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Prostaglandin E2 contributes to the synthesis of brain-derived neurotrophic factor in primary sensory neuron in ganglion explant cultures and in a neuropathic pain model

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) exists in small to medium size neurons in adult rat dorsal root ganglion (DRG) and serves as a modulator at the first synapse of the pain transmission pathway in the spinal dorsal horn. Peripheral nerve injury increases BDNF expression in DRG neurons, an event involved in the genesis of neuropathic pain. In the present study, we tested the hypothesis that prostaglandin E2 (PGE2) overproduced in injured nerves contributes to the up-regulation of BDNF in DRG neurons. Two weeks after partial sciatic nerve ligation (PSNL), BDNF levels in the ipsilateral L4–L6 DRG of injured rats were significantly increased compared to the contralateral side. Perineural injection of a selective cyclooxygenase (COX2) inhibitor or a PGE2 EP4 receptor antagonist not only dose-dependently relieved PSNL elicited mechanical hypersensitivity, but also suppressed the increased BDNF levels in DRG neurons. PSNL shifted BDNF expression in the ipsilateral DRG from small to medium and larger size injured neurons. BDNF is mainly co-expressed with the EP1 and EP4 while moderately with the EP2 and EP3 receptor subtypes in naïve and PSNL rats. PSNL also shifted the expression of EP1–4 receptors to a larger size population of DRG neurons. In DRG explant cultures, a stabilized PGE2 analog 16,16 dimethyl PGE2 (dmPGE2) or the agonists of EP1 and EP4 receptors significantly increased BDNF levels and the phosphorylated protein kinase A (PKA), extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK) and cAMP response element binding protein (CREB). The EP1 and EP4 antagonists, a sequester of nerve growth factor (NGF), the inhibitors of PKA and MEK as well as CREB small interfering RNA suppressed dmPGE2-induced BDNF. Taken together, EP1 and EP4 receptor subtypes, PKA, ERK/MAPK and CREB signaling pathways as well as NGF are involved in PGE2-induced BDNF synthesis in DRG neurons. Injured nerve derived-PGE2 contributes to BDNF up-regulation in DRG neurons following nerve injury. Facilitating the synthesis of BDNF in primary sensory neurons is a novel mechanism underlying the role of PGE2 in the genesis of neuropathic pain.

Introduction

Chronic neuropathic pain is a serious clinical concern which affects more than 6% of the population in the developed countries. This pain condition is usually caused by direct injury or diseases of the nervous systems responsible for pain transmission and perception. Neuropathic pain is generally manifested as spontaneous pain, hyperalgesia (exaggerated response to painful stimulation) and allodynia (painful response to innocuous stimulation). Due to poorly understood underlying mechanisms, the treatment of neuropathic pain is too often unsatisfactory. Thus vigorous research has focused on its central and peripheral mechanisms to uncover novel therapeutic targets. As a peripheral mechanism, the role of the inflammatory responses occurring in injured nerves has drawn great attention. We and others have previously shown that cyclooxygenase 2 (COX2), the rate limiting enzyme in the synthesis of the well-known pain mediator prostaglandin E2 (PGE2), was dramatically increased in invading macrophages (Ma and Eisenach, 2002, 2003a) and Schwann cells (Muja and Devries, 2004; Takahashi et al., 2004) of injured nerves. Levels of PGE2 in injured nerves are consequently elevated (Ma and Quirion, 2005; Muja and Devries, 2004; Schafers et al., 2004). Four PGE2 EP receptors, particularly EP1 and EP4, are up-regulated in injured nerves (Ma and Eisenach, 2003b) and DRG neurons (Ma et al., 2010a).

Abbreviations: BDNF, brain derived neurotrophic factor; CGRP, calcitonin gene-related peptide; COX2, cyclooxygenase 2; CREB, cAMP response element binding protein; DMEM, Dulbecco Modified Eagle Medium; DRG, dorsal root ganglion; dmPGE2, 16,16-dimethyl PGE2; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FG, fluorogold; FR, fluororuby; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HBSS, Hank's balanced buffered saline; IL-6, interleukin-6; IR, immunoreactivity or immunoreactive; MAPK, mitogen activated protein kinase; MEK, mitogen activated protein kinase kinase; NGF, nerve growth factor; NHS, normal horse serum; PGE2, prostaglandin E2; PKA, protein kinase A; PSNL, partial sciatic nerve ligation; siRNA, small interfering RNA; SP, substance P; TBS + T, Tris buffered saline containing Triton-X100 or Tween-20; trk, tyrosine receptor kinase; TRPV1, transient receptor potential vanilloid-1.

