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Research Paper

A new model of nerve injury in the rat reveals a role of Regulator of G protein Signaling 4 in tactile hypersensitivity

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ABSTRACT

Tactile hypersensitivity is one of the most debilitating symptoms of neuropathic pain syndromes. Clinical studies have suggested that its presence at early postoperative stages may predict chronic (neuropathic) pain after surgery. Currently available animal models are typically associated with consistent tactile hypersensitivity and are therefore limited to distinguish between mechanisms that underlie tactile hypersensitivity as opposed to mechanisms that protect against it. In this study we have modified the rat model of spared nerve injury, restricting the surgical lesion to a single peripheral branch of the sciatic nerve. This modification reduced the prevalence of tactile hypersensitivity from nearly 100% to approximately 50%. With this model, we here also demonstrated that the Regulator of G protein Signaling 4 (RGS4) was specifically up-regulated in the lumbar dorsal root ganglia and dorsal horn of rats developing tactile hypersensitivity. Intrathecal delivery of the RGS4 inhibitor CCG63802 was found to reverse tactile hypersensitivity for a 1 h period. Moreover, tactile hypersensitivity after modified spared nerve injury was most frequently persistent for at least four weeks and associated with higher reactivity of glial cells in the lumbar dorsal horn. Based on these data we suggest that this new animal model of nerve injury represents an asset in understanding divergent neuropathic pain outcomes, so far unravelling a role of RGS4 in tactile hypersensitivity. Whether this model also holds promise in the study of the transition from acute to chronic pain will have to be seen in future investigations.

1. Introduction

The search for the cellular and/or molecular underpinnings of persistent pain states has intensified over the past decades. The advances in our mechanistic understanding of chronic pain, which affects 30–50% of the general population (Bouhassira et al., 2008; Torrance et al., 2006) are heavily based on animal models of peripheral nerve injury (Gregory et al., 2013). Chronic pain often has neuropathic characteristics (Lavand'homme, 2011) and, as chronic pain, neuropathic pain is highly intractable (Baron et al., 2010). Nevertheless, nerve lesions do not always lead to persistent pain as illustrated by half the surgical amputees who do not develop chronic postoperative pain (Kehlet et al., 2006). We still know only little about the factors that facilitate versus the factors that protect against a transition from acute to chronic pain (Deumens et al., 2013).

Longitudinal studies on postoperative patients show a 10–50% prevalence of chronic pain, strongly associated with nerve lesions (Kehlet et al., 2006). Clinical data suggest that tactile hypersensitivity early after surgery holds predictive value for chronic (neuropathic) postoperative pain (Lavand'homme et al., 2005; Martinez et al., 2012). While tactile hypersensitivity does not consistently develop after surgery or nerve injury in human, most animal models of peripheral nerve injury show consistent tactile hypersensitivity (Gregory et al., 2013). Interestingly, the prevalence of tactile hypersensitivity after injury to the rat spinal cord was found to depend on the extent of tissue trauma (Kloos et al., 2005). This made us wonder whether restricting the extent of tissue

Abbreviations: CB_1 receptor, cannabinoid type-1 receptor; DRG, dorsal root ganglion; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adapter molecule 1; MPE, maximum-possible-effect; mSNI, modified spared nerve injury; PWT, paw withdrawal threshold; RGS4, Regulator of G protein Signaling 4; SNI, spared nerve injury; SSI, static sciatic index.

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trauma after peripheral nerve injury could reduce the prevalence of tactile hypersensitivity as well.

The prevalence of neuropathic pain-like behaviours such as tactile hypersensitivity may also depend on the choice of rat strain (Yoon et al., 1999). Nearly 50% of Holtzman rats were found to be protected against tactile hypersensitivity through an engagement of inhibitory projections descending from the brainstem to the spinal cord and modulating local nociceptive networks in the dorsal horn (De Felice et al., 2011). The failure to engage such inhibitory systems in the other 50% of rats remains largely unexplained, but disinhibition within the nociceptive system has been repeatedly documented after nerve injury, at multiple locations of the neuraxis (Blom et al., 2014; von Hehn et al., 2012).

A multitude of neuropathic mechanisms may cause disinhibitory states in the central nervous system (CNS), both of neuronal and non-neuronal (immune) origin. We recently demonstrated that the signaling efficacy of the analgesic cannabinoid type-1 (CB_1) receptors was reduced in the spinal cord of nerve injured rats due to an injury-induced up-regulation in Regulator of G protein Signaling 4 (RGS4) (Bosier et al., 2015). RGS is a family of multifunctional proteins that promote the termination of signaling through G protein-coupled receptors, and previous work already linked spinal RGS4 with a loss of opioid receptor signaling efficacy after peripheral nerve injury in the rat (Garnier et al., 2003).

In the present study, we modified the model of spared nerve injury in the rat in order to limit the extent of tissue trauma. Tactile hypersensitivity, which occurred in only 50% of rats with modified spared nerve injury, was found to be associated with an increased expression of RGS4.

