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Research Report

A comparison of spinal Iba1 and GFAP expression in rodent models of acute and chronic pain

Alfonso Romero-Sandoval[a,*], *Nu Chai*[a], *Nancy Nutile-McMenemy*[a], *Joyce A. DeLeo*[a,b,*]

[a]*Department of Anesthesiology, Neuroscience Center at Dartmouth, Dartmouth Medical School, Dartmouth College, 1 Medical Center Drive, Lebanon, NH 03756-1000, USA*

[b]*Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, USA*

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ABSTRACT

The treatment of acute and chronic pain is still deficient. The modulation of glial cells may provide novel targets to treat pain. We hypothesize that astrocytes and microglia participate in the initiation and maintenance of both, acute surgical and chronic neuropathic pain. Rats underwent paw incision, L5 nerve exposure or L5 nerve transection surgery. Behavioral mechanical allodynia was assessed using von Frey filaments. Immunohistochemistry was performed using anti-ionized calcium binding adaptor protein, Iba-1 (microglia), and anti-Glial Fibrillary Acidic Protein, GFAP (astrocytes) on day 1, 4 and 7 after surgery. Following paw incision and at spinal L5 segment GFAP expression was increased in laminae I–II and Iba1 in deep laminae on day 1, in the entire dorsal horn on day 4 and dissipated on day 7 after paw incision in parallel with the allodynia. L5 nerve transection induced mechanical allodynia from day 1 to 7 which correlated with Iba-1 increases on day 1, 4 (entire dorsal horn) and day 7 after nerve injury (deep laminae of the dorsal horn) at spinal L5 segment. Conversely, GFAP increased at later time points from day 4 (deep laminae) and on day 7 (entire dorsal horn). Our data demonstrates that astrocytes (GFAP expression) play a role in the initiation of acute pain and the maintenance of chronic pain while Iba-1 increases closely correlated with the early phase of neuropathic pain. Iba1 and GFAP increased rostrally, at L3 segment, after paw incision (day 4) and only Iba1 increased following L5 nerve transection (day 7).

1. Introduction

Despite the considerable advancements in our understanding of the mechanisms of acute and chronic pain, inadequate pain relief remains a considerable issue in acute postoperative and chronic (pathologic) pain conditions. A vast percentage of patients (~40%) receiving analgesics continue to experience moderate to extreme postoperative pain (Brennan et al., 2007; Dolin et al., 2002). Additionally, about 25% of patients receiving analgesic treatment after surgery show symptoms compatible with pain medication-related side-effects (Dolin and Cashman, 2005; Warfield and Kahn, 1995). Furthermore, acute postoperative pain may lead to persistent postsurgical pain (20–50%) and disability (5–10%) in common surgical procedures such as amputation, breast surgery, thoracotomy or coronary artery bypass surgery (Kehlet et al., 2006). In most patients, postsurgical chronic pain manifests similarly to neuropathic pain (Jung et al., 2003; Kehlet et al., 2006). In addition, pain affects 70% of the

* *Corresponding authors*. Department of Anesthesiology, Dartmouth-Hitchcock Medical Center, HB 7125, 1 Medical Center Drive, Lebanon, New Hampshire, 03756-1000, USA. Fax: +1 603 650 8449.
E-mail addresses: Edgar.A.Romero-Sandoval@Dartmouth.edu (A. Romero-Sandoval), Joyce.A.DeLeo@Dartmouth.edu (J.A. DeLeo).

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10 million cancer patients diagnosed each year and 60–100% of patients suffering from HIV/AIDS will experience pain during their illness (Cousins et al., 2004). Neuropathic pain, resulting from nerve injury, is often the most difficult to treat. A new treatment strategy is necessary because the majority of analgesics possess modest effectiveness, induce physical dependence, produce significant side effects or are not effective in all types of pain (Dworkin et al., 2003). A better understanding of the mechanisms underlying acute and chronic pain will aid in the development of more effective and safer drugs for pain syndromes.

The recognition of the critical role of glia in the pathophysiological process in acute and chronic pain conditions opens a new and promising avenue to new pain treatment strategies (Scholz and Woolf, 2007). It is generally believed that glial cells contribute to hypersensitivity mainly in chronic pain conditions. However, spinal microglia and astrocytes play a major role in the development and maintenance of allodynia and hyperalgesia in acute, subacute and chronic pain states. Microglia and astrocytes react to peripheral insults such as paw incision (Obata et al., 2006; Romero-Sandoval and Eisenach, 2007), carrageenan-induced paw inflammation (Hua et al., 2005), complete Freunds adjuvant-induced paw inflammation (Raghavendra et al., 2004), complete Freunds adjuvant-induced monoarthritis (Sun et al., 2007), zymosan-induced peripheral nerve inflammation (Ledeboer et al., 2005) or peripheral nerve injury (Jin et al., 2003; Tanga et al., 2005; Tsuda et al., 2003). These studies suggest that different types of pain may share a common pathophysiological mechanism, and that this may lie in the spinal cord immune response.

