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Longitudinal projections of primary afferents from the single dorsal root ganglion of the cervical or lumbosacral enlargements in chickens

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HIGHLIGHTS

- Primary afferents of a single dorsal root ganglion are studied in the chicken.
- Central projections from the cervical or lumbosacral enlargement are described.
- There is little overlap in central projections from both the enlargements.
- Long ascending fibers project to the rostral lamina III in addition to the medulla.

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ABSTRACT

Central projections originated from a single dorsal root ganglion (DRG) were studied in the chicken focusing on the rostrocaudal extension of primary afferents in each lamina by using anterograde labeling by lectin-HRP injection into either the 15th or the 24th DRG. In the injection into the 15th DRG, labeled fibers (LFs) were found in a wide rostrocaudal range of laminas IV (the spinal segment (SS) 1–20) and V (SS 4–18) and in a narrow range of other laminas. In the injection into the 24th DRG, LFs were distributed in a similar rostrocaudal range in all laminas except for laminas VIII and IX. LFs in laminas VIII and IX were restricted in the tracer injected segment. LFs in the lateral funiculus derived from both the enlargements projected into the rostral lamina III in addition to the lower medulla oblongata. There was little overlap in the extent of the primary terminal areas from both the enlargements.

1. Introduction

In the mammalian spinal cord, the process of transganglionic tracer transport has mainly been used to identify the central projections of primary afferents following peripheral injection of a tracer. Since anterograde labeling methods after injection into a single dorsal root ganglion (DRG) are relatively limited, only a few studies have examined the longitudinal extension of the primary terminal area of a single DRG origin.

Little is known about the central projections of primary afferents in the avian spinal cord, and most previous studies have employed classic transganglionic transport of tracers. Few studies have investigated the anatomical organization of primary afferents of a single DRG including embryonic studies [3,4] and a study using the degeneration method [10].

The gray matter of the chicken spinal cord has layers. The head of the dorsal horn consists of laminas I–IV. In mammals and pigeons, laminas I–IV piles up dorso-ventrally in order. The chicken dorsal horn head is unique with lamina II in a lateral position and lamina III in a medial position. Laminas II and III contact each other dorsally and are ventrally separated by lamina IV [2,12,24].

The aim of the present study was to elucidate (1) the central projections of primary afferents in each lamina, especially in the dorsal horn which is unique in lamination and (2) the longitudinal extension of the primary terminal area from a single DRG of the 15th or 24th spinal segment in the cervical and lumbosacral enlargements in the chicken spinal cord.

2. Materials and methods

2.1. Chickens

Eleven laying chickens (all male aged 45–60 days after hatching) (Ghen Corporation, Gifu, Japan) were used in this study. All the chickens provided with commercial food and water was provided

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ad libitum. The Institutional Animal Care and Use Committee of Tottori University, Tottori, Japan, approved experimental procedures.

2.2. Experimental design

The cervical or lumbosacral enlargement was exposed by laminectomy after deep anesthesia with xylazine (8 mg/kg), midazolam (2 mg/kg) and ketamine (25 mg/kg). Injections (0.05 μl) of 5% horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP; Toyobo, Oosaka, Japan) dissolved in distilled water was injected into the 15th DRG in 4 chickens, the 24th DRG of 4 chickens and the 25th DRG of one chicken via a glass micropipette cemented to a 1 μl Hamilton syringe. 0.05 μl of 3% cholera toxin B-HRP (B-HRP, Toyobo, Osaka, Japan) dissolved in distilled water was injected into the 15th or 24th DRG in each chicken. The DRG injections were all unilateral (right). After 2 days, the chickens were re-anesthetized deeply and perfused through the left ventricle with cold 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (PB). Transverse frozen sections (100 μm) were serially cut through the spinal segments (SS) 1, 2, 4, 6, 8, 10, 12, 13, 14, 15, 16, 18, 20, and 22 and the medulla oblongata in the chicken receiving DRG injection into the cervical enlargement or the SS 1, 2, 4, 8, 12, 16, 18, 20, 22, 23, 24, 25, 26, 28, and 30 and the medulla oblongata in the chicken receiving DRG injection into the lumbosacral enlargement.

2.3. Histochemical procedure

Transverse sections 100 μm thick were processed histochemically for HRP by using 3,3′,5,5′-tetramethybenzidine as the chromogen [14]. The transverse sections of the medulla were counterstained with thionin. The distribution of labeled fibers (LFs) was plotted using a drawing tube. The locations of LFs were described with reference to the descriptions of the avian medulla oblongata [8,24] and of the cytoarchitectonic lamination of the gray matter of the chicken spinal cord [2,12,25]. The chicken spinal gray matter consists of 10 laminas. However, lamina VI is found only in the cervical and lumbosacral enlargements, SS 13–16 and SS 21–28, respectively.

