Received: 23 March 2022 | Revised: 18 August 2022 | Accepted: 21 August 2022

DOI: 10.1111/bph.16003

RESEARCH ARTICLE

The sigma-1 receptor curtails endogenous opioid analgesia during sensitization of TRPV1 nociceptors

M. Carmen Ruiz-Cantero[1,2,3] | Elsa Cortés-Montero[4] | Aakanksha Jain[5,6] | Ángeles Montilla-García[1,2] | Inmaculada Bravo-Caparrós[1,2] | Jaehoon Shim[5,6] | Pilar Sánchez-Blázquez[4] | Clifford J. Woolf[5,6] | José M. Baeyens[1,2,3] | Enrique J. Cobos[1,2,3,7]

[1]Department of Pharmacology, Faculty of Medicine, University of Granada, Granada, Spain

[2]Institute of Neuroscience, Biomedical Research Center, University of Granada, Granada, Spain

[3]Biosanitary Research Institute ibs. GRANADA, Granada, Spain

[4]Department of Translational Neurosciences, Neuropharmacology, Cajal Institute, CSIC, Madrid, Spain

[5]Department of Neurobiology, Harvard Medical School, Boston, MA, USA

[6]F.M. Kirby Neurobiology Center, Boston Children's Hospital, Boston, MA, USA

[7]Teófilo Hernando Institute for Drug Discovery, Madrid, Spain

Correspondence
Enrique J. Cobos, Department of Pharmacology and Institute of Neuroscience, Faculty of Medicine, University of Granada, Tower B, 11th Floor, Avenida de la Investigación, 11, 18016 Granada, Spain.
Email: ejcobos@ugr.es

Funding information
Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía, Grant/Award Number: CTS109; European Regional Development Fund; Secretaría de Estado de Investigación, Desarrollo e Innovación, Grant/Award Numbers: FPU 15/02356 (E Cortés-Montero), FPU16/03213 (MC Ruiz-Cantero), PID2019-108691RB-I00, SAF2016-80540-R; Universidad de Granada,

Abstract

Background and Purpose: Peripheral sensitization contributes to pathological pain. While prostaglandin E2 (PGE2) and nerve growth factor (NGF) sensitize peptidergic C-nociceptors (TRPV1+), glial cell line-derived neurotrophic factor (GDNF) sensitizes non-peptidergic C-neurons (IB4+). The sigma-1 receptor (sigma-1R) is a Ca^{2+}-sensing chaperone known to modulate opoid analgesia. This receptor binds both to TRPV1 and the μ opioid receptor, although the functional repercussions of these physical interactions in peripheral sensitization are unknown.

Experimental Approach: We tested the effects of sigma-1 antagonism on PGE2-, NGF-, and GDNF-induced mechanical and heat hyperalgesia in mice. We used immunohistochemistry to determine the presence of endomorphin-2, an endogenous μ receptor agonist, on dorsal root ganglion (DRG) neurons. Recombinant proteins were used to study the interactions between sigma-1R, μ- receptor, and TRPV1. We used calcium imaging to study the effects of sigma-1 antagonism on PGE2-induced sensitization of TRPV1+ nociceptors.

Key Results: Sigma1 antagonists reversed PGE2- and NGF-induced hyperalgesia but not GDNF-induced hyperalgesia. Endomorphin-2 was detected on TRPV1+ but not on IB4+ neurons. Peripheral opioid receptor antagonism by naloxone methiodide or administration of an anti-endomorphin-2 antibody to a sensitized paw reversed the antihyperalgesia induced by sigma-1 antagonists. Sigma-1 antagonism transfers sigma-1R from TRPV1 to μ receptors, suggesting that sigma-1R participate in TRPV1-μ receptor crosstalk. Moreover, sigma-1 antagonism reversed, in a naloxone-sensitive manner, PGE2-induced sensitization of DRG neurons to the calcium flux elicited by capsaicin, the prototypic TRPV1 agonist.

Abbreviations: DRG, dorsal root ganglion; GDNF, glial cell line-derived neurotrophic factor; NGF, nerve growth factor; NMDAR, *N*-methyl-D-aspartate receptors; PGE2, prostaglandin E2; RTX, resiniferatoxin; TRPV1, transient receptor potential vanilloid-1; sigma-1R, sigma-1 receptor.

Grant/Award Number: PPJIB2019.11; Universidad de Granada / CBUA; Harvard Medical School - Blavatnik Sensory Disorders Research Award

Conclusion and Implications: Sigma-1 antagonism harnesses endogenous opioids produced by TRPV1+ neurons to reduce hyperalgesia by increasing μ receptor activity.

KEYWORDS
endomorphin-2, resiniferatoxin, Sigma-1 receptors, TRPV1, μ-opioid receptors

1 | INTRODUCTION

The **sigma-1 receptor** (sigma-1R) is a ligand-operated chaperone that, in response to the increase in the intracellular Ca^{2+} concentration, physically interacts with several different receptors and channels (Su et al., 2016). *N*-methyl-D-aspartate receptors (NMDAR) are major protein targets of sigma-1R (Sánchez-Fernández et al., 2017) and sigma-1 antagonism decreases NMDAR activity in the spinal cord to inhibit central sensitization and ameliorate neuropathic pain (Zamanillo et al., 2013). Neuropathic pain is an intended primary indication of the selective sigma-1R antagonist **S1RA**, which has been successfully tested in a phase IIa clinical trial on chemotherapy-induced neuropathic pain (Bruna et al., 2018), after phase I studies which demonstrated its safety and tolerability in healthy people (Abadias et al., 2013).

The secondary intended indication of S1RA is the enhancement of opioid analgesia (Vela et al., 2015), as there is overwhelming preclinical evidence for the increase of the antinociceptive effect induced by several clinically relevant opioid drugs (such as morphine) by sigma-1 antagonism (Sánchez-Fernández et al., 2017). Sigma-1R participates in the crosstalk between the **μ-opioid receptor** and NMDAR to modulate opioid analgesia at the CNS. **Ca^{2+}-activated calmodulin** (CaM) is a negative regulator of NMDAR and sigma-1R competes with CaM for the binding to NMDAR; therefore, the binding of the sigma-1R to NMDAR reduces CaM-induced NMDAR inhibition, which decreases μ receptor actions. In the presence of a sigma-1 antagonist, sigma-1Rs dissociate from the NMDAR and transfer to the μ receptor, allowing CaM to bind NMDAR, and enhancing μ receptor activity (Rodríguez-Muñoz, Cortés-Montero, et al., 2015; Rodríguez-Muñoz, Sánchez-Blázquez, et al., 2015). Modulation of μ receptor-mediated analgesia by the sigma-1R has been classically attributed to actions in the CNS (Mei & Pasternak, 2007); however, we more recently reported that sigma-1 antagonism also enhances peripheral antinociception induced by opioid drugs, including not only opioid analgesics such as morphine and fentanyl, but also the peripheral μ receptor agonist loperamide, used clinically as an antidiarrheal drug (Sánchez-Fernández et al., 2013 and 2014). In fact, sigma-1 antagonism is even able to induce peripheral antihyperalgesic effects by the potentiation of endogenous opioid peptides derived from immune cells in peripheral inflamed tissue (Tejada et al., 2017).

Transient receptor potential vanilloid-1 (TRPV1) is another more recently identified protein target of sigma-1Rs (Cortés-Montero et al., 2019; Ortíz-Rentería et al., 2018). In adult mice, TRPV1 receptors are mostly concentrated in peptidergic C-nociceptors, which can express neuropeptides such as **calcitonin gene-related peptide** (CGRP) or substance P (Priestley, 2009; Renthal et al., 2020), and play an important role in peripheral sensitization in response to algogenic ligands such as **prostaglandin E2** (PGE2) (Moriyama et al., 2005) and **nerve growth factor** (NGF) (Zhang et al., 2005). Non-peptidergic C-nociceptors do not express TRPV1 but can be labelled with Isolectin B4 (IB4) and sensitized by other factors, such as **glial cell line-derived neurotrophic factor** (GDNF) (Álvarez et al., 2012). These peripheral sensitizers are produced in a variety of pathological pain states and play a pivotal role in pain generation (Ji et al., 2016; Kotliarova & Sidorova, 2021). We and others have recently reported that sigma-1Rs are present in every DRG neuron (Bravo-Caparrós et al., 2020; Montilla-García et al., 2018; Shin et al., 2020), although the role of these receptors in the periphery has not been well studied. We aimed here to test whether sigma-1 antagonists ameliorate the hyperalgesia induced by peripheral sensitizers. As we found that the antihyperalgesic effect of sigma-1 antagonists on sensitizers of TRPV1+ nociceptors involves activation of the endogenous opioid system, we sought to determine the endogenous opioid peptide involved and its cellular source, and also tested whether there might be crosstalk between μ receptors and TRPV1 with the participation of sigma-1Rs, similar to the one described for the NMDAR in the CNS.

What is already known

- Sigma-1 antagonists enhance analgesia induced by morphine and other opioid drugs.

What does this study add

- Sigma-1 antagonism harnesses the analgesic potential of opioid peptides released from sensitized TRPV1+ sensory neurons.

What is the clinical significance

- Sigma-1 antagonism induces opioid analgesia exclusively at the painful site without administration of opioid drugs.

2 | METHODS

2.1 | Experimental animals

Most experiments were done in female CD-1 mice (Charles River, Barcelona, Spain), weighing 25–30 g. Animals were housed in colony cages (10 mice per cage), in a temperature-controlled room (22 ± 2°C) with an automatic 12-h light/dark cycle (08:00–20:00 h). An igloo and a plastic tunnel were placed in each housing cage for environmental enrichment. Some experiments were performed on male mice from the same strain. It is known that male mice are more aggressive to other mice than female animals (Edwards, 1968) and that the stress induced by fights with the alpha male can induce opioid analgesia (Miczek et al., 1982). We considered that this behaviour of male mice might be a confounder in our experiments in the context on the modulation of endogenous opioid analgesia by sigma-1 receptors. Therefore, we performed most experiments in female animals. However, we also tested male mice in some key experiments (see Section 3) to explore a possible sexual dimorphism in sigma-1-mediated modulation of hyperalgesia induced by peripheral sensitizers. Animals were fed a standard laboratory diet and tap water ad libitum until the beginning of the experiments. The behavioural experiments were carried out during the light phase (from 9:00 a.m. to 3:00 p.m.). The mice were randomized to treatment groups, testing each day a balanced number of animals from several experimental groups, and female mice were also tested randomly throughout the oestrous cycle. Mice were handled in accordance with international standards (European Communities Council directive 2010/63), and the experimental protocols were approved by regional (Junta de Andalucía) and Institutional (Research Ethics Committee of the University of Granada) authorities. To decrease the number of animals in this study, we used the same mice for behavioural studies, FACS analysis and immunostaining, when possible. Mice that were not used for in vitro studies were killed by cervical dislocation. Animal studies are reported in compliance with the ARRIVE guidelines and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020; Percie du Sert et al., 2020).

2.2 | Peripheral sensitizers, drugs and in vivo antibodies

The peripheral sensitizers, PGE2, NGF, and GDNF, were injected intraplantarly (i.pl.) into the right hind paw in a volume of 20 µl, using a 1710 TLL Hamilton microsyringe (Teknokroma, Barcelona, Spain) with a $30^{1/2}$-gauge needle. PGE2 (Tocris Cookson Ltd., Bristol, United Kingdom) and NGF (Sigma-Aldrich, Madrid, Spain) were dissolved in sterile physiological saline (0.9% NaCl), and GDNF (Preprotech, London, UK) was dissolved in 0.1% bovine serum albumin (Sigma-Aldrich) in sterile physiological saline. Stock PGE2, NGF or GDNF solutions were stored at −20°C and further dilutions were performed to obtain the appropriate final concentrations for the different experiments, just before administration. PGE2 (0.5 nmol), NGF (1 µg), and GDNF (40 ng) were i.pl. injected at 10 min, 3 h, and 20 min before the behavioural evaluation, respectively. These doses and times after administration were selected from their dose–response effects and time-courses (Figure S1A–F).

We used three sigma-1R ligands: S1RA (E-52862.HCl; 4-[2-[[5-methyl-1-(2-naphthalenyl)-1H-pyrazol-3-yl]oxy]ethyl] morpholine) (DC Chemicals, Shanghai, China) and **BD-1063** (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride) (Tocris Cookson Ltd.) were used as selective sigma-1 antagonists, and **PRE-084** (2-[4-morpholinethyl]1-phenylcyclohexanecarboxylate hydrochloride) (Tocris Cookson Ltd.) was used as a selective sigma-1 agonist (Cobos et al., 2008). In addition, we used several opioid receptor ligands. These include the prototypic µ receptor agonist morphine hydrochloride (General Directorate of Pharmacy and Drugs, Spanish Ministry of Health), the central penetrant opioid antagonist **naloxone hydrochloride** and its quaternary derivative naloxone methiodide (both from Sigma-Aldrich), which was used as a peripherally restricted opioid antagonist. Finally, the µ receptor antagonist cyprodime, the κ receptor antagonist nor-binaltorphimine and the δ receptor antagonist naltrindole (all from Tocris Cookson Ltd.) were used as selective antagonists for the three major opioid receptor subtypes. All drugs were dissolved in sterile physiological saline. To study the effect of systemic treatments, drugs were injected subcutaneously (s.c.) into the interscapular zone in a volume of 5 ml kg^{-1}. When the effect of the association of two drugs was tested, each drug was injected into a different area of the interscapular zone. To test for the effects of local treatments, drugs or their solvents were administered i.pl. in a volume of 20 µl. The sigma-1 antagonists or morphine were administered 30 min before the behavioural evaluation. The sigma-1 agonist PRE-084 or the opioid antagonists were injected 5 min before the sigma-1 antagonists or morphine (35 min before the behavioural evaluation).

The dose of PRE-084 used (32 mg kg^{-1}, s.c.), was selected based on our previous studies (Bravo-Caparrós et al., 2019; Entrena et al., 2009; Montilla-García et al., 2018), as well as the doses of naloxone (1 mg kg^{-1}, s.c.) and naloxone methiodide (2 mg kg^{-1}, s.c.) (Montilla-García et al., 2018; Sánchez-Fernández et al., 2014; Tejada et al., 2017). The doses of cyprodime (10–15 mg kg^{-1}, s.c.) used to reverse the effects of the sigma-1 antagonists were selected based on the reversion of the antihyperalgesic effect of the prototypic µ opioid agonist morphine (see Section 3 for details) and are in a range similar to the ones used in previous studies (Hutcheson et al., 1999). Nor-binaltorphimine (10 mg kg^{-1}, s.c.) and naltrindole (5 mg kg^{-1}, s.c.) have been repeatedly reported to reverse opioid effects at the same doses used in our study or lower (Baamonde et al., 2005; Hutcheson et al., 1999).

To block the effects of the endogenous opioid peptides (EOPs), 20 µl of a solution containing 3-E7 monoclonal antibody (Millipore Cat# MAB5276, RRID:AB_95197), which recognizes the pan-opioid sequence Tyr-Gly-Gly-Phe at the N-terminus of most EOPs (Rittner et al., 2001) was administered i.pl. at a dose of 1 µg. The i.pl. administration of this antibody has been previously shown to abolish the effects of sigma-1 antagonism on carrageenan-induced hyperalgesia (Tejada et al., 2017). As other EOPs, such as **endomorphin-2** (END2) (Alexander, Christopoulos, et al., 2021), lack this consensus sequence

we used the i.pl. administration (1 μg) of a specific antibody (Phoenix Pharmaceuticals, Cat# G-044-11, RRID:AB_2909534) to block its effects in vivo. When these antibodies and the sigma-1 antagonists were associated i.pl., they were dissolved in the same solution and injected together to avoid paw lesions due to multiple injections in the same paw.

