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Injured nerve-derived COX2/PGE2 contributes to the maintenance of neuropathic pain in aged rats

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Abstract

Neuropathic pain (NeP) is a debilitating disease afflicting mostly the aged population. Inflammatory responses in injured nerves play a pivotal role in the pathogenesis of NeP. Injured nerve derived cyclooxygenase 2/prostaglandin E2 (COX2/PGE2) contributes to the genesis of NeP at the early stage in young rats. Here we show that COX2/PGE2 is involved in the maintenance of NeP at a chronic stage in aged rats. Eighteen months after partial sciatic nerve ligation (PSNL), NeP remained prominent in aged rats. COX2 expressing macrophages and PGE2 levels were increased in injured nerves. PGE2 receptors (EP1 and EP4) and pain-related ion channel transient receptor potential vanilloid-1 (TRPV1) were increased in the ipsilateral dorsal root ganglion (DRG) neurons of aged PSNL rats. Perineural injection of a selective COX2 inhibitor NS-398 relieved NeP, reversed PSNL increased expression of EP1, EP4 and TRPV1 and suppressed the levels of pain-related peptide substance P and calcitonin gene-related peptide in DRG neurons. These data suggest that injured nerve-derived PGE2 contributes to the maintenance of NeP at the chronic stage in aged rats. Chronically facilitating the synthesis of pain-related molecules in nociceptive DRG neurons is a novel mechanism underpinning the contribution of PGE2.

Keywords: Substance P; Calcitonin gene-related peptide; Transient receptor potential vanilloid 1; Dorsal root ganglion; Aging; Nerve injury; Chronic pain

1. Introduction

Neuropathic pain (NeP), a common type of chronic pain, inflicts more than 3% of the population which is mostly composed of the elderly. NeP negatively impacts the quality of life of patients and imposes a heavy financial burden on medical care system. NeP can be elicited by physical damage or diseases of pain signalling pathways in the peripheral or central nervous system. Treating NeP is rather challenging due to unclear mechanisms. Inflammatory responses in injured nerves are believed to contribute to the pathogenesis of NeP (Moalem and Tracey, 2006). Sensitization of dorsal root ganglion (DRG) neurons by inflammatory mediators is a prerequisite of central sensitization and long-term plasticity, events involved in the maintenance of NeP. Prostaglandins are well defined pro-inflammatory and proalgesic mediators abundantly produced by inflammatory cells during inflammation. We and others have shown that cyclooxygenase 2 (COX2) and its main end product prostaglandin E2 (PGE2) are up-regulated in invading macrophages in injured nerves in rats and humans (Ma and Eisenach, 2002, 2003a; Muja and Devries, 2004; Takahashi et al., 2004; Ma and Quirion, 2005; Durrenberger et al., 2006). Local injection of non-selective COX inhibitors attenuated NeP within 1 month post-lesion (Syriatowicz et al., 1999; Ma and Eisenach, 2002, 2003a), suggesting that injured nerve derived COX2/PGE2 is involved in the maintenance of NeP at this early stage.

Since NeP affects mostly the aged populations (Davis and Srivastava, 2003), it is important to uncover the mechanisms governing the maintenance of NeP at more chronic stages in aged rats. Partial sciatic nerve ligation (PSNL) is a widely used model of NeP (Seltzer et al., 1990). An initial study documented NeP experienced by PSNL rats as late as 7 months post-lesion (Seltzer et al., 1990). It is unclear

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whether NeP persists when PSNL rats become older. Thus the first aim in the current study was to address whether PSNL rats still experience NeP at 10 months (middle-aged) and 18 months (aged) post-lesion. Although COX2 expressing invading macrophages have been reported to be present in injured human nerves for 2 years (Durrenberger et al., 2006), it is unknown whether the up-regulation of COX2/PGE2 in invading macrophages is a long-term event in injured nerves of aged rats and contributes to the maintenance of NeP at chronic stages. Therefore, the second aim was to determine the presence of COX2 expressing macrophages in injured nerves 18 months after PSNL as well as the effectiveness of perineural injection of a selective COX2 inhibitor NS-398 to relieve NeP at the other hand. Although mRNA of four PGE2 EP receptors are expressed in DRG neurons (Vanegas and Schaible, 2001; Fehrenbacher et al., 2005), little is known regarding protein distribution of these receptors in naïve and nerve injured rats. Therefore, the third aim was to examine the protein distribution of the four EP receptors in aged naïve rats and PSNL rats. Since the maintenance of NeP largely depends on the long-term expression or up-regulation of pain-related molecules in DRG neurons after nerve injury, the fourth aim was to determine the long-term effects of injured nerve derived PGE2 on the expression of pain-related peptide, substance P (SP) and calcitonin gene-related peptide (CGRP), and ion channel transient receptor potential vanilloid 1 (TRPV1) in DRG neurons of aged PSNL rats, 18 months post-lesion. This study has been partially published in abstract form (Ma et al., 2007).

