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PC1, a non-peptide PKR1-preferring antagonist, reduces pain behavior and spinal neuronal sensitization in neuropathic mice

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ABSTRACT

Peripheral neuropathy is characterized by abnormal pain responses triggered by the release of several mediators and neuronal hyperexcitability at the spinal cord level. Emerging evidence indicates that the enhanced activity of dorsal horn neurons requires communication with glia and microglia, cells that are physiologically involved in neuronal wellbeing. Prokineticins (PKs), which include PK1 and PK2, represent a novel family of chemokines characterized by a unique structural motif comprising five disulfide bonds. They are expressed in the peripheral and central nervous system. PKs bind two G protein coupled receptors, PKR1 and PKR2, and participate in the regulation of several biological processes, including pain sensation. This study aimed to investigate the anti-nociceptive effect of PC1, a non-peptide PKR1-preferring antagonist, in a mouse model of neuropathic pain. To do this, we assessed the activity of spinal cord nociceptive neurons as well as astrocyte and microglia phenotypes after repeated administration of PC1 in vivo. PC1 treatment strongly delayed the development of thermal hyperalgesia and tactile and mechanical allodynia. It also reduced spinal microglial and glial activation 8 days post injury in spared nerve injury (SNI) mice. Neuropathic mice showed an increased level of PK2 protein in the spinal cord, mostly in astrocytes. PC1 treatment completely reversed the increased responsiveness to mechanical stimuli, the decreased threshold of neuronal activation, and the increased spontaneous activity that were observed in nociceptive specific (NS) neurons of SNI mice.

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Introduction

Neuropathic pain is a debilitating pain occurring after nerve injury and is usually resistant to available treatments, including opioids. This pathological condition is characterized by abnormal pain perception, such as hyperalgesia and allodynia, and neuronal over-sensitization occurring at the spinal level and leading to abnormal pain transmission [1–3]. Emerging evidence indicates that the enhanced activity of dorsal horn neurons requires communication with glial cells (microglia and astrocytes), which are physiologically involved in the maintenance of brain homeostasis by providing support and protection for neuronal cells. In pathological conditions, i.e., chronic pain state, glial cells become activated [4–7]. They proliferate, change morphology, and release pro-inflammatory mediators that promote neuronal sensitization [8–12].

Prokineticins (PKs), consisting of PK1 and PK2, represent a novel family of cysteine-rich secreted proteins. They bind two endogenous G protein coupled receptors, PKR1 and PKR2, which are involved in several biological functions [13]. Both receptors have been identified in peripheral tissues and in the central nervous system (CNS) of humans and rodents [14–18] Besides several regulatory physiological functions indicated for PKs, their involvement in nociception has been described in numerous studies [19–23]. PKR1 and PKR2 are highly expressed in specific CNS areas controlling pain, particularly in the dorsal horn of the spinal cord and in dorsal root ganglia, in nociceptive neurons also express the

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transient receptor potential vanilloid receptor-1 (TRPV1), suggesting a PKR/TRPV1 cooperative interaction in nociceptor sensitization [16,17,24,25]. The activation of PKRs can elicit nociception, contributing to nociceptor sensitivity to different stimuli ([19,21]). In a previous study, we have suggested that modulating prokineticin system may represent a promising strategy for reducing primary afferent nociceptor sensitization and thus inhibiting inflammatory pain [16,17]. In addition to the effects on sensory neurons, PKs have been shown to exert modulatory effects in peripheral inflammatory cells. It has been suggested that inflammation induces upregulation of PK2 at lesion sites, where it exerts chemoattractant properties mainly through PKR1, thus supporting a pivotal role of the PK2/PKR1 pathway in inflammation as well [26].

We have recently shown the capability of PC1, a non-peptide PKR1-preferring antagonist, to exert analgesic effects associated with a restoration of the physiological levels of pro-inflammatory and anti-inflammatory cytokines in mice with chronic constriction of the sciatic nerve (CCI) [27]. Based on these findings, we considered it interesting to evaluate the capability of PC1 to counteract behavioral and electrophysiological changes induced by spared nerve injury which is cause of long lasting chronic pain, comparable to an advanced stage of neuropathy in human (see [28] for review).

We have here demonstrated: (i) an enduring anti-allodynic effect, (ii) a reversal of the spinal cord specific nociceptive (NS) neuron sensitization, (iii) a change in the mRNA and protein levels of PK2 and PKR1, and eventually (iv) amelioration of the dorsal horn spinal cord microglia and astrocyte phenotypes.

