Investigation for sensory innervation of the dorsal portion of the rat Co5/6 coccygeal intervertebral disc

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Introduction.
Developing a rat disease model for degenerative intervertebral disc compression using lumbar discs is technically impractical because of their location. Coccygeal intervertebral discs are more readily accessible and several reports of morphological evaluation of degenerative coccygeal intervertebral discs using compression devices exist. However, their sensory innervation and properties have not yet been characterized.

Objective.
To investigate the properties of the sensory innervation of the coccygeal (Co) 5/6 intervertebral disc in rats using a retrograde neurotracing method and immunohistochemistry.

Methods.
FluoroGold neurotracer (FG) was applied to the Co5/6 intervertebral discs of intraperitoneally anesthetized Sprague Dawley rats (n = 10); subsequently the discs and the L1–S4 dorsal root ganglia (DRGs) were resected and sectioned. The discs were double-stained for immunoreactivity to the neuronal marker β-tubulin (Tuj-1) and biotin-labeled isolectinB4 (IB4), a neuropathic pain marker, or Tuj-1 and calcitonin gene-related peptide (CGRP), an inflammatory pain marker. The DRGs were double-stained for IB4-binding and CGRP immunoreactivity. The proportions of IB4-binding or CGRP immunoreactive (-IR) DRG neurons were assessed by cell counting and compared.

Results.
The disc immunohistochemistry showed evidence of sensory nerve fibers lying in the outermost layer of the annulus fibrosus(Fig.1). These nerve fibers were not seen in the inner layer of the annulus fibrosus or in the nucleus pulposus. Among all the nerve fibers, the number of the CGRP-IR and Tuj1-IR nerve fibers was significantly higher than that of IB4-binding Tuji-IR fibers (P < 0.05)(Fig. 2) FG labeled DRG neurons mainly derived from S1–S3 DRGs, especially significant in S2 and S3 (P < 0.01). No labeled neurons were observed in the S4 DRG. The histochemistry of the DRGs showed a significant predominance (P<0.01) of CGRP-IR DRG neurons (3.5 ± 1.7% IB4-binding and 15.4 ± 5.6% CGRP-IR on average) (Fig. 3).

Discussion.
The results of the present study have a very important significance in the points that it provides the evidence of the innervation of Co5/6 coccygeal intervertebral disc. The major benefits of using coccygeal intervertebral discs in researches of degenerative discs are: (1) their easier accessibility than that of lumbar ones, (2) availability of compression devices widely protruding outside the body, which are unusable for lumbar discs. Existing papers have mainly discussed coccygeal intervertebral discs in a histological point of view. The present study enables us to research the nervous characteristics of coccygeal intervertebral discs in a histological point of view. The major benefits of using coccygeal intervertebral discs are: (1) their easier accessibility than that of lumbar ones, (2) availability of compression devices widely protruding outside the body, which are unusable for lumbar discs. Existing papers have mainly discussed coccygeal intervertebral discs in a histological point of view. The present study enables us to research the nervous characteristics of coccygeal intervertebral discs in a histological point of view.

Conclusions.
The present study showed evidence for nerve fibers in the discs and predominant innervation by CGRP-IR DRG neurons. The neurons innervating the discs mostly derived from S1–S3 DRGs, especially S2 and S3. These findings may be useful in developing rat models of disease involving degenerative intervertebral disc compression.