

Nociceptive phenotype of dorsal root ganglia neurons innervating the subchondral bone in rat knee joints

(ラット膝の軟骨下骨を支配する後根神経節ニューロンの侵害受容性フェノタイプ)

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1. Introduction

The subchondral bone of the distal femur is a source of pain caused by osteoarthritis (OA) or spontaneous osteonecrosis of the knee. To date, nociceptive phenotype of dorsal root ganglia (DRG) neurons innervating the subchondral bone in rat knee joints has not been evaluated. The purpose of this study was to clarify the distribution and nociceptive phenotype markers (calcitonin gene-related peptide (CGRP), tyrosine receptor kinase A (TrkA), neurofilament 200 (NF200) and isolectin B4 (IB4)) of DRG neurons innervating the subchondral bone of the distal femur in rats. Furthermore, we examined the differences in nociceptive phenotype markers between the subchondral bone and the knee joint afferents.

2. Materials and methods

2.1 Animals

Male Sprague-Dawley rats (3 weeks old) were used in this study.

2.2 Retrograde labeling

Retrograde labeling was used to identify afferents innervating the subchondral bone of the distal femur and the knee joint in rats. 1.5 μ l FB (10mg/ml in saline) was injected into the bilateral distal femoral epiphyses, while 10 μ l DiI (5 mg/mL in N, N dimethylformamide) was injected into the bilateral knee joints. At fourteen days after FB and DiI injection, the bilateral lumbar DRGs (L1-L6) were obtained. The DRGs were placed in 4% paraformaldehyde and 30% sucrose overnight and frozen in -80 degrees Celsius. Fourteen-micrometer frozen sections were then cut using a cryostat.

2.3 Immunohistochemistry

The sections were incubated in rabbit anti CGRP antibody, goat anti TrkA antibody, and mouse anti-NF200 overnight. The next day, secondary detection was performed with goat anti-rabbit IgG-FITC for CGRP, rabbit anti-goat IgG-FITC for TrkA and Alexa 488-conjugated donkey anti-mouse IgG for NF200. The sections for IB4 were incubated in IB4-FITC conjugate (10 μ g/ml).

2.4 Microscopic observation

The segmental distribution and the soma size of FB and DiI-labeled neurons were examined. For

each FB and DiI-labeled neuron, CGRP, TrkA, NF200 expression, and IB4 binding were quantified as the percentage of total FB and DiI-labeled neurons.

3. Results

3.1 Retrograde labeling

Of the FB-labeled neurons (subchondral bone afferents), 60% were localized in L3 DRGs. Of the DiI-labeled neurons (knee joint afferents), 67% were distributed in L3 and L4 DRGs. There were no significant differences in soma size distribution between FB and DiI labeled neurons.

3.2 Immunohistochemistry

The percentage of CGRP and TrkA-immunoreactive (IR) neurons in FB-labeled neurons (subchondral bone afferents) was significantly higher than DiI-labeled neurons (knee joint afferents), respectively ($p < 0.05$). On the other hand, the percentage of NF200-IR neurons in DiI-labeled neurons (knee joint afferents) was significantly higher than FB-labeled neurons (subchondral bone afferents) ($p < 0.05$). IB4 binding neurons were hardly ever present in FB and DiI-labeled neurons. There were no significant differences in soma size distribution of CGRP-IR and TrkA-IR neurons between FB and DiI-labeled neurons, whereas NF200-IR neurons among DiI-labeled neurons (knee joint afferents) were significantly larger than FB-labeled neurons (subchondral bone afferents) ($p < 0.05$).

4. Discussion

This is the first study to evaluate nociceptive phenotype of DRG neurons innervating the subchondral bone of the distal femur in rats. Our results showed significant differences in nociceptive phenotype between the subchondral bone and the knee joint afferents. As a whole, the majority of DRG neurons innervating the subchondral bone and the knee joint were CGRP-IR and TrkA-IR. IB4 binding neurons were hardly ever present. The percentage of CGRP-IR and TrkA-IR neurons innervating the subchondral bone was significantly higher than the knee joint. The peptidergic neurons containing neuropeptides such as CGRP are considered particularly important in transmitting inflammatory pain. Our results suggested the subchondral bone afferents could respond more sensitively to inflammation than the knee joint afferents.

In conclusion, we clarified nociceptive phenotype of DRG neurons innervating the subchondral bone in rat knee joints. It is expected that therapeutic approaches targeting CGRP and TrkA could attenuate pain from the subchondral bone in knee joints.