2. Materials and methods

The detailed procedure regarding materials and methods in this study is provided in supplemental information.

2.1. Partial sciatic nerve ligation and behavioural testing

Sprague–Dawley rats (male, 200–250 g) were used. Animal care and maintenance were performed in accordance to the guidelines approved by McGill University Animal Care Committee and the Canadian Council for Animal Care. Briefly, the left sciatic nerve of anesthetized rats was exposed at the upper thigh level. One-third to one-half of the nerve was tightly ligated with silicon-treated silk suture (size 6–0). All rats were allowed to survive under optimal conditions for various time points: 4 weeks, 10 and 18 months after PSNL. Heat hyperalgesia was measured using Hargreave's test while tactile allodynia was tested using von Frey filaments.

2.2. Perineural (i.pn.) injection of NS-398

A total volume of 0.2 ml of solution of saline ($n = 27$) or NS-398 (60 μg/rat, $n = 29$) was injected around injured sciatic nerve of rats 18 months after PSNL $p < 0.05$ or uninjured sciatic nerve of age-matched naïve rats ($n = 15$). Additional age-matched naïve rats ($n = 18$) were used as additional controls. Heat and mechanical hypersensitivities were measured 1 day before injection and 2, 24 and 48 h after injection.

2.3. 3,3′-Diaminobenzidine (DAB)-based immunostaining of DRG

Deeply anesthetized rats were perfused with paraformaldehyde. Sciatic nerve segments and L4-6 DRG of both sides were removed and cut on a cryostat. DRG sections were incubated in the following rabbit polyclonal antisera: anti-CGRP (1:4000), anti-SP (1:4000), anti-TRPV1 (1:1000) and anti-EP1-4 (1:500). Subsequently, the sections were incubated in biotinylated goat anti-rabbit (1:200) and further processed using Elite Vectastain ABC kit. Finally, the immunoprecipitates were developed by DAB enhanced by the glucose oxidase–nickel–DAB method. The detailed procedure to quantify the mean percents of EP1-4-, SP-, CGRP- and TRPV1-IR DRG neurons as well as EP1 translocation into plasma membrane is provided in supplemental information.

2.4. Double immunofluorescent staining

Sciatic nerve sections were incubated in the following primary antisera: rabbit anti-COX2 (1:200), mouse anti-ED1 (1:100) and mouse anti-S100 (1:100). DRG sections were incubated in the following primary antisera: rabbit polyclonal anti-EP1-4 (1:100), guinea pig anti-CGRP (1:500), guinea pig anti-SP (1:500) and guinea pig anti-TRPV1 (1:500). Sections were then incubated in biotinylated goat anti-mouse or goat anti-guinea pig IgG (1:200) and then in goat anti-rabbit IgG conjugated with Alexa Fluor 488 (1:200) and StreptAvidin conjugated with Alexa Fluor 568 (1:200). Sections were examined under a confocal microscope.

2.5. Enzyme Linked Immunosorbent Assay (ELISA) for PGE2, CGRP and SP

PSNL rats with i.pn. injection of saline or NS-398 and age-matched naïve rats were decapitated. Sciatic nerve segments and L4-6 DRG of both sides were removed, weighed, homogenized and centrifuged. Total protein content in supernatants was determined for all samples using BCA protein assay. Commercially available ELISA kits for were used to assay PGE2, SP and CGRP in supernatants of all groups based on the manufacturer's instructions. The microplates was read using a colorimetric microplate reader. The mean value (pg/100 mg wet weight protein) of PGE2 or CGRP and SP was compared statistically using one-way ANOVA with Dunnet multiple comparison methods. The significance level was set at $p < 0.05$.