Intracellular Activity of Motoneurons of the Rostral Nucleus Ambiguus During Swallowing in Sheep

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Zoungrana, Ouinlassida R., Mohamed Amri, Alexandre Car, and Claude Roman. Intracellular activity of motoneurons of the rostral nucleus ambiguus during swallowing in sheep. J. Neurophysiol. 77: 909-922, 1997. The nervous mechanisms that generate swallowing are still largely unknown. It has been suggested that a central pattern generator that contains a serial network of linked neurons must produce the successive excitation of motoneurons (Mns) and then the sequential activation of muscle through excitatory connections. Inhibitory connections have also been envisioned but never evidenced at the membrane level of the swallowing neurons. We investigated, by intracellular recordings, the behavior of 96 Mns in the rostral nucleus ambiguus during swallowing induced by application of superior laryngeal nerve stimulation to anesthetized sheep. The Mns were identified by antidromic activation following stimulation of glossoapharyngeal, pharyngoesophageal, or cervical vagal nerves. Nine Mns showed a bell-shaped depolarization during the buccal or the early pharyngeal stage of swallowing. They probably projected to muscles of the soft palate (palatopharyngeal) and upper pharynx (stylopharyngeal, hyopharyngeal). Thirty-eight Mns exhibited a chloride-dependent hyperpolarization, indicating that they were under an active inhibition throughout the buccopharyngeal stage of swallowing. These Mns constitute a heterogeneous pool: some of them, producing spontaneous inspiratory discharges, probably innervated laryngeal or pharyngeal muscles; others might also be Mns of the esophagus, whose swallowing pattern was modified because of the anesthesia (suppression of the esophageal peristalsis). Forty-nine Mns showed a chloride-dependent hyperpolarization with a variable duration at the onset of swallowing, followed by a depolarization that could take place during either the buccopharyngeal (HD1-Mns) or the esophageal (HD2- and HD3-Mns) stage of deglutition. HD1-Mns probably projected to the median and inferior constrictors of the pharynx. HD2-Mns produced depolarizations with longer latencies and durations than those of the HD1-Mns. They probably projected to either the superior esophageal sphincter or the cervical esophagus (CE). HD3-Mns showed a buccopharyngeal hyperpolarization that was followed first by a lower-amplitude hyperpolarization accompanying the proximal CE contraction and then by a delayed depolarization. These Mns probably innervated the inferior CE or thoracic esophagus. We conclude that the initial inhibition exerted on the HD-Mns, by delaying the excitation of Mns, may play a role in the nervous mechanisms involved in temporal organization of the swallowing motor sequence. We suggest that swallowing disorders in humans such as dysphagia by failure of cricopharyngeal relaxation, diffuse esophageal spasm, and achalasia might be caused by impaired inhibitory mechanisms.

INTRODUCTION

Swallowing is a centrally programmed motor sequence arising from the activity of hindbrain neurons belonging to the “swallowing center” (Car and Amri 1982; Car and Roman 1970a; Doty 1968; Dubner et al. 1978; Jean 1972, 1990; Kessler and Jean 1985; Miller 1982; Roman 1967, 1986). This center is composed of two intimately connected half-centers, and is located in the medulla and pons. It has three functional components: an afferent input system, an efferent system of motoneurons (Mns), and an organizing system of interneurons (Fig. 1). The peripheral afferents involved in swallowing are mainly located in the superior laryngeal nerve (SLN). They converge onto the solitary tract in the caudal region of the medulla. The Mns lie in motor nuclei of several cranial nerves, including trigeminal, facial, and hypoglossal nuclei, and the nucleus ambiguus (NA) in the case of the glossoapharyngeal nerve (IX) and vagal nerve (X) Mns projecting to striated muscles. The interneuron system sets up and programs the entire swallowing motor sequence (buccopharyngeal and esophageal stages) and is located in the solitary tract nucleus and the surrounding reticular formation. Neurons in this region exhibit a sequential firing pattern very similar to the sequential activity observed in the muscles.

The central mechanisms that generate the sequential firing of swallowing neurons are still largely unknown. It has been suggested (Jean 1972, 1990; Roman 1967) that the solitary tract nucleus and the surrounding reticular formation must contain a serial network of linked neurons with a rostrocaudal organization such that, once activated at its beginning, this network, also called a central pattern generator, can produce the successive excitation of Mns and then the sequential activation of muscles of deglutition. In addition to excitatory connections, inhibitory links have also been envisioned to exist between swallowing neurons and to play a role in the mechanisms of sequencing. However, direct evidence of inhibitory phenomena at the membrane level of swallowing neurons is still lacking.

