Original article

Primary sensory neurons with dichotomizing axons projecting to the facet joint and the low back muscle in rats

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Abstract

Background. Clinically, the origin of low back pain is unknown. The pain may originate from the lumbar muscles directly, or it may be referred pain from the spine. Dorsal root ganglion (DRG) neurons with dichotomizing axons have been reported in several species and are thought to be related to referred pain. However, these neurons, which have dichotomizing axons to the lumbar facet joints and to the lumbar muscle, have not been fully investigated.

Methods. Two neurotracers — 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI) and fluoro-gold (FG) — were used in the present double-labeling study. DiI crystals were placed in the right L5/6 facet joint, and FG was applied to right multifidus muscles at the L5 level in 10 rats. Two weeks later, bilateral DRGs from L1 through L6 were harvested, sectioned, and observed under a fluorescence microscope.

Results. DiI-labeled DRG neurons innervating the L5/6 facet joint (5.17% of the total DRG neurons) were distributed from L1 to L6. FG-labeled DRG neurons innervating the lower back muscle (15.9% of the total) were also distributed from L1 to L6. Double-labeled DRG neurons were found from L1 to L6. The ratio of total double-labeled total DiI-labeled DRG neurons was 17% and that of total double-labeled total FG-labeled DRG neurons was 7%. Approximately 17% of all DRG neurons innervating the facet joints had other axons that extended to the lower back muscle.

Conclusions. This finding provides a possible neuroanatomical explanation for referred low back muscle pain from the lower facet joints.

Introduction

Clinically, the origin of low back pain is unknown. Many studies have reported that lumbar muscles, intervertebral discs, and facet joints are a source of low back pain. Low back pain may originate from the lumbar muscles directly, although there is a possibility that it is referred pain from the spinal structure, such as an intervertebral disc or facet joint.

Some authors have reported a sensory innervation pattern from low back muscle and facet joint to dorsal root ganglion (DRG) and spinal dorsal horn in animal models. Gillette et al. have reported analyzing the spinal projections of nociceptive afferents arising from lumbar spinal joints and muscles using an anterograde neurotracer. Labeled neurons innervating lumbar facet joints and lumbar muscle were similarly distributed in multiple layers of the spinal cord bilaterally in the lumbar, sacral, and thoracic spinal cord. Using a neurotracer, the distribution of stained DRG cells innervating a low back muscle (multifidus) at L5 level in the rat were at a maximum at L3, and overall were distributed from the L1 to the L6 level. We have reported that DRG neurons innervating the lumbar multifidus muscles at the L4 level are present in the DRGs from L1 to L6. In facet joints in rats, L5/6 facet joints are innervated by DRG neurons from L1 to L6 multisegmentally. These reports suggest that sensory innervation of multifidus muscles and facet joints at lower levels show a similar pattern and are multisegmental.

With a double fluorescence labeling technique, DRG neurons with dichotomizing axons projecting to two different peripheral nerves have been found in divergent body areas in different species. Thus, dichotomizing nerve fibers of sensory neurons are considered to be a possible substratum of referred pain. However, these neurons, which have dichotomizing axons to the lumbar facet joints and to the lumbar multifidus muscles, have not been fully investigated.
The purpose of the present study was to investigate DRG neurons with dichotomizing axons projecting to both lumbar multifidus muscles and facet joints in rats using double fluorescence retrograde neurotracing labeling techniques.

**Materials and methods**

All protocols for the animal procedures were approved by the Ethics Committees of our institutions following the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (1996 revision). Ten male Sprague-Dawley (SD) rats weighing 250–300 g were used. They were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and treated aseptically throughout the experiments. A midline dorsal longitudinal incision and paraspinal incision were made over the lumbar spine. The right multifidus muscles at the L5 level and right L5/6 facet joint were exposed under a microscope. A 26-gauge needle with a tip filled with 1 mg of fluorogold (FG) (Fluoro-Gold Fluorochrome, Denver, CO, USA) crystals was advanced into the multifidus muscles. A 26-gauge needle with a tip filled with 1 mg of 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI) (Molecular Probes, Eugene, OR, USA) was advanced into the L5/6 facet joint. The fascia and skin were then closed.

Twelve days after surgery, these 10 rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and perfused transcardially with 0.9% saline, followed by 500 ml of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). Bilateral DRGs from levels L1 to L6 were resected. The specimens were immersed in the same fixative solution overnight at 4°C. After storing in 0.01 M phosphate buffer saline (PBS) containing 20% sucrose for 20 h at 4°C, each DRG was sectioned at a thickness of 20 μm on a cryostat. The number and percentage of DiI-labeled, FG-labeled, or double-labeled DRG neurons were evaluated under a fluorescence microscope. We analyzed 20 serial sections that were obtained from each DRG. Positive or negative labeling was blinded and observed by three orthopedic spine surgeons. If at least two of the observers were in agreement, their evaluation was used. These observers did not see the level of the DRGs.

The cross-sectional areas of the labeled neurons in all the rats was measured and their distributions compared. Cells were classified into three groups according to size as either small (0–600 μm²), medium (601–1000 μm²), or large (1001–3200 μm²) according to a classification system derived from conduction velocities in rats.

**Statistical analysis**

The data were compared using a nonpaired t-test. \( P < 0.05 \) was considered statistically significant.

**Results**

The FG-labeled DRG neurons innervating right multifidus muscles at the L5 level were distributed from L1 to L6. The DiI-labeled DRG neurons innervating the right L5/6 facet joint were also distributed from L1 to L6 (Fig. 1). There were no labeled neurons in the left side from L1 to L6. The total number of FG-labeled neurons was 866, and the total number of DiI-labeled neurons was 339 in 10 rats (Fig. 2). The peak level of FG- and DiI-labeled neurons was observed at the L2 level. The ratio of FG-labeled neurons/total DRG neurons investigated \((n = 5446)\) was 15.9%. The ratio of DiI-labeled neurons to total DRG neurons investigated \((n = 6557)\) was 5.17%.

![FG](A.png) ![DiI](B.png)

**Fig. 1.** Fluorescence photomicrographs showing fluorogold (FG)-labeled (A) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI)-labeled (B) dorsal root ganglion (DRG) neurons at the L2 level. A and B are the same sections. A FG-labeled neuron innervating the right multifidus muscles at the L5 level. B DiI-labeled neuron innervating the right L5/6 facet joint. The arrows indicate the neurons in A and B that are both FG- and DiI-labeled neurons.