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Nerve supply to the posterior longitudinal ligament and the intervertebral disc of the rat vertebral column as studied by acetylcholinesterase histochemistry.

II. Regional differences in the distribution of the nerve fibres and their origins

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INTRODUCTION

In our preceding paper, the outline of the nerve supply to the posterior longitudinal ligament (PLL) and the intervertebral disc (IVD) of the lumbar vertebrae in the rat was described, using a very sensitive acetylcholinesterase (AchE) enzyme histochemical method. It was revealed that there are two nerve fibre networks, which show separate distributions and morphological characteristics. However, the function of these nerve fibres still remains unclear.

In the present study, we investigated the regional differences in the pattern of the nerve supply at different vertebral levels and the origin of these nerve fibres. These results may allow us to determine a possible role of these nerves in the movement as well as in the pathology of the vertebral column.

MATERIALS AND METHODS

Thirty five male Wistar rats weighing 300–500 g were used. The nerve staining technique was based upon Tago's method (Tago, Kimura & Maeda, 1986) as described in detail in our preceding paper.

Firstly, the distribution of the nerve fibres at different vertebral levels was investigated on the PLL and the IVD of the cervical, thoracic and lumbar vertebrae of seven rats. Secondly, in order to investigate the origin of the nerve fibres observed, an experimental study was performed as follows. Twenty eight rats were deeply anaesthetised with an intraperitoneal injection of sodium pentobarbitone and laid in a prone position on an operating table. A median longitudinal skin incision was made in the low back, the paravertebral muscles were elevated subperiosteally and the vertebral lamina was exposed. The lamina was partially removed with an electric dental drill and unilateral or bilateral nerve roots and spinal ganglia of the first, second and third lumbar nerves were exposed. Following this, three types of surgery were performed: (i) Spinal ganglia of the first, second and third lumbar nerves were resected on one side (11 rats). (ii) The spinal ganglia of the first, second and third lumbar nerves were resected on both sides (10 rats). (iii) The ventral nerve roots of the first, second and third lumbar nerves were transected on both sides (7 rats). Each rat was killed one week after the operations, the PLL and the IVD from the operated level were dissected free and AchE staining was performed.