2.3 | In vivo ablation of TRPV1-expressing nociceptors

We used **resiniferatoxin** (RTX, Tocris Cookson Ltd) as a "molecular scalpel" to selectively ablate TRPV1-expressing neurons. RTX was dissolved in vehicle (10% Tween 80 and 10% ethanol in physiological saline). Animals received two doses of RTX i.p., on two consecutive days (25 μg kg^{-1} each dose). This total dose (50 μg kg^{-1}) has been previously reported to ablate all peripheral TRPV1+ neurons (González-Cano et al., 2020; Hsieh et al., 2008; Montilla-García et al., 2017, 2018), but we divided it into two doses to minimize distress. The control group received a double injection with an equal volume of vehicle. To minimize suffering, all procedures were done under isoflurane anaesthesia in oxygen (IsoVet®, B. Braun, Barcelona, Spain). The initial isoflurane dose was 4% for the induction of general anaesthesia, during 5 min. Then, RTX (or its solvent) was injected and anaesthesia was maintained for 10 min with isoflurane 2%. The efficacy of the treatment was determined by immunohistochemical assays of L4 DRGs (see Section 3 for details). Animals were placed in their home cages for 5 days after the first i.p. injection before behavioural testing and sample collection.

2.4 | Assessment of hyperalgesia

The animals were placed in the experimental room for a 1 h acclimatisation period before starting the experiments. For the assessment of either mechanical or thermal hyperalgesia, the mice were gently pincer grasped between the thumb and index fingers by the skin above the interscapular area and underwent sensory stimulation. All mice were used in only one experimental procedure (mechanical or thermal stimulation). The experimenters who evaluated the behavioural responses were blinded to the treatment group of each experimental animal.

Mechanical hypersensitivity was assessed with the paw pressure test following a previously described protocol (Menéndez et al., 2002; Tejada et al., 2017), with slight modifications. At the appropriate time after drug administration, mechanical stimulation was applied to the right hindpaw with an Analgesimeter (Model 37215, Ugo-Basile, Varese, Italy). A blunt cone-shaped paw-presser was applied at a constant intensity of 100 g to the dorsal surface of the hindpaw until the animal showed a struggle response. The struggle latency was measured with a chronometer. The test was done three times with a 1-min interval between stimulations, and the mean value of the three trials was recorded as the animal's struggle latency.

Thermal hypersensitivity was assessed with the unilateral hot plate test following a previously described protocol (Menéndez et al., 2002; Montilla-García et al., 2018), with slight modifications. After the appropriate time after drug administration, the plantar side of the stimulated hindpaw was placed on the surface of a thermal analgesimeter (Model PE34, Series 8, IITC Life Science Inc., Los Angeles, USA) previously set at 42 ± 1°C until the animal showed a paw withdrawal response. The latency in seconds from paw stimulation to the behavioural response was measured with a digital chronometer. The test was done three times with a 1-min interval between stimulations, and the mean value of the three trials was recorded as the animal's paw withdrawal latency. Only a clear unilateral withdrawal of the paw was recorded as response. We avoided simultaneous heat stimulation in both hind paws by placing the plantar side of the tested hind paw on the hot plate while the other hind paw was placed on filter paper (off the hot plate) during observations (see Supplementary Video 2 in Montilla-García et al., 2018).

2.5 | Immunohistochemistry

Mice were anaesthetised with 4% isoflurane (in oxygen) and perfused transcardially with 0.9% saline solution followed by 4% paraformaldehyde (Sigma-Aldrich). The L4 DRGs were dissected and post-fixed for 1 h in the same paraformaldehyde solution. Embedding procedures differed depending on the staining to be performed, as not all antibodies used showed optimal results in all embedding media. Samples for sigma-1R or CGRP immunostaining were dehydrated and embedded in paraffin. Tissue sections 5 μm thick were cut with a sliding microtome, mounted on microscope slides (Sigma-Aldrich), deparaffinized in xylol (Panreac Quimica, Castellar del Vàlles, Spain) and rehydrated before antigen retrieval (steam heating for 22 min with 1% citrate buffer, pH 8). Samples for END2 staining were incubated for 48 h in 30% sucrose (Sigma-Aldrich) at 4°C to be embedded in O.C.T Tissue-Tek medium (Sakura Finetek, Barcelona, Spain), and frozen and stored at −80 ° C until immunohistochemical study. Sections of DRG 15 μm thick were cut with a cryostat and thaw-mounted onto Superfrost Plus microscope slides (Thermo Fisher Scientific).

Tissue sections were incubated for 1 h in blocking solution with 5% normal goat or donkey serum, depending on the experiment, 0.3% Triton X-100, and 0.1% Tween 20 in Tris buffer solution. Then, the slides were incubated with the primary antibodies in blocking solution. The primary antibodies used were: mouse anti-sigma-1R (1:200, Santa Cruz Biotechnology Cat# sc-137,075, RRID:AB_2285870), rabbit anti-PGP9.5 (1:400, Millipore Cat# AB1761, RRID:AB_91019), rabbit anti-CGRP (1:800, BMA Biomedicals Cat# T-4032, RRID:AB_518147), goat anti-TRPV1 (1:100, Santa Cruz Biotechnology Cat# sc-12,498, RRID:AB_2241046), and rabbit anti-END2 (15 μg ml^{-1}, Phoenix Pharmaceuticals, Cat# G-044-11, RRID:AB_2909534). Incubation with the primary antibodies for sigma-1R, PGP9.5, CGRP, and TRPV1 was for 1 h at room temperature (RT), whereas incubation with the primary antibody for END2 was overnight at 4°C. When staining for END2 were performed, sections were incubated with the anti-END2

antibody overnight, and the next day, after washing three times for 10 min, the samples were incubated with the anti-TRPV1 antibody for 1 h. After incubation with the primary antibodies, sections were washed again three times for 10 min and incubated with the appropriate secondary antibodies: Alexa Fluor-488 goat anti-mouse (Thermo Fisher Scientific Cat# A-11029, RRID:AB_2534088), Alexa Fluor-594 goat anti-rabbit (Thermo Fisher Scientific Cat# A-11012, RRID:AB_2534079), Alexa Fluor-647 donkey anti-mouse (Thermo Fisher Scientific Cat# A-31571, RRID:AB_162542), Alexa Fluor-488 donkey anti-mouse (Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607), Alexa Fluor-488 donkey anti-goat (Thermo Fisher Scientific Cat# A-11055, RRID:AB_2534102), or Alexa Fluor-647 donkey anti-rabbit (Thermo Fisher Scientific Cat# A-31573, RRID:AB_2536183) (all 1:500). We also stained tissue sections with *Bandeiraea simplicifolia* lectin I, isolectin B4 (IB4) conjugated with Dylight-594 (1:100, Vector Laboratories Cat# DL-1207, RRID:AB_2336415). Finally, slides were washed three times for 10 min before the mounting procedure and they were coverslipped with ProLong Gold Antifade mounting medium (Thermo Fisher Scientific). Images were acquired with a confocal laser-scanning microscope (Model A1, Nikon Instruments Europe BV, Amsterdam, Netherlands).

To illustrate the overlap between different markers, we performed Venn diagrams. To construct these diagrams, an experimenter blinded to the treatments counted the neurons stained in four DRG slices per animal. There was a minimum separation between slices of 15 µm. The values from five different animals were averaged to construct the Venn diagrams.

The immunohistochemistry procedures used in the present study comply with the recommendations made by the *British Journal of Pharmacology* (Alexander et al., 2018).

2.6 | Fluorescence-activated cell sorting (FACS) analysis

We used plantar tissue from mice i.pl. injected with 20 µl of a solution containing PGE2 (0.5 nmol), GDNF (40 ng), or NGF (1 µg). Samples were collected at 10 min, 20 min, and 3 h after the injection of PGE2, GDNF, or NGF, respectively. As a positive control for neurotrophil recruitment, we used samples from mice i.pl. treated with λ-carrageenan (50 µl, 1% wt vol^{-1} in saline; Sigma-Aldrich) 3 h before sample collection (Tejada et al., 2017). Mice were killed by cervical dislocation and plantar tissue was dissected and digested with collagenase IV (1 mg ml^{-1}, LS004188, Worthington, Lakewood, NJ, USA) and DNAse I (0.1%, LS002007, Worthington) for 1 h at 37°C with agitation. Samples were filtered (pore size 70 µm) and the rat anti-CD32/16 antibody (1:100, 20 min, Biolegend Cat# 553141, RRID:AB_394656) was used to block Fc-γRII (CD32) and Fc-γRIII (CD16) binding to IgG. Cells were incubated with antibodies recognizing the haematopoietic cell marker CD45 (1:200, 103108, clone 30-F11, BioLegend Cat# 103108, RRID:AB_312973), the myeloid marker CD11b (1:100, BioLegend Cat# 101227, RRID:AB_893233), and the neutrophil-specific marker Ly6G (1:100, BioLegend Cat# 127617, RRID:AB_1877262), together with a viability dye (1:1000, 65-0865-14; Thermo Fisher Scientific), for 30 min on ice. The population of neutrophils (CD45+ CD11b+ Ly6G+ cells) was determined in cells labelled with the viability dye. Before and after incubation with the antibodies, the cells were washed three times in 2% fetal bovine serum (FBS)/PBS (FACS buffer). Cells were fixed with 2% paraformaldehyde for 20 min, and on the next day samples were assayed with a BD FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, USA). Compensation beads were used as compensation controls, and fluorescence minus one (FMO) controls were included to determine the level of nonspecific staining and autofluorescence associated with different cell subsets. All data were analysed with FlowJo 2.0 software (Treestar, Ashland, OR, USA).

2.7 | Recombinant protein expression

The coding region of the full-length murine sigma-1R (AF004927) and the *C*-terminal (Ct) regions of the µ receptor (AB047546: residues 286–398) and TRPV1 (NP_542437; residues 680–839) were amplified by reverse transcription polymerase chain reaction (RT-PCR) using total RNA isolated from the mouse brain as the template.

Specific primers containing an upstream Sgf I restriction site and a downstream Pme I restriction site were used, as described previously (Rodríguez-Muñoz, Sánchez-Blázquez, et al., 2015). The PCR products were cloned downstream of the glutathione S-transferase (GST)/HaloTag® coding sequence (Flexi® Vector, Promega, Spain) and the tobacco etch virus (TEV) protease site, and when sequenced, the proteins were identical to the GenBank™ sequences. The vector was introduced into the *Escherichia coli* BL21 (KRX L3002, Promega), and clones were selected on solid medium containing ampicillin. After 3 h of induction at RT, in the presence of 1-mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.1% rhamnose, the cells were collected by centrifugation (9720 × g for 15 min) and maintained at −80°C. The fusion proteins were purified under native conditions on GStrap FF columns (17-5130-01; GE Healthcare, Spain) or with HaloLink Resin (G1915; Promega). When necessary, the fusion proteins retained were cleaved on the column with ProTEV protease (V605A; Promega) and further purification was achieved by high-resolution ion exchange (780-0001Enrich Q; BioRad, Spain). Sequences were confirmed by automated capillary sequencing. Recombinant calmodulin (CaM, 208694) was purchased at Merck Millipore (Spain).

2.8 | In vitro interactions between recombinant proteins: Pull-down of recombinant proteins and the effect of drugs on sigma-1R/TRPV1/µ receptor Ct interactions

Having demonstrated that the sigma-1R does not bind to GST (Z02039; GenScript Co., USA) (Cortés-Montero et al., 2019), we assessed the association of GST-free sigma-1Rs with the GST-tagged TRPV1 Ct or µ receptor Ct sequences. The *C*-terminal domains of

TRPV1 (100 nM) and μ receptor (200 nM) were immobilized through covalent attachment to N-Hydroxysuccinimide (NHS)-activated Sepharose 4 fast flow (4FF, 17-0906-01; GE) according to the manufacturer's instructions. Recombinant sigma-1R (200 nM) was then incubated with either NHS-blocked Sepharose 4FF (negative control) or with the immobilized TRPV1/ μ receptor sequence in 200 μl of a buffer containing 50-mM Tris–HCl (pH 7.5), 0.2% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) and 3 mM $CaCl_2$. The samples were mixed by rotation for 30 min at RT, and the sigma-1Rs bound to TRPV1/μ receptor-Sepharose 4FF were recovered by centrifugation (4300 × g for 5 min) and washed three times. To study whether the drugs used provoked changes in the TRPV1/ μ receptor-sigma-1R association, the agarose-attached TRPV1–sigma-1R complexes were incubated for a further 30 min at RT with rotation in the presence of the drugs and with or without the other receptor (μ receptor or TRPV1) in a final reaction volume of 300 μl of 50-mM Tris–HCl (pH 7.5), 3-mM $CaCl_2$, and 0.2% CHAPS. In some experiments, we added CaM (200 nM) after the incubation of TRPV1–sigma-1R complexes with the drugs and the respective washes and the mix were incubated for a further 30 min at RT with rotation. Agarose pellets containing the bound proteins were obtained by centrifugation, and they were washed three times in the presence of 3-mM $CaCl_2$ and then solubilized in 2× Laemmli buffer, analysing the sigma-1R/CaM content in Western blots. The compounds studied were S1RA and BD1063 (10 nM) and were dissolved in aqueous solution.

The sigma-1R/CaM bound to the Sepharose-TRPV1/ μ receptor sequences were resolved with Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in 4–12% Bis–Tris gels (NP0341; Invitrogen, Fisher Scientific, Spain), with 2-(N-morpholino) ethanesulfonic acid SDS (MES SDS) as the running buffer (NP0002; Invitrogen). The proteins were transferred onto 0.2 μm Polyvinylidene difluoride (PVDF) membranes (162-0176; BioRad) and probed overnight at 6°C with primary antibodies diluted in Tris-buffered saline (pH 7.7) (TBS) + 0.05% Tween 20 (TTBS): anti-sigma-1R (Thermo Fisher Scientific Cat# 42-3300, RRID:AB_2533521), anti-CaM (Millipore Cat# 05-173, RRID:AB_309644). All primary antibodies were detected using the appropriate horseradish-peroxidase-conjugated secondary antibodies.

The blot areas containing the corresponding sizes of cloned target proteins were selected for image capture and analysis. The Western blot images were visualized by chemiluminescence (170-5061; BioRad) and recorded on an ImageQuant™ LAS 500 (GE). For each blot, the area containing the target cloned protein was typically selected. The device automatically captures the selected area and the associated software automatically calculated the optimal exposure time to provide the strongest possible signal from which the rest of the signals could be accurately quantified. For each group of immunosignals derived from the same cloned protein, the area of the strongest signal was used to determine the average optical density of the pixels within the object area mm^{-2} of all the signals (AlphaEase FC software). The grey values of the means were then normalized within the 8 bit/256 Gy levels [(256 − computed value)/computed value].

The Western blotting procedures used in the present study comply with the recommendations made by the *British Journal of Pharmacology* (Alexander et al., 2018).

