Neuropharmacology 125 (2017) 231–242

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Grip strength in mice with joint inflammation: A rheumatology function test sensitive to pain and analgesia

Ángeles Montilla-García [a, b, 1], Miguel Á. Tejada [a, b, 1], Gloria Perazzoli [b, c], José M. Entrena [b, d], Enrique Portillo-Salido [h], Eduardo Fernández-Segura [b, e, f], Francisco J. Cañizares [b, e, f], Enrique J. Cobos [a, b, e, g, *]

[a] Department of Pharmacology, Faculty of Medicine, University of Granada, 18071 Granada, Spain
[b] Institute of Neuroscience, Biomedical Research Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain
[c] Department of Anatomy and Embryology, School of Medicine, University of Granada, 18071 Granada, Spain
[d] Animal Behavior Research Unit, Scientific Instrumentation Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain
[e] Biosanitary Research Institute, University Hospital Complex of Granada, 18012 Granada, Spain
[f] Department of Histology, Faculty of Medicine, University of Granada, 18071 Granada, Spain
[g] Teófilo Hernando Institute for Drug Discovery, 28029 Madrid, Spain
[h] Drug Discovery and Preclinical Development, ESTEVE, Parc Científic de Barcelona, Baldiri Reixac 4-8, Barcelona, Spain

ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form
11 July 2017
Accepted 26 July 2017
Available online 29 July 2017

Keywords:
Grip strength
Functional disability
Animal model
Joint pain
Periarticular inflammation
Analgesia

ABSTRACT

Grip strength deficit is a measure of pain-induced functional disability in rheumatic disease. We tested whether this parameter and tactile allodynia, the standard pain measure in preclinical studies, show parallels in their response to analgesics and basic mechanisms. Mice with periarticular injections of complete Freund's adjuvant (CFA) in the ankles showed periarticular immune infiltration and synovial membrane alterations, together with pronounced grip strength deficits and tactile allodynia measured with von Frey hairs. However, inflammation-induced tactile allodynia lasted longer than grip strength alterations, and therefore did not drive the functional deficits. Oral administration of the opioid drugs oxycodone (1–8 mg/kg) and tramadol (10–80 mg/kg) induced a better recovery of grip strength than acetaminophen (40–320 mg/kg) or the nonsteroidal antiinflammatory drugs ibuprofen (10–80 mg/kg) or celecoxib (40–160 mg/kg); these results are consistent with their analgesic efficacy in humans. Functional impairment was generally a more sensitive indicator of drug-induced analgesia than tactile allodynia, as drug doses that attenuated grip strength deficits showed little or no effect on von Frey thresholds. Finally, ruthenium red (a nonselective TRP antagonist) or the *in vivo* ablation of TRPV1-expressing neurons with resiniferatoxin abolished tactile allodynia without altering grip strength deficits, indicating that the neurobiology of tactile allodynia and grip strength deficits differ. In conclusion, grip strength deficits are due to a distinct type of pain that reflects an important aspect of the human pain experience, and therefore merits further exploration in preclinical studies to improve the translation of new analgesics from bench to bedside.

1. Introduction

Pain is an important global health problem, and there is a need for new analgesics (Goldberg and McGee, 2011). However, despite major advances in our understanding of pain mechanisms in recent decades, there has been little translation of new analgesics from bench to bedside (Barrett, 2015; Kissin, 2010; Mao, 2009).

The predictive validity of animal models of pain has been intensely debated, and one possible reason for the limited

Abbreviations: ANOVA, analysis of variance; CFA, complete Freund's adjuvant; COX-2, cyclooxygenase-2; DRG, dorsal root ganglion; HPMC, hydroxypropyl methylcellulose; NSAID, nonsteroidal antiinflammatory drug; p.o., orally; QST, quantitative sensory testing; RR, ruthenium red; RTX, resiniferatoxin; s.c., subcutaneous; SC, spinal cord; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid 1.

* Corresponding author. Department of Pharmacology, Faculty of Medicine, University of Granada, Avenida de la Investigación 11, 18071 Granada, Spain.
E-mail address: ejcobos@ugr.es (E.J. Cobos).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuropharm.2017.07.029

translation of pain research is the differences in outcome measures used to evaluate pain and analgesia in experimental animals and human patients (Cobos and Portillo-Salido, 2013; Mogil and Crager, 2004; Negus et al., 2006; Negus, 2013; Percie and Rice, 2014). Ideally, for the purposes of translation, animal testing should mimic as closely as possible routine clinical practice and clinical trials. Standard outcome measures used in preclinical chronic pain research have been adapted from quantitative sensory testing (QST) designed for the evaluation of patients with chronic pain, and von Frey filaments are one of the most widely used QST instruments to determine the mechanical pain threshold in preclinical research. In human patients, QST procedures are used to detect sensory alterations during neuropathic pain (e.g. Bennett, 2001; Bouhassira et al., 2005; Moharic et al., 2012). However, the use of QST in patients with rheumatic diseases is rare. To our knowledge only three published studies used von Frey filaments in human patients with rheumatoid arthritis (Hendiani et al., 2003; Morris et al., 1997; van Laarhoven et al., 2013), one of the most worrisome painful conditions that occurs with joint inflammation (Lee, 2013; Scott et al., 2000). This low number of clinical reports with von Frey testing is in marked contrast to the hundreds of preclinical studies that have used this technique in animal models of joint pain.

