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Swallow-related inhibition in laryngeal motoneurons

Takeshi Suzuki[a,b], Ken Nakazawa[a,*], Keisuke Shiba[c,d]

[a] Department of Integrative Neurophysiology, Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chiba City, Chiba 260-8670, Japan
[b] Department of Otolaryngology, Graduate School of Medicine, Chiba University, Chiba City, Chiba 260-8670, Japan
[c] Chiba Medical Center, Tsubakimori 4-1-2, Chiba City, Chiba 260-8606, Japan
[d] Hikifune Otolaryngology Clinic, Kyojima 1-1-1-213, Sumida, Tokyo 131-0046, Japan

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ABSTRACT

Inhibitory postsynaptic potentials (IPSPs) of laryngeal motoneurons (LMs) are essential for narrowing the glottis at just the right time during swallowing, which prevents aspiration. To examine the property of IPSPs of LMs during swallowing, we monitored the effects of intracellular application of chloride ion and extracellular application of inhibitory neurotransmitter antagonists on the membrane potential trajectories of LMs during fictive swallowing in decerebrate, paralyzed cat. Adductor LMs hyperpolarized briefly at the beginning of the pharyngeal stage of swallowing (PS) and then depolarized explosively during the remaining part of the PS. Abductor LMs exhibited various patterns during swallowing; hyperpolarization during the PS followed by depolarization at the offset of the PS, slight depolarization, or plateau potentials. Chloride-dependent IPSPs were revealed during the initial part of PS in adductor LMs and during the whole PS in abductor LMs. The swallow-related IPSPs were depressed by iontophoretic extracellular application of bicuculline in both adductor and abductor LMs, but they were not modified by strychnine application. It is concluded that the swallow-related inhibition of both adductor and abductor LMs is chloride-dependent IPSPs mediated through $GABA_A$ receptors, not through glycine receptors.

1. Introduction

Swallowing movements result from spatially and temporally coordinated activations of oral, pharyngeal, laryngeal, and esophageal muscles. In particular, the larynx plays a critical role in swallowing. Glottic closure and laryngeal elevation during swallowing prevents aspiration of food and facilitates bolus transfer from the pharynx to the esophagus. Motoneurons of the upper airway and esophageal muscles receive the sequential inhibitory and excitatory postsynaptic inputs from the central pattern generator of swallowing (Gestreau et al., 2000; Shiba et al., 1999; Sumi, 1969; Tomomune and Takata, 1988; Zoungrana et al., 1997). Almost all these motoneurons initially receive inhibitory inputs and then receive following excitatory ones. The inhibitory postsynaptic potentials (IPSPs) evoked in these motoneurons delay the motoneuronal excitation and thus determine the timing of its initiation during swallowing (Jean, 2001; Zoungrana et al., 1997). Laryngeal muscle motoneurons also receive a complex combination of excitatory and inhibitory synaptic inputs (Gestreau et al., 2000; Shiba et al., 1999; Zoungrana et al., 1997). Excitatory postsynaptic potentials evoked in laryngeal motoneurons are certainly essential for the swallow-related laryngeal activity, but IPSPs also play important roles in determining the timing and amplitude of the excitation of laryngeal motoneurons during swallowing (Zoungrana et al., 1997). The present study focused on the IPSPs evoked in laryngeal motoneurons during swallowing.

Little is known about the property of swallow-related hyperpolarization in laryngeal motoneurons except that swallow-related hyperpolarization in some laryngeal motoneurons are known to be chloride-dependent (Gestreau et al., 2000; Numasawa et al., 2004). γ-Aminobutyric acid (GABA) and glycine are thought to be candidates for the neurotransmitters mediating IPSPs in laryngeal motoneurons during swallowing, because it is reported that these transmitters mediate the respiratory-related IPSPs (Haji et al., 1990, 1992) and IPSPs evoked by laryngeal afferent stimulation (Haji et al., 1996; Yasuda, 1997) in vagal and laryngeal motoneurons. In the present study, we compared the changes in membrane potential trajectories of laryngeal motoneurons during fictive swallowing between before and after intracellular application of chloride ion in decerebrate, paralyzed cats to reveal the nature of their central inhibitory drives during swallowing. Furthermore, to determine whether GABA or glycine receptors mediate the swallow-evoked IPSPs in laryngeal motoneurons, we examined the effects of iontophoretic extracellular application of GABA and glycine antagonists on the membrane potential trajectories of laryngeal motoneurons during swallowing.

