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Research report

Innervation of the rat trachea by bilateral cholinergic projections from the nucleus ambiguus and direct motor fibers from the cervical spinal cord: a retrograde and anterograde tracer study

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Abstract

A tract–tracer method was employed to examine the innervation of the rat trachea. Cholera toxin β subunit (CTB) was injected into the following locations in separate groups of rats: (1) ventral trachea, (2) lateral trachea, (3) ventral trachea after the excision of the nodose ganglion, and (4) ventral trachea after the transection of C1–C2 spinal nerves. CTB injection in the ventral trachea showed bilateral labeling of neurons in the nucleus ambiguus (NA), medial subnucleus of the nucleus of the solitary nucleus, dorsal motor nucleus of the vagus (DMV), and lamina IX of C1–C6. CTB injection in the lateral trachea showed significant ipsilateral predominance of neuronal labeling in the NA and lamina IX of C1–C2 segments. CTB injection in rats after the excision of the nodose ganglion revealed no labeling in the ipsilateral DMV and NA and a significant reduction of neuronal labeling in C1. CTB injection in rats after the transection of C1–C2 spinal nerves showed a significant decrease in the number of labeled neurons in ipsilateral NA, C1, and C2 and no labeling of fibers in C1–C2. The combination of retrograde fluorogold labeling and choline acetyltransferase (ChAT) immunostaining revealed that all fluorogold-labeled neurons in the NA and lamina IX of C1–C2 colocalized with ChAT. The injection of biotinylated dextran amine in NA produced labeling in axonal terminals on postganglionic neurons, but not in other regions of the trachea. Our findings indicate that the rat trachea is innervated bilaterally by cholinergic motor neurons in NA and C1–C2, while those traveling through the spinal nerves project directly to the trachea.

Theme: Motor systems and sensorimotor integration
Topic: Spinal cord and brainstem

Keywords: Airway; Nerve transection; Sensory innervation; Cholera toxin; Dextran amine

1. Introduction

Autonomic innervation of the airway includes the parasympathetic and sympathetic nervous systems, which function to control smooth muscle tension, secretion of mucosal glands, vascular tone, and tracheobronchial reflexes [3,7]. The preganglionic parasympathetic neurons innervating the trachea originate from the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus (NA) [5,8]. Postganglionic parasympathetic neurons are located in the peritracheal ganglion or submucosal plexus [1,9,11,14]. Sensory fibers that are distributed in the tracheal epithelium project to the nucleus of the solitary tract (NTS) through neurons located in the nodose or jugular ganglion.

The innervation pattern of vagal motor neurons to the lung shows species differences: NA in the cat innervates the lung contralaterally [8], whereas in the rat, it innervates the lung bilaterally [12]. The contralateral pathway in bilateral

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innervation undergoes decussation inside the thorax in the rat [12]. In the trachea, however, the issue of whether the trachea is innervated bilaterally or contralaterally remains to be resolved, although both DMV and NA participate in tracheal innervation [5].

A tract-tracing study using cholera toxin β subunit (CTB) showed that neurons in the dorsomedial part of the ventral horn in C1 and C2 participate in the innervation of the trachea in the dog, ferret, and rat, in addition to NA and DMV [5]. Furthermore, the authors found CTB-labeled fibers in the ventral funiculus of the upper cervical segments in the dog and in the cervical dorsal horn in the ferret. The study suggested that the upper cervical spinal cord participates partially in the control of the trachea.

Sensory or parasympathetic nerve fibers are distributed in the epithelium or lamina submucosa of the trachea of the ferret and rat [4,9,13]. Pérez-Fontán and Velloff [11] reported that the injection of CTB in the tracheal lumen resulted in labeling neurons in NA and DMV by trans-epithelial transport to intra- or subepithelial space, where labeled vagal motor neurons project their axons in the rat. The authors hypothesized that vagal motor neurons in DMV and NA project their axons directly to the epithelium or submucosa without interposition of intrinsic neurons, which are located in the peritracheal parasympathetic ganglion. This hypothesis is contradictory to the classic concept of the airway parasympathetic transmission; preganglionic vagal neurons synapse on postganglionic neurons in the local ganglion, and, in turn, the latter exert their effects on smooth muscles, glandular cells, and epithelial cells [7,10]. However, their experiment was carried out under the presence of the classic parasympathetic rely [11]. Therefore, the hypothesis of a direct vagal motor innervation to the trachea remains to be confirmed.

