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Opioids and TRPV1 in the peripheral control of neuropathic pain – Defining a target site in the injured nerve

Dominika Labuz, Viola Spahn, Melih Özgür Celik, Halina Machelska*

Department of Anesthesiology and Critical Care Medicine, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12203 Berlin, Germany

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ABSTRACT

Targeting peripheral neuropathic pain at its origin may prevent the development of hypersensitivity. Recently we showed this can be mediated by opioid receptors at the injured nerve trunk. Here, we searched for the most relevant peripheral site to block transient receptor potential vanilloid 1 (TRPV1), and investigated analgesic interactions between TRPV1 and opioids in neuropathy. In a chronic constriction injury (CCI) of the sciatic nerve in mice, we assessed the effects of μ-, δ- and κ-opioid receptor agonists and TRPV1 antagonist (SB366791) injected at the CCI site or into the injured nerve-innervated paw on spontaneous paw lifting, heat and mechanical sensitivity. We also examined TRPV1 expression in total membrane and plasma membrane fractions from nerves and paws. We found that opioids and SB366791 co-injected in per se nonanalgesic doses at the CCI site or into the paw diminished heat and mechanical sensitivity. SB366791 alone dose-dependently alleviated heat and mechanical sensitivity. TRPV1 blockade in the paw was more effective than at the CCI site. None of the treatments diminished spontaneous paw lifting. TRPV1 expression analysis suggests that the levels of functional TRPV1 do not critically determine the TRPV1 antagonist-mediated analgesia. Together, the identification of the primary action site in damaged nerves is crucial for effective pain control. Contrary to opioids, the TRPV1 blockade in the injured nerve peripheral terminals, rather than at the nerve trunk, appears promising against heat pain. Opioid/TRPV1 antagonist combinations at both locations partially reduced neuropathy-triggered heat and mechanical pain.

1. Introduction

Analgesic efficacy of most painkillers is limited by adverse effects, which are often produced at sites remote from pain source. Neuropathic pain is a debilitating condition, frequently caused by disease or trauma to peripheral nerves (e.g., diabetes, amputation, compression, transection) (Baron et al., 2010). Current pharmacotherapy is hindered by nausea, constipation, fatigue, sleep disturbance, cognitive impairment, dependence and addiction resulting from actions of antidepressants, antiepileptics and opioids in the gut or brain (Sindrup and Jensen, 1999; Stein and Kopf, 2009; Stein et al., 2010). In neuropathy, not only pain induction but also maintenance, including central sensitization, is driven by the input from peripheral sensory afferents (Costigan et al., 2009; Baron et al., 2013). This implies that targeting peripheral nerves may prevent the noxious drive and plasticity within the central nervous system, and provide analgesia devoid of untoward effects. Accordingly, local anesthetic nerve blocks are broadly used for pain management (Bonica, 1984; Stein and Kopf, 2009; Haroutounian et al., 2014). Also opioids applied in low, systemically inactive doses to injured tissue produce analgesia by activating peripheral opioid receptors (Kalso et al., 2002; Stein et al., 2003; Stein and Machelska, 2011; Sawynok and Liu, 2014).

Clearly, there is a growing interest in approaches to peripheral pain inhibition, also suggested for cannabinoids, blockers of sodium channels or transient receptor potential (TRP) channels (Patapoutian et al., 2009; Sawynok and Liu, 2014; Waxman and

Abbreviations: cAMP, cyclic adenosine monophosphate; CCI, chronic constriction injury; DAMGO, [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin; DMSO, dimethyl sulfoxide; DPDPE, D-Pen^2,D-Pen^5-enkpephalin; DRG, dorsal root ganglion; I.pl., intraplantar; PKA, protein kinase A; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid 1; SB366791, 4′-chloro-3-methoxycinnamanilide; U50,488H, *trans*-(±)3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide.

* Corresponding author.
E-mail addresses: dominika.labuz@charite.de (D. Labuz), viola.spahn@charite.de (V. Spahn), ozgur.celik@charite.de (M.Ö. Celik), halina.machelska@charite.de (H. Machelska).

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Zamponi, 2014). In animal neuropathic pain models, the drugs are typically applied to damaged nerve-innervated paws, i.e. tissue remote from the nerve lesion site (Stein and Machelska, 2011; Labuz and Machelska, 2013). However, for efficient pain relief it is important to precisely determine the most relevant locus along the damaged nerve (Kissin, 2008). Indeed, we have recently found that opioids were particularly effective when applied at the nerve injury site. In a chronic constriction injury (CCI) of the sciatic nerve, μ-, δ- and κ-opioid receptor agonists administered at the CCI site fully blocked mechanical and heat sensitivity. In contrast, opioids injected into the injured nerve-innervated paw were only weakly or not effective at all, with particularly poor actions in heat sensitivity (Labuz and Machelska, 2013). Therefore, in this study we investigated potential functional interactions between opioid receptors and TRP vanilloid 1 (TRPV1), and searched for the most relevant peripheral site to target TRPV1 for analgesia in neuropathy.