A direct comparison of glial reactivity between acute vs. chronic rodent pain models has not been done thus far. The current study tests the hypothesis that microglia and astrocytes participate in the development and maintenance of both acute postoperative and chronic neuropathic pain states. We compared the L5 nerve transection chronic neuropathic pain model with the acute paw incision model. Our laboratory has extensively characterized the glial responses in the L5NT model. We have demonstrated that microglial reactivity is involved not only in the initiation (Raghavendra et al., 2003) but also in the long-term maintenance of L5NT-induced hypersensitivity (Tawfik et al., 2007), and that astrocytes are mainly reactive in later phases of behavioral hypersensitivity following L5NT (Raghavendra et al., 2003). The paw incision pain model induces acute hypersensitivity by at least day 1 and persists three-four days after surgery. This hypersensitivity spontaneously dissipates by day 7 after paw incision (Brennan et al., 1996). We and others have shown that paw incision induces microglial reactivity (Obata et al., 2006; Romero-Sandoval and Eisenach, 2007; Zhu et al., 2003).

2. Results

2.1. *Paw incision- and L5 nerve transection-induced allodynia*

Paw incision induced mechanical allodynia on day 1 and 4 and by day 7 the mechanical withdrawal thresholds were not significantly different from baseline values (Fig. 1A). Normal animals and the contralateral side to paw incision surgery showed similar mechanical withdrawal thresholds (data not shown). L5 spinal nerve exposure sham surgery did not alter mechanical withdrawal thresholds at any time points tested (Fig. 1B). L5NT induced mechanical allodynia from day 1 to 7 (Fig. 1C).

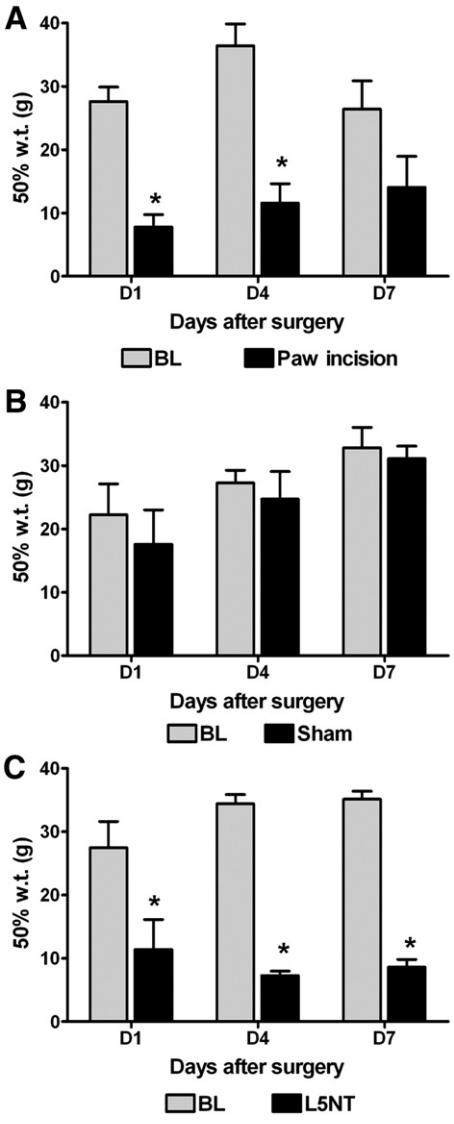

Fig. 1 – 50% withdrawal thresholds ipsilateral to paw incision (A), L5 exposure sham surgery (B) or L5 nerve transection (L5NT, C) before (base line = BL) and one, four and seven days (D1, D4 and D7 respectively) after surgery. *$P<0.05$ vs. base line value, *t* tests or Mann–Whitney *U* test when normality failed. $N=4$ for all groups.

2.2. *Iba1 expression*

At day 1 post paw incision surgery, we observed a significant increase in Iba1 expression in the deep laminae (but not laminae I–II) of the dorsal horn compared to the dorsal horn contralateral to the incision or to normal naive tissue (Figs. A and B). One day after surgery, Iba1 expression was significantly increased throughout the dorsal horn (laminae I–II and deep