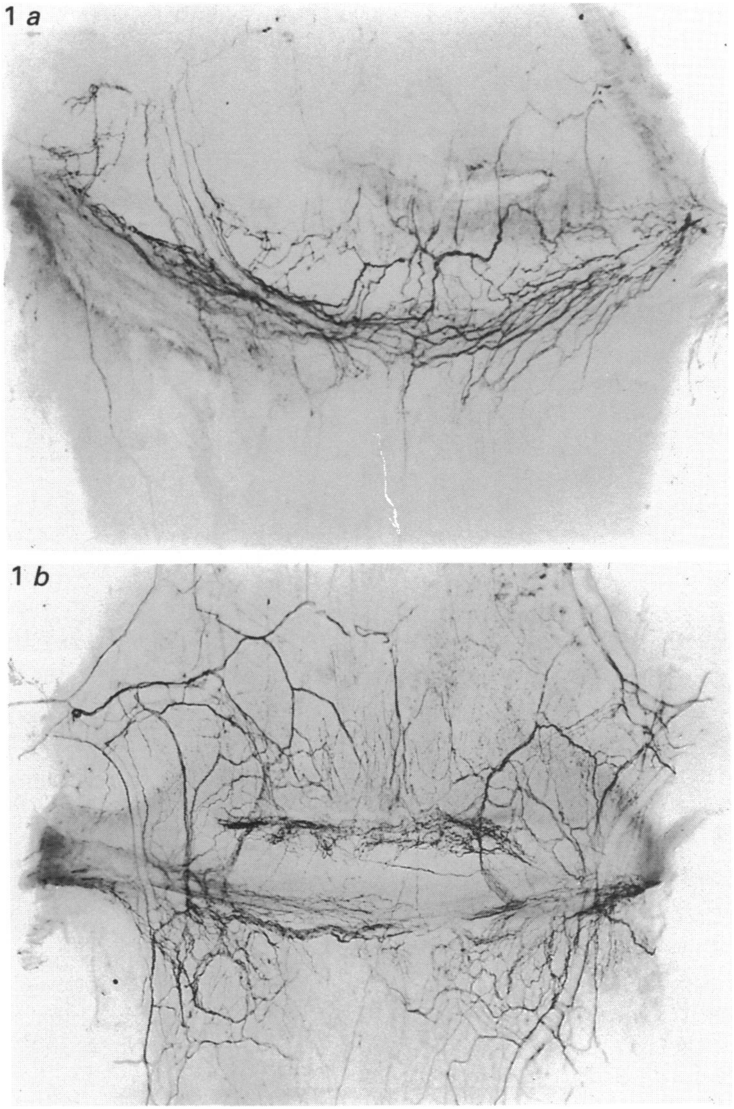


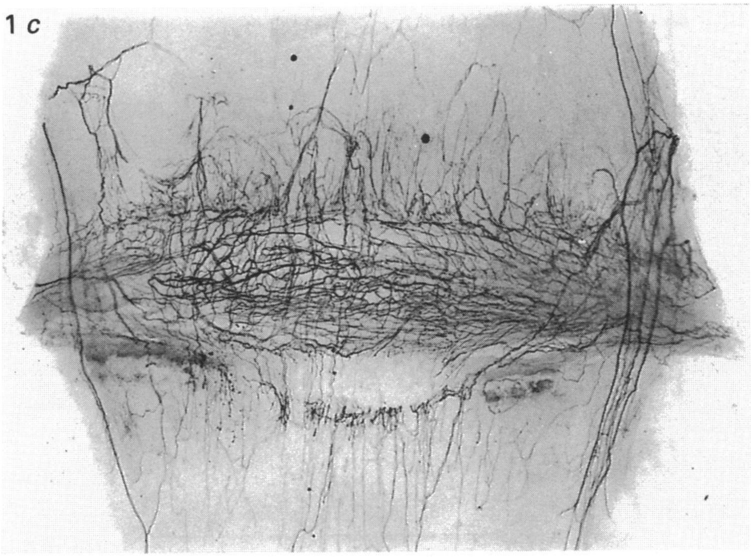
Fig. 1 (*a–d*). Acetylcholinesterase-positive nerve networks at different vertebral levels. (*a*) At the cervical level, both the superficial and deeper nerve networks show a low density of nerve fibres in comparison with that of the lumbar vertebrae. (*b*) At the middle thoracic level, the superficial nerve network becomes better developed, while the deeper nerve network is hardly seen. (*c*) At the lower thoracic and upper lumbar levels, both nerve networks are the most dense and have a wide area of distribution. (*d*) At the lower lumbar level, the density of the nerve fibres becomes less marked in both nerve networks. Whole-mount preparation. (*a*) × 40; (*b–d*) × 30.

RESULTS

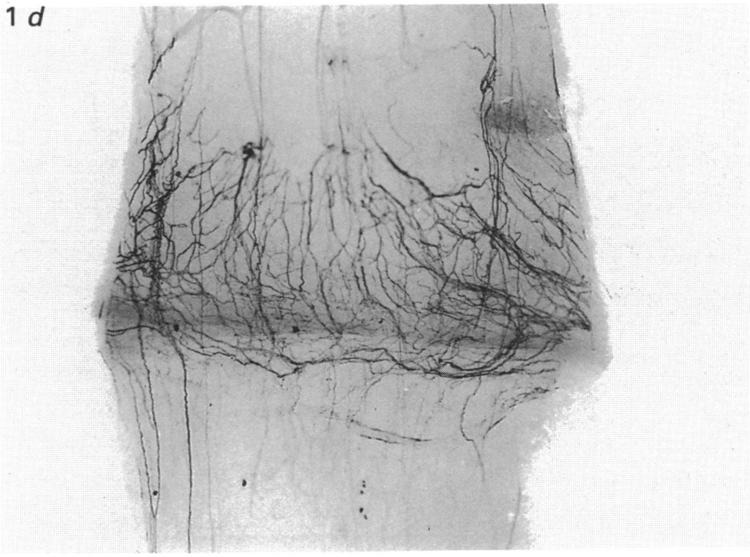
Distribution of the nerve fibres at different vertebral levels

As described in the previous report, two distinct nerve fibre networks could be recognised in the PLL and the IVD of the upper lumbar vertebrae: one is the superficial nerve network supplied by the meningeal branch of the spinal nerve (sinu-vertebral nerve), the other is the deeper nerve network supplied by nerve fibres coming