2.9 | Calcium imaging

Thoracic and lumbar DRGs were dissected and transferred into 10% FBS and 1% penicillin–streptomycin supplemented DMEM 1× (10-013-CV; Thermo Fisher Scientific) at 4°C. DRGs were then digested in 5 mg ml^{-1} collagenase and 1 mg ml^{-1} Dispase II (Roche, Indianapolis, IN) and then titrated using fire-polished glass Pasteur pipettes of decreasing sizes in the presence of DNAse I inhibitor (50 U) using standard procedures (Wainger et al., 2015). Cells were centrifuged (200 × g for 12 min) through 10% BSA (Sigma-Aldrich) and resuspended in 1-ml Neurobasal (Sigma-Aldrich) containing B27 supplement (Invitrogen), penicillin and streptomycin, 2 ng ml^{-1} GDNF, 10-μM arabinocytidine (all from Sigma-Aldrich). Cells were plated on poly-D-lysine (500 μg ml^{-1}) and laminin (5 mg ml^{-1}) coated 35 mm tissue culture dishes at 5000–8000 per dish, 5% carbon dioxide, at 37°C.

After 48 h, neurons were incubated for 50 min with 4 μg ml^{-1} Fura2-AM (Invitrogen) at RT. Cell were washed out with standard extracellular solution (SES) (Boston BioProducts) and images were acquired on a Nikon Eclipse Ti microscope (Nikon, Melville, NY) with standard 340- and 380-nm filters controlled by a Ludl Mac6000 shutter using Nikon Elements software. Images were taken every 3 s.

Cells were incubated in SES for 2 min. Then, a first exposure to a low concentration (0.05 μM) of the TRPV1 agonist capsaicin was made for 30 s. Cells were incubated with PGE2 (10 μM) for 7.5 min (from 11:30 to 19:00 min), and exposed to a second application of the same low concentration of capsaicin (0.05 μM) at the end of PGE2 incubation (from 18:30 to 19:00 min). When the effects of S1RA (10 μM) were tested, it was added to the solution 3 min before PGE2 application, and remained in the medium for the whole duration of PGE2 incubation (from 8:30 to 19:00 min). This concentration of S1RA is approximately the same than the plasma concentration of neuropathic patients treated with the sigma-1R antagonist (Abadias et al., 2013; Bruna et al., 2018). In experiments where naloxone (1 μM) was used to reverse the effect of S1RA, the opioid antagonist was added to the medium 1 min before S1RA application (from 7:30 to 19:00 min). A high concentration of **capsaicin** (1 μM) was applied at 24 min for 30 s, to determine all capsaicin-sensitive neurons. Finally cells were exposed to KCl (80 mM) for 10 s at 29:30 min, as a positive control to determine the total number of viable neurons at the end of the experiment. After the application of each compound, cells were washed appropriately.

PGE2-induced sensitization was defined as the increase in $F_{340/380}$ obtained with the low dose of capsacin during the application of PGE2 minus the value obtained in the first application of capsaicin. We selected plates with at least six neurons, which responded to both capsaicin (1 μM) and KCl and had a stable signal during the total exposure time.

2.10 | Data and statistical analysis

Studies were designed to generate groups of equal size. Statistical analysis was undertaken only for studies where each group size was at least n = 5. Most statistical analysis was carried out with one-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test. We examined whether data were normally distributed (Shapiro–Wilk test) and the homogeneity of variances (Brown–Forsythe test) before the ANOVA was performed. The post hoc test was run only if F achieved the necessary level of statistical significance ($P < 0.05$) and data fulfilled the assumptions of the ANOVA. In some experiments, the original data were log-transformed to meet the ANOVA assumptions. Results from Western blot assays were analysed using a Kruskal–Wallis test, which is suitable for nonparametric data, followed by a Student–Newman–Keuls post hoc test. The signals from the Western blot were expressed as the change relative to the assay-matched controls, which were assigned an arbitrary value of 1. Statistical analyses were performed with the SigmaPlot 14.5 program. The differences between values were considered significant when the *P* value was below 0.05. Sample sizes for experiments were estimated based on our previous studies (Cortés-Montero et al., 2019; Tejada et al., 2017; Wainger et al., 2015). The declared group size is the number of independent values, and that statistical analysis was done using these independent values. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2022).

2.11 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos, et al., 2021; Alexander, Kelly, et al., 2021; Alexander, Mathie, et al., 2021).

3 | RESULTS

3.1 | Involvement of TRPV1+ nociceptors in the hyperalgesia induced by PGE2, NGF and GDNF

We first studied the distribution of several neuronal markers in the DRG from intact female mice. Specifically, we stained for sigma-1R, CGRP, TRPV1, and IB4. The sigma-1R stained numerous cells with neuronal morphology (Figure 1a); in fact, the double labelling of sigma-1Rs with the pan-neuronal marker PGP9.5 showed that sigma-1Rs were present in most, if not all, DRG neurons (PGP9.5+ cells) (Figure S2). CGRP+ cells accounted for 28% of sigma-1R+-neurons (Figure 1a,b). Double labelling of CGRP and TRPV1 showed that both neuronal populations markedly overlap, as most CGRP+ neurons express TRPV1, and most TRPV1+ neurons express CGRP (Figure 1c,d). On the other hand, staining for TRPV1 and IB4 showed minimal overlap among DRG neurons, and each population

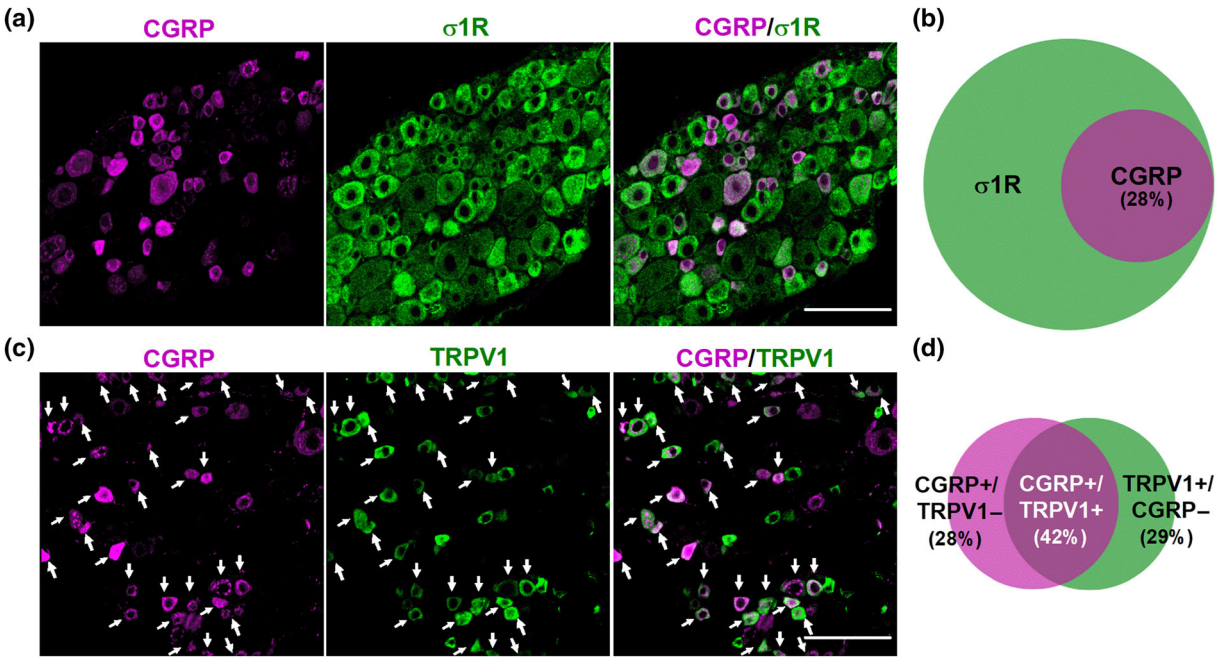

FIGURE 1 Double labelling of CGRP in combination with the sigma-1 receptor (σ1R) and TRPV1. Immunostaining was performed in the L4 dorsal root ganglion (DRG) from female mice. (a) Representative images from the double labelling of CGPR (magenta) and σ1R (green). Scale bar 50 µm. (b) Venn diagram showing the overlap between CGRP+ and σ1R+ neurons. (c) Representative images from the double labelling of CGPR (magenta) and TRPV1 (green). White arrows indicate co-localization of CGRP and TRPV1 markers. Scale bar 50 µm. (d) Venn diagram displaying the percentage of CGRP+, TRPV1+ and CGRP+/TRPV1+ neurons among the total number of neurons labelled with any of these markers. Samples from five mice were used to construct the Venn diagrams.

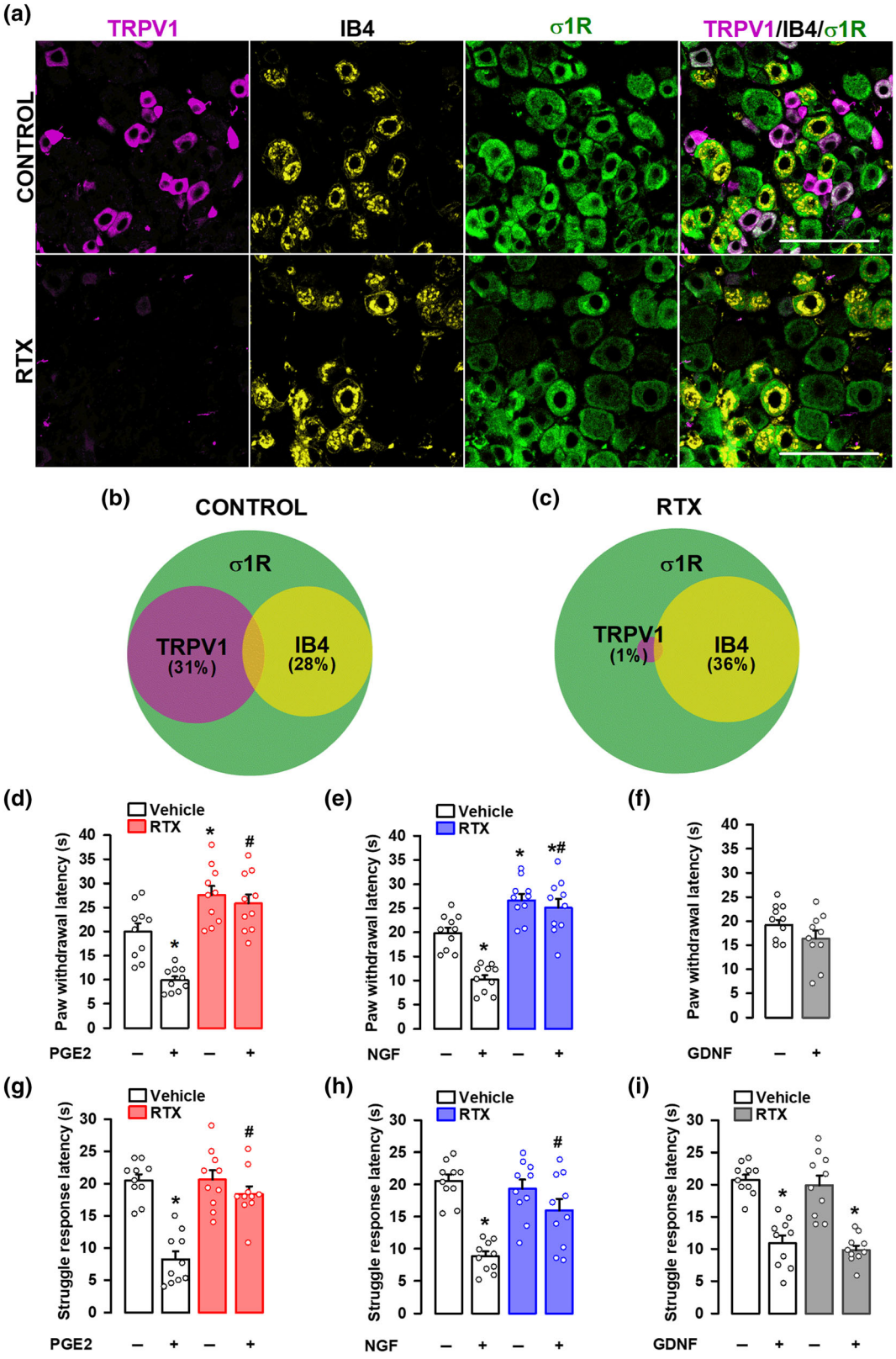

FIGURE 2 Legend on next page.

FIGURE 2 Effect of the ablation of TRPV1-expressing neurons on mechanical hyperalgesia induced by PGE2, NGF and GDNF. Female mice were treated intraperitoneally (i.p.) with resiniferatoxin (RTX, 25 μg kg^{-1} for two consecutive days) or its vehicle 5 days before obtaining samples or performing the behavioural experiments. (a) Triple labelling of TRPV1 (magenta), isolectin B4 (IB4, yellow) and sigma-1 receptor (σ1R, green) in the L4 dorsal root ganglion (DRG). Top panels: samples from vehicle-treated mice (control). Bottom panels: samples from mice treated with resiniferatoxin (RTX). Scale bar, 100 μm. (b,c) Venn diagrams showing the overlap between TRPV1+, IB4+ and σ1R+ neurons in samples from control mice (b) and from mice treated with RTX (c). Samples from five mice per group were used to construct the Venn diagrams. (d–i) the behavioural results represent the latency to paw withdrawal evoked by a heat stimulus of 42 ± 1°C (d–f), and the latency to struggle response evoked by a mechanical stimulus of 100 g (g–i) in mice treated intraplantarly (i.pl.) with PGE2 (0.5 nmol) (d,g), NGF (1 μg) (e,h), GDNF (40 ng) (f,i), or their solvents. Values are the mean ± SEM (10 animals per group). Statistically significant differences between the values obtained in control non-sensitized animals and mice treated with the peripheral sensitizer, $^{*}P < 0.05$; and between the values obtained in animals sensitized with PGE2 or NGF, and administered with RTX or its vehicle, $\#P < 0.05$ (one-way ANOVA followed by Bonferroni test). Data shown in H and I were log-transformed to meet the ANOVA assumptions.

constituted about one-third of sigma-1R+ cells (see top panels of Figure 2a for representative images, and Figure 2b). Treatment with RTX virtually abolished TRPV1 labelling, but IB4 staining was still readily detectable (Figure 2a, bottom panels). In fact, the proportion of IB4+ neurons in the remaining sigma-1R+ cells was even increased, since when TRPV1+ population is ablated, the remaining IB4 neurons represent a higher percentage considering the number of surviving neurons as the 100% (compare Figure 2b,c). These results confirm the specificity of the ablation of TRPV1+ neurons by RTX.

We next aimed to study the effects of the in vivo ablation of TRPV1-expressing neurons by RTX on behavioural responses to sensory stimulation after the administration of several peripheral sensitizers, as well as in non-sensitized animals. PGE2 and NGF induced a marked decrease in paw withdrawal latency to a contact heat stimulus (42 ± 1°C) in comparison to saline-injected mice, denoting development of heat hyperalgesia (Figure 2d,e). RTX increased the response latency in non-sensitized animals as well as in mice sensitized with PGE2 or NGF (Figure 2d,e). We also tested the effect of GDNF on heat sensitivity. In our experimental conditions, this neurotrophin (in contradistinction to the other peripheral sensitizers tested) was unable to induce heat hyperalgesia (Figure 2f).