* Corresponding author. Fax: +81 43 227 3442.
E-mail address: nakazak@faculty.chiba-u.jp (K. Nakazawa).

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2. Materials and methods

All of the procedures used in this study conform to the Physiological Society of Japan Guiding Principles for the Care and Use of Animals, and were approved by the University Committee for the Use of Animals in Research.

Data were obtained from 25 adult cats of either sex. The animals were initially anesthetized with halothane (1.0–3.0%) vaporized in 50% nitrous oxide/50% oxygen and were decerebrated at the pre-collicular level after bilateral ligation of the common carotid arteries. Dexamethasone (1 mg/kg, i.m.) and atropine (0.1 mg/kg, i.m.) were administrated to minimize brain edema and to reduce secretion in the airways, respectively. The trachea was intubated, and cannulae were placed in the femoral artery to monitor blood pressure and in the femoral veins for drug administration. Mean blood pressure was maintained above 90 mm Hg using d.i.v. infusion of epinephrine in saline at the rate of 0.01 mg/kg/min when necessary. Animals were placed in a stereotaxic frame and the dorsal surface of the medulla was exposed for recording. The caudalmost 5 mm of cerebellum was aspirated to facilitate insertion of electrodes. Rectal temperature was kept at 36–37.5 °C using a heating lamp. Anesthesia was discontinued following the completion of all surgical procedures and at least one hour prior to data collection. The animal was paralyzed with pancuronium bromide (Mioblock®, Japan Organon Ltd., Tokyo, Japan; initially 0.3 mg/kg i.v. then 0.15 mg/kg/h) and artificially ventilated with room air (18–24 cycles/min). End-tidal CO_2 was kept at 3–5%. Bilateral pneumothoraces were made to reduce respiratory movements of the brainstem. At the end of each experiment, the animal was killed by an overdose of sodium pentobarbital.

Bipolar silver cuff electrodes were placed around the C5 phrenic and pharyngeal branch of the vagus nerves (Ph-X) for recording, around both superior laryngeal nerves (SLNs) for stimulation, and around both recurrent laryngeal nerves (RLNs) for both recording and stimulation. Activities of these nerves were amplified, full-wave rectified, and low-pass filtered (time constant, 1 ms).

To reveal the presence of IPSPs that might hyperpolarize laryngeal motoneurons during particular phases of swallowing, we attempted to reverse postsynaptic hyperpolarizations to depolarizations by intracellular application of chloride ion. To record the membrane potential of the laryngeal motoneurons, laryngeal motoneurons were impaled using a glass electrode containing 3 M KCl (tip impedance 10–25 MΩ). Laryngeal motoneurons were located from 0.5 mm caudal to 2.5 mm rostral to the obex, 2.7–3.5 mm lateral from midline and 2.5–3.5 mm below the dorsal surface. Laryngeal motoneurons were identified by antidromic activation from the RLN. Laryngeal motoneurons activated antidromically from the RLN were classified into expiratory laryngeal motoneurons (ELMs) and inspiratory laryngeal motoneurons (ILMs) according to their expiratory and inspiratory depolarization, respectively (Barillot et al., 1990; Shiba et al., 1999). For each motoneuron, the membrane potential was defined as the difference between the intracellular and extracellular potentials, using a single grounded Ag/AgCl electrode inserted into the temporalis muscle as a reference. Fictive swallowing was evoked by electrical stimulation of the SLN (pulse duration, 0.2 ms; frequency, 8–14 Hz; intensity, 40–150 μA) and identified by bursting activities in the Ph-X or RLN (Umezaki et al., 1998). The period of the bursting activities corresponds to the pharyngeal stage of swallowing. Chloride ion was injected into motoneurons by intracellular application of continuous negative currents (5–25 nA, 5–20 min). Membrane potentials and nerve activities were sampled at 20 and 2 kHz, respectively, using a Cambridge Electronic Design (Cambridge, UK) 1401-plus data interface and Spike 2 software in conjunction with a computer.

