Neurones in the dorsal root ganglia of T13, L1 and L2 innervate the dorsal portion of lower lumbar discs in rats

A STUDY USING DiI, AN ANTEROGRADE NEUROTRACER

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Based on a study using a retrograde neurotracer, we have previously found that the dorsal portion of the L5/6 disc in the rat is multisegmentally innervated by dorsal root ganglia (DRG) from the level of T13 to L6, and that sensory nerve fibres from DRG of T13, L1 and L2 pass through the paravertebral sympathetic trunks. In this study in newborn rats, we injected crystals of 1,1'-dioctadecyl-3,3',3'-tetramethylnedocarbocyanine perchlorate (DiI) into the DRG of T13, L1 and L2 and showed DiI-labelled sensory nerve fibres in the dorsal portion of the discs from the level of T13/L1 to L5/6. Our results show that the dorsal portion of the lumbar discs is innervated by the DRG from levels T13 to L2.

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The vertebra, intervertebral disc, facet joints, posterior longitudinal ligament (PLL) and dura mater are segmentally innervated by the dorsal ramus and the sinuvertebral nerves from the spinal nerve at corresponding levels.¹⁻⁹ Many studies have described the presence of sensory nerve endings in the annulus fibrosus.¹⁰⁻¹² It is believed they originate from the sinuvertebral nerves from the ventral ramus of the spinal nerve and the ramus communicans at the corresponding level.^{2,3,8,12}

Sensory innervation of the dorsal portion of the intervertebral disc has been thought to be segmental. Patients with lesions of the lower disc, however, sometimes experi-

ence diffuse pain in the lower back, the anterior thigh or the inguinal region. These latter are in the L1 or L2 dermatomes, which do not correspond to the segmental innervation of the dorsal portion of the disc.

Recently, we described that in the rat the L5/6 facet joint is multisegmentally innervated by the dorsal root ganglia (DRG) from L1 to L6 and that some nerve fibres pass through the paravertebral sympathetic trunks.^{13,14} The ventral portion of the L5/6 disc is innervated only by the DRG of L1 and L2¹⁵ and the dorsal portion by DRG from T13 to L6.¹⁶ The investigations were based on methods of retrograde transport in which neurotracers were injected into the facet joints or intervertebral discs only at the level of L5/6. The innervation of other segmental discs by upper DRG was not confirmed.

Our aim in this study was to clarify the existence of neurones in upper lumbar DRG, which send axons to the dorsal portion of lumbar discs, by using an anterograde transport method with fluorescent carbocyanine dye.

Materials and Methods

Eighty-five newborn Sprague-Dawley (SD) rats were anaesthetised with intraperitoneal sodium pentobarbital (40 mg/kg) on the day of birth and perfused transcardially with 30 ml of 4% paraformaldehyde in phosphate buffer (0.1M, pH 7.4). A midline dorsal longitudinal incision was made over the lumbar spine. The lamina was gently exposed between the spinal muscles and laminectomy was performed microscopically. The DRG were exposed and a sagittal incision of approximately 0.5 mm was made. Crystals of 1,1'-dioctadecyl-3,3',3'-tetramethylnedocarbocyanine perchlorate (DiI) were applied to the incised DRG and the incision sealed with cyanoacrylate to prevent leakage. There were three groups (n = 80) as follows: in group 1 DiI was applied to the ipsilateral T13 DRG, in group 2 to the L1 DRG, and in group 3 to the L2 DRG. The wound was then closed and postfixed in 4% paraformaldehyde in phosphate buffer (0.1M, pH 7.4) at 4°C.

Eight weeks after application of DiI, we obtained specimens of spines from the level of T11 to S2 including the DRG at the injected level. These were immersed in the same fixative solution overnight at 4°C. After storing in 0.01M phosphate-buffered saline (PBS) containing 20%

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Fig. 1

Figure 1a – Photomicrograph showing nerve bundles in the dorsal portion of the vertebral body and intervertebral disc at the level of L5/6. Bilateral thick nerve bundles from the intervertebral foramen run at the margin of the PLL craniocaudally (arrows). Some thin nerve bundles are seen in the posterior annulus fibrosus (arrowheads) (bar = 1 mm; AChE). Figure 1b – DiI-labelled nerve bundles are seen in the dorsal portion of the vertebral body (arrows) and intervertebral disc (arrowheads) at the level of L5/6. The labelled nerve bundles originated from the L2 DRG (bar = 1 mm).