1 *c*



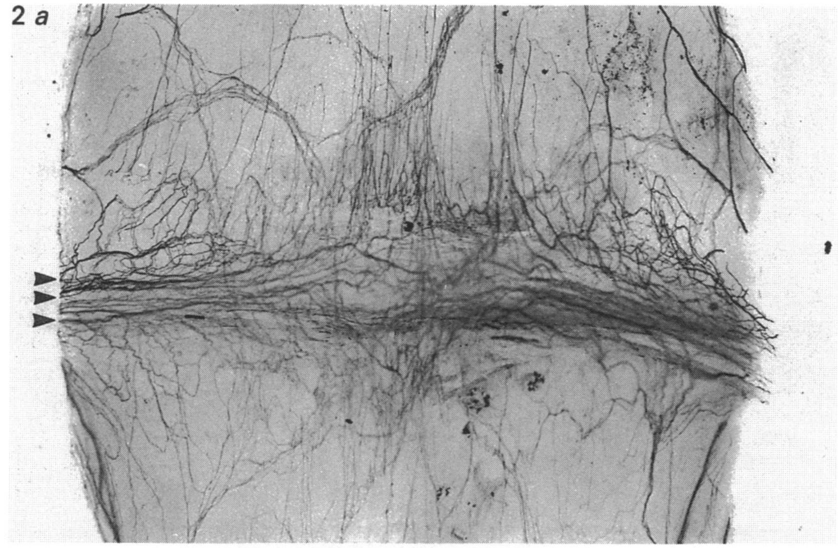
1 *d*



through the posterolateral portion of the annulus fibrosus (AF) apart from the meningeal branch. This is the basic pattern of the nerve supply but it is modified according to the vertebral level.

In the cervical region, both the superficial and deeper nerve networks showed a low density of nerve fibres in comparison with that of the lumbar vertebrae (Fig. 1 *a*). At the middle thoracic level, the superficial nerve network became better developed, while the deeper nerve network, which was observed at the cervical and lumbar levels, was hardly seen (Fig. 1 *b*).

At the lower thoracic and upper lumbar levels, both nerve networks were most developed in density as well as in the distribution area of the nerve fibres. The characteristic feature in this region was that the mesh of the nerve networks



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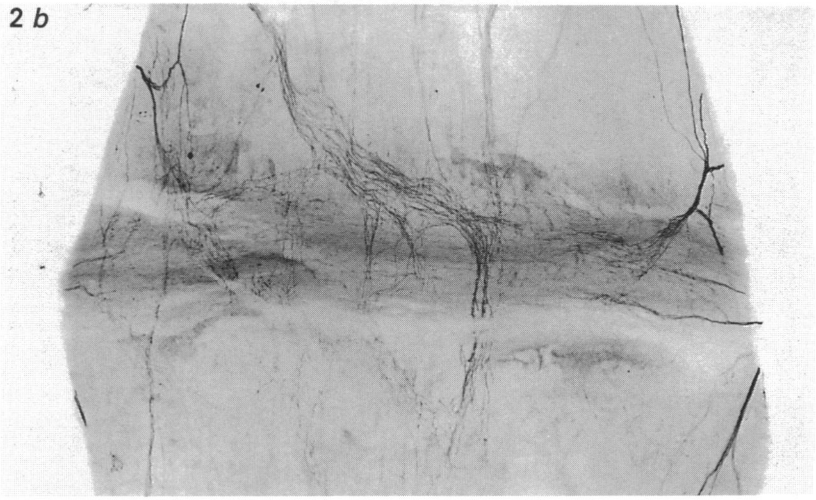

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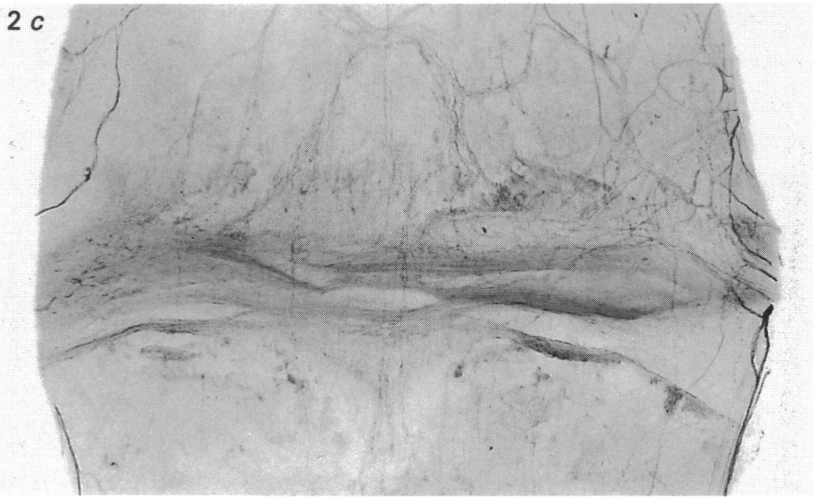