In the present study, intracellular recordings were obtained from Mns in the rostral part of NA, with a view to investigating their behavior during swallowing. These Mns project to the striated muscles of the soft palate, pharynx, and esophagus (Bieger and Hopkins 1987; Grêlot et al. 1989a; Hudson 1986; Kobler et al. 1994; Lawn 1966a,b; Yoshida et al. 1980, 1981). An attempt was made to classify these Mns on the basis of several criteria, including 1) the swallowing-induced changes in membrane potential, 2) the patterns of spike discharge, and 3) the peripheral projection of the axons. In addition, we assessed the short-latency postsynaptic potentials [excitatory postsynaptic potentials (EPSPs) and/or in-
hibitory postsynaptic potentials (IPSP) induced by application of stimulation to the peripheral afferents involved in swallowing control (SLN, IX, and X). A preliminary report of these experiments has been previously presented (Car et al. 1992).

METHODS

Surgery
Experiments were carried out on 18 adult ewes (merino breed; 20–30 kg) of various ages (mostly between 2 and 6 yr). Anesthesia was induced with a short-lasting barbiturate anesthetic, thiopental sodium (Nesdonal, 40 mg/kg body wt iv) to enable cannulation of the trachea, femoral artery, and saphenous vein. The anesthesia was then maintained with halothane (Fluothan, 1–2%) throughout the experiment. The trachea was cannulated to allow for artificial ventilation (air or oxygen) with a respirator (Bird Mark 8) and maintenance of the anesthesia. Respiratory movements of the brain stem were sometimes prevented or reduced by a bilateral pneumothorax, and a positive end-expiratory pressure of 2–5 cm of water was then applied to prevent the lungs from collapsing. The femoral artery and vein were catheterized to monitor the arterial blood pressure and administer solutions, respectively. The mean arterial pressure was ±80 mmHg in all the experiments. The depth of anesthesia was adjusted to keep the pupillary constriction and heart rate typically <100 bpm. Before surgery, two broad-spectrum antibiotics, a short-lasting one (Cortexiline) and a long-lasting one (Procaciline), were administered simultaneously to prevent any infectious phenomena. The rectal temperature was kept at 38°C.

The geniohyoid muscle (GH) was exposed close to its point of insertion on the hyoid bone (Fig. 2) via a medial incision of the skin under the jaw. A ventromedial incision made from the stylohyoid to the midportion of the neck gave access to swallowing control (SLN, IX, and X). A preliminary report of these experiments has been previously presented (Car et al. 1992).

Stimulation
Platinum bipolar electrodes were used to stimulate SLN (swallow triggering) and motor nerves (IX, PET, PEB, X) to test...
whether neurons were antidromically activated. The electrodes were connected to a constant-current stimulator (WPI instruments) through a high-frequency isolation unit. The stimulator delivered rectangular pulses that were adjustable in their strength (intensity or voltage) and duration. Stimulation parameters were as follows: motor nerves, 0.5–3 V, 0.05–0.3 ms; SLN, 100–300 μA, 0.1–0.3 ms. Swallowing was usually induced by short trains of 5–10 pulses at 100–300 Hz.

**Recording**

For the electromyographic (EMG) recordings, bipolar electrodes consisting of enameled copper wire were inserted into the ipsilateral GH and PH through a hypodermic needle. The electrode (120 μm diam; insulation removed over 1 mm at the tip; interpolar distance 2–3 mm) was bent at the tip so as to form a hook in the muscle when the hypodermic needle was removed. The EMG of the CE was recorded with the use of a bipolar recording electrode held on the esophageal wall by suction. Neurons were impaled with single glass micropipettes (beveled or not), filled with 3 M potassium chloride solution, which were used for both the intracellular recordings and the chloride ion injections. The microelectrode resistance (tip broken or not) ranged from 15 to 30 MΩ (measured at 100 Hz). The micropipettes were inserted vertically through a small circular pressure device (2–3 mm diam) gently applied to the dissected patch of medullary dorsal surface. Mns whose activity was synchronous with the EMG discharges accompanying swallowing were located 4.5–6.5 mm rostral to the obex, 4.5–6 mm lateral to the midline, and 4–7 mm deep.

Intracellular potentials were amplified with the use of a high-impedance device with capacity compensation, DC offset, and bridge circuit for current injection across the recording electrode (Transidyne model 1600). DC recordings of membrane potentials together with the EMGs of the swallowing muscles (GH, PH, CE) were continuously displayed on oscilloscope (Tektronix 565) and chart recorder (Gould TA 2000). Oscilloscope screens were photographed with a camera when required (Grass model C4 N). The intracellular signals were also available as X-Y plots on a pen recorder (7004b X-Y Recorder, Hewlett-Packard) fed with the output from a digital oscilloscope (Nicolet 3991). All the data were stored on a tape recorder with the use of the video pulse code modulation system at a sampling rate of 11, 22, or 44 kHz per channel (Neurocorder, Neurodata).

**Chloride iontophoresis and current injection**

To determine whether the neuronal hyperpolarization during swallowing was chloride dependent, we attempted to shift the equilibrium potential of this ion to less negative values to reverse the hyperpolarization. Chloride ions were injected into cells by iontophoresis via a negative current delivered in successive steps until a steady state of 1–5 nA was reached after several minutes. In some cells, negative current pulses (1–9 nA) were injected manually to study the intrinsic properties of the neuronal membrane, such as the presence of a postinhibitory rebound.

