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Functional and morphological organization of the nucleus tractus solitarius in the fictive cough reflex of guinea pigs

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Abstract

Projection of the superior laryngeal nerve (SLN) afferent fibers into the nucleus tractus solitarius (NTS) was investigated using a fluorescent tracer in guinea pigs. High density of fluorescence was detected in the ipsilateral NTS extending from 0.5 mm caudal to 1.2 mm rostral to the obex. At coronal slices, the fluorescent granules, lines and patches were located in the interstitial, medial and dorsal regions of NTS. Fluorescence was also found in the dorsal region of contralateral commissural NTS. Microstimulation of the rostral NTS, which corresponded to the region showing the strong fluorescence, induced an increase in the inspiratory discharge of phrenic nerve that was immediately followed by a large burst discharge of the iliohypogastric nerve in decerebrate, paralyzed and artificially ventilated guinea pigs. This serial response of the two nerves was identical to that induced by electrical stimulation of the SLN. Intravenous injection of codeine suppressed both NTS and SLN-induced responses. The SLN-induced response was inhibited by microinjection of codeine into the ipsilateral NTS and abolished by lesion of the ipsilateral NTS. These results suggest that the NTS has an integrative function in production of cough reflex and is possible sites of action of central antitussive agents.

Keywords: Cough reflex; Superior laryngeal nerve; Microstimulation of the nucleus tractus solitarius; Nerve recording; Fluorescent tracing; Codeine

1. Introduction

A fictive cough model of the cat has been usually employed to investigate the neuronal mechanisms underlying cough reflex (Tomori and Widdicombe, 1969; Leith et al., 1986; Bolser, 1991; Oku et al., 1994; Grélot and Bianchi, 1997; Shannon et al., 1997, 1998, 2000). In the cough reflex pathway, the nucleus tractus solitarius (NTS) is though to be the first relay nucleus for the tussigenic afferent information from the upper airway (Widdicombe, 1998; Sant'Ambrogio and Widdicombe, 2001), since the histological studies have revealed that the afferent fibers of superior laryngeal nerve (SLN) are terminated in the ventrolateral, interstitial and commissural regions of NTS (Kalia and Mesulam, 1980; Lucier et al., 1986). Furthermore, Gestreau et al. (1997) demonstrated that Fos-like protein, as a marker of neuronal activation, was expressed in the ventrolateral division of NTS after cough-inducible laryngeal stimulation. This expression of Fos-like protein was inhibited by systemic injection of codeine. Moreover, local injection of codeine into the NTS suppressed the cough reflex induced by electrical stimulation of the SLN and mechanical stimulation of the tracheal mucosa (Kito et al., 1977a). Together with electrophysiological results that microstimulation of the NTS could induce a cough-like response (Borison, 1948; Kase et al., 1970; Mori and Sakai, 1972; Chuo and Wang, 1975), it seems likely that the NTS plays roles not only in relay but also in integration of cough-related inputs arising in peripheral receptors (Shannon et al., 1998, 2000; Bolser and Davenport, 2002).

The guinea pig is the most suitable among inbred small experimental animals for investigating cough reflex that can take the place of the cat because the rat and mouse hardly show cough reflex (Belvisi and Bolser, 2002; Tanaka et al.,

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2002; Ohi et al., 2004). Up to now, however, the guinea pig is merely used for the plethysmographic evaluation of the antitussive action of the drugs (Bolser et al., 1993; Yasumitsu et al., 1996; Kotzer et al., 2000; Morita et al., 2002; Tanaka and Maruyama, 2003; Brown et al., 2004). This may be due to the fact that the central mechanisms underlying cough reflex remain largely unsolved in guinea pigs. To overcome this disadvantage, we developed a new fictive cough model of the decerebrate, paralyzed and artificially ventilated guinea pig that showed a cough reflex in response to electrical stimulation of the SLN as well as to chemical and mechanical stimulation of the upper airway (Ohi et al., 2004). The behaviors of the inspiratory (phrenic; PN) and expiratory (iliohypogastric; IHN) nerves during the fictive cough in this model paralleled with those in the cat's model. The efficacy of codeine was also identical between guinea pigs and cats.

