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Glial cell activation in the spinal cord and dorsal root ganglia induced by surgery in mice

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ABSTRACT

In rodents, surgery and/or remifentanil induce postoperative pain hypersensitivity together with glial cell activation. The same stimulus also produces long-lasting adaptative changes resulting in latent pain sensitization, substantiated after naloxone administration. Glial contribution to postoperative latent sensitization is unknown. In the incisional pain model in mice, surgery was performed under sevoflurane+remifentanil anesthesia and 21days later, 1 mg/kg of (−) or (+) naloxone was administered subcutaneously. Mechanical thresholds (Von Frey) and glial activation were repeatedly assessed from 30 min to 21days. We used ionized calcium binding adaptor molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) to identify glial cells in the spinal cord and dorsal root ganglia by immunohistochemistry. Postoperative hypersensitivity was present up to 10 days, but the administration of (−) but not (+) naloxone at 21days, induced again hyperalgesia. A transient microglia/macrophage and astrocyte activation was present between 30 min and 2days postoperatively, while increased immunoreactivity in satellite glial cells lasted 21days. At this time point, (−) naloxone, but not (+) naloxone, increased GFAP in satellite glial cells; conversely, both naloxone steroisomers similarly increased GFAP in the spinal cord. The report shows for the first time that surgery induces long-lasting morphological changes in astrocytes and satellite cells, involving opioid and toll-like receptors, that could contribute to the development of latent pain sensitization in mice.

1. Introduction

Nociceptive hypersensitivity after tissue injury and/or opioid administration has been widely investigated in animal models and man (Célérier et al., 2001; Hay et al., 2009; Richebé et al., 2005; Silverman, 2009). Its relevance is partially related to the fact that in surgical patients, the extent and duration of postoperative pain hypersensitivity seems to be a critical factor contributing to the development of chronic post-surgical pain in genetically predisposed individuals (Kehlet et al., 2006; Macrae, 2008). In general, and depending on the surgery, healing of the wound occurs after a period of days–weeks during which pain and hyperalgesia gradually disappear. After tissue repair, silent (latent) long-lasting plastic adaptations in neuronal/non-neuronal systems remain, and exposure to new stimulus (nociceptive/non-nociceptive) precipitate again hyperalgesia in animal models of nociception (Campillo et al., 2011; Le Roy et al., 2011; Rivat et al., 2007).

This phenomenon known as latent pain sensitization or long-time pain vulnerability, could be the basis for the progression from acute to chronic pain, but the precise events underlying this transformation are poorly understood. In a rat model of incisional pain, the preoperative administration of nefopam prevented latent pain sensitization (Laboureyras et al., 2009), although it is not yet know if the drug could prevent chronic post-surgical pain in humans. Other reports have shown in rodents that NMDA antagonists (Rivat et al., 2002), or protein kinase M zeta inhibitors (Asiedu et al., 2011) can also block this latent sensitized state, suggesting that different mechanism are involved in the development of persistent pain.

Among the multiple potential mechanism implicated in pain and opioid-induced hypersensitivity, spinal cord (SC) glial cell activation has been consistently reported after inflammation, nerve injury (Peters et al., 2010; Raghavendra et al., 2004; Romero-Sandoval et al., 2008) and/or opioid exposure (Garrido et al., 2005; Horvath et al., 2010). It is also interesting that acute opioid administration also activates satellite glial cells (SGCs) in the dorsal root ganglia (DRG), implicating the peripheral nervous system in opioid analgesia (Berta et al., 2012). Animal studies have shown that spontaneous activity in the DRG after peripheral injury and inflammation or manipulation of the DRG, may change the input to the spinal cord modifying spinal neuronal/glial activity (Xie et al., 2009).

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Since tissue injury and opioid administration each induce glial activation, we hypothesized that surgery performed under remifentanil anesthesia, as routinely done in clinical practice, would induce robust changes in glial cell activity in the postoperative period, correlating with pain hypersensitivity. We also proposed that after the initial injury/activation, latent adaptative changes in glial cells would remain and the exposure to harmful or detrimental stimulus could induce glial re-activation. We used a challenge with naloxone to test for latent pain sensitization, as previously described by our group (Campillo et al., 2011; Romero et al., 2011).

Given the increased relevance of the DRG as the first anatomical site where modulation of sensory information occurs, we assessed glial cell activity in the DRG and the dorsal horn of the SC, ipsilateral to the surgery. Our results could be helpful to establish future cellular and anatomical targets to prevent the development of latent pain sensitization after surgery, and the transformation of acute into chronic pain.

2. Material and methods

2.1. Animals

Swiss CD1 male mice weighing 25–30 g obtained from Charles-River (CRIFFA, France) were used in all experiments. All procedures and animal handling met the guidelines of the European Communities directive 86/609/EEC regulating animal research. The protocol was approved by the institutional review board of our institution (Comité Ético de Experimentación Animal – Parc de Recerca Biomèdica de Barcelona, Spain). All the experimental procedures were carried out in the animal facilities located in the Parc de Recerca Biomèdica de Barcelona (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care since June 2010). Animals were housed four per cage with autoclaved poplar soft wood bedding (Souralit S.L., Barcelona, Spain) and maintained in a room under a 12 h light/dark cycle (lights on at 8 AM), at controlled temperature (21 ± 1 °C) and relative humidity ($55 \pm 10\%$). Food and water were available ad libitum except during behavioral evaluation.