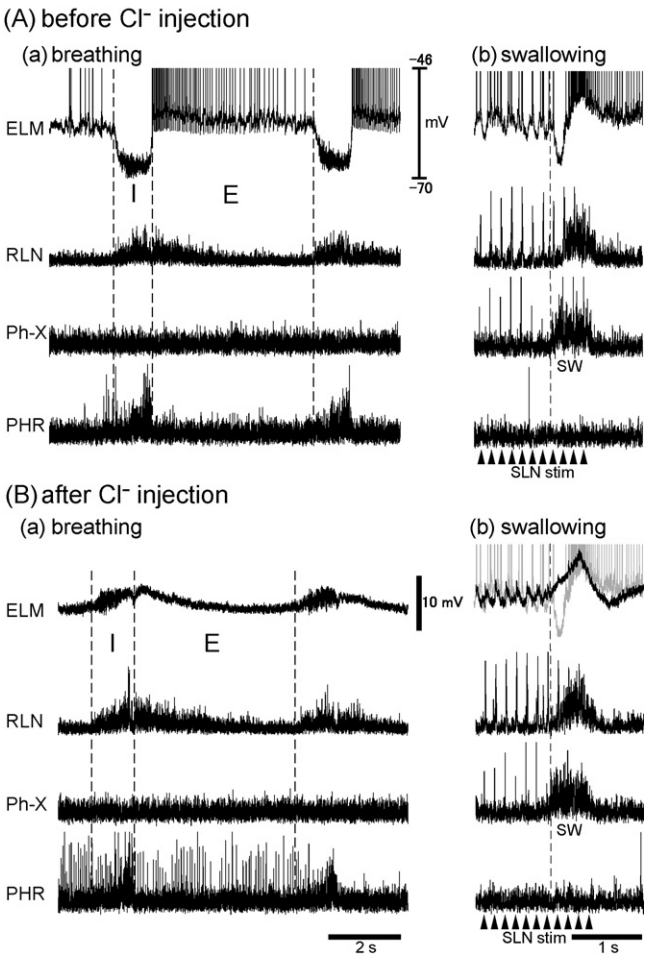

Fig. 1. Membrane potential changes in an expiratory laryngeal motoneuron (ELM) during fictive breathing (a) and swallowing (b) before (A) and after (B) intracellular chloride iontophoresis. (A) During fictive breathing, the membrane potential of ELM was rapidly depolarized at the transition from the inspiratory (I) to expiratory (E) phase, followed by a slow repolarization during the expiratory phase (decrementing pattern), and then it was hyperpolarized during the inspiratory phase. The inspiratory and expiratory phases were identified by phrenic nerve activity (PHR) during fictive breathing. During fictive swallowing (SW), the membrane potential of this neuron fell at the initiation of the pharyngeal stage (dotted line) identified by the burst of the pharyngeal branch of the vagus nerves (Ph-X), and then it abruptly depolarized for the remainder of the Ph-X burst. Fictive swallowing was induced by stimulation of the superior laryngeal nerve (SLN) (indicated by arrowheads), and identified by the burst of the recurrent laryngeal nerve (RLN) or Ph-X. (B) Reversal of inhibitory postsynaptic potentials (IPSPs) occurred after 10 min of intracellular iontophoresis of chloride ion (Cl^-); the current was off during recordings. Cl^- injection reversed the inspiratory-related hyperpolarization during breathing. The swallow-related hyperpolarization at the initiation of the pharyngeal stage was also reversed after chloride injection. The swallow-related membrane potential trajectories before Cl^- injection (illustrated as gray line) were superimposed on that after Cl^- injection (shown in (B)(b)).

We used a double-barrel electrode to examine the effects of juxtacellular application of GABA and glycine antagonists to laryngeal motoneurons on the swallow-evoked IPSPs. Two glass pipettes were glued together so that the tip of one barrel (tip impedance 8–25 MΩ) was 40–100 μm longer than the other one (tip impedance 5–15 MΩ). The longer barrel was filled with 2 M K citrate and used for intracellular recording, while shorter one was filled with bicuculline methiodide (5 mM in saline solution) or strychnine sulfate (5 mM in saline solution) and used for iontophoresis of these drugs. Bicuculline and strychnine were ejected with positive currents (50–100 nA; 1–10 min). The duration and