**Curarization**

To prevent the medullary neurons from being activated by sensory feedback resulting from contraction of muscles during swallowing (see Car and Amri 1982), the animals were paralyzed with gallamine triethiodure (Flaxedil). The curarization was generally kept light (0.5–0.7 mg/kg) to maintain a weak EMG activity in the swallowing muscles. Deep curarization (2 mg/kg) abolishing EMG activity was sometimes used, but always after checking the closely correlated neuronal discharge and reflex swallowing.
RESULTS

Swallowing patterns of NA neurons

Stable intracellular recordings were successfully obtained with 96 Mns of the rostral NA. These Mns were identified by checking that antidromic invasion occurred in response to stimulation applied to IX, PE, and X. These nerves contain motor fibers of the pharynx (IX, PE), larynx (X), and esophagus (PE, X). Moreover, PE was stimulated at the level of PET and PEB (see METHODS). The Mns activated only by stimulation of PET were therefore pharyngeal Mns, whereas those responding to both PET and PE stimulation were mainly esophageal Mns. In the following, the former group is referred to as PET Mns and the latter as PE Mns. Lastly, Mns innervating the CE and thoracic esophagus are located in PEB and cervical X, respectively.

On the basis of the correlation that exists between membrane potential variations in NA Mns and motor events in swallowing (EMG of GH, PH, and CE), three types of Mns could be distinguished. Some Mns were depolarized during GH EMG activity (D-Mns), whereas others were hyperpolarized (H-Mns). A third type of Mn (hyperpolarized-depolarized; HD-Mns) showed a more complex pattern, consisting of a hyperpolarization during GH EMG followed by a depolarization during the pharyngeal (HD1-Mns) or esophageal (HD2- and HD3-Mns) components of deglutition. None of these patterns showed any change after motor paralysis by curarization, which indicates that the membrane potential changes were of central origin.

D-Mns

These Mns (N = 9), which were antidromically activated when stimulation was applied to PET (latency 1.3 ± 0.3 ms, mean ± SD; N = 6; Fig. 3A2) or IX (latency 1.2–3 ms; N = 3; Figs. 3B4 and 4A2), displayed a bell-shaped depolarization during the buccopharyngeal stage of swallowing. The depolarization, which usually started at the beginning of GH activity and ended during PH discharge, reached 6–17 mV (12.6 ± 4 mV, N = 5) in amplitude and 170–300 ms (197 ± 6.9 ms, N = 7) in duration (Figs. 3, A1 and B1, and 4, A1 and B1). Injection of chloride ions into five D-Mns had no effect on the shape of the depolarization.

Three D-Mns that spontaneously fired action potentials (spikes) at a rate of 5–20 Hz exhibited an increase in their spiking frequency during swallowing-induced depolarization (Fig. 4, A1 and B1). Lastly, four other D-Mns, two of which were spontaneously spiking, exhibited slow rhythmic fluctuations in their membrane potential, consisting of gradually increasing and rapidly decreasing depolarizations (maximal amplitude 12 mV, duration < 7 s) occurring during expiratory (N = 3; Fig. 4B) or inspiratory (N = 1) movements. These neurons were antidromically activated when PET (N = 3) and IX (N = 1), respectively, were stimulated.

In addition to the long-lasting swallowing depolarization, application of stimulation to peripheral afferents in SLN or IX generally induced EPSPs in D-Mns (Table 1; Fig. 3, B2 and B3) with latencies of 5.8 ± 0.7 ms (N = 8) and 5–7 ms (N = 3), respectively, whereas stimulation applied to X had no effect.

H-Mns

These Mns (N = 38, 7 of which fired spontaneously) showed a membrane hyperpolarization during the buccopharyngeal stage of swallowing (Figs. 5, A and C, and 6A); they were antidromically activated in response to stimulation applied to PET (latency 2.4 ± 0.5 ms, N = 5; Fig. 5B1), PEB (latency 2.7 ± 0.6 ms, N = 16), IX (latency 1.4 ms, N = 1; Fig. 6B1), and X (latency 3.4 ± 0.6 ms, N = 16; Fig. 5D1). The membrane hyperpolarization of the H-Mns...
lasted 150–300 ms (207.2 ± 25.6 ms, N = 21) and had an amplitude ranging between 10 and 25 mV (14.3 ± 3.8 mV, N = 13). This hyperpolarization wave coincided with the EMG activity of GH or started a little sooner (20–30 ms), and stopped during the EMG activity of the PH muscle (Fig. 5, A and C1). Moreover, intracellular iontophoresis of chloride ions through the microelectrode shifted the equilibrium potential for chloride to a less negative potential. This typically reversed the swallowing-induced hyperpolarization to a depolarization, indicating that a chloride-dependent inhibitory mechanism was involved (Fig. 5C2).