PGE2 and NGF also induced mechanical hyperalgesia, decreasing the struggle latency to paw pressure in comparison to saline-injected mice (Figure 2g,h). RTX treatment did not affect the responses to mechanical stimulation in non-sensitized animals but abolished PGE2- and NGF-induced mechanical hypersensitivity (Figure 2g,h). Therefore, while TRPV1-expressing neurons are dispensable for mechanical nociceptive pain, they are essential for the mechanical hyperalgesia induced by these two chemical algogens. GDNF also induced significant mechanical hypersensitivity, which remained in spite of the ablation of TRPV1+ neurons by RTX treatment (Figure 2i). Therefore, although mechanical hypersensitivity can be triggered by PGE2, NGF and GDNF, only that induced by PGE2 and NGF is dependent on TRPV1+ nociceptors.

3.2 | The antihyperalgesic effects of sigma-1 antagonism and the peripheral opioid system

As all three peripheral sensitizers induced mechanical hyperalgesia, we first tested the effects of sigma-1 antagonists on this sensory modality, in female animals. The systemic (subcutaneous, s.c.) administration of the sigma-1 antagonists S1RA and BD-1063 did not modify the struggle latency to mechanical stimulation in non-sensitized animals (Figure S3), but induced a dose-dependent increase in the response latency in PGE2- or NGF-treated mice, reaching values similar to control animals (i.e., a full antihyperalgesic effect) at the highest dose tested (Figure 3a,b). However, neither S1RA nor BD-1063, at doses that fully reversed hyperalgesia induced by PGE2 or NGF, were able to induce any effect on GDNF-induced mechanical hyperalgesia (Figure 3c). The s.c. administration of the sigma-1 agonist PRE-084 did not alter the struggle response to mechanical stimulation in non-sensitized animals (Figure S3), but reversed the effect of both S1RA and BD-1063 on PGE2-induced hyperalgesia (Figure 3d,e, respectively). These results support the selectivity of the effects induced by the sigma-1 antagonists on the receptor. Mirroring the results in female mice, S1RA induced a full antihyperalgesic effect to the mechanical stimulus in male mice sensitized with PGE2, and this effect was reversed by PRE-084 (Figure S4A). In addition, S1RA was unable to induce any effect on GDNF-induced mechanical hyperalgesia in male mice (Figure S4B). These results suggest that the overall effect of sigma-1 antagonism on peripheral sensitization is preserved in both sexes in the mouse.

The ameliorative effects induced by S1RA or BD-1063 on PGE2-induced hyperalgesia in female mice were also reversed not only by the opioid antagonist naloxone but also by its peripherally restricted analogue naloxone methiodide (Figure 3d,e). Similarly, the effects induced by S1RA on PGE2-induced hyperalgesia in male mice were also fully reversed by naloxone methiodide (Figure S4A). These results suggest the involvement of the peripheral opioid system in the effects induced by sigma-1 antagonism in mice from both sexes. To identify which opioid receptor subtype was participating in the antihyperalgesic effects induced by S1RA and BD-1063 in female mice, we used antagonists with selectivity for the opioid receptor subtypes. The antihyperalgesic effect induced by the sigma-1 antagonists was abolished by the μ receptor antagonist cyprodime, but not the δ receptor antagonist naltrindole or the κ receptor antagonist nor-binaltorphimine (Figure 3d,e). We also tested the effects of S1RA and BD-1063 on NGF-induced hyperalgesia, with equivalent results (i.e., the effects of the sigma-1 antagonists were reversed by PRE-084, naloxone, naloxone methiodide and cyprodime, but not by naltrindole or nor-binaltorphimine) (Figure 3f,g). These results suggest that the effect of systemically administered sigma-1 antagonists on PGE2- and

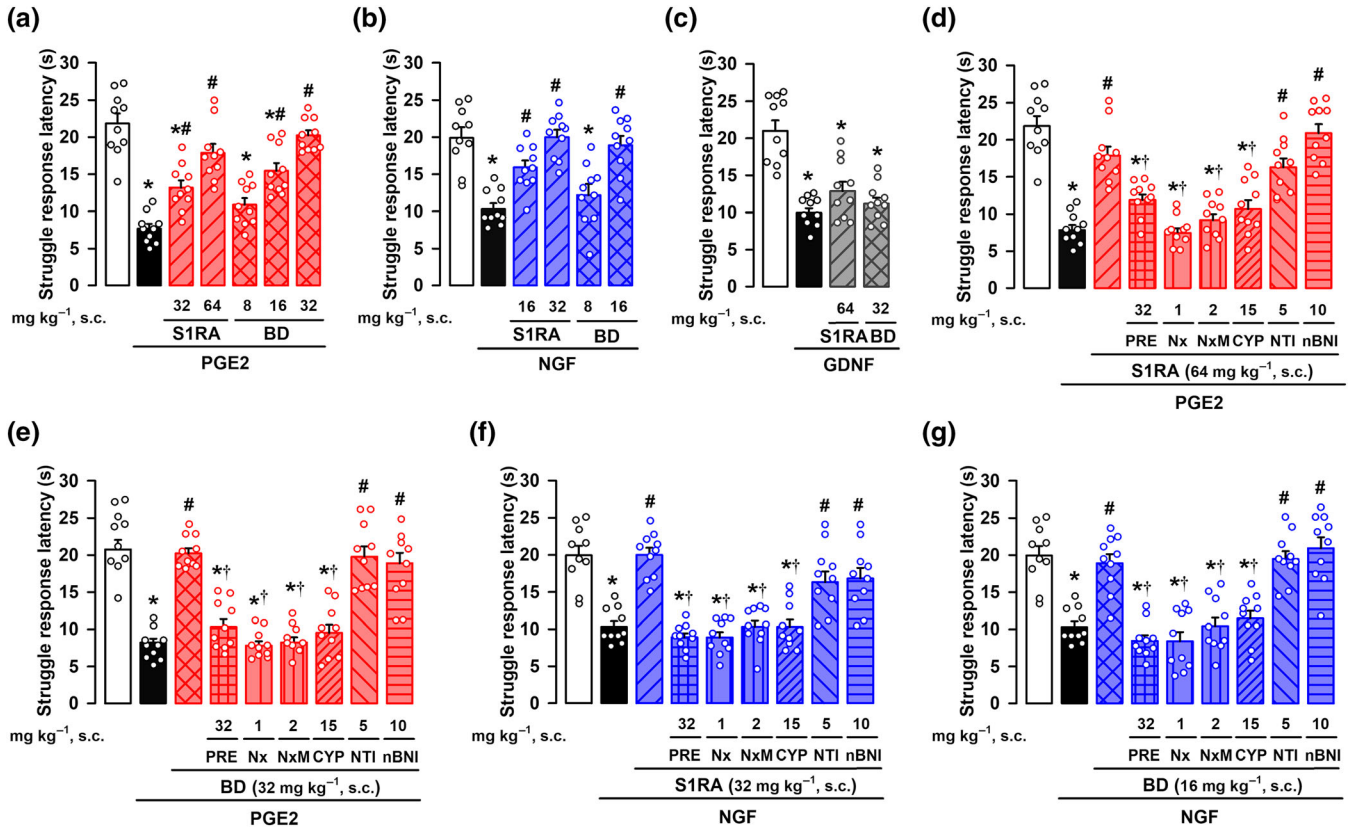

FIGURE 3 Effects of the systemic administration of the sigma-1 antagonists S1RA and BD-1063 (BD) on mechanical hyperalgesia induced by PGE2, NGF, or GDNF. The results represent the latency to struggle response evoked by a mechanical stimulus of 100 g in female mice administered subcutaneously (s.c.) with S1RA, BD or saline, and intraplantarly (i.pl.) with (a) PGE2 (0.5 nmol), (b) NGF (1 μg), (c) GDNF (40 ng), or their solvents. Animals treated with S1RA or BD or their solvent also received s.c. administration of PRE-084 (PRE), naloxone (Nx), naloxone methiodide (NxM), cyprodime (CYP), naltrindole (NTI), nor-binaltorphimine (nBNI), or saline and were tested on mechanical hyperalgesia induced by PGE2 (d,e) and NGF (f,g). Values are the mean ± SEM (10 animals per group). Statistically significant differences between the values obtained in control non-sensitized animals (white bars) and the other experimental groups, $^{*}P < 0.05$; between the values obtained in PGE2-, NGF-, or GDNF-treated animals administered with S1RA, BD or their solvent, $\#P < 0.05$; and between PGE2- or NGF-treated animals administered with S1RA or BD alone or their association with PRE, Nx, NxM, or CYP, $\dagger P < 0.05$ (one-way ANOVA followed by Bonferroni test). Data shown in (c) and (e) were log-transformed to meet the ANOVA assumptions.

NGF-induced hyperalgesia involves the activation of peripheral μ receptors, but not other opioid receptor subtypes. The s.c. administration of S1RA and BD-1063 also induced robust antihyperalgesic effects to a heat stimulus in mice sensitized with PGE2 and NGF, and these were reversed by both the sigma-1 agonist PRE-084 and the peripheral opioid antagonist Nx-M (Figure S5), mirroring the peripheral opioid effects induced by sigma-1 antagonism on mechanical hyperalgesia induced by these sensitizers of TRPV1+ neurons.

We also tested the effects of PRE-084 and the opioid antagonists on the antihyperalgesic effect induced by morphine in female mice, the prototypic opioid agonist. Systemic (s.c.) administration of morphine induced a dose-dependent antihyperalgesic effect in animals sensitized with PGE2 and tested with the mechanical stimulus (Figure S6A). The antihyperalgesic effect of morphine was not modified by the sigma-1 agonist PRE-084 (Figure S6B) (at the same dose that reverses the antihyperalgesic effect induced by the sigma-1 antagonists). The antihyperalgesic effect of morphine was fully reversed by the opioid antagonist naloxone and its quaternary derivative naloxone methiodide (Figure S6B), indicating that these effects were mediated peripherally. Morphine effects were also dose-dependently and fully reversed by cyprodime (at the same dose used to reverse the effect of sigma-1 antagonists), but not by naltrindole or nor-binaltorphimine (Figure S6B).

We also tested the effect of morphine on GDNF-induced hyperalgesia in female mice. This opioid also induced a dose-dependent antihyperalgesic effect (Figure S6C), which was reversed by naloxone but not by PRE-084 (Figure S6D). In contrast to the results on PGE2-induced hyperalgesia, naloxone methiodide did not modify the antihyperalgesic effect of morphine after sensitization with GDNF (Figure S6D), pointing to central actions of morphine as responsible for this antihyperalgesic effect. These results highlight the differences of opioid effects depending on the peripheral sensitizer used, which might be related to the different neuronal populations sensitized by each algogen.

In summary, both the systemic administration of sigma-1 antagonists and morphine induced antihyperalgesic effects, which are mediated by peripheral μ receptor activation, in animals sensitized with PGE2 or NGF. The opioid mediated antihyperalgesic effects of sigma-1 antagonism were absent though on GDNF-induced hypersensitivity.

Moreover, morphine's antihyperalgesic effect on GDNF-induced hyperalgesia does not depend on peripheral opioid receptors.

3.3 | Sigma-1 antagonism and endogenous opioid peptides

We hypothesized that sigma-1 antagonists might induce peripheral opioid effects at the peripheral terminal sensitized site, where the animals receive sensory stimulation. The i.pl. administration of the sigma-1 antagonists S1RA or BD-1063 dose-dependently fully reversed mechanical hyperalgesia induced by either PGE2 (Figure 4a) or NGF (Figure 4b) in female mice, without altering the response latency of non-sensitized animals (Figure S7).

To neutralize the actions of endogenous opioid peptides at the sensitized site, we administered i.pl. a monoclonal antibody, 3-E7, which recognizes the pan-opioid sequence Tyr-Gly-Gly-Phe at the *N*-terminus of most opioid peptides (see Methods). The i.pl. administration of 3-E7 did not modify the antihyperalgesic effect of S1RA or BD-1063 on the mechanical hyperalgesia induced by either PGE2 (Figure 4c) or NGF (Figure 4d), suggesting that opioid peptides containing the target sequence of the 3-E7 do not mediate the effect

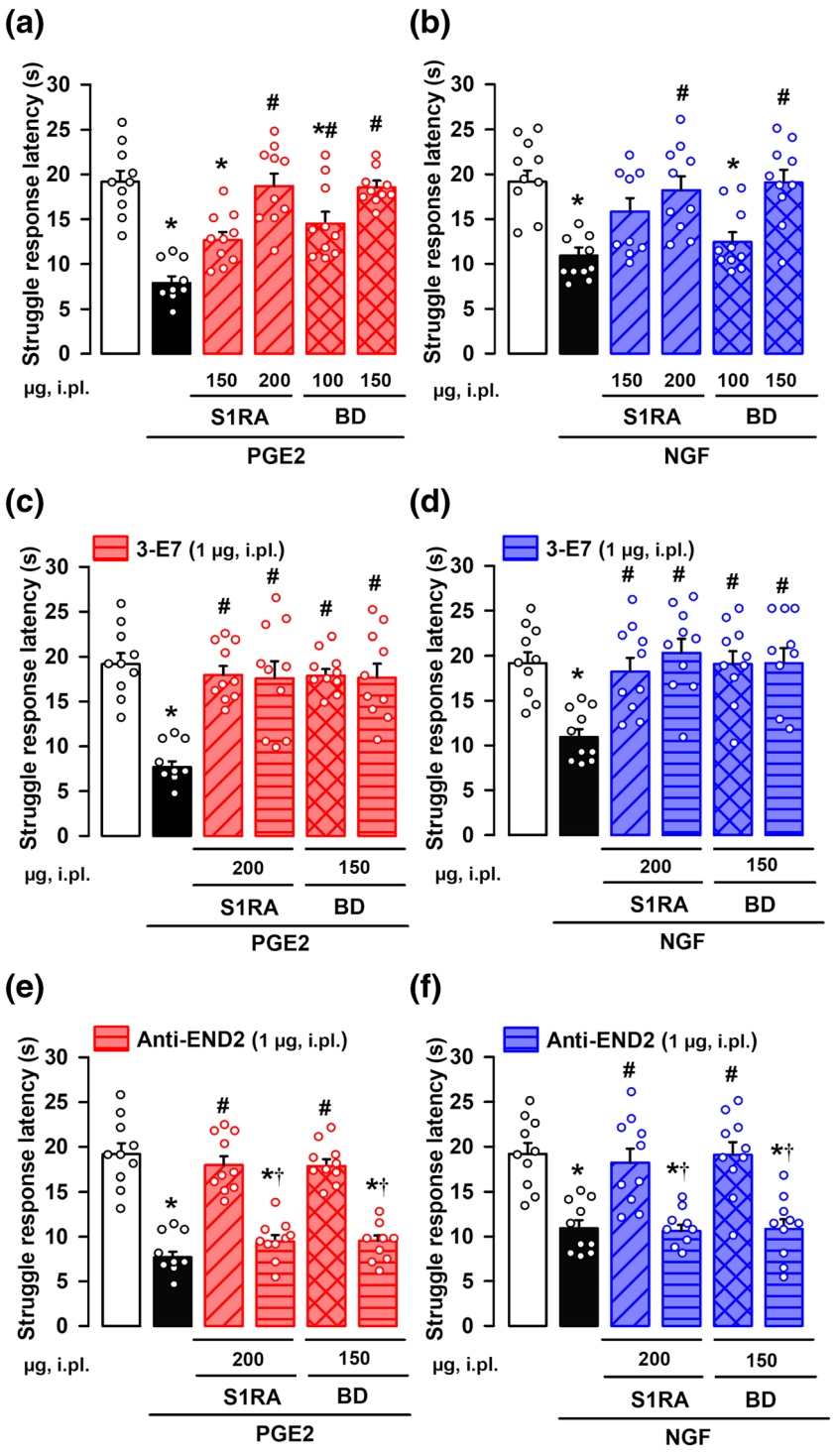

FIGURE 4 Effects of the local administration of the sigma-1 antagonists S1RA and BD-1063 (BD) on mechanical hyperalgesia induced by PGE2 or NGF. The results represent the latency to struggle response evoked by a mechanical stimulus of 100 g in female mice administered intraplantarly (i.pl.) with S1RA, BD or saline, and with (a,c,e) PGE2 (0.5 nmol), (b,d,f) NGF (1 μg) or their solvent. Animals i.pl. treated with S1RA or BD were also coadministered with antibodies for endogenous opioid peptides: 3-E7 (c,d) and anti-endomorphin-2 (anti-END2) (e,f), and tested on mechanical hyperalgesia induced by PGE2 (c,e) and NGF (d,f). Values are the mean ± SEM (10 animals per group). Statistically significant differences between the values obtained in control non-sensitized animals (white bars) and the other experimental groups, $^{*}P < 0.05$; between the values obtained in PGE2- or NGF-treated animals administered with S1RA, BD or their solvent $\#P < 0.05$; and between PGE2- or NGF-treated animals administered with S1RA or BD alone or coadministered with the anti-END2 antibody, $\dagger P < 0.05$ (one-way ANOVA followed by Bonferroni test). Data shown in C were log-transformed to meet the ANOVA assumptions.

observed. The administration of this antibody did not alter the response latency of non-sensitized animals (Figure S7).

