Satellite glial cells in dorsal root ganglia are activated in experimental autoimmune encephalomyelitis

Rebekah A. Warwick, Craig J. Ledgerwood, Talma Brenner, Menachem Hanani

Laboratory of Experimental Surgery, Hadassah-Hebrew University Medical Center, Mount Scopus, Jerusalem 91240, Israel
Department of Neurology, and the Agnes Ginges Center for Human Neurogenetics, Hadassah-Hebrew University Medical Center, Jerusalem 91120, Israel

HIGHLIGHTS

- Ten days post EAE induction, mice had reduced pain thresholds.
- GFAP expression was increased in SGCs in DRG of 10-day EAE mice.
- Coupling among SGCs was increased in DRG of 10-day EAE mice.

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ABSTRACT

Pain is a serious and common problem with patients suffering from multiple sclerosis (MS). Very little has been done to investigate the peripheral mechanisms of pain in MS. Here we used a mouse model of experimental autoimmune encephalomyelitis (EAE) to investigate the possible contribution of satellite glial cells (SGCs) to pain in MS. EAE mice had reduced pain thresholds 10 days after disease induction. We examined dorsal root ganglia and found increased expression of glial fibrillary acidic protein in SGCs, a marker of SGC activation, and increased coupling among SGCs, a known component of activated SGCs. Activated SGCs have previously been shown to contribute to pain in other classical neuropathic pain models, suggesting that pain in multiple sclerosis has a peripheral component.

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1. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS). Pain is a common problem for MS patients, with reported prevalence of between 43% and 92% [1,2], and in 23% of MS patients, pain was present even at the onset of the disease [3]. Pain in MS patients has been classified into three groups; central neuropathic pain (which includes trigeminal neuralgia, Lhermitte’s sign and dysesthetic pain in the extremities), musculoskeletal pain and headaches [4].

Experimental autoimmune encephalomyelitis (EAE) is the animal model used for the study of MS, which shares many pathological features of the disease, such as inflammation, demyelination, and impaired locomotor activity [5,6]. A small number studies have utilised the EAE model for investigating pain in MS. Mechanical hyperalgesia and thermal cold alldynia was shown in a rat EAE model [7] and in a mouse EAE model [8].

MS is considered a CNS disease, however recent research using the EAE animal models has shown that the peripheral nervous system (PNS) is also affected. Expression of brain-derived neurotrophic factor (BDNF) and the cytokine, tumour necrosis factor alpha (TNFα), were both significantly increased in dorsal root ganglia (DRG) of EAE models, and this increase was positively correlated with neurological disability [9,10].

Ectopic neuronal activity originating from the sensory ganglia has long been implicated in the initiation and maintenance of chronic pain [11]. More recently, satellite glial cells (SGCs), which surround neurons in the sensory ganglia, have also been shown to contribute to pain in nerve injury and in other neuropathic pain models [12–14].

Gap junctional coupling among SGCs was found to be increased in pain models. Administration of gap junction blockers to animal pain models reduced pain behaviour [12,14]; suggesting that
increased gap-junctional coupling among SGCs contributes to pain. It has been proposed that gap junction blockers may have a potential in pain therapy [12].

With the aim of investigating the possible contribution of SGCs to pain in MS, here we investigate whether SGCs in DRG of an EAE mouse model are activated and undergo the same changes seen in other classical pain models.

2. Methods

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 International Association for the Study of Pain.

EAE was induced in 8-week-old female C57BL/6 mice as previously described with some modifications [15]. Briefly, 250 μg of MOG35–55 (Sigma, St Louis, USA) emulsified in complete Freund’s adjuvant (CFA) containing 5 mg/ml heat-killed Mycobacterium tuberculosis was injected s.c. into the left para-lumbar region. Immediately thereafter, and again at 48 h, 0.5 ml of pertussis toxin (350 ng) was injected i.p. into the mice. Disease severity was evaluated daily according to the following scale: (0) without disease, (1) tail weakness, (2) hind limb weakness, sufficient to impair righting, (3) one limb plegic, and (4) paraplegia with forelimb weakness. According to the animal ethics agreements mice were euthanised at stage 4.

