Location of Parasympathetic Preganglionic Neurons in the Sacral Spinal Cord of the Domestic Fowl

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The pudendal nerve in the domestic fowl arises from the pudendal plexus near the caudomedial margin of the kidney, runs down along the ureter and extends to the cloacal region. This nerve is said to contain parasympathetic preganglionic fibers. Therefore, it is presumed that the pudendal nerve controls both the excretory function such as defecation and micturition and the reproductive function such as copulation, ejaculation and oviposition. Thus, in the present study horseradish peroxidase (HRP) was applied to the pudendal nerve in order to determine the distribution of parasympathetic preganglionic neurons in the sacral spinal cord of the domestic fowl, *Gallus domesticus* L.

Materials and Methods

Thirteen fowls (Brown Leghorn) of both sexes weighing an average of 1.54 kg were used. Under sodium pentobarbiturate anesthesia (20 mg/kg), either pudendal plexus at the caudomedial margin of the kidney or pudendal nerve near by the urodeum along the ureter was transected unilaterally. The central stump of the transected nerve was sucked into a short polyethylene tube. The tubing was then filled with 25% w/v saline solution of HRP (Boehringer Grad I) and sealed at both ends with silicone grease.

After survival time of two days the fowls were anesthetized and perfused through the left common carotid artery with physiological saline, followed by a double aldehyde mixture, at a pressure of 100–120 mmHg. The fixative contained 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Finally the bird was perfused with 10% sucrose in phosphate buffer at 4°C. The spinal cord was then dissected out and immersed in phosphate buffered 30% sucrose until sectioning.
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The spinal cord was cut on a cryostat at 50 µm in a transverse or horizontal plane. All sections were processed with the tetramethylbenzidine as chromogen according to the method of MESULAM. They were mounted on glass slides coated with chrome alum-gelatine and counterstained with 1% neutral red solution.

Results and Discussion

It was easy to find HRP-labeled cells filled with a black granular reaction product. Labeled cells were ipsilaterally found in the operation side. In application of HRP to the pudendal plexus, labeled neurons were observed in two areas of the sacral spinal cord; one was located in the most ventrolateral part of the ventral horn and the other was situated in the intermediomedial region of the gray matter. Applying HRP to the pudendal nerve, HRP-labeled neurons were only seen in the intermediomedial region. Since it is well known that somatomotor neurons to muscles of the limb are localized in the ventrolateral part of the ventral horn, it was concluded that labeled cells in the intermediomedial region were parasympathetic preganglionic neurons sending axons into the pudendal nerve.

In transverse sections a tight cluster of labeled neurons were most frequently observed in the intermediomedial region dorsolateral to the central canal (Fig. 1, A). These cells were generally oval to trigonal in shape. Just a few labeled cells were detected in both intercalated and intermediolateral regions and along the lateral border of the dorsal horn. In horizontal sections labeled neurons were found to form

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Fig. 1. Parasympathetic preganglionic neurons labeled by the retrograde transport of HRP in the pudendal nerve. A: Transverse section through the spinal segment 31. Labeled cells are located in the intermediomedial region dorsolateral to the central canal. B: Horizontal section through the spinal segment 31. Labeled cells form a cell column along the midline. Calibrations 100 µm.
a cell column along the midline (Fig. 1, B). Its length was approximately 10 mm, extending from spinal segment 30 to 33. The cells had fusiform or polygonal profiles, and their major dendrites were often longitudinally arranged. The number of labeled preganglionic neurons varied in different fowls from 800 to 1,500.

Only a few labeled axons could be occasionally observed running obliquely from the intermediomedial region through the gray matter to the ventral horn where they joined the axons of the somatomotor neurons, and presumably leaving the spinal cord through the ventral roots. However, their direct connection with the perikarya of the preganglionic neurons could not be noted.

Previous HRP studies concerning the location of parasympathetic preganglionic neurons in the mammalian sacral spinal cord have consistently shown that these cells have the appearance of an inverted "L" in the intermediolateral region, but not in the intermediomedial region dorsolateral to the central canal5-9). On the contrary, the present study indicates that preganglionic neurons of the pudendal nerve in the domestic fowl lie in the intermediomedial region of the spinal segments 30-33. A distinct difference between mammals and birds with regard to the location of parasympathetic preganglionic neurons in the sacral spinal cord can be observed. The reason why there is such a difference between both species has remained unsolved. The intermediomedial location of preganglionic neurons in the domestic fowl is probably characteristic of autonomic preganglionic cells in birds. Because sympathetic preganglionic neurons in the pigeon have been shown to form an intermediomedial cell column (column of TERNI) dorsolateral to the central canal10).

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References