Taking into account that the antihyperalgesic effect of sigma-1 antagonism appeared to be mediated exclusively by peripheral μ receptor activation (as described in the preceding section), that endomorphins have a high affinity and selectivity for μ receptors, and that these endogenous opioid peptides differ from most opioid peptides in their *N*-terminal sequence (Horvath, 2000; Machelska, 2011) (and therefore are not susceptible to the neutralizing effects of 3-E7), they are candidates for the antihyperalgesic effects induced by sigma-1 antagonists. Supporting this, i.pl. administration of a monoclonal antibody against endomorphin-2 did not alter the response latency of non-sensitized animals (Figure S7), but fully reversed the local antihyperalgesic effect of both S1RA and BD-1063 after sensitization with either PGE2 (Figure 4e) or NGF (Figure 4f).

We then aimed to identify the source of the endomorphin-2 responsible for the antihyperalgesic effects induced by sigma-1 antagonism, and tested immune cells and peripheral sensory neurons as possible sources of this endogenous opioid peptide.

We first tested in female mice whether the i.pl. administration of the peripheral sensitizers recruited immune cells to the injected site. We used samples from mice administered with carrageenan, a pro-inflammatory agent, as a positive control. Although we found a prominent increase in immune cells (stained with the pan-haematopoietic cell marker CD45) in paw tissue after carrageenan injection, we did not find any accumulation of these cells after the injection of PGE2, NGF or GDNF (Figure 5a,b). As neutrophils are known to be recruited early to the inflamed site (Rittner et al., 2001), we also tested for possible increases of this specific immune cell population. We found prominent neutrophil (CD45+ CD11b+ Ly6G+ cells) recruitment in paw tissue after carrageenan injection, but we did not find any accumulation of these immune cells after the injection of any of the algogenic compound tested (Figure 5c,d). Therefore, it is unlike that immune cells would constitute the cells harbouring the endogenous opioid peptides responsible of the peripheral opioid effects induced by sigma-1 antagonism against PGE2- and NGF-induced hyperalgesia.

We then tested whether endomorphin-2 was present in peripheral sensory neurons from female mice. Using the same antibody which administered in vivo was able to abolish the antihyperalgesic effects of sigma-1 antagonists, we found endomorphin-2 immunoreactivity in DRG samples, and interestingly, the majority of this labelling was found in TRPV1+ nociceptors, with a virtual absence of endomorphin-2 staining on IB4+ neurons (see left panels of Figure 6a and Figure 6b). A few additional larger cells with neuronal morphology (TRPV1-/IB4- cells) were also found to be endomorphin-2+ (see left panels of Figures 6a and S8 for representative images of high and low magnification, respectively). As a proof of the specificity of the expression of endomorphin-2 by TRPV1+ nociceptors, we performed immunostaining experiments after RTX administration. Treatment with this toxin completely ablated TRPV1 staining and most of endomorphin-2 immunoreactivity (Figure 6c), which remained only in some larger neurons, whereas IB4 labelling was globally preserved (see right panels of Figures 6a and S8), and in fact constituted most of the labelled (CGRP+, IB4+ or TRPV1+) neurons after the ablation of TRPV1+ nociceptors (Figure 6c). These results suggest that most of the neurons which express endomorphin-2 correspond to peptidergic (TRPV1+) nociceptors. This pattern for the expression of endomorphin-2 agrees with the naloxone-sensitive effect of sigma-1 antagonists on hyperalgesia induced by sensitizers of TRPV1+ nociceptors, such as PGE2 or NGF.

3.4 | Sigma-1R: A link between TRPV1 and the μ receptor

As previously commented, the modulation of μ receptor antinociception by sigma-1R at the CNS involves the participation of NMDARs (see the Introduction section). Taking into account that the opioid-dependent antihyperalgesic effect of Sigma-1 antagonism appears to involve peptidergic (TRPV1+) nociceptors, we hypothesized that the central NMDAR mechanism might have a peripheral analogue based on interactions of TRPV1, sigma-1Rs, and μ receptor.

Using recombinant proteins, we found that the sigma-1R and CaM each strongly interact with the C-terminal segment of TRPV1 in the presence of calcium (see lanes one and seven in Figure 7a). However, when the sigma-1R and CaM are present together, sigma-1R binds to TRPV1 and this does not allow CaM to bind the C-terminal domain of TRPV1 (Figure 7a, lane six). This CaM binding site is important for TRPV1 desensitization (Numazaki et al., 2003). The presence of either S1RA or BD-1063 hinders the interaction between sigma-1R and TRPV1 (Figure 7a, lanes two and four), allowing CaM to bind to the TRPV1 channel (Figure 7a, lanes three and five). We also examined the influence of μ receptors on the interaction between sigma-1Rs and TRPV1 and found that the presence of the C-terminus of the μ receptor, which contains the binding site for sigma-1Rs (Rodríguez-Muñoz, Cortés-Montero, et al., 2015; Rodríguez-Muñoz, Sánchez-Blázquez, et al., 2015) enhanced the dissociation of sigma-1Rs from the C-terminal domain of TRPV1 that is induced by S1RA (Figure 7b). In the presence of S1RA, the interaction between the sigma-1R and the C-terminal domain of the μ receptor is markedly increased, in spite of the presence of the C-terminus of TRPV1 (Figure 7c). In other words, sigma-1 antagonism promotes the transfer of sigma-1Rs from the C-terminal domain of TRPV1 to the C-terminus of the μ receptor, and this facilitates the binding of CaM to the C-terminus of TRPV1.

Our results indicate that sigma-1Rs are a key player in the cross-talk between the μ receptor and TRPV1. We then explored the functional consequences of the interactions between sigma-1Rs, TRPV1 and opioid receptors for nociceptor sensitization. We performed calcium-imaging experiments on cultured capsaicin-sensitive DRG neurons from adult female mice, sensitized with PGE2. The application of a low concentration of capsaicin (0.05 μM) produced a hardly measurable increase in intracellular calcium concentration. However, after application of PGE2, these neurons showed a robust increase in intracellular calcium in response to the same low

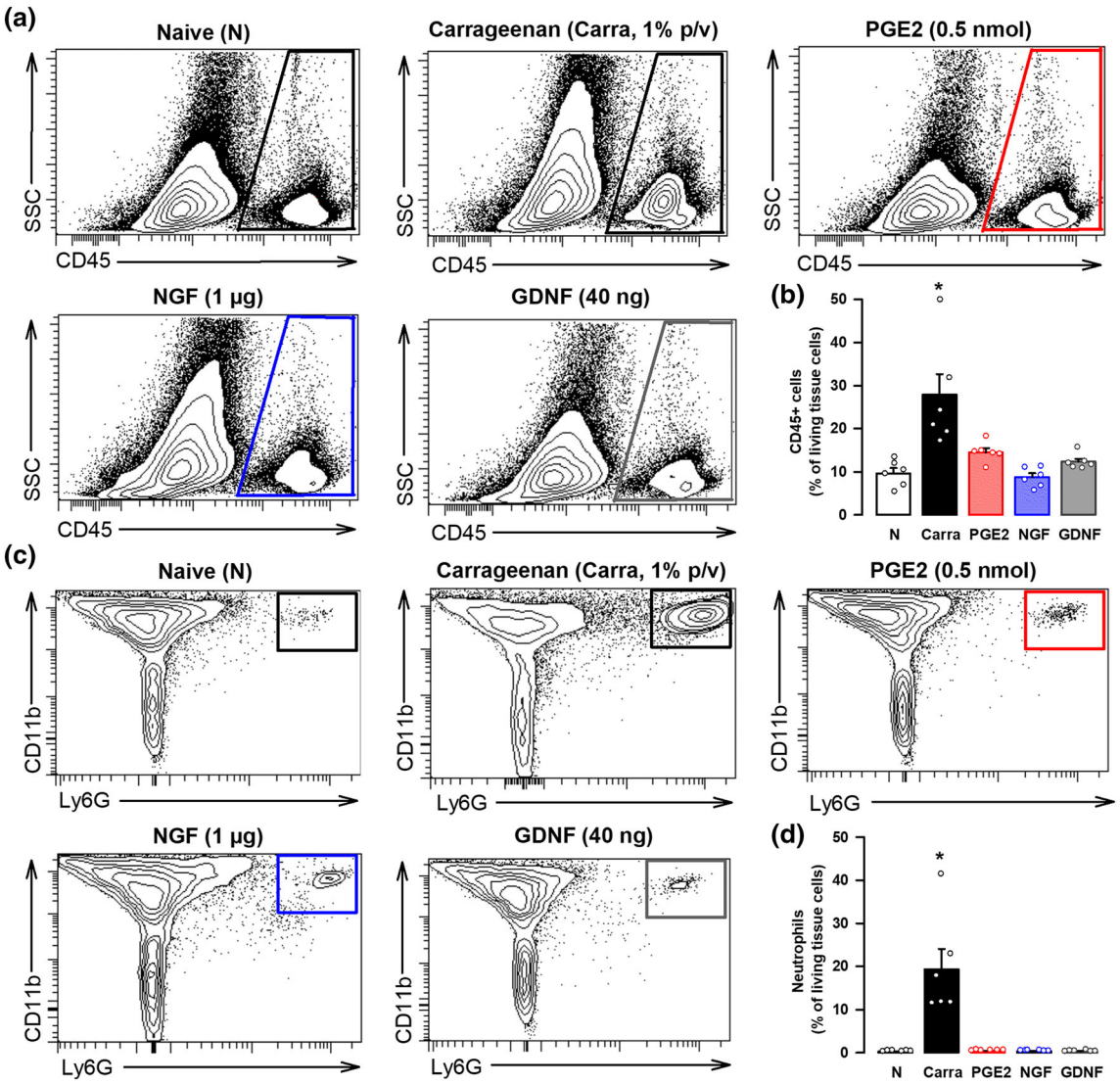

FIGURE 5 The in vivo treatment with PGE2, NGF, or GDNF does not produce significant immune cell recruitment at the injection site. (a) Representative side scatter (SSC) versus CD45 plots showing that cells from haematopoietic lineage (CD45+ cells) do not increase in the paw from female mice after intraplantar (i.pl.) administration of PGE2 (0.5 nmol), NGF (1 µg) or GDNF (40 ng), in comparison to naïve (N) mice, whereas this cell population greatly increases after the i.pl. administration of a solution containing 1% carrageenan (carra). Gating for CD45+ cells is shown as a trapezoid in the right side of each FACS diagram. (b) Quantification of CD45+ cells with respect to the number of living cells in the paw from naïve mice and after the i.pl. treatments. (c) Representative FACS diagrams, gated from CD45+ cells, showing that neutrophils (CD11b + Ly6G+ cells) greatly increase in the paw from female mice after the i.pl. administration of carra but not after the injection of any of the three peripheral sensitizers tested. Gating for neutrophil quantification is shown as a square in the right corner of each FACS diagram. (d) Quantification of neutrophils with respect to the number of living cells in the paw from naïve mice and after the i.pl. treatments. (b,d) values are the mean ± SEM (six animals per group). Statistically significant differences between naïve and carrageenan-treated animals: $^*P < 0.05$. There were no significant differences between naïve animals and those treated with PGE2, NGF, and GDNF (one-way ANOVA followed by Bonferroni test). Data shown in (b) and (d) were log-transformed to meet the ANOVA assumptions.

concentration of capsaicin (see Figure 7d for a representative recording), indicating the sensitization of TRPV1+ nociceptors by this algogen. We then measured the effect of S1RA on the PGE2-induced sensitization, and found that application of the sigma-1 antagonist greatly decreased the peak amplitude of PGE2-sensitized neurons responses to capsaicin (Figure 7e). This effect was reversed by the opioid antagonist naloxone (Figure 7e), which indicates that S1RA acts through opioid activation to reverse TRPV1+ neuron sensitization.

4 | DISCUSSION

TRPV1+ nociceptors can be sensitized by PGE2 and NGF to express the endogenous µ receptor agonist endomorphin-2. We show that sigma-1Rs are also expressed by these neurons and participate in the crosstalk between TRPV1 and the µ receptor, tonically limiting the antihyperalgesic effect of the endogenous opioid peptide.