In 12 H-Mns displaying no spontaneous activity and sending their axons to X (N = 5), PEB (N = 5), or PET (N = 2), a weak burst of one to three spikes occurred at the end of the hyperpolarization wave, or just before the return of the membrane potential to its resting level (Fig. 5, A and C1). In the last case, the repolarization phase usually showed a clear-cut inflection point (Fig. 5A, †), after which the membrane repolarization immediately began to occur more rapidly. In addition, it should be stressed that intracellularly injected chloride ions that reversed the swallowing hyperpolarization induced a small depolarization at the time when spikes were observed before current injection (Fig. 5C2, †). In contrast, in seven spontaneously firing H-Mns, which fired tonically at a rate of 15 Hz, no change in the spiking frequency was noted after the end of the swallowing hyperpolarization. These Mns’ spontaneous discharge was only transiently interrupted during the swallowing hyperpolarization (Fig. 6A).

Furthermore, 12 H-Mns that were silent at rest, and that hyperpolarized during the buccopharyngeal stage of swallowing, exhibited cyclic depolarizations that were synchronous with the inspiratory movements. These depolarizations, which had maximal amplitudes ranging from 7 to 23 mV (16.2 ± 5.8 mV), displayed rapidly increasing and long-lasting (1.5–2 s) decreasing phases.

Lastly, stimulation of peripheral afferents (see Table 1) induced EPSPs, EPSP-IPSP sequences (Fig. 6B2), and most frequently, pure IPSPs (Fig. 5, B2 and D2) in H-Mns. These IPSPs occurred in response to the different stimulations and had latencies of 6.5 ± 1.2 ms (SLN; N = 26), 7.9 ± 1.3 ms (IX; N = 22), and 12.7 ± 2.7 ms (X; N = 8).

**HD-Mns**

These Mns (N = 49) were all hyperpolarized during the EMG activity of GH. The hyperpolarization was followed, however, by a depolarization with a variable duration and latency; depolarization occurred, for example, either during the buccopharyngeal stage of swallowing synchronously with the EMG of PH (HD1-Mns; Fig. 7, A1 and B1) or at different periods in the esophageal stage, during the EMG of the CE (HD2-Mns; Fig. 9, A1 and B1) or even much later (HD3-Mns; Fig. 10, A1 and B1).

In addition, two HD1-Mns (1 in PET and 1 in PEB) and six HD2-Mns at rest exhibited cyclic depolarizations (6–15 mV in amplitude and 1.5–2 s in duration) during inspiratory movements.

Moreover, all three types of synaptic responses (EPSPs, IPSPs, EPSP-IPSPs) were recorded from HD-Mns on stimulation of peripheral afferents. The latencies of these responses were similar to those mentioned above in the case of other Mns (Figs. 7B3; 8, 2; 9, A2 and B3; and 10, A and B1). It is worth noting, however (see Table 1), that 1) IPSPs were the predominant response, and were obtained consistently on the HD2- and HD3-Mns in response to the different stimulations; 2) EPSPs were mainly produced by the HD2-Mns when stimulation was applied to SLN; and 3) application of stimulation to X had no effect on the HD1-Mns.

**HD1-Mns.** These neurons (N = 9) were all silent at rest and were antidromically activated when stimulation was applied to IX (N = 1, latency 3 ms), PET (N = 1, latency 2 ms), and X (N = 7, latencies 3–5 ms).
TABLE 1. Amplitude and duration of synaptic responses induced in different types of NA motoneurons by stimulation of peripheral afferents