Pain thresholds were assessed by observing withdrawal responses to mechanical stimulation of the plantar skin of hind paws using von Frey hairs (Stoelting, Wood Dale, IL, USA), as described previously [14]. Hairs of 0.07–2 g were applied 10 times at intervals of 5–20 s in ascending order. Sharp retraction of the stimulated hind paw marked a response. The threshold for withdrawal response (pain threshold) was 6 out of 10 responses. Data were analysed using t-test.

Immunohistochemistry was carried out as follows. Mice were killed by CO2 inhalation, and the DRGs were removed and placed in 4% paraformaldehyde for 2 h at room temperature. The DRGs were then washed in 0.1 M phosphate buffered saline (PBS) before incubation in 30% 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 (Leica CM 1950, Leica Microsystems, Germany) and thaw mounted on SuperFrostPlus slides (Menzel, Braunschweig, Germany). Sections were washed in PBS and incubated in blocking solution containing 3% bovine serum albumin (BSA) in PBS with 0.3% Triton X-100 for 60 min at room temperature. Primary antibody against GFAP (rabbit anti–GFAP, Dako, Copenhagen, Denmark) was diluted 1:400, in PBS containing 1% BSA and incubated overnight at 4 °C. Sections were washed in PBS and incubated with the secondary antibody, donkey anti-rabbit conjugated to DyLight 549-TPF ester (Jackson ImmunoResearch, West Grove, PA, USA) diluted 1:400 in PBS with 10 μmol/L of the fluorescent dye 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) and 1% BSA for 2 h at room temperature. Finally, sections were washed in PBS. Controls omitted the primary antibody. Sections were imaged using an upright microscope (Axioskop FS2, Zeiss, Jena, Germany), equipped with fluorescent illumination and a digital camera (Pixera penguin 600CI, Los Gatos, CA, USA). Data were analysed using t-test.

Intracellular labelling was carried out as previously described [14]. Briefly, individual SGCs in excised DRGs were injected with the fluorescent dye Lucifer yellow (LY, Sigma), 3% in 0.5 mol/L LiCl solution from sharp glass microelectrodes. The dye was passed by hyperpolarising current pulses, 100 ms in duration; 0.5–1 nA in amplitude at 5 Hz for 1–2 min. Dye injections were made under visual inspection to allow cell identification. At the end of the injection of each cell, the coupling incidence was determined by the number of glial envelopes belonging to neighbouring neurons that were labelled as the result of the injection of a single SGC. Data were pooled for each group from multiple experiments and analysed using Fisher’s exact test.

3. Results

3.1. EAE mice

EAE mice were monitored for 19 days after disease induction and were given a behavioural score according to disease severity (see Section 2; Fig. 1a).

3.2. Pain threshold

We measured the pain thresholds of EAE mice, every 2 days after EAE induction up to 10 days, at the time point when behavioural signs started (for example tail weakness). Ten days after EAE induction pain thresholds were significantly lowered (Fig. 1b).

3.3. Expression of GFAP in SGCs

A useful marker for SGC activation is the increased expression of GFAP. Under normal conditions GFAP expression in SGCs is low, and is only detected by immunohistochemistry in a small percentage of cells. After nerve injury and in pain models GFAP expression in SGCs is increased and a higher percentage SGCs are immunoreactive for GFAP.