3. Results

3.1. General observations

Although the density of LFs differed from case to case, the exact patterns of labeling were similar in all cases investigated. Therefore, this description focuses mainly on the heavily labeled cases giving consideration to all cases. Ventral roots labeled by diffusion of the tracer were observed in 4 of 6 chickens in the 15th DRG injection group and in 2 of 5 chickens in the 24th DRG injection group. However, observations of the primary afferent fibers were not disturbed, since labeled motoneurons were a few, restricted to the ventral horn at the level corresponding to the injected DRG and were morphologically distinguishable from the labeled afferent fibers. Furthermore, there was no labeling in any dorsal and ventral root except for the injected segment.

LFs appeared only on the injected side of the spinal cord except for the most rostral segments (SS 1 and 2) and the medulla oblongata in which LFs were bilaterally observed in all cases including the B-HRP injection cases. In cross section, dorsal roots entering the cord bifurcated into the medial and lateral bundles (M- and L-bundles, respectively) to the dorsal horn. The M-bundle emitted fibers that entered the dorsal horn from the dorsal funiculus. The L-bundle divided into the main and minor branches. The main branches entered the Lissauer's tract to ascend or descend and gave rise to collaterals into the dorsal horn running along the ventral border. The minor branches entered directly into the dorsal part of lamina II. There was no difference in the longitudinal extension of the primary terminal area between the major and minor branches. Since lamina I was a very thin layer covering the dorsal horn, we could not distinguish terminals in lamina I from those in laminas II and III.

In primary afferent projections from spinal nerves in rats, WGA-HRP and B-HRP labeled different subpopulations of fibers, with the B-HRP-labeled subpopulation biased toward afferents of large diameter [9,13]. In birds, dorsal root fibers divide into a coarse-fibred medial and a thin-fibred lateral bundle just after entering into the spinal cord [18]. L- and M-bundles were labeled in the same fashion by WGA-HRP and B-HRP, respectively. Furthermore, LFs by both tracers were similar in distribution in each lamina of the dorsal horn.

3.2. The 15th DRG projections to the spinal cord and the medulla oblongata (Fig. 1)

(1) SS 15 corresponding to the injected DRG showed the heaviest labeling through the spinal cord. LFs of the main branch of the L-bundle were found in the lateral part of lamina II and observed running into the adjacent area of laminas IV and V. LFs of the minor branch entered lamina II and subsequently lamina IV. The M-bundle entered laminas III and V densely in all cases and faintly in lamina IV. In heavily labeled cases, LFs radiated from lamina V to laminas VI–IX.

(2) SS 14 and 16: Although label density was lower than that at the injected level (SS 15), these SS were also heavily labeled and the distribution pattern of LFs was also similar to that of SS 15. LFs increased in number in the dorsal funiculus, especially in its ventral part, and in lamina V in comparison with SS 15.

(3) SS 12 and 13: LFs were primarily found in laminas II, IV, and V in SS 12 and in laminas II and IV–VI in SS 13 (lamina VI was not found in SS 12 or in more rostral SS). LFs from the major branch of the L-bundle in lamina II decreased in density and were mainly located in the lateral borders of lamina II. The minor branch of the L-bundle had few or lightly LFs. LFs from the M-bundle in laminas IV and V increased or were similar in density to those in SS 14. Lamina VII was free of LFs except for the narrow adjacent area of lamina V (SS 12) or lamina VI (SS 13).

(4) SS 1, 2, 4, 6, 8 and 10: LFs were concentrated in the medial part of lamina IV through these SS and found in the medial part of lamina V in SS 6–10. Although LFs in lamina III disappeared in SS 6–12, these fibers reappeared in medial lamina III in SS 1–4. In SS 1 and 2, a few LFs also appeared in the contralateral dorsal funiculus. LFs in the dorsal funiculus were ventrally located in SS 4–10 as well as dorsally in SS 1 and 2 along the midline.

(5) SS 18 and 20: Labeling density decreased prominently in SS 18 and was low or nonexistent in SS 20. LFs were found in laminas II–V and VII in SS 18, and laminas II and IV in SS 20. LFs from the main branch of the L-bundle appeared to decrease in number. LFs from the minor branch entered the lateral border of lamina II. LFs from the M-bundle entered lamina IV and then ran ventrolaterally into lamina V. In the dorsal funiculus, LFs were located in its ventral part.

(6) Medulla oblongata: LFs were observed bilaterally but more prominently in the ipsilateral side. LFs were concentrated in the dorsal funicular nucleus in the lower medulla and additionally in the solitary nucleus and the external cuneate nucleus in the more rostral medulla. LFs in the medulla were markedly more abundant than those in the dorsal funiculi of the upper cervical cord.