Neuroscience 274 (2014) 209–217

SYSTEMIC INFLAMMATION ALTERS SATELLITE GLIAL CELL FUNCTION AND STRUCTURE. A POSSIBLE CONTRIBUTION TO PAIN

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Abstract—Local peripheral injury activates satellite glial cells (SGCs) in sensory ganglia, which may contribute to chronic pain. We hypothesized that systemic inflammation affects sensory ganglia like local injury. We induced systemic inflammation in mice by injecting lipopolysaccharide (LPS) intraperitoneally, and characterized SGCs and neurons in dorsal root ganglia (DRG), using dye injection, calcium imaging, electron microscopy (EM), immunohistochemistry, and electrical recordings. Several days post-LPS, SGCs were activated, and dye coupling among SGCs increased 3–4.5-fold. EM showed abnormal growth of SGC processes and the formation of new gap junctions. Sensitivity of SGCs to ATP increased twofold, and neuronal excitability was augmented. Blocking gap junctions reduced pain behavior in LPS-treated mice. Thus, changes in DRG due to systemic inflammation are similar to those due to local injury, which may explain the pain in sickness behavior and in other systemic diseases.

Key words: satellite glial cell, dorsal root ganglion, purinergic receptors, gap junction, glial activation, sickness behavior.

INTRODUCTION

Chronic pain is a major public health problem with a prevalence of about 20% in the adult population (Breivik et al., 2006), but in a large percentage of cases therapy for it is not effective. Studies on animal models have contributed greatly to the progress in this field (Colleoni and Sacerdote, 2010; Jaggi et al., 2011), particularly in elucidating cellular and molecular aspects. Currently, most pain models in animals are based on local injury (axotomy, inflammation, etc.), and were designed to promote the understanding of localized pain. However, chronic pain in systemic disease is common (Borsook, 2012), but has received much less attention. In diseases such as systemic infection, diabetes, and sickness behavior, pain can be a prominent symptom (Marcus, 2005; Koroschetz et al., 2011). Immune activation is a common feature of these diseases; for example, it occurs in type 2 diabetes (Donath and Shoelson, 2011), in which neuropathic pain is highly prevalent (Callaghan et al., 2012).

Sickness behavior is caused by systemic inflammation, triggered by infection (Watkins and Maier, 2000; Dantzer et al., 2008), and is characterized by malaise, depression, and pain. The proposed mechanism of sickness behavior is the elevation in the blood levels of inflammatory mediators (mainly cytokines), followed by their downstream effects on both the central and peripheral nervous systems. The pain symptoms associated with systemic disease may be severe, but this aspect has received only little study. The pain mechanisms, when discussed, are believed to be centrally mediated.

An important station in the pain pathways are sensory ganglia, which contain the somata of sensory neurons and satellite glial cells (SGCs) that surround the neurons, but sometimes clusters containing two or more neurons share a common SGC sheath (Hanani, 2005; Pannese, 2010). Neurons in these ganglia generate ectopic firing in a variety of pain models, and thus can contribute to chronic pain (LaMotte et al., 1996; Devor, 2006). Although central changes take place in pain states, the single most important factor in the generation and maintenance of chronic neuropathic pain are changes in excitability of primary nociceptive afferents (Scadding and Koltzenberg, 2006). Indeed, there is evidence for ectopic firing in primary afferents in patients suffering from chronic pain and in animal models of pain (Devor, 2006). Therefore, a likely focus for research of inflammatory systemic pain is sensory ganglia.

Current pain treatment is directed at neurons, but there is evidence that spinal glial cells are involved in immune response and play crucial roles in chronic pain, largely by their ability to regulate the activity of spinal neurons (Milligan and Watkins, 2009; Ji et al., 2013; Tsuda et al., 2013). Likewise, SGCs in sensory ganglia play an important role in chronic pain. We found that gap junction-mediated coupling among SGCs is greatly augmented in pain models, and proposed a role for these cells in chronic pain (Hanani et al., 2002; Ledda et al., 2009; Huang et al., 2010). Reports from other laboratories confirmed this idea (Takeda et al., 2009; Durham and Garrett, 2010; Jasmin et al., 2010; Huang et al., 2013).

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Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DRG, dorsal root ganglia; EM, electron microscopy; GFAP, glial fibrillary acidic protein; IUR, isotropic uniform random; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SGCs, satellite glial cells.

http://dx.doi.org/10.1016/j.neuroscience.2014.05.029
0306-4522/

Following local peripheral nerve injury, SGCs display augmented levels of glial fibrillary acidic protein (GFAP), (Woodham et al., 1989; Li and Zhou, 2001) and an increased production of cytokines (Takeda et al., 2009). These changes are the hallmarks of immune activation in astrocytes, indicating a similar immune role for SGCs.