Fig. 2

Figure 2a – Photomicrograph of the DRG of L2 labelled with DiI. There is no leakage of DiI. The arrows indicate the surface of the DRG. Yellow neurones are strongly labelled and red neurones moderately or weakly labelled (bar = 800 μ m). Figure 2b – A higher magnification of Figure 2a. DiI was taken up in the small (arrowheads) and large neurones (arrows) (bar = 200 μ m).

sucrose for 24 hours at 4°C, the dorsal portion of the vertebral column and DRG at the injected level were sectioned in the coronal plane at a thickness of 40 μ m on a cryostat. Slices of the dorsal portion of the vertebral bodies, intervertebral discs and DRG were placed under cover slips and observed by fluorescence microscopy. We performed analyses using specimens obtained without leakage of DiI from DRG at the injected level. In the remaining five rats the specimens were stained *en bloc* using an AChE histochemical method to investigate all the nerve fibres present in the dorsal portion of the lumbar discs.

Under anaesthesia, as before, rats were perfused with the same fixative solution and the whole lumbar spine was obtained. The endogenous activity of tissue peroxidase was quenched by soaking the lumbar spine for 30 minutes in 0.3% hydrogen peroxide solution in 0.01M PBS. The specimens were incubated in reactive solution containing 0.1 mmol/l of sodium citrate, 0.03 mmol/l of cupric sulphate, 4 μ mol/l of potassium ferricyanide, 0.02 mmol/l of

acetylthiocholine iodide, and 0.3% Triton-X in 0.1mol/l of sodium hydrogen maleate buffer (pH 6.0) for six hours at room temperature. After staining, they were rinsed in PBS and observed by stereomicroscopy.

Results

In all five rats AChE-stained sparse nerve bundles were observed on the dorsal portion of the vertebral bodies and intervertebral discs. Thick nerve bundles from the intervertebral foramen ran at the margin of the PLL craniocaudally. Thin nerve bundles were observed on the posterior annulus fibrosus of the L5/6 intervertebral disc (Fig. 1a).

Of the 80 rats to which DiI had been applied, 60 showed no leakage, 20 rats each in groups 1, 2 and 3 (Fig. 2a). They were used for the present analysis. DiI was taken up in both large and small neurones in each DRG (Fig. 2b). Some DiI-labelled nerve fibres were observed in the dorsal portion of the intervertebral discs or vertebral bodies at

Table I. Distribution of the DiI-labelled nerve bundles in the dorsal portion of the vertebral bodies and intervertebral discs.

	Level of the DRG		
	T13	L1	L2
Level of the vertebral body			
T11	0	0	0
T12	0	0	0
T13	1	0	0
L1	1	0	0
L2	2	2	3
L3	0	0	3
L4	1	3	2
L5	1	2	2
L6	1	2	2
S1	0	0	0
S2	0	0	0
Total	7	9	12
Level of the disc			
T11/12	0	0	0
T12/13	0	0	0
T13/L1	1	1	0
L1/2	1	1	1
L2/3	0	1	3
L3/4	0	2	2
L4/5	1	2	3
L5/6	1	2	2
L6/S1	0	0	0
S1/2	0	0	0
Total	4	9	11

different levels. Figure 1b shows DiI-labelled nerve bundles running at the margin of the PLL and transversely at the L5/6 intervertebral disc. The course of the DiI-labelled nerve fibres was similar to that of AChE-stained nerve fibres. In total, 52 DiI-labelled nerve bundles were observed in seven rats in group 1, ten in group 2 and 12 in group 3. In group 1, they were seen in the annulus fibrosus at the levels of T13/L1, L1/2, L4/5 and L5/6 and at the margin of the PLL in the vertebral body at the levels T13, L1, L2, L4, L5 and L6, and in group 2 in the annulus fibrosus at the levels of T13/L1, L1/2, L2/3, L3/4, L4/5 and L5/6 and in the vertebral body at the levels of L2, L4, L5 and L6. In group 3, they were seen in the annulus fibrosus at the levels of L1/2, L2/3, L3/4, L4/5 and L5/6 and in the vertebral body at the levels of L2, L3, L4, L5 and L6 (Table I). DiI-labelled nerve bundles were not seen on the contralateral side.