2.2. Surgery

We used the incisional postoperative pain mouse model previously described (Brennan et al., 1996) and validated in our laboratory (Célérier et al., 2006). Animals were anesthetized with sevoflurane delivered for 30 min via a nose mask (induction, 3.5% v/v; surgery, 3.0% v/v) in a sterile operating room. A 0.7 cm longitudinal incision was made with a number 20 blade through the skin and fascia of the plantar surface of the right hind paw, starting 0.3 cm from the proximal edge of the heel extending toward the toes. The underlying plantaris muscle was exposed and incised longitudinally, keeping the muscle origin and insertion intact. After hemostasis with slight pressure, the skin was closed with two 6–0 silk sutures and the wound covered with povidone–iodine antiseptic ointment. After surgery, the animals were allowed to recover under a heat source in cages with sterile bedding.

2.3. Behavioral testing

Punctate mechanical stimulus (referred as hyperalgesia throughout the text) served as a measure of nociception. Before the experiments, animals were habituated to the equipment for 2–3 days (without nociceptive stimulation). All behavioral experiments were performed between 9:00 AM and 4:00 PM. Hyperalgesia was measured by the hind paw withdraw response to von Frey filament stimulation. Animals were placed in methacrylate cylinders (30 cm high, 9 cm diameter; acquired from Servei Estació, Barcelona, Spain) with a wire grid bottom through which the von Frey filaments were applied (bending force range from 0.008 to 2 g; North Coast Medical, Inc., San Jose, CA). Animals were allowed to habituate for 2 h before testing, to achieve immobility. The filament force was increased or decreased according to the response. The upper limit value (2 g) was assigned when there was no response and the threshold of response was calculated using the up–down method (Chaplan et al., 1994). Paw shaking or licking were considered nociceptive-like responses. Both hind paws were alternately tested.

2.4. Perfusion, tissue processing and immunohistochemistry

For each time point, 4–5 mice were deeply anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneal) and perfused intracardially with phosphate-buffered saline (PBS; 0.1 M, pH 7.4) followed by a fixative containing 10% formalin. The spinal cord and the dorsal root ganglia (ipsilateral and contralateral) from the L4–L6 segments were removed and post-fixed for 2 h. Tissues were removed and then cryoprotected for 24 h at 4 °C in 20% sucrose in 0.1 M PBS. After that, tissues were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Zoeterwoude, The Netherlands), and transverse sections (25 or 13 μm thickness, respectively) were cut using a cryostat (Leica, Madrid, Spain) at −21 °C. Then, six or seven sections of each tissue were serially cut, placed on gelatinized slides, and processed for immunohistochemistry. Every fifth section was picked from a series of consecutive tissue sections, preventing significant overlap of neuronal profiles and minimizing repeated counting of the same neuronal profile across multiple sections.

Non-specific binding was blocked with 5% normal goat serum (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Tissue sections were processed for 36 h at 4°C, for ionized calcium binding adaptor molecule 1 (Iba1), glial fibrillary acidic protein (GFAP) immunohistochemistry, according to the following primary antibody dilutions: Iba1 (1:2000, Wako Chemical, Richmond, VA), GFAP (1:500, DakoCytomation, Denmark). Sections were then incubated in secondary antibody AlexaFluor 488 (1:2000; Molecular Probes Europe BV, The Netherlands) or Cy3 (1:2000, Chemicon International, Temecula, CA) goat anti-rabbit IgG, for 1 h at room temperature in goat serum in PBS (5% goat serum and 1% Triton-X-100, Sigma). For control samples, the primary antibody was omitted. All sections were then mounted with Vectashield® + Dapi (Vector Laboratories, Burlingame, CA) and cover-slipped.

2.5. Quantification

To avoid variability in the staining procedure, all the sections to be compared were processed together, and images were acquired under the same exposure conditions. Fluorescence images were acquired with a microscope (Leica DMR, Madrid, Spain) outfitted with a filter set for Alexa 488 and Cy3, and equipped with a digital camera (Leica DFC 300 FX, Madrid, Spain). Spinal cord images were captured and recorded under 4x or 20x objectives, and the dorsal root ganglia under a 10x objective. Immunoreactive images were counted in a blinded manner on randomly selected spinal cord and DRG from L4–L5 ($n = 4$–5 animals per experimental group and time point). For qualitative analysis, we used samples from each group and time point from at least 4 slices per animal; values were averaged and compared with the control group. All images were analyzed with Image J software (version 1.44, NIH, Bethesda, MD). Quantification of Iba1 and GFAP was performed by determining the immunofluorescence intensity of cells within a fixed area in both