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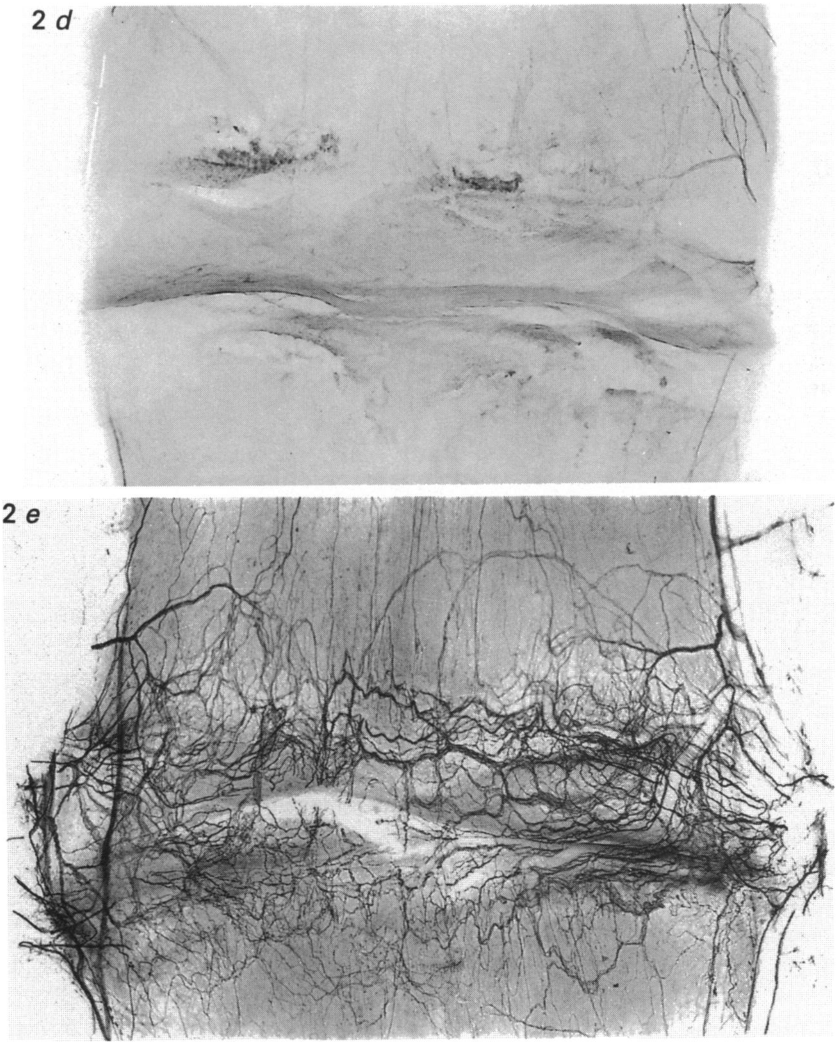

Fig. 2. (*a–e*). Results of resection experiments. (*a*) Unilateral ganglionectomy (Group 1). Bilateral diminution of the nerve fibres was found in the superficial nerve network, while a unilateral decrease was prominent in the ipsilateral deeper nerve network in all the operated segments. The arrowheads show the operated side. (*b–d*) Bilateral ganglionectomy (Group 2). The deeper nerve network disappeared almost completely in every operated segment. In the superficial nerve network, in the most cranially operated segment (*b*), the density of the nerve fibres decreased moderately. In the next segment (*c*), the number of nerve fibres decreased to a greater extent. In the most caudal segment (*d*), the nerve fibres disappeared almost completely. (*e*) Bilateral ventral rhizotomy (Group 3). The nerve fibres of both nerve networks remained intact. Whole-mount preparation. × 30.

innervating the intervertebral portion of the PLL was much denser cranial to the AF and less dense caudal to it (Fig. 1*c*).