Most CGRP+ DRG neurons express TRPV1 and vice versa, as shown here and in previous studies (Priestley, 2009). On the other

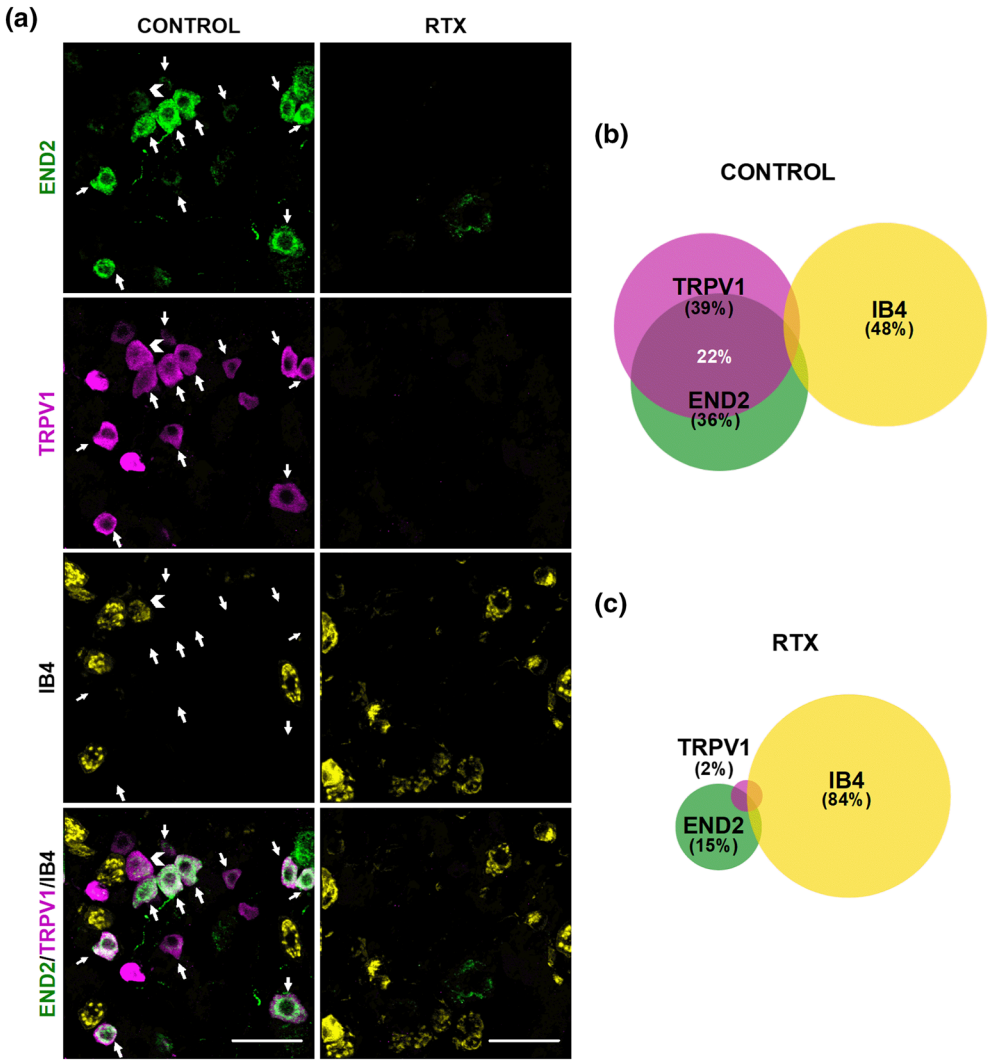

FIGURE 6 Endomorphin-2 (END2) is present in TRPV1+ but not in IB4+ neurons. (a) Triple labelling of endomorphin-2 (END2, green), TRPV1 (magenta), and isolectin B4 (IB4, yellow) in L4 DRG from female mice. Left panels: samples from solvent-treated mice (control). Right panels: samples from mice treated with resiniferatoxin (RTX). White arrows indicate co-localization of END2 and TRPV1 markers. White arrowhead indicates co-localization of TRPV1 and IB4 markers. Scale bar 50 μm. (b,c) Venn diagrams displaying the percentage of TRPV1+, IB4+, and END2+ neurons among the total number of neurons labelled with any of these markers in samples from control mice (b) and from mice treated with RTX (c). Samples from five mice per group were used to construct the Venn diagrams.

hand, TRPV1+ and IB4+ neurons constitute separate cellular populations (peptidergic and non-peptidergic C-nociceptors, respectively) with only occasional overlap, as shown in the current and previous studies using several mouse strains (Sheehan et al., 2019; Woodbury et al., 2004; Zwick et al., 2002). The in vivo ablation of peptidergic (TRPV1+) C neurons by RTX increased the response latency to heat stimulus in non-sensitized animals and in mice sensitized with PGE2 or NGF, in agreement with the known role of TRPV1+ neurons in the coding of heat nociception or hypersensitivity (Cavanaugh et al., 2009). We also show that RTX-sensitive neurons are dispensable for mechanical nociceptive pain, in agreement with previous studies (Montilla-García et al., 2018; Zhang et al., 2013), but they are essential for the mechanical hyperalgesia induced by PGE2 or NGF. These latter results can be explained by the fact that both algogenic compounds induce mechanosensitivity in those nociceptors, which are normally mechanically insensitive (Emery et al., 2016; Prato et al., 2017), and this might be dependent on a phenotypic switch of peptidergic C-nociceptors (Prato et al., 2017).

The systemic administration of the sigma-1 antagonists S1RA and BD-1063 reversed mechanical and heat hyperalgesia induced by PGE2 and NGF. Interestingly, the opioid antagonist naloxone and its peripherally restricted analogue naloxone methiodide abolished the effects of not only morphine, used as a reference opioid analgesic, but also those of the sigma-1 antagonists, indicating that these effects were opioid in nature and mediated peripherally. These peripheral opioid-dependent effects induced by sigma-1 antagonism are seen in mice from both sexes. In addition, the antihyperalgesic effects of sigma-1 antagonists and morphine to the mechanical stimulus involved the activation of the μ receptor, but not other opioid receptor subtypes, as they were fully reversed by cyprodime, but not by naltrindole or nor-binaltorphimine (at doses known to inhibit μ, δ, and κ opioid receptor responses, respectively) (Baamonde et al., 2005; Hutcheson et al., 1999). Although it seems evident to attribute the effect of morphine to direct actions on the μ receptor (Matthes et al., 1996), S1RA or BD-1063 lack any affinity for the μ receptor (Sánchez-Fernández et al., 2013). Since sigma-1 antagonism is known to potentiate opioid analgesia (Sánchez-Fernández et al., 2017), we hypothesized that the peripheral opioid-like effects of sigma-1 antagonists on PGE2- and NGF-induced hyperalgesia might be the result of the potentiation of endogenous opioid peptides released at the

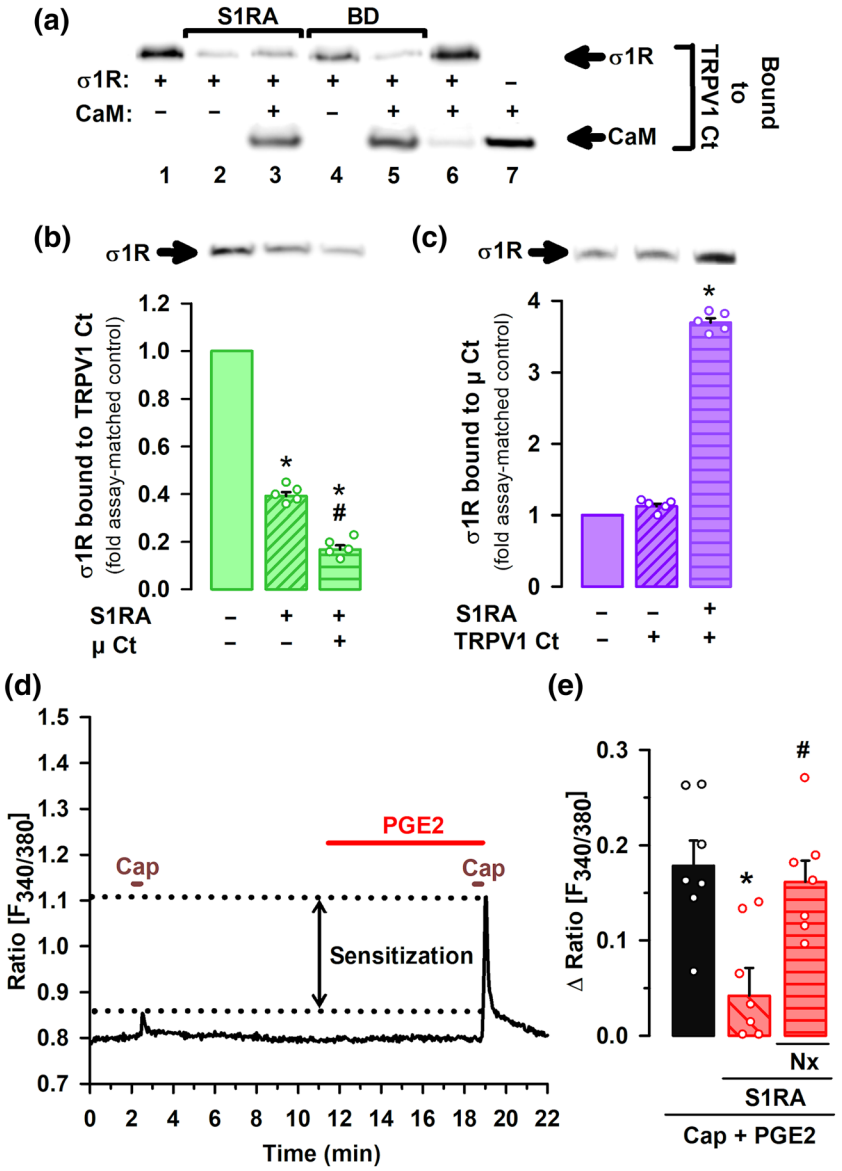

FIGURE 7 Influence of sigma-1 antagonism on the interaction of the sigma-1 receptor (σ1R) with TRPV1 and μ opioid receptors (μ), and the effect on PGE2-induced sensitization of TRPV1 neurons. (a–c) Experiments were performed in the presence of $CaCl_2$. Blots shown are representative of three experiments. Gels were cropped to show bands under investigation only and full-length gels are provided in Figure S9A–C. (a) Effect of S1RA and BD-1063 (BD) on the in vitro interaction of the σ1R and calmodulin (CaM) with the C-terminus (Ct) of TRPV1. σ1R and CaM were incubated with TRPV1 Ct immobilized in N-hydroxysuccinimide (NHS)-activated Sepharose. The bands represent σ1R and CaM that remained bound to the TRPV1 Ct after incubation with the sigma-1 antagonists or their solvent. (b) Immobilized TRPV1 Ct was incubated with σ1R with and without μ receptor Ct. The blots represent σ1R that remained bound to the TRPV1 Ct after incubation with S1RA or its solvent. (c) Immobilized μ receptor Ct (μ Ct) was incubated with σ1R with and without TRPV1 Ct. The blots represent σ1R that remained bound to the μ receptor Ct after incubation with S1RA or its solvent. (b,c) The signals from the blots were expressed as the change relative to the controls, which were assigned an arbitrary value of 1. Values are the mean ± SEM (five determinations per group). Statistically significant differences between the values obtained in the control (solid bars) and the other experimental groups (striped bars), $^*P < 0.05$; and between the values of the group incubated with S1RA alone or with μ receptor Ct, $\#P < 0.05$ (Kruskal–Wallis test followed by Student–Newman–Keuls test). (d) Representative calcium imaging recording (ratio F340/380) of a cultured mouse DRG neuron treated with 0.05-μM capsaicin (Cap) before and after treatment with PGE2. Sensitization of calcium flux by PGE2 is shown between dotted lines. (e) Mean response amplitudes for the increase in the ratio F340/380 in response to capsaicin in cultured neurons from female mice, sensitized with PGE2 and incubated with S1RA, naloxone (Nx) or their solvents. Values are the mean ± SEM of the values found in seven different culture dishes, each obtained from a different mouse. Statistically significant differences between the capsaicin response in PGE2-sensitized neurons incubated with S1RA or its solvent, $^*P < 0.05$; and between the responses from sensitized neurons treated with S1RA alone or associated with Nx, $\#P < 0.05$ (one-way ANOVA followed by Bonferroni test).

sensitized site. The local administration of sigma-1 antagonists abolished PGE2- and NGF-induced mechanical hyperalgesia, and this was not reversed by the administration of 3-E7, a monoclonal antibody which recognizes the *N*-terminus of most endogenous opioid peptides. However, the antihyperalgesic effects of sigma-1 antagonists were reversed by an antibody against endomorphin-2, which lacks the consensus *N*-terminus of other opioid peptides and, in agreement with the previously commented μ-opioid selectivity of the effects induced by sigma-1 antagonists, is a selective μ-opioid agonist (Horvath, 2000; Machelska, 2011).

Immune cells can produce endomorphins (Labuz et al., 2006; Mousa et al., 2002), and we recently described that sigma-1 antagonism enhances the opioid analgesia induced by opioid peptides released by immune cells during inflammation (Tejada et al., 2017). We show here that PGE2 or NGF did not recruit immune cells which might account for the antihyperalgesic effect of sigma-1 antagonists. However, we found endomorphin-2 immunoreactivity in mouse DRG neurons, in agreement with previous studies in the rat which show that endomorphin-2 (but not endomorphin-1) is produced by peripheral sensory neurons (Fichna et al., 2007; Sanderson et al., 2004; Scanlin et al., 2008). Importantly, we show that endomorphin-2 is mostly expressed by peptidergic (TRPV1+) nociceptors, a result which also agrees with the previously described distribution of this endogenous opioid peptide in the rat DRG, as it was shown to colocalize with substance P and CGRP (Sanderson et al., 2004). Therefore, the sensory neurons required for PGE2- and NGF-induced hyperalgesia are the same neuronal subtype which expresses endomorphin-2. In spite of the well-known analgesic actions of endomorphin-2 (Fichna et al., 2007), the production of this endogenous opioid peptide by TRPV1+ nociceptors seems not enough to counterbalance sensitization by PGE2 or NGF, except when potentiated by sigma-1 antagonists. Our results might be explained by the contribution of an autocrine mechanism, in the peripheral terminal of the nociceptor, in the antihyperalgesic effect of sigma-1 antagonism.

The modulation of μ receptor-mediated analgesia by sigma-1Rs in the CNS relies on the binding of the sigma-1R to the NMDAR, physically preventing the binding of CaM to the NMDAR, and hence reducing the inhibition of channel activity. Sigma-1 antagonism dissociates Sigma-1Rs from NMDAR and transfer them to the μ receptor. In this situation, CaM gains access to NMDARs to curtail channel activity and consequently enhancing μ receptor actions (Rodríguez-Muñoz, Cortés-Montero, et al., 2015 and b). TRPV1 is another protein partner of sigma-1Rs (Cortés-Montero et al., 2019; Ortíz-Rentería et al., 2018), and similar to NMDARs, it is a Ca^{2+} channel regulated by CaM, and binding of CaM to the C-terminus of TRPV1 promotes the desensitization of the channel (Numazaki et al., 2003). We showed here that sigma-1 antagonism promotes the transfer of sigma-1Rs from the C-terminal domain of TRPV1 to the C-terminus of the μ receptor, and this facilitates the binding of CaM to the C-terminus of TRPV1. Therefore, we show for the first time that the mechanism for the modulation of opioid analgesia by sigma-1Rs based on the cross-talk between NMDARs and μ receptors has an analogue on peptidergic C neurons that uses TRPV1 instead of NMDARs. TRPV1 has multiple known protein partners able to alter channel function (Zhao & Tsang, 2016). It may be worth testing in future studies whether sigma-1R influence other components of the interactome of TRPV1 in addition to CaM binding.