<table>
<thead>
<tr>
<th>Motoneurons</th>
<th>Effects</th>
<th>SLN</th>
<th>X</th>
<th>IX</th>
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<tr>
<td>D-Mns</td>
<td>EPSP</td>
<td>7.9 ± 4.2 mV</td>
<td>6–10 mV</td>
<td>15–30 ms</td>
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<td></td>
<td>IPSP</td>
<td>4.2 mV</td>
<td>25 ms / 5–3 ms</td>
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<td></td>
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<td>(8)</td>
<td>(3)</td>
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<tr>
<td>H-Mns</td>
<td>EPSP</td>
<td>6.6–12 mV</td>
<td>4.5 ± 1.9 mV</td>
<td>15–50 ms</td>
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<td></td>
<td>IPSP</td>
<td>8.5 ± 5.5 mV</td>
<td>7.4 ± 3.6 mV</td>
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<td></td>
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<td>15–50 ms / 41.2 ± 12 ms</td>
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<td>(5)</td>
<td>(4)</td>
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<td></td>
<td>EPSP + IPSP</td>
<td>5 mV; 2.5 ms</td>
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<td>7.5 mV; 35 ms</td>
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<td>HD1-Mns</td>
<td>EPSP</td>
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<td></td>
<td>IPSP</td>
<td>1–7 mV</td>
<td>20–30 ms</td>
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<td>(1)</td>
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<td>HD2-Mns</td>
<td>EPSP</td>
<td>10.5 ± 1.5 mV</td>
<td>6 mV</td>
<td>3.9 mV; 13.5 ms</td>
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<td></td>
<td>IPSP</td>
<td>3.5 ± 1.4 mV</td>
<td>3.5 ± 15.1 mV</td>
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<td></td>
<td></td>
<td>2.5 ± 9.8 ms</td>
<td>5.5 mV; 6 ms</td>
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<td>11–38 ms / 4.5 mV; 43 ms</td>
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<td>(7)</td>
<td>(1)</td>
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<tr>
<td>HD3-Mns</td>
<td>EPSP</td>
<td>/</td>
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<td></td>
<td>IPSP</td>
<td>7.3 ± 3.9 mV</td>
<td>9.7 ± 3.5 mV</td>
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<td>3.9 ± 15.1 ms</td>
<td>33.9 ± 7 ms</td>
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<td>(12)</td>
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<td>EPSP + IPSP</td>
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<td>2 mV; 13.5 ms</td>
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Values with ± are means ± SD. Values in parentheses are numbers of motoneurons. NA, nucleus ambiguus; SLN, superior laryngeal nerve; X, vagal nerve; IX, glossopharyngeal nerve; D-Mns, depolarized motoneurons; H-Mns, hyperpolarized motoneurons; HD1-Mns, motoneurons that depolarized during the buccopharyngeal stage of deglutition; HD2- and HD3-Mns, motoneurons that depolarized during the esophageal stage of deglutition; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential. / Lack of response. * Values not measured.

and PEB (N = 7, latency 2.1 ± 0.3 ms; Fig. 7, A2 and B2). The swallowing hyperpolarization, which had an amplitude of 3–14 mV (7.4 ± 3 mV, N = 7), started with the EMG activity of GH, lasted for 100–200 ms (154 ± 25 ms, N = 5), and ended before the offset of the GH activity (Fig. 7, A1 and B1). The depolarization that immediately followed had a duration of 120–250 ms (173.3 ± 41.5 ms, N = 6) and an amplitude of 6–16 mV (12.2 ± 2.9 mV, N = 7), reaching its maximum at the end of the GH activity or during the period of PH EMG activity. In three of nine cases, the depolarization wave gave rise to action potentials (Fig. 7 B1).

HD2-Mns. These Mns (N = 28) often showed spontaneous spiking (N = 12), which was sometimes accompanied by a respiratory modulation. They were antidromically activated in response to stimulation of PEB (Figs. 8, 1, and 9B2). During swallowing, HD2-Mns exhibited a buccopharyngeal hyperpolarization with a higher amplitude (15.2 ± 2.4 mV, N = 15) and a longer duration (204.4 ± 41.6 ms, N = 24) than observed in the case of the HD1-Mns. The hyperpolarization started in phase with the EMG activity of GH and ended either at the same time as this EMG activity or during that of PH (Fig. 9, A1 and B1); it was immediately followed by a depolarization with a lower amplitude (7.6 ± 2.9 mV, N = 10) and a longer duration (550 ± 206.5 ms) than those of the HD1-Mns. The depolarization, which sometimes generated spikes (N = 1–15, mean frequency 15–50 Hz; Fig. 9, A1 and B1), usually ended during or just after the EMG activity of the CE. In one neuron, chloride injection caused the hyperpolarization to reverse and increased the depolarization amplitude.

The spiking of the spontaneously active Mns (5–20 Hz) stopped during hyperpolarization and resumed with a transient acceleration (up to 50 Hz) during depolarization. This acceleration coincided with EMG activity of the lower pharynx and/or proximal esophagus (Fig. 8, 1).

HD3-Mns. These Mns (N = 12) were all silent at rest and were antidromically activated in response to stimulation of PEB or X, showing latencies of 2.5–4 ms (3.3 ± 0.6 ms,
FIG. 5. Mns hyperpolarized during the buccopharyngeal stage of swallowing (H-Mns). A and B and C and D correspond to 2 different neurons. A: ipsilateral SLN stimulation (100 μA, 0.2 ms, 4 shocks at 30 Hz) evoked short-latency inhibitory postsynaptic potentials (IPSPs) followed by a slow hyperpolarization during swallowing-induced GH EMG activity. Note the fast repolarization (↑) triggering an action potential during EMG of PH. B1: antidromic potentials (latency 2.4 ms) elicited by PET stimulation (2 pulses at 500 Hz; 2 superposed traces). B2: IPSPs evoked by SLN stimulation (4 pulses at 30 Hz) in the absence of swallowing. C1: 2 SLN stimulations (6 pulses at 250 Hz) induced IPSPs followed by swallowing-induced hyperpolarizations with spike discharges at the end of the repolarizations occurring during PH EMG activity (slightly curarized animal). C2: membrane potential changes observed after intracellular chloride injection (negative current with an intensity of 2 μA during 5 min). Note reversal of hyperpolarizing waves and the slight depolarization (↓) in the area where spikes occurred in C1. D1: antidromic potentials (latency 2.7 ms) elicited by X stimulation (2.5 V, 0.2 ms, 2 pulses at 150 Hz; 2 superposed traces). D2: stimulation applied to X (single pulse or 2 pulses at 300 Hz) induced IPSPs (latency 12 ms; ↑) that were mixed with hyperpolarizing postpotentials of antidromic spikes and that were facilitated by 2 pulses.