TRPV1 is a calcium-permeable, non-selective cation channel activated by capsaicin, protons, membrane-derived lipids and noxious heat (Tominaga et al., 1998). TRPV1 is predominantly expressed in peripheral nociceptive neurons and was originally proposed to primarily mediate inflammatory heat hyperalgesia (Caterina et al., 1997, 2000). Nevertheless, in pharmacological approaches, systemically or centrally applied TRPV1 antagonists also attenuated heat sensitivity in neuropathic and postoperative pain (Pomonis et al., 2003; Vilceanu et al., 2010; Uchytilova et al., 2014). Although mechanistically less clear, there is accumulating evidence on the role of TRPV1 in mechanotransmission. Electrophysiological studies have shown that TRPV1-expressing sensory neurons process mechanical stimuli (McGaraughty et al., 2008; Brenneis et al., 2013) and capsaicin can lower mechanical thresholds *in vivo* (Honore et al., 2005; Binshtok et al., 2007), while TRPV1 knockout mice exhibited higher mechanical thresholds in longer-lasting peripheral inflammation and nerve injury (Szabó et al., 2005; Kim et al., 2012). Although not all (Wu et al., 2008; Uchytilova et al., 2014), several other studies reported reduction of mechanical sensitivity by TRPV1 antagonists injected systemically or centrally in inflammatory, neuropathic and postoperative pain models (Pomonis et al., 2003; Honore et al., 2005; Kanai et al., 2005; Christoph et al., 2007; McGaraughty et al., 2008; Watabiki et al., 2011). Additionally, TRPV1 antagonists decreased side effects and improved analgesic actions of morphine following systemic or spinal injections in acute or bone cancer pain models (Chen et al., 2008; Niiyama et al., 2009; Nguyen et al., 2010). However, possible peripheral analgesic effects of TRPV1 antagonists and interactions with opioids in neuropathy have not been addressed.

Here we asked whether weak opioid analgesia at the peripheral endings of injured nerves (Labuz and Machelska, 2013) can be enhanced by concomitant TRPV1 blockade, assessing neuropathy-induced spontaneous, heat and mechanical pain. While examining opioid–TRPV1 interactions, we were intrigued that, in contrast to opioids, the TRPV1 antagonist was less effective at the injured axons than at their peripheral terminals innervating paw. Hence, we systematically examined the TRPV1 antagonist analgesic effects and the expression of TRPV1 at both locations.

2. Materials and methods

2.1. Animals

Experiments were approved by the State animal care committee (Landesamt für Gesundheit und Soziales, Berlin, Germany) and were performed according to the *Guide for the Care and Use of Laboratory Animals* adopted by the U.S. National Institutes of Health, and the ARRIVE guidelines (Kilkenny et al., 2010). Male C57BL/6J mice (25–30 g, 6–8 weeks old; Harlan Laboratories, Horst, The Netherlands; bred at the Charité-Campus Benjamin Franklin, Berlin, Germany) were kept in groups of 3–5 per cage, with free access to food and water, in environmentally controlled conditions (12 h light/dark schedule, light on at 7:00 h; 22 ± 0.5 °C; humidity 60–65%). Animals were randomly placed in cages by an animal caretaker who was not involved in the study. After completion of *in vivo* experiments and for tissue collection for *ex vivo* experiments animals were killed with isoflurane-overdose (Abbott, Wiesbaden, Germany). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Neuropathy

CCI was induced in deeply isoflurane-anesthetized mice by exposing the sciatic nerve at the level of the right mid-thigh and placing three loose silk ligatures (4/0) around the nerve with about 1-mm spacing; the ligatures were tied until they elicited a brief twitch in the respective hind limb. Sham operation was performed in a similar manner but without nerve ligation. The wound was closed with silk sutures (Labuz et al., 2009, 2010; Labuz and Machelska, 2013).

2.3. Assessment of nociception

In all experiments, animals were habituated to the test cages daily (1–2 times for 15 min), starting 6 days prior to nociceptive testing. During the testing, the sequence of paws was alternated between animals to avoid “order” effects. Six to nine animals per group were used. The experimenter was blinded to the treatments; substances were prepared in separate, coded vials by a colleague not involved in *in vivo* testing. The codes were broken after completion of experiments.

2.3.1. Heat sensitivity (Hargreaves test)

Mice were individually placed in clear Plexiglas chambers positioned on a stand with glass surface (Model 336; IITC Life Sciences, Woodland Hills, CA). Radiant heat was applied to the plantar surface of hind paws from underneath the glass floor with a high-intensity projector lamp bulb and paw withdrawal latency was evaluated using an electronic timer. The withdrawal latency was defined as the average of two measurements separated by at least 10 s. The heat intensity was adjusted to obtain baseline withdrawal latency of about 10–12 s in uninjured paws, and the cut-off was 20 s to avoid tissue damage (Labuz and Machelska, 2013).

2.3.2. Mechanical sensitivity (von Frey test)

Animals were individually placed in clear Plexiglas cubicles located on a stand with anodized mesh (Model 410; IITC Life Sciences). The following calibrated von Frey filaments were used: 0.078 mN (0.0056 g), 0.196 mN (0.0076 g), 0.392 mN (0.041 g), 0.686 mN (0.059 g), 1.569 mN (0.14 g), 3.922 mN (0.28 g), 5.882 mN (0.54 g), 9.804 mN (0.66 g), 13.725 mN (1.15 g), 19.608 mN (2.35 g), and 39.216 mN (4.37 g) (Stoelting, Wood Dale, IL). The filaments were applied until they bowed, for approximately 3 s, to the plantar surface of hind paws. The up-down method was used to estimate 50% withdrawal thresholds (Chaplan et al., 1994). Testing began using a 3.922 mN (0.28 g) filament. If the animal withdrew the paw the just preceding weaker filament was applied. In the case of no withdrawal the next stronger filament was applied. The maximal number of applications was 6–9, and the cut-off was 39.216 mN (4.37 g) because an uninjured paw could be elevated with the next filament (58.82 mN or 5.3 g), according to our previous studies (Labuz et al., 2009, 2010; Labuz and Machelska, 2013).