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Increased PGE2 level in injured nerves is a long-lasting event in rats and human with nerve injury (Durrenberger et al., 2006; Ma et al., 2010a) and thus likely exerts long-term effects on EP receptor bearing cells, *e.g.* invading macrophages through autocrine or paracrine pathways, and on nociceptive DRG neurons (nociceptors) by stimulating *en passant* injured or spared axons, a hypothesis that we previously formulated (Ma and Quirion, 2008). Of these chronic effects, facilitating the production of pain-related mediators such as neuropeptides, growth factors, ion channels, cytokines and chemokines in injured nerves as well as in DRG neurons is highly possible. In fact, the maintenance of neuropathic pain mainly depends on the long-term up-regulation of these pain-related molecules (Scholz and Woolf, 2007). Therefore, the persistent facilitation of the synthesis of pain-related mediators in injured nerves and DRG might be one of the mechanisms underlying the role of injured nerve-derived COX2 and PGE2 in the initiation and maintenance of neuropathic pain. Indeed, we have previously shown that injured nerve derived-COX2 and PGE2 facilitate the up-regulation of pro-inflammatory cytokine interleukin-6 (IL-6) (Ma and Quirion, 2005) and pain peptide calcitonin gene-related peptide (CGRP) (Ma and Quirion, 2006; Ma et al., 2010b) in invading macrophages following partial sciatic nerve ligation (PSNL). We recently showed that injured nerve-derived COX2 and PGE2 are involved in the up-regulation of IL-6 (St-Jacques and Ma, 2011) and in the production of pain-related peptide substance P (SP) and CGRP as well as transient receptor potential vanilloid-1 (TRPV1) in DRG neurons (Ma, 2010; Ma et al., 2010a) following PSNL. In the current study, we explored the contributing role of injured nerve derived COX2 and PGE2 in the up-regulation of the pain-related neurotrophin brain-derived neurotrophic factor (BDNF) in DRG neurons of rats experiencing neuropathic pain elicited by PSNL.

The role of BDNF in adulthood to serve as a pain modulator at spinal dorsal horn level has been well documented (Merighi et al., 2008; Pezet et al., 2002; Thompson et al., 1999). BDNF exists in small to medium size DRG neurons of naïve adult rats (Zhou et al., 1999). It can be anterogradely transported to the superficial spinal dorsal horn and released as a neuromodulator to act on its high-affinity tyrosine kinase receptor, trkB, present in nociceptive neurons, thus modulating the synaptic efficacy of the first synapse in the nociception pathway (Merighi et al., 2008; Pezet et al., 2002; Thompson et al., 1999). BDNF is up-regulated in DRG neurons following peripheral inflammation and nerve injury (Obata and Noguchi, 2006). A growing body of evidence has shown that the increased levels of BDNF in DRG neurons and spinal dorsal horn contribute to the genesis of neuropathic pain through BDNF/trkB signaling (Deng et al., 2000; Fukuoka et al., 2001; Quintao et al., 2008; Wang et al., 2009; Yajima et al., 2002, 2005; Zhou et al., 2000)