N = 4) and 2.5–4.5 ms (3.8 ± 0.5 ms, N = 8), respectively. Swallowing triggered a hyperpolarization in the HD3-Mns, the characteristics of which (amplitude 19.2 ± 4.8 mV, duration 207.1 ± 37.8 ms) were very similar to those observed in the case of the HD2-Mns (Fig. 10, A1 and B1). This buccopharyngeal hyperpolarization was often followed by a further low-amplitude (5 mV) hyperpolarization with a variable duration (100–500 ms), coinciding roughly with the EMG activity of the CE (Fig. 10A1). Intracellular injection of chloride ions into three Mns resulted in the reversal or abolition of both hyperpolarization waves (Fig. 10B2). After the second hyperpolarization wave, a long-lasting delayed depolarization was always observed with a variable amplitude (6.2 ± 4 mV) and duration (1,000 ± 266.6 ms, N = 9), starting 500–1,200 ms (767.7 ± 216.2 ms, N = 9) after the onset of the buccopharyngeal hyperpolarization. In 7 of 12 Mns the depolarization wave generated a burst of action potentials at a mean frequency of 20–30 Hz. In addition, when

FIG. 6. Spontaneously firing Mn inhibited during the buccopharyngeal stage of swallowing. A: effects of 2 SLN stimulations (100 μA, 0.3 ms, 7 shocks at 160 Hz) inducing IPSPs only, and IPSPs followed by a swallowing hyperpolarization and interrupting the spontaneous discharge during swallowing-induced EMG activity of GH. B1: antidromic potential (latency 1.3 ms) and orthodromic IPSP (latency 5.5 ms; ↑) elicited by IX stimulation (1.5 V, 0.3 ms, single pulse). B2: repetitive SLN stimulation (160 Hz) inducing series of EPSP-IPSPs followed by a hyperpolarization beginning at the onset of swallowing-induced GH EMG activity (↑).
**FIG. 7.** Mns showing a hyperpolarization-depolarization sequence during the buccopharyngeal stage of swallowing (HD1-Mns). **A1:** cricopharyngeal muscle (superior esophageal sphincter). **A1** and **B1:** membrane potential changes in 2 different Mns during swallowing elicited by ipsilateral SLN stimulation (100 μA, 0.2 ms, short train of pulses at 250 Hz). Initial hyperpolarizations were followed by short-lasting depolarizations occurring before (**A1**) or during (**B1**) the PH EMG activity and triggering spikes in **B1**. **A2** and **B2:** antidromic invasion of the same Mns induced by stimulating (4 V, 0.1 ms) PEB (latencies 1.8 ms in A2, 2.6 ms in B2). **B3:** IPSPs (latencies 8 ms) elicited in response to stimulation (3 V, 0.1 ms, single pulse) applied to IX and SLN from top to bottom, respectively.

**FIG. 8.** Membrane potential changes in a PEB Mn projecting to the superior esophageal sphincter. Slightly curarized animal. **1:** effects of 2 SLN stimulations (250 μA, 0.2 ms, 5 pulses at 300 Hz) on Mn activity. Spontaneous firing was inhibited (strong hyperpolarization) during GH EMG swallowing activity, i.e., during the sphincter opening phase (see Fig. 7A1), and enhanced (depolarization corresponding to closure phase of the sphincter) just before the CE EMG activity. **2:** application of stimulation to ipsilateral SLN (1 pulse) triggered supraliminal or infraliminal EPSPs (3 trials; latency 5 ms). **3:** antidromic potentials (3 traces; latency 2.6 ms) elicited by PEB stimulation (3.5 V, 0.1 ms, single pulse).
primary peristalsis (that occurring during deglutition) was facilitated by inflating a balloon (10 ml) in the esophageal area to which the impaled Mn projected, the amplitude of the depolarization wave increased (up to 30 mV) and action potentials often occurred. Finally, when the balloon was inflated to 20–30 ml and kept in the same esophageal area, a series of rhythmic contractions was reflexly induced with corresponding rhythmic neuron depolarizations (Fig. 10 B3).