2. Materials and methods

2.1. Animals: models of peripheral nerve injury

A total of 149 adult female Sprague Dawley rats, 10–12 weeks old, were used, 139 under ethical approval of the Belgium authority on animal experimentation (LA2230419) and 10 under ethical approval of the Italian authority on animal experimentation (the Scuola Internazionale Superiore di Studi Avanzati (SISSA) ethics committee). All experiments were conducted under strict regulations, respecting the European Community Council directive of 24 November 1986 (86–609/ECC) and the decree of 20 October 1987 (87–848/EEC). The animals were kept in groups of 2–3 animals per standard makrolon cage with ad libitum access to food at a regular 12:12 h light-dark cycle. An exception to this rule of social housing applied to animals receiving an indwelling intrathecal catheter, i.e. a total of n = 24 rats that were individually housed. Animals were either subjected to nerve injury (n = 124) or sham-surgery (n = 15) using methods reported previously (Decosterd and Woolf, 2000), but with slight modifications. In brief, the sciatic nerve was exposed *at random* on either the left or the right side under sevoflurane anaesthesia (6% in oxygen for induction; 2–3% in oxygen for maintenance) and aseptic conditions. Then, the three peripheral nerve branches of the sciatic nerve (i.e. tibial, common peroneal and sural nerve branches) were exposed through blunt dissection and freed from the surrounding connective tissue. Animals were *ad random* divided into three groups: (1) spared nerve injury (SNI; n = 21), (2) modified SNI (mSNI; n = 103), and (3) sham surgery (n = 15). For SNI, the tibial and common peroneal nerve branches were injured while for mSNI only the common peroneal nerve branch was injured. Injury was inflicted using a non-serrated nerve clamp, i.e. the De Beer clamp (Honer Medizin-Technik GmbH & Co., Spaichingen, Germany) exerting a force of 54 N over a period of 30 s (Luis et al., 2007). In both mSNI and SNI the sural nerve branch was left intact (spared). Sham surgery involved skin incision and the sciatic nerve branches were dissected free, but were not crushed. Then, wounds were closed using 4/0 prolene sutures and animals were returned to their home cage. Postoperative care did not include pain medication as this might interfere with the primary study outcome, i.e. the development of neuropathic pain-like behaviour.

2.2. Electrophysiology

Adult female rats were anaesthetized with CO_2 and then sacrificed through CO_2 asphyxiation followed by cervical dislocation in line with the guidelines provided by the Italian Animal Welfare act, following the European Directive for animal experiments 2010/63/EU. Sciatic nerves were carefully dissected out from dorsal vertebrae to the ankle. One sciatic nerve per animal was used and each time the side (right or left leg) was randomly selected (n = 10 animals; 5 right, 5 left). At the end of the experiment, a precision caliper was used to carefully measure the mean lengths of the three peripheral branches of the sciatic nerve (tibial branch = 40.85 ± 2.31 mm; common peroneal branch 34.60 ± 2.15 mm; sural branch = 28.62 ± 2.942 mm (n = 10)).

As for electrophysiological recordings, monopolar glass suction electrodes were used to draw in the peripheral extremity of each branch, while stimuli were delivered to the sciatic nerve using a concentric bipolar electrode (see cartoon in Fig. 1A). Signals were recorded in AC, amplified 1000 times (DP-304®, Warner Instruments, CT), digitalized (250 KHz, Digidata® 1440 A, Molecular Devices Corporation, CA) and stored in a personal computer for further analysis. Single pulses (total width = 0.2 ms) were delivered as cathodic-first charge-balanced biphasic rectangular current injections without a delay between cathodal and anodal phases. In order to obtain input/output curves, we delivered a train of stimuli (0.33 Hz) of increasing amplitude (80 stimuli, 5 pulses for each steps from 10 to 160 μA). The stimulating threshold was defined as the minimum pulse strength able to evoke an appreciable response (for tibial = 20.00 ± 1.67 μA; common peroneal = 23.33 ± 1.67 μA; sural = 23.33 ± 1.67 μA; n = 10). Nerve injury was inflicted onto both tibial and common peroneal branches (see cartoon in Fig. 1B) as described earlier. In order to prevent decay of signal amplitude with the slightest movements of nerve extremities during clamp manipulation, we released all suctions after control recordings. After lesion, the nerve clamp was removed and new suctions were performed with the same glass electrodes, to obtain an identical seal as in control conditions. The same procedures were performed on the unlesioned (spared) sural nerve, which served as an internal control. Time to peak was calculated as the time spanning from the first stimulation artifact to the peak of response. At least five traces were averaged for each stimulation intensity. Conduction velocity was expressed in m/s and resulted from a division of fiber length by the mean time to peak for the maximal stimulation strength applied (160 μA).

2.3. Algesimetry

After habituating the animals to the experimenter (R.D.), the animals were placed in transparent plastic chambers without floor, positioned on an elevated wire mesh. Acclimatization was allowed for a period of about 20 min after which the von Frey test was performed. Herein, a set of eight calibrated von Frey hair filaments (Stoelting, Wood Dale, IL, US) was used (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5, 15.1 g). Filaments were applied to the plantar hind paw surface and held in a slightly buckled position for a period of around 8 s, starting with the 2 g-filament. The choice for the following filament was based on the response to the previous filament application, being the closest-lower filament in case of a positive withdrawal response ('x') or the closest-higher filament in case of a negative withdrawal response ('o'). A positive withdrawal response was defined by a paw withdrawal associated with aversive behaviour, such as keeping the stimulated paw elevated, licking the paw, or attacking or biting of the filament. This method of filament application was continued until a sequence of six filament applications was acquired starting either with 'o-x' or with 'x'. In case the upper-end filament (15.1 g) or the lower-end filament (0.4 g) was