. Our results suggest that RF does not have significant direct pro-inflammatory effects on human adult microglia *in vitro*. However, RF may transiently increase basal BDNF production in microglia and regulate the release of other chemokines. These data suggest a potential modulatory role of RF in neuroinflammation and pain perception.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

The authors thank Dr David Álvarez-Carbonell, Case Western Reserve University, Cleveland, OH, USA, for sharing the C20 cell line; Mr Mario Amici, Section of Physiscs, Neuroscience Department, Università Cattolica del Sacro Cuore (UCSC), Rome, Italy for technical assistance with SEM; and Denis Mariano, a native English speaking teacher for the careful language revision of the manuscript. The study was in part supported by UCSC Research Funds (D1-2017 to CDR, D1-2020 to LS, D1-2020 to ET) and by MI-UR-PRIN Grant (201789LFKB) awarded to ET.

References

1) Berhardt LV. Textbook of advances in medicine and biology. Nova Science Publishers, 2019.
2) Servin FS, Billard V. Remifentanil and other opioids. Handb Exp Pharmacol 2008; 182: 283-311.

3) Spampinato SM. Overview of genetic analysis of human opioid receptors. Methods Mol Biol 2021; 2201: 3-13.
4) Arout CA, Edens E, Petrakis IL, Sofuoglu M. Targeting opioid-induced hyperalgesia in clinical treatment: neurobiological considerations. CNS Drugs 2015; 29: 465-486.
5) Fletcher D, Martinez V. Opioid-induced hyperalgesia in patients after surgery: a systematic review and a meta-analysis. Br J Anaesth 2014; 112: 991-1004.
6) Ferrini F, De Koninck Y. Microglia control neuronal network excitability via BDNF signalling. Neural Plast 2013; 2013: 429815.
7) Ferrini F, Trang T, Mattioli TA, Laffray S, Del'Guidice T, Lorenzo LE. Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl^- homeostasis. Nat Neurosci 2013; 16: 183-192.
8) Roeckel LA, Le Coz GM, Gavériaux-Ruff C, Simonin F. Opioid-induced hyperalgesia: Cellular and molecular mechanisms. Neuroscience 2016; 338: 160-182.
9) Aguado D, Abreu M, Benito J, García-Fernández J, Gómez de Segura IA. Amitriptyline, minocycline and maropitant reduce the sevoflurane minimum alveolar concentration and potentiate remifentanil but do not prevent acute opioid tolerance and hyperalgesia in the rat: a randomised laboratory study. Eur J Anaesthesiol 2015; 32: 248-254.
10) Yang T, Du S, Liu X, Ye X, Wei X. Withdrawal from spinal application of remifentanil induces long-term potentiation of c-fiber-evoked field potentials by activation of Src family kinases in spinal microglia. Neurochem Res 2018; 43: 1660-1670.
11) Smith AM, Dragunow M. The human side of microglia. Trends Neurosci 2014; 37: 125-135.
12) Garcia-Mesa Y, Jay TR, Checkley MA, Luttge B, Dobrowolski C, Valadkhan S, Landreth GE, Karn J, Alvarez-Carbonell D. Immortalization of primary microglia: a new platform to study HIV regulation in the central nervous system. J Neurovirol 2017; 23: 47-66.
13) Aceto P, Dello Russo C, Lai C, Perilli V, Fucci N, De Giovanni N, Piras A, Navarra P, Sollazzi L. Relationship between blood remifentanil concentration and stress hormone levels during pneumoperitoneum in patients undergoing laparoscopic cholecystectomy. Eur Rev Med Pharmacol Sci 2017; 21: 4419-4422.
14) Cappoli N, Mezzogori D, Tabolacci E, Coletta I, Navarra P, Pani G, Dello Russo C. The mTOR kinase inhibitor rapamycin enhances the expression and release of pro-inflammatory cytokine interleukin 6 modulating the activation of human microglial cells. EXCLI J 2019; 18: 779-798.
15) Dello Russo C, Lisi L, Tringali G, Navarra P. Involvement of mTOR kinase in cytokine-dependent microglial activation and cell proliferation. Biochem Pharmacol 2009; 78: 1242-1251.
16) Dello Russo C, Cappoli N, Coletta I, Mezzogori D, Paciello F, Pozzoli G, Navarra P, Battaglia A. The human microglial HMC3 cell line: where do we stand? A systematic literature review. J Neuroinflammation 2018; 15: 259.
17) Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, Jaeger BN, O'Connor C, Fitzpatrick C, Pasillas MP, Pena M, Adair A, Gonda DD, Levy ML, Ransohoff RM, Gage FH, Glass CK. An environment-dependent transcriptional network specifies human microglia identity. Science 2017; 356: eaal3222.
18) Davis RL, Buck DJ, McCracken K, Cox GW, Subhas Das S. Interleukin-1β-induced inflammatory signaling in C20 human microglial cells. Neuroimmunol Neuroinflammation 2018; 5: 50.
19) Pozzo ED, Tremolanti C, Costa B, Giacomelli C, Milenkovic VM, Bader S, Wetzel CH, Rupprecht R, Taliani S, Settimo FD, Martini C. Microglial pro-inflammatory and anti-inflammatory phenotypes are modulated by translocator protein activation. Int J Mol Sci. 2019; 20: 4467.
20) Cappoli N, Tabolacci E, Aceto P, Dello Russo C. The emerging role of the BDNF-TrkB signaling pathway in the modulation of pain perception. J Neuroimmunol 2020; 349: 577406.
21) Ding H, Chen J, Su M, Lin Z, Zhan H, Yang F, Li W, Xie J, Huang Y, Liu X, Liu B, Zhou X. BDNF promotes activation of astrocytes and microglia contributing to neuroinflammation and mechanical allodynia in cyclophosphamide-induced cystitis. J Neuroinflammation 2020; 17: 19.
22) Yin R, Zhao S, Qiu C. Brain-derived neurotrophic factor fused with a collagen-binding domain inhibits neuroinflammation and promotes neurological recovery of traumatic brain injury mice via TrkB signalling. J Pharm Pharmacol 2020; 72: 539-550.