**DISCUSSION**

Previous studies on sheep have demonstrated the existence of a variety of swallowing neurons located in the medulla (Jean 1972, 1990; Roman 1986). To our knowledge, these neurons have been investigated only by means of extracellular microelectrodes (Amri and Car 1988; Amri et al. 1984; Jean 1972, 1978; Kessler and Jean 1985; Sumi 1963, 1964). Only two intracellular studies have been performed on a few swallowing neurons located in the hypoglossal motor nucleus (Sumi 1969; Tomomune and Takata 1988). The present study is the first in which extensive analysis was carried out on the membrane potential changes occurring in NA Mns during swallowing.

**Different patterns of membrane potential change during swallowing**

**D-Mns.** The D-Mns were depolarized during the buccal stage (EMG of GH) or at the beginning of the pharyngeal stage of swallowing. With the use of EMG methods, Doty and Bosma (1956) provided evidence that a series of muscles contracts simultaneously at the very beginning of swallowing, and constitutes the so-called “leading complex.”

These muscles include GH, the mylohyoid, the palatopharyngeal, and the superior constrictor of the pharynx. Given the timing of their discharge and their axonal targets (PET or IX), it is therefore likely that the D-Mns innervate the muscles of the upper pharynx, such as the stylopharyngeal and hyopharyngeal (superior constrictor) muscles, or those of the soft palate, such as the palatopharyngeal muscle (Dougherty et al. 1958; Kitamura and Ogata 1990; Kitamura et al. 1991). Judging by the antidromic invasions, the D-Mns of PET were presumably more numerous than those of IX (6 vs. 3); this assumption fits neuroanatomic data showing that a large proportion of the pharyngeal Mns send their axons into the pharyngeal branch of X in the cat (Grélot et al. 1989a), and that IX provides only the motor innervation of the stylopharyngeal muscle in the rat (Kobler et al. 1994).
FIG. 10. Mns showing a double hyperpolarization followed by a delayed depolarization during thoracic esophagus contraction (HD3-Mns). A1 and B1: membrane potential changes in 2 Mns of PEB (A1) and X (B1) after ipsilateral SLN stimulation (200 μA, 0.1 ms, 10 pulses at 300 Hz) in slightly curarized animals. Note, in addition to IPSPs and strong buccopharyngeal hyperpolarization, the slight hyperpolarization (•) that occurred during the CE EMG activity followed by long-lasting depolarization triggering spikes in A1. A2 and A3: IPSPs elicited by stimulation (1 V, 0.2 ms) of cervical X (single pulse: A2) and IX (train of pulses at 500 Hz: A3). B2: effects of intracellular chloride injection (negative 2-nA current applied for 5 mn). Note the disappearance of the buccopharyngeal hyperpolarization, the reversal of the esophageal hyperpolarization (•), and the increase in the final depolarization. B3: slow depolarizations induced in the Mn by inflating (†) an intraesophageal balloon placed in the superior thoracic esophagus.

Four D-Mns furthermore showed a respiratory modulation occurring during either expiration (N = 3) or inspiration (N = 1). The latter, having its axon within IX, might be an Mn projecting to the stylopharyngeal muscle, because this muscle is innervated by IX, which contains mostly inspiratory fibers (Grèlot et al. 1989b). The former group of three D-Mns with axons in PET might correspond to Mns innervating the superior and middle pharyngeal constrictors, because expiratory activities have been recorded in the hypopharyngeal and thyropharyngeal muscles in dogs (Kawasaki et al. 1964) as well as in cats (Murakami and Kirchner 1974; Sherrey and Megirian 1974, 1975). In addition, Grèlot et al. (1989b) have mentioned that pharyngeal expiratory Mns of NA exhibited a depolarization (5–7 mV, 320 ms) during swallowing in response to SLN stimulation.

Lastly, applying stimulation to peripheral afferents of SLN and IX (but not X) generally induced EPSPs in D-Mns with latencies (4.5–7 ms) similar to those reported in previous studies on NA Mns (Barillot et al. 1984; Mori 1973; Porter 1963). These EPSPs arising from the Mn pool may be the central sign that “elementary reflexes” have been produced in the pharyngeal musculature in response to SLN and IX stimulation (Doty 1968; Doty and Bosma 1956; Dubner et al. 1978). The D-Mns having relationships with the proximal alimentary canal and discharging at the onset of swallowing may therefore mainly receive excitatory inputs from buccopharyngeal afferents.

H-Mns. During the buccopharyngeal stage of swallowing, these Mns showed a long-lasting hyperpolarization that was chloride dependent, indicating that a strong active inhibition was involved. Twelve of these Mns gave off one or a few spikes at the end of the hyperpolarization wave, or even before the membrane potential had returned to its resting level. This phenomenon might be attributable to the intrinsic properties of the neurons and/or to some synaptic effects. The firing of action potentials observed at the end of the membrane hyperpolarization may have originated from the so-called “postinhibitory rebound,” which is an excitation induced by withdrawal of inhibition. Dean et al. (1989) and Johnson and Getting (1991) have reported that 81% and 58% of NA Mns tested in the rat and guinea pig, respectively, exhibited postinhibitory rebounds when they were hyperpolarized with negative current pulses (−0.5 nA). However, the spikes generated before the end of hyperpolarization may have resulted from a mechanism similar to that first described in inferior olivary neurons (Llinas and Yarom 1981a,b, 1986) and found to exist in other neurons in the CNS (Llinas 1988). It was suggested (Llinas 1988) that these neurons might possess oscillatory properties involving low-threshold and voltage-dependent calcium conductances.