Fig. 1. Pain thresholds in EAE mice. (A) EAE mice were given a behavioural score every day for 19 days following induction of EAE. EAE mice began to show behavioural signs 9 days after EAE induction. Data were collected from 5 to 10 mice. (B) Ten days after EAE induction pain thresholds were significantly reduced. Data were collected from 6 to 8 mice. *p < 0.05.
GFAP expression was increased in SGCs in DRGs of 10-day EAE mice compared with control mice. Neurons that were surrounded by GFAP-immunoreactive SGCs (GFAP-IR SGCs) by more than 50% of their circumference were counted and expressed as a percentage out of the total number of neurons analysed. Neurons and SGCs were easily distinguished using DAPI staining because neuronal nuclei were larger and paler compared with SGC nuclei. In 10-day EAE mice the number of neurons surrounded by GFAP-IR SGCs was increased twofold compared with controls (Fig. 2).

### 3.4. Dye coupling between SGCs

LY is a small fluorescent molecule that can easily pass through gap junctions. It was injected into SGCs to assess the incidence of gap junction-mediated coupling between SGCs in DRGs of 10-day EAE and in controls. Two types of coupling are observed between SGCs, between SGCs surrounding a single neuron (Fig. 3a) and between SGCs surrounding more than one neuron (Fig. 3b–d).

Under normal conditions SGCs are more typically seen to be coupled around a single neuron (Fig. 3a), whereas in pain models, coupling between SGCs surrounding multiple neurons (Fig. 3b–d) is increased. We quantified the incidence of SGC coupling surrounding multiple neurons in L4/5 DRGs of 10-day EAE and control mice. The incidence of coupling between SGCs surrounding multiple neurons was significantly increased by over twofold in 10-day EAE mice compared with controls (Fig. 3e).

### 4. Discussion

In this study we used an EAE mouse model to investigate the possible contribution of SGCs to pain in MS. We showed that EAE mice had reduced pain thresholds 10 days after EAE induction. We examined SGCs in L4/5 DRGs of 10-day EAE mice, and found that...
the SGCs were activated (had increased expression of GFAP) and displayed augmented coupling. Activated SGCs were previously shown to contribute to pain in other neuropathic pain models [14].

Pain thresholds were measured using von Frey hairs in 10-day EAE mice, when the mice began to show behavioural signs of EAE, for example tail weakness and possibly hind limb weakness. These mice are still able to walk and use their hind legs, and so were able to respond to the von Frey hairs, allowing for accurate measurement of paw withdrawal thresholds. The early development of pain in EAE mice can be compared with pain in MS, where 23% of patients are in pain at the onset of the disease [3].

The mechanisms by which pain behaviour is altered in the EAE model are not clear, but our results demonstrate that peripheral changes that may contribute to pain, do take place in this model. The main outcome of the model is central demyelination, but there is evidence for peripheral changes in EAE animals [9,10], including activation of the peripheral immune system [16]. Thus it is conceivable that the results described here may be due to such events.

Astrocytes are known to be activated after nerve injury, ischaemia and inflammation. Activated astrocytes are characterised by hypertrophy, release of inflammatory mediators and up-regulation of the intermediate filaments GFAP and vimentin [17,18]. Similarly, after nerve injury, SGCs display a higher expression of GFAP in the corresponding ganglion, and are said to be activated [19]. Increased expression of GFAP in SGCs has also been shown in chemotherapy induced pain models [14,20]. Activated SGCs have an altered phenotype that includes increased synthesis of cytokines and augmented gap junctional coupling [14,21].

Augmented gap junctional coupling among SGCs has been observed in a variety of chronic pain models [12–14,22,23]. Administration of gap junction blockers to these models diminished pain behaviour [12,14,22]. A variety of gap junction blockers were shown in vitro to reduce excitability of DRG neurons from a mouse inflammatory pain model [12]. These reports suggest that increased gap junctional coupling between SGCs contributes to pain behaviour. It is possible that the increase in coupling among SGCs in the EAE mice contributes to the lowered pain thresholds observed in these mice. Further work is needed to establish whether gap junction blockers reduce pain behaviour in the EAE mice, and whether they have a potential in pain therapy for MS patients. This article not only sheds light on the potential mechanisms of pain in MS, but also provides further evidence for changes in the PNS in the EAE models, suggesting a peripheral component for pain in MS.

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References

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