At the lower lumbar level, the distribution pattern was similar to that in the upper lumbar region, but the density of the nerve fibres became lower in both nerve networks (Fig. 1*d*).

The origin of the AchE-positive nerve fibres in the PLL and the IVD

After resection of the spinal ganglia on one side (Group 1), the superficial and the deeper nerve networks decreased in nerve fibre density in different ways. Bilateral diminution of the nerve fibres was found in the superficial nerve network while a unilateral decrease was prominent in the ipsilateral deeper nerve network in all the operated segments (Fig. 2*a*).

After bilateral resection of the spinal ganglia (Group 2), the deeper nerve network disappeared almost completely in every operated segment and individual variations were negligible (Fig. 2*b–d*). On the other hand, in the superficial nerve network, the reduction of the nerve fibres varied considerably in intensity with the cranio-caudal level of the segment. In general, in the most cranially operated segment, the density of the nerve fibres moderately decreased (Fig. 2*b*). In the next segment, the number of nerve fibres decreased to a greater extent (Fig. 2*c*). In the most caudal segment, the nerve fibres almost disappeared, including those around the blood vessels in the marginal portion of the PLL (Fig. 2*d*). However, some individual variations were seen in the superficial nerve network. In two out of the ten cases, many nerve fibres still remained, even in the most caudal segment.

After bilateral transection of the ventral nerve roots (Group 3), the nerve fibres of both nerve networks remained intact (Fig. 2*e*).

DISCUSSION

The distribution of nerve fibres in the PLL and the IVD showed regional differences in accordance with the vertebral levels. This was particularly noticeable in the deeper nerve network. The density of nerve fibres in the deeper nerve network was very well-developed from the lower thoracic to the middle lumbar level and it was relatively low at the cervical and the lower lumbar levels. Moreover, at the mid-thoracic level, this nerve network was hardly seen. In a report about the biomechanical properties of the human PLL, the PLL of the lumbar vertebra showed the maximum extensibility to a stretching force but that of the thoracic vertebra was very low (Maeno *et al.* 1985). This result indicates a close relationship between the regional differences of the distribution of the nerve fibres in the PLL and those of its biomechanical properties. Namely, the dense deeper nerve network was seen at the lumbar level where the PLL showed marked extensibility. On the other hand, this nerve network was hardly seen at the middle thoracic level where the PLL showed a very low extensibility. It is further suggested on these grounds that this deeper nerve network may receive information about the extension of the PLL, transmit it to the central nervous system and consequently regulate the movement and posture of the vertebral column.

The present study revealed that the AchE-positive nerve fibres in the PLL and the IVD mostly originated from the spinal ganglia. The function of the nerve fibres distributed in the PLL and the IVD has been interpreted in the light of the morphological features of the nerve terminals or by the relationship with the tissues around the nerve fibres. It is considered that the unmyelinated nerve fibres of small calibre around the blood vessels are postganglionic sympathetic nerve fibres and have a vasomotor function and that the unmyelinated free nerve endings far from the blood vessels are associated with pain or thermal perception. The complex encapsulated or unencapsulated nerve endings of large diameter are associated with proprioception (Bogduk, Tynan & Wilson, 1981; Bogduk, 1983; Hirsch, Ingelmark & Miller, 1963; Malinsky, 1959). However, it is unreasonable to consider that all the nerve fibres distant from the blood vessels are sensory only by considering the morphological features of the nerve terminals. Furthermore, the present study showed only a few nerve terminals in the PLL and the IVD in contrast to the abundance of nerve fibres. Recently, a tracer study, using horseradish peroxidase (HRP) injected into the IVD, revealed that sensory nerve fibres exist in this region (Forsythe & Ghoshal, 1984). However, this result did not show how many sensory nerve fibres were included within the abundant nerve network in this region. Our experimental studies clarified this question and proved that the abundant AchE-positive nerve fibres in this region were almost derived from the spinal ganglia.