To understand the NTS function in generation of cough reflex in the guinea pig, three subjects of particular interest were focused in the present study. Firstly, we histologically investigated the projection of SLN afferent terminals in the NTS using a fluorescent tracer. Secondly, we compared the reflex discharges of PN and IHN induced by microstimulation of the NTS region with those by SLN stimulation. Furthermore, influences of the NTS lesion on the SLN-induced fictive cough were investigated. Finally, we examined effects of microinjection of codeine into the NTS on the fictive cough induced by SLN stimulation to clarify the site of the drug action. The present results provide evidence that the NTS plays an essential role in production of cough reflex by relaying and integrating the tussigenic signals from the upper airway and is the most possible site of action of the central antitussive agents, such as codeine.

2. Materials and methods

This study was conducted in accordance with Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

2.1. Histochemistry

For application of WGA-FL (WGA-Alexa Fluor 488 conjugate, Molecular Probes, Eugene, OR, USA), male Hartley guinea pigs (Japan SLC, Hamamatu, Japan) weighing 350–600 g were anesthetized with intramuscular injection of a mixed solution of ketamine (80 mg/kg) and xylazine (8 mg/kg). The right SLN was separated from the surrounding tissue and cut. The SLN in the guinea pig ramified into two branches; an internal branch and an external branch. The former is thought to convey sensory afferent signals and the latter, efferent motor signals, on the analogy of the cat SLN (Lucier et al., 1986). Therefore, the proximal cut end of the internal branch was soaked in a tracer solution, 3 μl of 5% WGA-FL in saline, for 90 min. After a survival time of 24 h, the animals were re-anesthetized and perfused with 200 ml of heparinized (5 U/ml) physiological saline, followed by 150 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The whole brainstem was dissected, postfixed for 60 min and placed in 10 mM phosphate buffered saline (PBS, pH 7.4) at 4 °C. Serial coronal slices with a 50 μm thickness were prepared with a vibratome (DTK1000, Dosaka EM, Kyoto, Japan). The slices were washed in 10 mM PBS and placed on slides to dry for 60 min at room temperature. The slides were coverslipped with 50% glycerol and 2.5% 1,4-diazabicyclo[2,2,2]octane (DABCO; Sigma, St. Louis, MO) in PBS to protect the fading of fluorescence. Sections were viewed under fluorescent epimicroscope attached on the ×4–20 objectives (Provis AX80TR, Olympus, Tokyo, Japan) with a CCD camera (DP70, Olympus). The fluorescence of WGA was observed by wavelength of 470–490 nm for excitation and 515–550 nm for absorption with a mirror unit of NIBA. The fluorescent and light micrographs at the same views were taken to determine anatomical location. The final images obtained were analyzed with computer software (Photoshop, Adobe Systems Incorporated, USA).

2.2. Electrophysiology

Male Hartley guinea pigs (400–600 g) were anesthetized with inhalation of halothane (1.5–2.0% in oxygen-enriched air). The trachea was intubated, and catheters were inserted into the femoral vein for drug administration and the femoral artery for monitoring blood pressure. Artificial ventilation was performed after the animals were paralyzed with intravenous injection of pancuronium bromide (2 mg/kg initially and 0.2 mg/kg hourly), and tracheal pressure was maintained between 1 and 6 cmH_2O. The right SLN was isolated from the surrounding tissue and bipolar cuff electrodes were attached. The head of animals was fixed on a stereotaxic frame in prone position and decerebration was performed by aspirating the brain rostral to the pre-collicular transection. Hemostasis was performed by emplacement of hemostatic material on brain lesions. The right PN and left IHN were cut distally and mounted on bipolar electrodes in mineral oil pool. A pneumothorax was performed to reduce movement of the rib cage associated with ventilation. After finishing the surgery, halothane anesthesia was discontinued and at least 3 h elapsed before the nerve discharges were recorded. End-tidal CO_2 and O_2 were maintained at 5–6 and 30–32%, respectively. Glucose–lactate Ringer solution was infused at the rate of 4 ml/(body h) to keep the mean blood pressure over 80 mmHg. Rectal temperature was kept at 36–38 °C by external heating. At the end of experiments, a lethal dose of pentobarbital (100 mg/kg) was injected intravenously to cause cardiac arrest and death.

The efferent discharges of PN and IHN were recorded to monitor the central inspiratory and expiratory outputs, respectively. Signals were amplified, rectified, filtered (30–3000 Hz) and integrated (0.1 s time constant). All