Discussion

Since the first description of the sinuvertebral nerve by Luschka in 1850,⁹ many investigators have reported that the lumbar intervertebral disc is innervated by sinuvertebral nerves consisting of spinal sensory fibres from the adjacent DRG and postganglionic sympathetic fibres.²⁻⁸ Recent studies have shown that the L5/6 facet joint and the ventral and dorsal portions of the intervertebral disc are innervated

by DRG at corresponding levels and by the DRG of T13, L1 and L2, the nerve fibres of which pass through the paravertebral sympathetic trunks.¹³⁻¹⁸ These results, however, were demonstrated using retrograde transport methods in which labelled cells in DRG were studied after a neuro-tracer had been injected into the facet joint or intervertebral disc only at L5/6. In this study, using an anterograde transport method with DiI, we have clarified that the upper DRG supply sensory nerve fibres to the dorsal portion of the lower lumbar discs as well as the upper lumbar discs. DiI was taken up in the DRG neurone and transported on the membrane of the nerve fibres to the peripheral nerve endings. Previous retrograde studies and this study have therefore shown that there are neurones of DRG of T13, L1 and L2 innervating the dorsal portion of the lumbar lower discs.

We have shown in the rat that the sensory fibres of DRG from T13 to L2 innervate multisegmental lumbar discs. Furthermore, we suggest that upper DRG supply the sensory nerve fibres to the discs multisegmentally from one upper segment to four or five lower. These observations indicate that in the lumbar spine, there are more sensory fibres of the neurones of the upper DRG supplying the

Fig. 3

Diagram of the sensory pathways from the DRG of L1 to the dorsal portion of the lumbar discs. Two different pathways are postulated. One is the segmental-like pathway passing through the sinuvertebral nerves for discs at the corresponding or adjacent levels, and the other is the non-segmental pathway in the paravertebral sympathetic trunks for lower discs (DRG, dorsal root ganglion; PST, paravertebral sympathetic trunks; PLL, posterior longitudinal ligament; RC, ramus communicans; SVN, sinuvertebral nerve).

posterior portion in lower discs than in upper discs at the corresponding level.

We consider that the pathway from the upper DRG neurones to the posterior portion of the discs may consist of two distinct nervous systems: innervation to the corresponding and adjacent discs and that to distant segments. In the segmental-like innervation, sensory nerve fibres from the upper DRG are believed to pass through sinuvertebral nerves on the PLL to reach the upper discs. In the non-segmental innervation sensory nerve fibres from the DRG of L1 enter the paravertebral sympathetic trunks and reach the discs from L3/4 to L5/6 directly through each ramus (Fig. 3).

In the previous study, the sensory innervation of the dorsal portion of the lumbar disc had not been clearly demonstrated since the injection of tracer to the site without leakage to other portions was difficult in experiments on anterograde or retrograde transport. In this study to determine if there was leakage of DiI to other areas, we examined specimens histologically and confirmed that the DiI remained within the DRG. We could, however, only count 52 DiI-labelled nerve bundles in 60 rats. This may have been due to the small number of DRG neurones at the level of T13, L1 and L2 innervating the dorsal portion of the L5/6 disc, typically only two or three.¹⁶ Furthermore, the network of nerve fibres in the dorsal portion of the lumbar intervertebral disc is very sparse in newborn rats (Fig. 1a), and therefore most DiI-labelled nerve fibres may be invisible in this area.

In the case of degeneration of the lumbar intervertebral disc, pain is induced by chemical and mechanical factors from the degenerated disc. Blockade of the spinal nerves at the same level has been effective for some patients with discogenic disorders, but for others blockade of L2 spinal nerves or the paravertebral sympathetic trunk is effective.^{17,19,20} Our findings that neurones of the upper DRG innervate the posterior portion of the lumbar intervertebral disc multisegmentally may explain these clinical events if a similar pattern of innervation is present in man.

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