Materials and methods

Animals

Male CD-1 mice (35–40 g) were housed five per cage under controlled illumination (12:12 h light:dark cycle; light on 06.00 h) and environmental conditions (room temperature 20–22 °C, humidity 55–60%) for at least 1 week before the experiments. Food and water were available ad libitum. The Animal Ethics Committee of “Sapienza,” University of Rome, approved the experimental procedures. Animal care complied with the IASP and European Community (E.C. L358/1 18/12/86) guidelines on the use and protection of animals in experimental research. All efforts were made to minimize animal suffering and the number of animals used.

Drug treatments

PC1, a non-peptide PKR1-preferring antagonist, was synthesized by Balboni et al. [29]. SNI mice received PC1 (150 μg/kg, s.c.) or saline (50 μl/10 g body weight, s.c.) twice per day (10:00 a.m. and 19:00 p.m.). The treatment was performed starting from day 1 (1 day after the surgery) up to day 8. Testing was performed chronically every morning before PC1 treatment for 18 days. A group of treated mice were sacrificed on the day 9 for biochemical, electrophysiological and immunohistochemical evaluations. The doses were chosen according to Maftei et al. [27].

Neuropathic pain model

Spared nerve injury (SNI)

Mononeuropathy was induced according to the method of Decosterd and Woolf [30]. Mice were anesthetized by intraperitoneal injection of ketamine xylazine (60 mg/kg + 10 mg/kg). The sciatic nerve was exposed at the level of its trifurcation into sural, tibial, and common peroneal nerves. The tibial and common peroneal nerves were ligated tightly with 5.0 silk thread and then transected just distal to the ligation, leaving the sural nerve intact. Sham mice were anesthetized and the sciatic nerve was exposed at the same level, but it was not transected.

Pain related behavior

Measurement of thermal nociception

Plantar test. Thermal hyperalgesia was evaluated using the plantar test (Ugo Basile) [31] and the hot plate test (Ugo Basile, Italy). Mice were placed in clear Plexiglas chambers; a mobile infrared radiant heat source was focused under the plantar surface of the hind paws. Latency to paw withdrawal was determined by a timer. The stimulus intensity was preset to obtain a paw withdrawal latency ranging from 9 to 11 s. Withdrawal threshold of the hind paws was defined as the mean of three measurements.

Measurement of allodynia

The mechanical hypersensitivity was assessed by using Von Frey and Dynamic plantar aesthesiometer. The Von Frey is commonly tool used to evaluate the nocifensive responses of neuropathic pain state in preclinical and clinical research. In this system, the filaments are manually held and they can be adjusted according to the paw posture. Dynamic plantar aesthesiometer is an automatic instrument with a single filament able to record the force and rate of change in force [32]. Both apparatus are appropriate to evaluate pain behavior in SNI model. In particular, Von Frey is commonly used to assess tactile allodynia whereas Dynamic plantar aesthesiometer is used to measure mechanical allodynia.

von Frey test. Tactile allodynia was assessed by calibrated von Frey filaments (2 Biological Instruments, Italy), as previously described [33]. Animals were placed in individual Plexiglas boxes on a raised metal mesh surface and allowed to acclimatize for 30 min before the test. Testing was initiated with a medium-sized filament, which was applied for 7 s to the plantar area (plantar territory of the sural nerve) until the filament bent slightly. If the mouse withdrew or lifted the paw, the response was considered positive and a filament one size smaller was tried. Conversely, if no response was observed, a filament one size larger was tried. The protocol was repeated until five changes in behavior had been observed. The 50% withdrawal threshold was determined according to the following equation:

$$Xf + kD$$

where Xf is the value of the last von Frey filament used, k is the Dixon value for the positive/negative pattern, and D is the logarithmic difference between stimuli.

Dynamic aesthesiometer

Mechanical allodynia was assessed using the Dynamic Plantar Aesthesiometer (Ugo Basile, Italy). This apparatus employs a single non-flexible filament (0.5-mm diameter) to apply an increasing force to the plantar surface of the mouse hind paw. Animals were placed in a test cage with a wire mesh floor and allowed to acclimatize for 30 min before testing. The filament was applied to the skin of the plantar area (plantar territory of the sural nerve) of the hind paw, and it began to exert an increasing upward force, reaching a maximum of 30 g in 10 s, until the paw was withdrawn. The withdrawal threshold was defined as the force, in grams, at which the mouse withdrew its paw. Withdrawal thresholds of ipsilateral and contralateral paws were measured thrice, and the reported value is the mean of the three evaluations.

RNA extraction and RT-PCR

Total RNA was extracted from dorsal spinal cord at L4–L6 level using an RNeasy Kit (QIAGEN) according to the manufacturer’s

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