Changes In Synaptic Transmission Of Substantia Gelatinosa Neurons In A Rat Model Of Lumbar Radicular Pain

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Introduction: Radicular pain is commonly encountered as a symptom of neuropathic pain in patients with lumbar spinal canal stenosis or lumbar disc herniation. To date, little is known about the pathophysiological mechanisms of radicular pain or why it develops into chronic neuropathic pain. We made the animal model of radicular pain in which a nerve root is ligated proximal to DRG. This model produces long-lasting mechanical allodynia and thermal hyperalgesia, which mimic clinical symptoms of lumbar radicular pain.

The substantia gelatinosa (SG; lamina II) in the spinal cord dorsal horn receives primary afferent inputs from Aδ and C fibers, which predominantly convey nociceptive sensations. Nociceptive information is modified and integrated in the SG, which consequently regulates the outputs of projection neurons located in lamina I and lamina VI-V, suggesting that the SG may be a therapeutic target for treating radicular pain.

The purpose of this study was to investigate changes in synaptic transmission of SG neurons following nerve root injury and to clarify the pathophysiological mechanisms of radicular pain using in vivo patch-clamp recording.

Methods: 1. Experimental animals: Six-week-old male Sprague-Dawley rats (n=85) weighing 160-180g at the beginning of the experiments were used.

2. Surgical procedures for producing the radicular pain model: Animals were randomly assigned to two different surgical groups: the root constriction group and the control group. In the root constriction group, the right L5 spinal root was tightly ligated extradurally with 8-0 nylon sutures proximal to the DRG.

3. Evaluation of mechanical hypersensitivity: Mechanical stimuli were produced using a 3.8-g von Frey filament. Mechanical sensitivity was evaluated as the frequency of withdrawal responses. The mechanical withdrawal frequencies of each rat were expressed as the number of responses from the non-injured side subtracted from the number of responses from the injured side. This procedure was performed 1 day before and 4, 7, 10, 13, 16, 19, 22, 25, and 28 days after surgery.

4. Evaluation of thermal hyperalgesia: Thermal hyperalgesia was evaluated as thermal withdrawal thresholds in response to noxious heat stimuli. The withdrawal latency was defined as the time from the onset of radiant heat to the withdrawal of the tested foot. The thermal withdrawal latency of each rat was defined as the differential score, which was calculated by subtracting the withdrawal latency of the non-injured side from that of the injured side.

5. Estimation of spontaneous pain-related behaviors: The degree of spontaneous behavior related to spontaneous pain was evaluated using a numerical scale as modified from a method described previously. The scale used in this study was as follows: 0 = the paw of the operated side was pressed...
normally on the floor, 1=the paw rested lightly on the floor, 2=only the internal edge of the paw was pressed on the floor; 3=only the heel was pressed on the floor, and the hind paw was in an inverted position; 4=the whole paw was elevated.

6. Electrophysiological procedures: We performed in vivo patch-clamp recordings according to a previous described method. The rats were used 11-15 days after surgery when mechanical hypersensitivity and thermal hyperalgesia had fully developed. In the voltage-clamp mode, the holding potentials (VH) were −70mV and 0mV, at which glycine-mediated and GABA-mediated IPSCs, and glutamate-mediated EPSCs, respectively, were negligible. Spontaneous excitatory postsynaptic potentials (EPSPs) and action potentials (APs) were recorded in the current-clamp mode. Whole cell patch-clamp recordings were made from SG neurons at the L4 and L5 segments of the spinal cord in the root constriction and control groups.

7. Stimuli to the receptive field: After determining the receptive field, the responses to non-noxious stimuli were assessed with a puff of air using a pico-injector. The noxious pinch stimuli were applied with toothed forceps. SG neurons exhibited several response profiles during air-puff or pinch stimuli in the current-clamp mode. Neurons are classified as multireceptive if they exhibit APs in response to non-noxious stimuli and noxious stimuli, and as nociceptive if they respond only to noxious stimuli.

Results: 1. Behavioral tests: The time courses of withdrawal frequencies are shown in Fig.1A. The differential score for noxious thermal stimuli was significantly lower in the root constriction group than in the control group from 4 days after the injury and thereafter (P<0.05, Fig.1B). Scale number to evaluate the degree of spontaneous behavior related to spontaneous pain 11-15 days after the root constriction or sham surgery is shown in Fig.1C.

2. In vivo patch-clamp recordings: A total of 141 SG neurons was recorded. The incidence of APs in SG neurons in the root constriction group recorded from the L5 segmental level of the spinal cord was higher than that in the control group (34% vs. 8%, P<0.05). The mean frequency of AP firings was also significantly higher in the root constriction group than in the control group (P<0.01). Receptive fields were found in all SG neurons recorded from control rats. In the root constriction group, SG neurons with no receptive field were seen and the proportion of these neurons was 16%. Examples of cell types recorded in SG neurons are shown in Fig.2A. The proportions of multi-receptive, nociceptive, and subthreshold neurons in the control group were 18%, 33%, and 49%, respectively (Fig.2B). In the root constriction group, with the exception of the neurons with no receptive field, the proportions of these neurons were 43%, 16%, 41%, respectively. A significant difference in the proportions of the cell types was observed between the two groups (P<0.05). The mean frequency of AP firings from the root constriction group was significantly higher than that from the control group (0.05±0.02Hz vs. 0.01±0.01Hz, P<0.01). There was one SG neuron (4%) with no receptive fields in the rats in the root constriction group. The proportions of multi-receptive, nociceptive, and subthreshold neurons in the control group were 17%, 33% and 50%, respectively. In the root constriction group, except for neurons with no receptive field, the proportions of these neurons were 15%, 30%, 55%, respectively. No significant difference was observed between the two groups. The mean frequencies and amplitudes of spontaneous EPSCs recorded at L4 and L5 segmental levels in SG neurons in the root constriction group were higher and larger than those at the same segmental levels in the control group (P<0.05, Fig.3). Spontaneous IPSCs were recorded from 13 neurons in the root constriction group and from 16 neurons in the control group at the L5 segmental level of the spinal cord. The mean frequency of spontaneous
IPSCs from the root constriction group was significantly lower than that from the control group (15±1Hz vs. 18±1Hz, P<0.05).

**Discussion:** The results of the present study indicated that injuring the nerve root led to characteristic excitatory changes in SG neurons at each segmental level and changed the properties of SG neurons. Central sensitization in SG neurons may contribute to the occurrence of radicular pain.

**Significance:** Further study is needed to investigate how to prevent the excitatory synaptic reorganization in SG neurons, and this would be of value for the treatment of radicular pain.
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Fig. 2

Fig. 3