PGE2 increased calcium flux induced by capsaicin, the prototype TRPV1 agonist (Wainger et al., 2015), and this was fully reversed by S1RA, and in a naloxone-sensitive manner. It is known that endomorphin-2 is released by DRG neurons in response to intracellular calcium increases (Scanlin et al., 2008), and this might account for the naloxone-sensitive effect of S1RA that we recorded. Altogether, our data and previous literature show that sensitization of TRPV1 results in enhanced Ca^{2+} influx, which promotes the release of endogenous opioid peptides (endomorphin-2) with the potential to induce

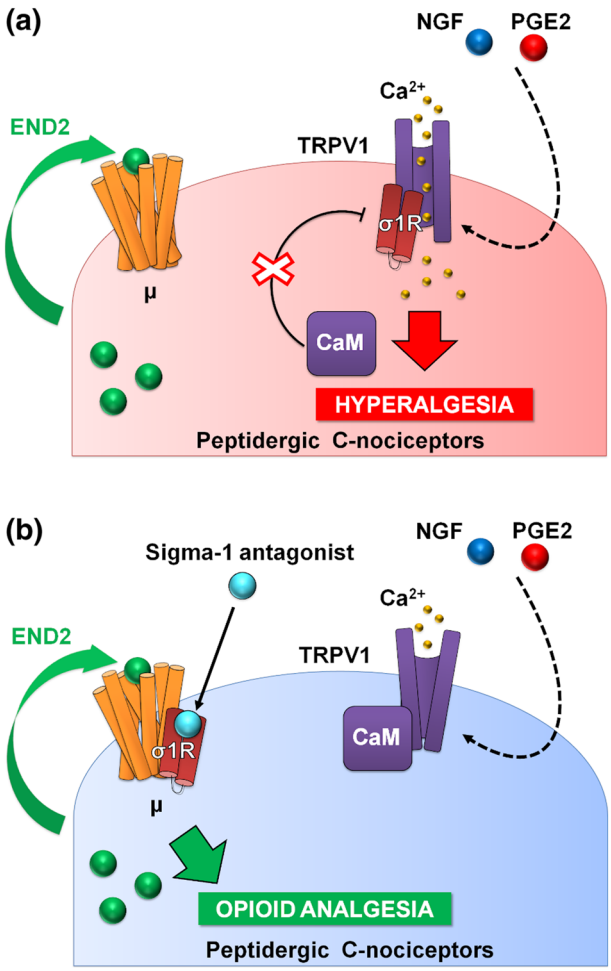

FIGURE 8 Proposed mechanism of action for the effects of sigma-1 antagonism on hyperalgesia induced by sensitization of TRPV1+ neurons. (a) Sensitization by algogenic chemicals (such as PGE2 and NGF) favours Ca^{2+} influx through TRPV1. In response to Ca^{2+}, the sigma-1 receptor (σ1R) binds to TRPV1 preventing calmodulin (CaM) binding (and therefore preventing desensitization of the channel). TRPV1+ neurons produce the endogenous opioid peptide endomorphin-2 (END2), whose effects are not sufficient to relieve hyperalgesia. (b) Sigma-1 antagonists transfer σ1R from TRPV1 to μ opioid receptors (μ), and this facilitates the interaction of CaM with the desensitization site of TRPV1 and the enhancement of the effects of END2, producing opioid-mediated antihyperalgesic effects during nociceptor sensitization.

analgesia through μ receptor activation. However, this analgesia through neuronally derived endogenous opioids might be curtailed by the binding of sigma-1R to TRPV1 (Figure 8a). Sigma-1R antagonists trigger the transfer of sigma-1Rs from the TRPV1 to the μ receptor, decreasing Ca^{2+} flux and enhancing the action of endomorphin-2 to induce opioid analgesia in the sensitized peripheral terminal, in the absence of an exogenous opioid drug (Figure 8b).

Although extracellular calcium influx through TRPV1 after capsaicin activation is the expected primary responsible for the increase in intracellular calcium we observed, other channels in the plasma membrane and in intracellular locations can also be activated after TRPV1 stimulation and participate in calcium flux (DuBreuil et al., 2021; Shah et al., 2020). Therefore, the modulation of TRPV1 by sigma-1 receptors may merit further study using electrophysiological recordings as a more direct approach to study channel functioning.

TRPV1 are relevant for both pain and itch (Roberson et al., 2013). As sigma-1 antagonism decreases sensitization of TRPV1+ neurons, it could be hypothesized that these drugs might induce antipruritogenic effects in addition to the antihyperalgesic effects showed here. This possibility will be addressed in future studies.

We also tested the effects of sigma-1 antagonism on the hyperalgesia induced by a different peripheral sensitizer: GDNF. This algogenic compound induced RTX-insensitive mechanical hyperalgesia without inducing significant heat hypersensitivity. The cellular targets of GDNF are non-peptidergic C-nociceptors (IB4+ neurons) (Álvarez et al., 2012), a neuronal population resistant to RTX (Montilla-García et al., 2018; Zhang et al., 2013) that although relevant for mechanical hypersensitivity, is dispensable for heat sensitivity (Cavanaugh et al., 2009), which explains the behavioural effects observed. We also show that sigma-1 antagonism was absolutely devoid of effect on GDNF-induced hyperalgesia in either female or male mice. It is relevant to note that IB4+ neurons do not express endomorphin-2. In addition, similar to PGE2 or NGF, GDNF failed to recruit immune cells at the site of injection that could harbour endogenous opioid peptides to be potentiated by sigma-1 antagonism. Therefore, our results suggest that sigma-1 antagonism requires the presence of an opioid agonist that can be potentiated in order to relieve hyperalgesia from peripheral sensitization, and point to the specificity in the modulation of the sensitization of TRPV1 neurons because of their content on endomorphin-2.

TRPV1+ nociceptors constitute a relatively small population of neurons in the mouse, but most human nociceptors express TRPV1 (Middleton et al., 2021). Therefore, it would be expected that the effects of sigma-1 antagonism on peripheral sensitization, which appear to be restricted to TRPV1+ neurons in the mouse, would be broader in humans.

In summary, sigma-1 receptors limit peripheral opioid analgesia during sensitization of peptidergic C nociceptors. Sigma-1 antagonists are able to harness neuronally derived endogenous opioids to reduce hyperalgesia at the pain site, by promoting TRPV1 desensitization and increasing μ receptor activity. The modulation of endogenous opioid analgesia by sigma-1 receptors might have potential clinical application for pain treatment.

ACKNOWLEDGMENTS

We thank Ana Santos Carro and Mohamed Tassi Mzanzi for their technical support with confocal microscopic image acquisition and Gustavo Ortíz Ferrón for his technical support in the FACs experiments. M.C. Ruiz-Cantero and E. Cortés-Montero were supported by the Training University Lecturers program (FPU) of the Spanish Ministry of Economy and Competitiveness (MINECO). This study was partially supported by the University of Granada (grant number PPJIB2019.11), the Spanish State Research Agency (10.13039/501100011033) under the auspices of MINECO (grant numbers SAF2016-80540-R and PID2019-108691RB-I00), the Andalusian Regional Government (grant CTS109), the European Regional Development Fund, and Harvard Medical School - Blavatnik Sensory Disorders Research Award. This research was done in partial fulfilment of the requirements for the doctoral thesis of M.C.R.-C. Funding for open access charge: University of Granada / CBUA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M Carmen Ruiz-Cantero: Conceptualization; funding acquisition; investigation; methodology; writing—original draft; writing—review and editing. **Elsa Cortés-Montero:** Investigation; methodology; writing—original draft; writing—review and editing. **Aakanksha Jain:** Investigation; methodology; supervision; writing—original draft; writing—review and editing. **Ángeles Montilla García:** Investigation; methodology; writing—original draft; writing—review and editing. **Inmaculada Bravo-Caparrós:** Investigation; methodology; writing—original draft; writing—review and editing. **Jaehoon Shim:** Investigation; methodology; writing—original draft; writing—review and editing. **Pilar Sánchez-Blázquez:** Investigation; methodology; writing—original draft; writing—review and editing. **Clifford Woolf:** Conceptualization; supervision; writing—original draft; writing—review and editing. **Jose M Baeyens:** Conceptualization; funding acquisition; supervision; writing—original draft; writing—review and editing. **Enrique J Cobos:** Conceptualization; funding acquisition; supervision; writing—original draft; writing—review and editing.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, Immunoblotting and Immunochemistry, and Animal experimentation, and as recommended by funding agencies, publishers, and other organizations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abadias, M., Escriche, M., Vaque, A., Sust, M., & Encina, G. (2013). Safety, tolerability and pharmacokinetics of single and multiple doses of a novel sigma-1 receptor antagonist in three randomized phase I studies. *British Journal of Clinical Pharmacology*, *75*(1), 103–117. https://doi.org/10.1111/j.1365-2125.2012.04333.x

Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Abbracchio, M. P., Alexander, W., Al-Hosaini, K., Bäck, M., Barnes, N. M., Bathgate, R., … Ye, R. D. (2021). The Concise Guide to PHARMACOLOGY 2021/22: G protein-coupled receptors. *British Journal of Pharmacology*, *178*-(Suppl 1), S27–S156. https://doi.org/10.1111/bph.15538

Alexander, S. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Buneman, O. P., Cidlowski, J. A., Christopoulos, A., Davenport, A. P., Fabbro, D., Spedding, M., Striessnig, J., Davies, J. A., Ahlers-Dannen, K. E., … Zolghadri, Y. (2021). The Concise Guide to PHARMACOLOGY 2021/22: Introduction and other protein targets. *British Journal of Pharmacology*, *178*(Suppl 1), S1–S26. https://doi.org/10.1111/bph.15537

Alexander, S. P., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Aldrich, R. W., Attali, B., Baggetta, A. M., Becirovic, E., Biel, M., Bill, R. M., Catterall, W. A., … Zhang, X. (2021). The Concise Guide to PHARMACOLOGY 2021/22: Ion channels. *British Journal of Pharmacology*, *178*(Suppl 1), S157–S245. https://doi.org/10.1111/bph.15539

Alexander, S. P. H., Roberts, R. E., Broughton, B. R. S., Sobey, C. G., George, C. H., Stanford, S. C., Cirino, G., Docherty, J. R., Giembycz, M. A., Hoyer, D., Insel, P. A., Izzo, A. A., Ji, Y., MacEwan, D. J., Mangum, J., Wonnacott, S., & Ahluwalia, A. (2018). Goals and practicalities of immunoblotting and immunohistochemistry: A guide for submission to the British Journal of Pharmacology. *British Journal of Pharmacology*, *175*(3), 407–411. https://doi.org/10.1111/bph.14112

Álvarez, P., Chen, X., Bogen, O., Green, P. G., & Levine, J. D. (2012). IB4(+) nociceptors mediate persistent muscle pain induced by GDNF. *Journal of Neurophysiology*, *108*(9), 2545–2553. https://doi.org/10.1152/jn.00576.2012

Baamonde, A., Lastra, A., Juarez, L., García, V., Hidalgo, A., & Menéndez, L. (2005). Effects of the local administration of selective μ-, δ- and κ-opioid receptor agonists on osteosarcoma-induced hyperalgesia. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *372*(3), 213–219. https://doi.org/10.1007/s00210-005-0013-6

Bravo-Caparrós, I., Perazzoli, G., Yeste, S., Cikes, D., Baeyens, J. M., Cobos, E. J., & Nieto, F. R. (2019). Sigma-1 receptor inhibition reduces neuropathic pain induced by partial sciatic nerve transection in mice by opioid-dependent and -independent mechanisms. *Frontiers in Pharmacology*, *10*, 613. https://doi.org/10.3389/fphar.2019.00613

Bravo-Caparrós, I., Ruiz-Cantero, M. C., Perazzoli, G., Cronin, S. J. F., Vela, J. M., Hamed, M. F., Penninger, J. M., Baeyens, J. M., Cobos, E. J., & Nieto, F. R. (2020). Sigma-1 receptors control neuropathic pain and macrophage infiltration into the dorsal root ganglion after peripheral nerve injury. *The FASEB Journal*, *34*(4), 5951–5966. https://doi.org/10.1096/fj.201901921R

Bruna, J., Videla, S., Argyriou, A. A., Velasco, R., Villoria, J., Santos, C., Nadal, C., Cavaletti, G., Alberti, P., Briani, C., Kalofonos, H. P., Cortinovis, D., Sust, M., Vaqué, A., Klein, T., & Plata-Salaman, C. (2018). Efficacy of a novel sigma-1 receptor antagonist for oxaliplatin-induced neuropathy: A randomized, double-blind, placebo-controlled phase IIa clinical trial. *Neurotherapeutics*, *15*(1), 178–189. https://doi.org/10.1007/s13311-017-0572-5

Cavanaugh, D. J., Lee, H., Lo, L., Shields, S. D., Zylka, M. J., Basbaum, A. I., & Anderson, D. J. (2009). Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(22), 9075–9080. https://doi.org/10.1073/pnas.0901507106

Cobos, E. J., Entrena, J. M., Nieto, F. R., Cendán, C. M., & Del Pozo, E. (2008). Pharmacology and therapeutic potential of sigma$_1$ receptor ligands. *Current Neuropharmacology*, *6*(4), 344–366. https://doi.org/10.2174/157015908787386113

Cortés-Montero, E., Sánchez-Blázquez, P., Onetti, Y., Merlos, M., & Garzón, J. (2019). Ligands exert biased activity to regulate sigma 1 receptor interactions with cationic TRPA1, TRPV1, and TRPM8 channels. *Frontiers in Pharmacology*, *10*, 634. https://doi.org/10.3389/fphar.2019.00634

Curtis, M. J., Alexander, S., Cirino, G., George, C. H., Kendall, D. A., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Patel, H. H., Sobey, C. G., Stanford, S. C., Stanley, P., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., & Ahluwalia, A. (2022). Planning experiments: Updated guidance on experimental design and analysis and their reporting III. *British Journal of Pharmacology*, *179*, 3907–3913. https://doi.org/10.1111/bph.15868

DuBreuil, D. M., Chiang, B. M., Zhu, K., Lai, X., Flynn, P., Sapir, Y., & Wainger, B. J. (2021). A high-content platform for physiological profiling and unbiased classification of individual neurons. *Cell Reports Methods*, *1*(1), 100004. https://doi.org/10.1016/j.crmeth.2021.100004

Edwards, D. A. (1968). Mice: Fighting by neonatally androgenized females. *Science*, *161*(3845), 1027–1028. https://doi.org/10.1126/science.161.3845.1027

Emery, E. C., Luiz, A. P., Sikandar, S., Magnusdottir, R., Dong, X., & Wood, J. N. (2016). In vivo characterization of distinct modality-specific subsets of somatosensory neurons using GCaMP. *Science Advances*, *2*(11), e1600990. https://doi.org/10.1126/sciadv.1600990

Entrena, J. M., Cobos, E. J., Nieto, F. R., Cendán, C. M., Gris, G., Del Pozo, E., Zamanillo, D., & Baeyens, J. M. (2009). Sigma-1 receptors are essential for capsaicin-induced mechanical hypersensitivity: Studies with selective sigma-1 ligands and sigma-1 knockout mice. *Pain*, *143*(3), 252–261. https://doi.org/10.1016/j.pain.2009.03.011

Fichna, J., Janecka, A., Costentin, J., & Do Rego, J. C. (2007). The endomorphin system and its evolving neurophysiological role. *Pharmacological Reviews*, *59*(1), 88–123. https://doi.org/10.1124/pr.59.1.3

González-Cano, R., Montilla-García, Á., Perazzoli, G., Torres, J. M., Canizares, F. J., Fernández-Segura, E., Costigan, M., Baeyens, J. M., & Cobos, E. J. (2020). Intracolonic mustard oil induces visceral pain in mice by TRPA1-dependent and -independent mechanisms: Role of tissue injury and P2X receptors. *Frontiers in Pharmacology*, *11*, 613068. https://doi.org/10.3389/fphar.2020.613068

Horvath, G. (2000). Endomorphin-1 and endomorphin-2: Pharmacology of the selective endogenous mu-opioid receptor agonists. *Pharmacology & Therapeutics*, *88*(3), 437–463. https://doi.org/10.1016/s0163-7258(00)00100-5