The nerve fibres around the blood vessels are considered to be postganglionic sympathetic nerve fibres as mentioned above. We have also recognised aminergic nerve fibres around blood vessels in the marginal portion of the PLL by fluorescent histochemistry (Kojima, unpublished data). However, the AchE-positive nerve fibres around the blood vessels also disappeared after the resection of the spinal ganglia. Thus, these AchE-positive nerve fibres are neither cholinergic nor sympathetic, unlike those around the cerebral blood vessels (Iwayama, Furness & Burnstock, 1970; Itakura *et al.* 1984), but they may play a role in vasosensation or in vasomotor function through the axon reflex. It is of great interest that not only the aminergic sympathetic nervous system but also the spinal ganglion-derived nervous system may be related in the regulation of the circulation in this region.

The present study also revealed that there was a slight difference in the pattern of the nerve supply in the superficial and the deeper nerve networks. The superficial nerve network was supplied by bilateral spinal ganglia which innervate both sides of not only the corresponding segment but also the adjacent upper and lower segments (mainly the lower two segments). On the other hand, the deeper nerve network was supplied only by the bilateral spinal ganglia of the corresponding segment (but predominantly by the unilateral ganglion) and not by the adjacent upper and lower spinal ganglia. In fact, the observation that these two nerve networks are derived through separate routes has already been made in our preceding paper (Kojima, Maeda, Arai & Shichikawa, 1990). Furthermore, this observation may reasonably explain the fact that these two nerve networks have different distribution patterns at different vertebral levels as found in the present study. Many reports suggest that the PLL and the IVD of humans are supplied bilaterally and polysegmentally by the spinal ganglia. For example, communication with the nerve fibres of the opposite side (Edgar & Nundy, 1966; Edgar & Ghadially, 1976; Kimmel, 1961; Lamb, 1979; Pedersen, Blunck & Gardner, 1956; Wiberg, 1949) and the nerve supply to the adjacent upper and lower segment by ascending and descending branches (Bogduk *et al.* 1981; Bodguk, 1983; Bridge, 1959; Kimmel, 1961; Lamb, 1979; Pedersen *et al.* 1956; Shimizu, 1978; Spurling & Bradford, 1939; Spurling & Grantham, 1940; Wiberg, 1949) have already been reported. Bilateral and polysegmental nerve supply were further confirmed by recent experimental results showing that HRP injected into the AF in the dog can be demonstrated in the spinal ganglia of both sides bisegmentally, cranial as well as caudal to the injected site, by retrograde axonal transport (Forsythe *et al.* 1984). However, it should be noted that individual variations are frequently seen in the polysegmental nerve supply as pointed out in the present study as well as in many other reports. The reason why many nerve fibres of the superficial nerve network remain, even in the most caudal segment, in two cases after resection of the bilateral spinal ganglia (Group 2) might be due to nerve fibres running upward from the lower spinal ganglia. The bilateral and polysegmental nerve supply to the PLL and the IVD may throw light on the pathogenesis of low back pain, originating from the IVD, that is not localised clearly but has a wide distribution, as discussed for many years (Edgar & Nundy, 1966; Edgar & Ghadially, 1976; Forsythe & Ghoshal, 1984; Hirsch *et al.* 1963).

Finally, the role of AchE in the sensory nervous system still remains unclear, since the peripheral sensory neurons may not function by using acetylcholine as a neurotransmitter. In this context, it is of great interest that this enzyme is related to the hydrolysis of certain neuropeptides such as substance P (Chubb, Hodgson & White, 1980; Lockridge, 1982; Chubb, Ranieri, White & Hodgson, 1983). Further investigation will be needed in the future.

SUMMARY

Regional differences of the distribution and the origin of the nerve fibres of the posterior longitudinal ligament (PLL) and the intervertebral disc (IVD) were investigated by acetylcholinesterase (AchE) enzyme histochemistry in the rat. The deeper nerve network was well-developed at the lower thoracic and the upper lumbar vertebral levels, but it was hardly seen at the mid-thoracic level. This difference can be correlated with the extensibility of the PLL. The experimental study revealed that the AchE-positive nerve fibres in the PLL and the IVD were mostly derived from the spinal ganglia, that the superficial nerve network was supplied by the spinal ganglia bilaterally and polysegmentally, and that the deeper nerve network was supplied bilaterally but unisegmentally.

REFERENCES

BOGDUK, N., TYNAN, W. & WILSON, A. S. (1981). The nerve supply to the human lumbar intervertebral discs. *Journal of Anatomy* **132**, 39–56.