Although it is generally agreed that BDNF is up-regulated in DRG neurons following nerve injury, the type of DRG neurons up-regulating BDNF levels remains controversial. After either complete or partial nerve injury, BDNF was shown to be down-regulated in small and medium neurons and *de novo* synthesized in medium and large neurons (Zhou et al., 1999), over-expressed in uninjured small neurons (Fukuoka et al., 2001), or increased in small, medium and large size DRG neurons (Ha et al., 2001). It is necessary to use tract tracing method to revisit this issue in the PSNL model in which injured and spared DRG neurons are intermingled. Most importantly, it is necessary to determine the factors which are responsible for the up-regulation of BDNF in DRG neurons following nerve injury. NGF has been well known to induce BDNF in trkA containing DRG neurons after nerve injury (Pezet and Mcmahon, 2006). PGE2 could be another candidate factor for the induction of BDNF as it was shown to induce NGF and BDNF in brain neurons (Rage et al., 2006) and astrocytes (Toyomoto et al., 2004). Thus, it is highly possible that injured nerve derived-COX2 and PGE2 contributes, directly or indirectly through NGF, to the up-regulation of BDNF in DRG neurons resulting from nerve injury.

Therefore, in the present study we attempted to address four specific aims to test this hypothesis. Our first aim was to identify the DRG neuron type(s) in which BDNF is up-regulated following PSNL. The second aim was to determine if blocking COX2 and PGE2 signaling suppresses the up-regulated levels of BDNF in DRG neurons. The third aim was to determine if exogenous PGE2 directly increases BDNF protein levels in cultured DRG neurons. The fourth aim was to determine if NGF is involved in PGE2-induced BDNF synthesis. Finally, we attempted to identify the EP receptor subtype(s) and signaling transduction pathways underlying PGE2-induced BDNF. Part of data in this study has been reported in abstract form (Cruz Duarte et al., 2010).

Materials and methods

Partial sciatic nerve ligation

Sprague–Dawley rats (male, 250–350 g in body weight, Charles River, St-Constant, Québec, Canada) were used in this study. Animal care and maintenance were in accordance with the protocols and guidelines approved by McGill University Animal Care Committee and the Canadian Council for Animal Care. Adequate measures have been taken to minimize pain and discomfort of animals. Anesthetic inhalation of isoflurane (5% for induction and 2% for maintenance, Animal Source Center, McGill University, Québec, Canada) was used. The left sciatic nerve was exposed at the high thigh level and one-third to one-half of the nerve was tightly ligated with silicon-treated silk suture (size 6–0) as described before (Seltzer et al., 1990). After surgery, all rats had free access to food and water and were allowed to live under optimal conditions for the required periods of time.

To trace injured and spared DRG neurons, the injections of two fluorescent dyes into injured sciatic nerves were performed immediately following PSNL as described before (Ma and Bisby, 1998b; St-Jacques and Ma, 2011). Briefly, 1 μl of 5% fluororuby (FR, Invitrogen Canada Inc., Burlington, ON, Canada), a red dye that was only taken up by injured axons to label injured DRG neurons, was injected into the nerve at the injury site. To avoid causing injury, 1 μl of 5% fluorogold (FG, Molecular Probes Inc.), was injected into the distal sciatic nerve. FG is a green dye that can be taken up by both injured and spared axons, but can only be transported to spared DRG neurons by spared axons when the injection site is distal to the nerve injury site. Thus, the labeling with FR (red) and FG (green) differentiated injured and spared DRG neurons, respectively, as revealed by fluorescence microscopy.

Perineural drug injection

Rats were briefly anesthetized with the inhalation of 5% isoflurane. Perineural injection was made in the hindlimb in which the sciatic nerve was partially ligated. The head of the femur was used to determine the sciatic nerve position at the high thigh level. A total volume of 0.2 ml of solution was injected. The accuracy of the perineural injection approach was demonstrated by a high success rate (>95%) in pre-experimental tests. Injection of 0.2 ml 1% lidocaine paralyzed the hindlimb of all rats. Injection of trypan blue dye using this approach in rats revealed that the upper sciatic nerve segment was completely immersed in the dye.

To determine if over-produced PGE2 and EP4 receptor signaling in injured nerves contributes to BDNF up-regulation in DRG neurons, a selective COX2 inhibitor NS-398 (100 μg/rat, n = 5, Cayman Chemical Inc., Ann Arbor, MI, USA) or a selective EP4 receptor antagonist AH23848 (50, 100, 250 and 500 μg, n = 5 in each dose, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) dissolved in 0.2 ml saline was perineurally injected to PSNL rats one to two weeks after PSNL. Vehicle (0.01% ethanol in saline) served as control injection.