Hsieh, Y. L., Chiang, H., Tseng, T. J., & Hsieh, S. T. (2008). Enhancement of cutaneous nerve regeneration by 4-methylcatechol in resiniferatoxin-induced neuropathy. *Journal of Neuropathology and Experimental Neurology*, *67*(2), 93–104. https://doi.org/10.1097/nen.0b013e3181630bb8

Hutcheson, D. M., Sánchez-Blázquez, P., Rodríguez-Díaz, M., Garzón, J., Schmidhammer, H., Borsodi, A., Roques, B. P., & Maldonado, R. (1999). Use of selective antagonists and antisense oligonucleotides to evaluate the mechanisms of BUBU antinociception. *European Journal of Pharmacology*, *383*(1), 29–37. https://doi.org/10.1016/s0014-2999(99)00611-1

Ji, R. R., Chamessian, A., & Zhang, Y. Q. (2016). Pain regulation by non-neuronal cells and inflammation. *Science*, *354*(6312), 572–577. https://doi.org/10.1126/science.aaf8924

Kotliarova, A., & Sidorova, Y. A. (2021). Glial cell line-derived neurotrophic factor family ligands, players at the Interface of neuroinflammation

and neuroprotection: Focus onto the glia. *Frontiers in Cellular Neuroscience*, *15*, 679034. https://doi.org/10.3389/fncel.2021.679034

Labuz, D., Berger, S., Mousa, S. A., Zollner, C., Rittner, H. L., Shaqura, M. A., Segovia-Silvestre, T., Przewlocka, B., Stein, C., & Machelska, H. (2006). Peripheral antinociceptive effects of exogenous and immune cell-derived endomorphins in prolonged inflammatory pain. *The Journal of Neuroscience*, *26*(16), 4350–4358. https://doi.org/10.1523/JNEUROSCI.4349-05.2006

Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P. H., Cirino, G., Docherty, J. R., George, C. H., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Sobey, C. G., Stefanska, B., Stephens, G., Teixeira, M., & Ahluwalia, A. (2020). ARRIVE 2.0 and the British Journal of Pharmacology: Updated guidance for 2020. *British Journal of Pharmacology*, *177*(16), 3611–3616. https://doi.org/10.1111/bph.15178

Machelska, H. (2011). Control of neuropathic pain by immune cells and opioids. *CNS & Neurological Disorders Drug Targets*, *10*(5), 559–570. https://doi.org/10.2174/187152711796234952

Matthes, H. W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dollé, P., Tzavara, E., Hanoune, J., Roques, B. P., & Kieffer, B. L. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature*, *383*(6603), 819–823. https://doi.org/10.1038/383819a0

Mei, J., & Pasternak, G. W. (2007). Modulation of brainstem opiate analgesia in the rat by sigma 1 receptors: A microinjection study. *The Journal of Pharmacology and Experimental Therapeutics*, *322*(3), 1278–1285. https://doi.org/10.1124/jpet.107.121137

Menéndez, L., Lastra, A., Hidalgo, A., & Baamonde, A. (2002). Unilateral hot plate test: A simple and sensitive method for detecting central and peripheral hyperalgesia in mice. *Journal of Neuroscience Methods*, *113*(1), 91–97. https://doi.org/10.1016/s0165-0270(01)00483-6

Miczek, K. A., Thompson, M. L., & Shuster, L. (1982). Opioid-like analgesia in defeated mice. *Science*, *215*(4539), 1520–1522. https://doi.org/10.1126/science.7199758

Middleton, S. J., Barry, A. M., Comini, M., Li, Y., Ray, P. R., Shiers, S., Themistocleous, A. C., Uhelski, M. L., Yang, X., Dougherty, P. M., Price, T. J., & Bennett, D. L. (2021). Studying human nociceptors: From fundamentals to clinic. *Brain*, *144*(5), 1312–1335. https://doi.org/10.1093/brain/awab048

Montilla-García, Á., Perazzoli, G., Tejada, M. Á., González-Cano, R., Sánchez-Fernández, C., Cobos, E. J., & Baeyens, J. M. (2018). Modality-specific peripheral antinociceptive effects of mu-opioid agonists on heat and mechanical stimuli: Contribution of sigma-1 receptors. *Neuropharmacology*, *135*, 328–342. https://doi.org/10.1016/j.neuropharm.2018.03.025

Montilla-García, Á., Tejada, M. Á., Perazzoli, G., Entrena, J. M., Portillo-Salido, E., Fernández-Segura, E., Cañizares, F. J., & Cobos, E. J. (2017). Grip strength in mice with joint inflammation: A rheumatology function test sensitive to pain and analgesia. *Neuropharmacology*, *125*, 231–242. https://doi.org/10.1016/j.neuropharm.2017.07.029

Moriyama, T., Higashi, T., Togashi, K., Iida, T., Segi, E., Sugimoto, Y., Tominaga, T., Narumiya, S., & Tominaga, M. (2005). Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Molecular Pain*, *1*, 3. https://doi.org/10.1186/1744-8069-1-3

Mousa, S. A., Machelska, H., Schafer, M., & Stein, C. (2002). Immunohistochemical localization of endomorphin-1 and endomorphin-2 in immune cells and spinal cord in a model of inflammatory pain. *Journal of Neuroimmunology*, *126*(1–2), 5–15. https://doi.org/10.1016/s0165-5728(02)00049-8

Numazaki, M., Tominaga, T., Takeuchi, K., Murayama, N., Toyooka, H., & Tominaga, M. (2003). Structural determinant of TRPV1 desensitization interacts with calmodulin. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(13), 8002–8006. https://doi.org/10.1073/pnas.1337252100

Ortíz-Rentería, M., Juárez-Contreras, R., González-Ramirez, R., Islas, L. D., Sierra-Ramírez, F., Islas, L. D., Sierra-Ramírez, F., Llorente, I., Simon, S. A., Hiriart, M., Rosenbaum, T., & Morales-Lazaro, S. L. (2018). TRPV1 channels and the progesterone receptor Sig-1R interact to regulate pain. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(7), E1657–E1666. https://doi.org/10.1073/pnas.1715972115

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*, *18*(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Prato, V., Taberner, F. J., Hockley, J. R. F., Callejo, G., Arcourt, A., Tazir, B., Hammer, L., Schad, P., Heppenstall, P. A., Smith, E. S., & Lechner, S. G. (2017). Functional and molecular characterization of mechanoinsensitive "silent" nociceptors. *Cell Reports*, *21*(11), 3102–3115. https://doi.org/10.1016/j.celrep.2017.11.066

Priestley, J. V. (2009). Neuropeptides: Sensory systems. In M. Malcangio (Ed.). *Synaptic plasticity in pain textbook* (pp. 935–943). Springer.

Renthal, W., Tochitsky, I., Yang, L., Cheng, Y. C., Li, E., Kawaguchi, R., Geschwind, D. H., & Woolf, C. J. (2020). Transcriptional reprogramming of distinct peripheral sensory neuron subtypes after axonal injury. *Neuron*, *108*, 128–144. https://doi.org/10.1016/j.neuron.2020.07.026

Rittner, H. L., Brack, A., Machelska, H., Mousa, S. A., Bauer, M., Schäfer, M., & Stein, C. (2001). Opioid peptide-expressing leukocytes: Identification, recruitment, and simultaneously increasing inhibition of inflammatory pain. *Anesthesiology*, *95*(2), 500–508. https://doi.org/10.1097/00000542-200108000-00036

Roberson, D. P., Gudes, S., Sprague, J. M., Patoski, H. A., Robson, V. K., Blasl, F., Duan, B., Oh, S. B., Bean, B. P., Ma, Q., Binshtok, A. M., & Woolf, C. J. (2013). Activity-dependent silencing reveals functionally distinct itch-generating sensory neurons. *Nature Neuroscience*, *16*(7), 910–918. https://doi.org/10.1038/nn.3404

Rodríguez-Muñoz, M., Cortés-Montero, E., Pozo-Rodrigálvarez, A., Sánchez-Blázquez, P., & Garzón-Nino, J. (2015). The ON:OFF switch, sigma1R-HINT1 protein, controls GPCR-NMDA receptor cross-regulation: Implications in neurological disorders. *Oncotarget*, *6*(34), 35458–35477. https://doi.org/10.18632/oncotarget.6064

Rodríguez-Muñoz, M., Sánchez-Blázquez, P., Herrero-Labrador, R., Martínez-Murillo, R., Merlos, M., Vela, J. M., & Garzón, J. (2015). The sigma1 receptor engages the redox-regulated HINT1 protein to bring opioid analgesia under NMDA receptor negative control. *Antioxidants & Redox Signaling*, *22*(10), 799–818. https://doi.org/10.1089/ars.2014.5993

Sánchez-Fernández, C., Entrena, J. M., Baeyens, J. M., & Cobos, E. J. (2017). Sigma-1 receptor antagonists: A new class of neuromodulatory analgesics. *Advances in Experimental Medicine and Biology*, *964*, 109–132. https://doi.org/10.1007/978-3-319-50174-1_9

Sánchez-Fernández, C., Montilla-García, Á., González-Cano, R., Nieto, F. R., Romero, L., Artacho-Cordón, A., Montes, R., Fernández-Pastor, B., Merlos, M., Baeyens, J. M., Entrena, J. M., & Cobos, E. J. (2014). Modulation of peripheral μ-opioid analgesia by σ1 receptors. *Journal of Pharmacology and Experimental Therapeutics*, *348*(1), 32–45. https://doi.org/10.1124/jpet.113.208272

Sánchez-Fernández, C., Nieto, F. R., González-Cano, R., Artacho-Cordon, A., Romero, L., Montilla-García, Á., Zamanillo, D., Baeyens, J. M., Entrena, J. M., & Cobos, E. J. (2013). Potentiation of morphine-induced mechanical antinociception by σ_1 receptor inhibition: Role of peripheral σ_1 receptors. *Neuropharmacology*, *70*, 348–358. https://doi.org/10.1016/j.neuropharm.2013.03.002

Sanderson, N. K., Skinner, K., Julius, D., & Basbaum, A. I. (2004). Co-localization of endomorphin-2 and substance P in primary afferent nociceptors and effects of injury: A light and electron microscopic study in the rat. *The European Journal of Neuroscience*, *19*(7), 1789–1799. https://doi.org/10.1111/j.1460-9568.2004.03284.x

Scanlin, H. L., Carroll, E. A., Jenkins, V. K., & Balkowiec, A. (2008). Endomorphin-2 is released from newborn rat primary sensory neurons in a frequency- and calcium-dependent manner. *The European Journal of Neuroscience*, *27*(10), 2629–2642. https://doi.org/10.1111/j.1460-9568.2008.06238.x

Shah, S., Carver, C. M., Mullen, P., Milne, S., Lukacs, V., Shapiro, M. S., & Gamper, N. (2020). Local Ca^{2+} signals couple activation of TRPV1 and ANO1 sensory ion channels. *Science Signaling*, *13*(629), eaaw7963. https://doi.org/10.1126/scisignal.aaw7963

Sheehan, K., Lee, J., Chong, J., Zavala, K., Sharma, M., Philipsen, S., Maruyama, T., Xu, Z., Guan, Z., Eilers, H., Kawamata, T., & Schumacher, M. (2019). Transcription factor Sp4 is required for hyperalgesic state persistence. *PLoS ONE*, *14*(2), e0211349. https://doi.org/10.1371/journal.pone.0211349

Shin, S. M., Wang, F., Qiu, C., Itson-Zoske, B., Hogan, Q. H., & Yu, H. (2020). Sigma-1 receptor activity in primary sensory neurons is a critical driver of neuropathic pain. *Gene Therapy*, *29*(1–2), 1–15. https://doi.org/10.1038/s41434-020-0157-5

Su, T. P., Su, T. C., Nakamura, Y., & Tsai, S. Y. (2016). The sigma-1 receptor as a pluripotent modulator in living systems. *Trends in Pharmacological Sciences*, *37*(4), 262–278. https://doi.org/10.1016/j.tips.2016.01.003

Tejada, M. Á., Montilla-García, Á., Cronin, S. J., Cikes, D., Sánchez-Fernández, C., González-Cano, R., Ruiz-Cantero, M. C., Penninger, J. M., Vela, J. M., Baeyens, J. M., & Cobos, E. J. (2017). Sigma-1 receptors control immune-driven peripheral opioid analgesia during inflammation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(31), 8396–8401. https://doi.org/10.1073/pnas.1620068114

Vela, J. M., Merlos, M., & Almansa, C. (2015). Investigational sigma-1 receptor antagonists for the treatment of pain. *Expert Opinion on Investigational Drugs*, *24*(7), 883–896. https://doi.org/10.1517/13543784.2015.1048334

Wainger, B. J., Buttermore, E. D., Oliveira, J. T., Mellin, C., Lee, S., Saber, W. A., Wang, A. J., Ichida, J. K., Chiu, I. M., Barrett, L., Huebner, E. A., Bilgin, C., Tsujimoto, N., Brenneis, C., Kapur, K., Rubin, L. L., Eggan, K., & Woolf, C. J. (2015). Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts. *Nature Neuroscience*, *18*(1), 17–24. https://doi.org/10.1038/nn.3886

Woodbury, C. J., Zwick, M., Wang, S., Lawson, J. J., Caterina, M. J., Koltzenburg, M., Albers, K. M., Koerber, H. R., & Davis, B. M. (2004). Nociceptors lacking TRPV1 and TRPV2 have normal heat responses. *The Journal of Neuroscience*, *24*(28), 6410–6415. https://doi.org/10.1523/JNEUROSCI.1421-04.2004

Zamanillo, D., Romero, L., Merlos, M., & Vela, J. M. (2013). Sigma 1 receptor: A new therapeutic target for pain. *European Journal of Pharmacology*, *716*(1–3), 78–93. https://doi.org/10.1016/j.ejphar.2013.01.068

Zhang, J., Cavanaugh, D. J., Nemenov, M. I., & Basbaum, A. I. (2013). The modality-specific contribution of peptidergic and non-peptidergic nociceptors is manifest at the level of dorsal horn nociresponsive neurons. *The Journal of Physiology*, *591*(4), 1097–1110. https://doi.org/10.1113/jphysiol.2012.242115

Zhang, X., Huang, J., & McNaughton, P. A. (2005). NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. *The EMBO Journal*, *24*(24), 4211–4223. https://doi.org/10.1038/sj.emboj.7600893

Zhao, R., & Tsang, S. Y. (2016). Versatile roles of intracellularly located TRPV1 channel. *Journal of Cellular Physiology*, *232*(8), 1957–1965. https://doi.org/10.1002/jcp.25704

Zwick, M., Davis, B. M., Woodbury, C. J., Burkett, J. N., Koerber, H. R., Simpson, J. F., & Albers, K. M. (2002). Glial cell line-derived neurotrophic factor is a survival factor for isolectin B4-positive, but not vanilloid receptor 1-positive, neurons in the mouse. *The Journal of Neuroscience*, *22*(10), 4057–4065. https://doi.org/10.1523/JNEUROSCI.22-10-04057.2002

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ruiz-Cantero, M. C., Cortés-Montero, E., Jain, A., Montilla-García, Á., Bravo-Caparrós, I., Shim, J., Sánchez-Blázquez, P., Woolf, C. J., Baeyens, J. M., & Cobos, E. J. (2023). The sigma-1 receptor curtails endogenous opioid analgesia during sensitization of TRPV1 nociceptors. *British Journal of Pharmacology*, *180*(8), 1148–1167. https://doi.org/10.1111/bph.16003