BOGDUK, N. (1983). The innervation of the spine. *Spine* **8**, 286–293.

BRIDGE, C. J. (1959). Innervation of spinal meninges and epidural structures. *Anatomical Record* **133**, 553–563.

CHUBB, I. W., HODGSON, A. J. & WHITE, G. H. (1980). Acetylcholinesterase hydrolyzes substance P. *Neuroscience* **5**, 2065–2072.

CHUBB, I. W., RANIERI, E., WHITE, G. H. & HODGSON, A. J. (1983). The enkephalins are amongst the peptides hydrolyzed by purified acetylcholinesterase. *Neuroscience* **10**, 1369–1377.

EDGAR, M. A. & NUNDY, S. (1966). Innervation of the spinal dura mater. *Journal of Neurology, Neurosurgery and Psychiatry* **29**, 530–534.

EDGAR, M. A. & GHADIALLY, J. A. (1976). Innervation of the lumbar spine. *Clinical Orthopaedics and Related Research* **115**, 35–41.

FORSYTHE, W. B. & GHOSHAL, N. G. (1984). Innervation of the canine thoracolumbar vertebral column. *Anatomical Record* **208**, 57–63.

HIRSCH, C., INGELMARK, B. & MILLER, M. (1963). The anatomical basis for low back pain. *Acta orthopaedica scandinavica* **33**, 1–17.

ITAKURA, T., NAKAKITA, K., KAMEI, I., NAKA, Y., NAKAI, K., KOMAI, N., IMAI, H., KIMURA, H. & MAEDA, T. (1984). Morphological study on innervation of the cerebrospinal blood vessels. *Neurological Surgery* **12**, 282–288.

IWAYAMA, T., FURNESS, J. B. & BURNSTOCK, G. (1970). Dual adrenergic and cholinergic innervation of the cerebral arteries of the rat. *Circulation Research* **26**, 635–646.

KIMMEL, D. L. (1961). Innervation of spinal dura mater and dura mater of the posterior cranial fossa. *Neurology* **11**, 800–809.

KOJIMA, Y., MAEDA, T., ARAI, R. & SHICHIKAWA, K. (1990). Nerve supply to the posterior longitudinal ligament and the intervertebral disc of the rat vertebral column as studied by acetylcholinesterase histochemistry. I. Distribution in the lumbar region. *Journal of Anatomy* **169**, 237–246.

LAMB, D. W. (1979). The neurology of spinal pain. *Physical Therapy* **8**, 971–973.

LOCKRIDGE, O. (1982). Substance P hydrolysis by human serum cholinesterase. *Journal of Neurochemistry* **39**, 106–110.

MAENO, M., MAKIKAWA, M., NISHIOKA, J., FUKUDA, S. & SHICHIKAWA, K. (1985). Difference of biomechanical characteristics between the enthesis and the other parts of the spinal posterior longitudinal ligaments. *Central Japan Journal of Orthopaedic and Traumatic Surgery* **28**, 1915–1917.

MALINSKY, J. (1959). The ontogenic development of nerve terminations in the intervertebral discs of man. *Acta anatomica* **38**, 96–113.

PEDERSEN, H. E., BLUNCK, C. F. J. & GARDNER, E. (1956). The anatomy of lumbosacral posterior rami and meningeal branches of spinal nerves (sinu-vertebral nerves). *Journal of Bone and Joint Surgery* **38 A**, 377–391.

SHIMIZU, T. (1978). Nerve distribution in human lumbar spine. *Acta medica* **48**, 523–552.

SPURLING, R. G. & BRADFORD, F. K. (1939). Neurologic aspects of herniated nucleus pulposus. *Journal of the American Medical Association* **113**, 2019–2022.

SPURLING, R. G. & GRANTHAM, E. G. (1940). Neurologic picture of herniations of the nucleus pulposus in the lower part of the lumbar region. *Archives of Surgery* **40**, 375–388.

TAGO, H., KIMURA, H. & MAEDA, T. (1986). Visualization of detailed acetylcholinesterase fiber and neuron staining in rat brain by a sensitive histochemical procedure. *Journal of Histochemistry and Cytochemistry* **34**, 1431–1438.

WIBERG, G. (1949). Back pain in relation to the nerve supply of the intervertebral disc. *Acta orthopaedica scandinavica* **19**, 211–221.