The aims of the present study were to determine whether NA and DMV innervate trachea bilaterally and whether the cervical motor neurons directly innervate the trachea. Experiments involved tract tracing using CTB and denervation of the vagus nerve and spinal nerves. In the latter experiment, we chose the cervical spinal cord, where autonomic innervation of both sympathetic and parasympathetic nerves is absent [2]. CTB was injected in the tracheal wall because previous studies reported that retrograde-labeled neurons were more numerous in NA and DMV than those obtained by injection into the tracheal lumen [5,11].

2. Materials and methods

A total of 52 Wistar rats (weight 250–300 g, 10–16 weeks, of both sexes) was used in the present study. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Faculty of Agriculture, Gifu University.

2.1. Injection of retrograde tracers in the trachea

Experiments consisted of five groups to clarify bilateral innervation of the medulla to the trachea and innervation of motor neurons in the cervical spinal cord to the trachea. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg body weight). Under sterile conditions, the cervical trachea was exposed. In rats of group 1 (n=5), 0.5% CTB (low salt, List Biological Laboratories, USA) in phosphate-buffered saline (PBS) was injected in the ventral midline of the trachea in the 5th to 14th intercartilagenous spaces, using a glass micropipette (a total of 10 injections of 400 nl each, total volume=4 μl). In rats of group 2 (n=5), the injection protocol was similar to that used for group 1, except that the injections were made in the left or right side of the trachea. In rats of group 3 (n=5), the left or right nodose ganglion was excised, followed 7–10 days later by tracheal injections under anesthesia of the same toxin solution used for group 1, at the same dose and in the same anatomical site. In rats of group 4 (n=5), 2 mm of the right C1 and C2 spinal nerves, just lateral to the intervertebrate foramen, was cut, followed 7–10 days later by tracheal injections under anesthesia of the same toxin solution used for group 1, at the same dose and in the same anatomical site. In rats of group 5, 0.5% fluorogold (Biotum, USA) in distilled water was injected in the ventral midline of the trachea in the 5th to 14th intercartilagenous spaces using a glass micropipette (a total of 10 injections of 400 nl each, total volume=4 μl) to detect colocalization with choline acetyltransferase (ChAT), similar to group 1.

Three controls of injections were done to examine whether tracers injected in the trachea were leaked into neighboring tissues, i.e., the m. sternohyoideus, which covers the ventral surface of the trachea. In rats of control 1 (n=3), after the m. sternohyoideus of both sides were removed between near origin and insertion, CTB was injected in the ventral midline of the trachea, in the 5th to 14th intercartilagenous spaces (a total of 10 injections of 400 nl each, total volume=4 μl). In rats of control 2 (n=3), CTB was injected in the m. sternohyoideus of both sides (each side received a total of 10 injections of 400 nl each, total volume=4 μl). In rats of control 3 (n=3), fluorogold was injected in the ventral midline of the trachea in the 5th to 14th intercartilagenous spaces (a total of 10 injections of 400 nl each, total volume=4 μl), while CTB was injected in the m. sternohyoideus of both sides (each side received a total of 10 injections of 400 nl each, total volume=4 μl).

On the 4th day after the injection of CTB or fluorogold, all rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal injection), perfused with Ringer's solution, followed by 4% formaldehyde in 0.1 M phosphate buffer at pH 7.4. The brainstem and cervical spinal cord were dissected out and postfixed in the same fixative for 2–3 days. Specimens were transferred to 30% sucrose in PBS at