In this work we asked whether systemic inflammation affects SGCs in mouse dorsal root ganglia (DRG) in the same manner as local insults. We found that pain induced by systemic inflammation, and pain caused by local injury share several essential features, which reside in sensory ganglia.

EXPERIMENTAL PROCEDURES

Animals and drug treatment

We used Balb/c mice, 2–5 months old, (males:females, 1:1), weighing 20–25 g. No differences between sexes were observed. The experiments were approved by the Institutional Animal Care and Use Committee of the Hebrew University-Hadassah Medical School and adhere to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. To induce systemic inflammation we used a single intraperitoneal (i.p.) E. coli lipopolysaccharide (LPS) injection, 2.5 mg/kg (Sigma St. Louis, MO, USA), dissolved in saline. Uninjected mice served as controls. In preliminary experiments we verified that there was no difference between behavioral responses of uninjected animals and saline-injected ones.

Behavioral testing

Mice were placed in a clear plastic box on a wire mesh floor and were allowed to accustom to their new environment for at least 20 min before behavioral testing. Pain thresholds were assessed by observing withdrawal responses to mechanical stimulation, using von Frey hairs (Stoelting, Wood Dale, IL, USA). Hairs of 0.07–6 g were applied 10 times at intervals of 5 and 20 s in ascending order. The von Frey hairs were pressed against the plantar skin of the hind paw until the hair buckled. Sharp retraction of the stimulated hind paw was considered as a response in the paw. Withdrawal responses to the application of von Frey hairs to the abdomen were also examined. We considered the appearance of any of the following behaviors on application of a hair as a withdrawal response: sharp retraction of abdomen, immediate licking or scratching of site of application of hair, and jumping. The threshold response was defined when six out of 10 responses occurred. Care was taken not to stimulate the same point on the skin in succession. The right and left hind paws were both checked and averaged.

The forced swim test was conducted at 1 week post-LPS injection. Mice were placed in a water bath maintained at 25 ± 1 °C, observed for 6 min and then returned to their home cage. The duration of immobility, swimming and climbing was evaluated during the last 5 min of this period. Immobility was defined as passive floating. The water was changed and the bath was cleaned thoroughly between testing sessions.

Immunohistochemistry

Animals were killed by CO_2 inhalation, DRG L4/5 were removed and placed in 4% paraformaldehyde in phosphate buffer (pH 7.3) for 90 min at room temperature, and were then washed in phosphate-buffered saline (PBS) followed by incubation in 20% sucrose in PBS overnight before freezing in Tissue-Tek embedding medium (Sakura Finetek, Torrance, CA, USA). Sections were cut 12-μm-thick using a cryostat (Jung CM3000, Leica Microsystems, Wetzlar, Germany) and thaw-mounted on slides. Sections were washed in PBS and incubated with 50 mmol/L ammonium chloride for 30 min. Sections were washed again in PBS and then incubated in a blocking solution containing 3% bovine serum albumin (BSA) in PBS with 0.3% Triton X-100 for 2 h at room temperature. Primary antibodies against GFAP (rabbit anti-GFAP, Dako, Copenhagen, Denmark) were diluted 1:400 in PBS containing 1% BSA and incubated overnight at 4 °C. Sections were washed in PBS and incubated with secondary antibodies, donkey anti-rabbit conjugated to DyLight 549-TFP ester (Jackson ImmunoResearch, West Grove, PA, USA), 1:400 in PBS with 10 mmol/L of the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma) and 1% BSA for 2 h at room temperature. Finally, sections were washed in PBS. Controls omitted the primary antibody. Sections were photographed using an upright microscope (Axioskop FS2, Zeiss, Jena, Germany), equipped with fluorescent illumination and a digital camera (Pixera Penguin 600CL, Los Gatos, CA, USA) connected to a personal computer. Neurons and SGCs could be distinguished using DAPI because neuronal nuclei were larger and paler than SGC nuclei. Neurons that were surrounded by GFAP-positive SGCs by more than 50% of their circumference were counted and expressed as a percentage of the total number of neurons present in the fields analyzed. This criterion was used because the SGC sheath can be partly very thin and invisible under light microscopy; see also Warwick and Hanani (2013). Data from each group were collected from four animals. Five randomly selected fields containing about 50 neurons per field were analyzed from each animal and averaged.

Intracellular labeling

DRG L4/5 were removed as described above and pinned to the bottom of a silicon rubber-coated dish using fine pins. The dish was placed on the stage of an upright microscope (Axioskop), equipped with fluorescent illumination and a digital camera. The dish was superfused with Krebs solution, which contained (in mmol/L): 118 NaCl, 4.7 KCl, 14.4 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 2.5 $CaCl_2$ and 11.5 glucose; pH 7.3. This solution was bubbled with a mixture of 95% O_2/5% CO_2. Individual SGCs in DRG were injected with the fluorescent dye Lucifer yellow (LY; Sigma), 3% in 0.5 mol/L LiCl solution from sharp glass microelectrodes,