Pain is a complex phenomenon. Part of the core of the human pain phenotype includes alterations in physical functioning, which negatively impacts several aspects of daily life in patients with painful diseases (Romera et al., 2011; Turner et al., 2005). Because of the important relationship between pain and physical functioning, one set of consensus-based recommendations advocates measuring physical function as one of the main outcomes in clinical trials of treatments for pain (Dworkin et al., 2008). In this connection, grip strength has been widely and routinely evaluated for decades in rheumatology as a functional measure in patients with joint inflammation (e.g. Bijlsma et al., 1987; Lee, 2013; Pincus and Callahan, 1992), and remarkably, it is known to correlate to pain (Callahan et al., 1987; Fraser et al., 1999; Overend et al., 1999). Despite the widespread use of grip strength in rheumatology, this outcome is poorly characterized as a pain measure in experimental animals. However, as noted above, preclinical studies of tactile allodynia are abundant. It is known that transient receptor potential (TRP) channels or TRP-expressing nociceptors participate in inflammatory cutaneous hypersensitivity (Szallasi et al., 2007), but much less is known about the neurobiological mechanisms leading to pain-induced functional disability.

In light of these antecedents, we aimed to compare the sensitivity of grip strength in mice with joint inflammation vs. inflammatory tactile allodynia to the effects of several analgesic drugs of different pharmacological classes, and tested whether the appearance of grip strength deficits and tactile allodynia arose from the same mechanisms.

2. Material and methods

2.1. Experimental animals

Experiments were done in 680 female CD1 mice (Charles River, Barcelona, Spain) weighing 28–30 g at the beginning of the study. We choose female animals because it has been reported that women may be at greater risk for pain-related disability than men (e.g. Unruh, 1996; Stubbs et al., 2010), but no previous studies have evaluated grip strength as a measure of pain-induced functional disability in female animals. Animals were tested randomly throughout the estrous cycle. They were housed in colony cages with free access to food and water prior to the experiments, and were kept in temperature- and light-controlled rooms (22 ± 2 °C, and light–dark cycle of 12 h). The experiments were done during the light phase (from 9:00 a.m. to 3:00 p.m.). All experimental protocols were carried out in accordance with international standards (European Communities Council directive 2010/63), and were approved by the Research Ethics Committee of the University of Granada. To decrease the number of animals in this study, we used the same mice for behavioral studies, histological analysis and immunostaining, when possible.

2.2. CFA-induced periarticular inflammation

Mice were injected periarticularly with complete Freund's adjuvant (CFA) (Sigma-Aldrich, Madrid, Spain) or sterile physiological saline (0.9% NaCl) as a control around the tibiotarsal joint. CFA (or saline) was administered subcutaneously in two separate injections to the inner and outer side of the joint in a volume of 10 or 15 μL/injection (20 or 30 μL/paw), to obtain homogeneous inflammation (Chen et al., 2009; Lolignier et al., 2011). We used a 1710 TLL Hamilton microsyringe (Teknokroma, Barcelona, Spain) with a 30½-gauge needle under isoflurane anesthesia (IsoVet®, B. Braun, Barcelona, Spain). CFA-treated mice had prominent inflammation that appeared to be restricted to the administration site and nearby areas (heel), whereas the paw pad did not appear to be affected. This allowed us to test the mechanical threshold in these two distinct areas. See "Results" for details. Because weight loss or delayed weight gain are considered signs of ongoing distress (Blackburn-Munro, 2004), body weight was monitored daily to ensure that our protocol did not induce excessive harm to the animals. Inflammatory edema was monitored by measuring ankle thickness with an electronic caliper (e.g. Croci and Zarini, 2007).

2.3. Drugs and drug administration

We used the following prototypic analgesics: the nonsteroidal antiinflammatory drug (NSAID) ibuprofen sodium salt (10–80 mg/kg), the cyclooxygenase-2 (COX-2) inhibitor celecoxib (40–160 mg/kg), and acetaminophen (40–320 mg/kg) (all from Sigma-Aldrich), and the opioids tramadol (10–80 mg/kg) and oxycodone hydrochloride (1–8 mg/kg) (supplied by Laboratorios Esteve, Barcelona, Spain). We also tested the effects of the antispastic baclofen (5–20 mg/kg) (Sigma-Aldrich). All drugs were dissolved in 0.5% hydroxypropyl methylcellulose (HPMC) with the exception of celecoxib and acetaminophen, which were suspended in HPMC supplemented with 1% Tween 80 (both from Sigma-Aldrich). These drugs or their solvents were administered orally (p.o.) in a volume of 10 mL/kg.

In addition, we also tested the effects of ruthenium red (1–2 mg/kg) (Sigma-Aldrich), a nonselective TRP antagonist (St Pierre et al., 2009). Ruthenium red was dissolved in saline and administered subcutaneously (s.c.) into the interscapular zone in a volume of 5 mL/kg. The control group received an equal volume of saline.

In all cases, behavioral evaluations after drug administration were recorded by an observer blinded to the treatment.

2.4. In vivo ablation of TRP vanilloid 1 (TRPV1)-expressing nociceptive neurons

We used resiniferatoxin (RTX) to selectively ablate TRPV1-expressing neurons. The drug (Tocris Cookson Ltd, Bristol, UK) was dissolved in 10% Tween 80 and 10% ethanol in normal saline. Animals received a single dose of RTX (50 μg/kg) via intraperitoneal injection, which has been previously reported to ablate all peripheral TRPV1+ neurons (Hsieh et al., 2012). The control group received an equal volume of vehicle. All procedures were done under isoflurane anesthesia to minimize distress, 5 days before behavioral testing or sample collection.