In addition to the above interpretations, the late firing of the H-Mns might also reflect the impact of excitatory inputs occurring subsequent to the inhibitory ones responsible for hyperpolarization in the series of buccopharyngeal events involved in swallowing. Some Mns, in fact, showed a clear-cut inflexion during the repolarization phase, with a fast return to the resting potential level, and often generated one or several spikes (Fig. 5A). Now, on injection of chloride into these Mns, a small depolarization was unmasked in the place corresponding exactly to the repolarizing inflexion (see Fig. 5C2). The physiological significance of this hypothetical synaptic excitation still remains to be elucidated.
As regards the possible function of the H-Mns, the antidromic activations recorded here showed that these Mns may project to the pharynx, the larynx, and even the esophagus. Some of them \((N = 12)\), sending their axons to PET and X, were obviously involved in inspiratory activity of pharyngeal and laryngeal muscles. In the cat, Grélot et al. (1989b) have recorded inspiratory discharges from nerve strands belonging to the pharyngeal branch of X innervating the pharyngeal constrictors. Barillot et al. (1984) have indicated that the laryngeal inspiratory Mns producing IPSPs in response to SLN stimulation had relationships with the posterior cricoarytenoid muscle (glottis abductor muscle). Several H-Mns may therefore belong to the central respiratory network, and the fact that they are hyperpolarized during swallowing might reflect the inhibition of respiration during deglutition (Doty 1968).

It is also possible that many H-Mns, particularly those sending their axons to PEB and X, may be swallowing Mns whose axons project to proximal or distal esophageal muscles. Their pattern of activity during swallowing might have been expected to be the same as that of the HD-Mns (see below). But in fact, the depolarization did not show up because the peristalsis was depressed or even abolished by anesthesia, as often occurred in our experiments.

**HD-Mns.** When swallowing occurred, the HD-Mns showed a chloride-dependent hyperpolarization followed by a depolarization, which could take place during either the buccopharyngeal (HD1-Mns) or esophageal (HD2- and HD3-Mns) stages of deglutition.

The HD1-Mns, having their axons within IX \((N = 1)\) or PET \((N = 8)\), probably innervated the pharyngeal musculature, particularly the median and inferior constrictor muscles, because PET contains efferent fibers that project to the thyropharyngeal and cricopharyngeal muscles (Dougherty et al. 1958). Moreover, in HD1-Mns the depolarization that occurred after a short-lasting hyperpolarization \((154 \pm 25 \text{ ms})\) peaked at the onset of PH EMG activity, and then after the depolarization of the D-Mns assumed to innervate muscles of the soft palate or upper pharynx (especially the superior constrictor, see above). EMG recordings performed on the pharyngeal muscles during swallowing in rabbits (Basmajian and Dutta 1961) and cats (Doty and Bosma 1956) have shown that the median and inferior constrictors start to contract successively at 190 and 260 ms, respectively, after the onset of the GH EMG activity. The above authors also noted that the EMGs of superior, median, and inferior constrictors overlapped. In addition, the inferior constrictor remained silent during a large part of the activity of the superior constrictor and soft palate musculature (Basmajian and Dutta 1961). In the same way, Doty and Bosma (1956) have indicated that the activity of the inferior constrictor was inhibited during that of the leading complex muscles (mylohyoid, GH, superior constrictor, and a few soft palate muscles). Our results are in keeping with the above EMG studies. The early hyperpolarization that characterized the HD1-Mns might in fact explain the phase of “electric silence” observed in the inferior constrictor muscle in EMG studies. On the other hand, the HD1-Mns depolarization had a short duration \((173.3 \pm 41.5 \text{ ms})\) and started before the end of that of the D-Mns, which might explain the overlapping of EMG activities of superior and inferior constrictors. Our data also indicate (see Table 1) that application of stimulation to SLN and IX, but not to cervical X, mainly induced IPSPs in HD1-Mns, which indicates that prominent peripheral inhibitory inputs are received by these Mns.

The HD2-Mns \((N = 28)\) had their axons within PEB and were depolarized with longer latencies and durations \((550 \pm 206 \text{ ms})\) than the HD1-Mns. They may therefore innervate the superior esophageal sphincter (SES; cricopharyngeal muscle) or the CE. Some of them \((N = 12)\), which were spontaneously active and sometimes showed a respiratory modulation \((N = 6)\), produced during swallowing an inhibition-excitation sequence that was very similar to the SES activity pattern. In several EMG studies on various species, it has indeed been reported that at rest, the cricopharyngeal muscle showed a tonic activity that disappeared during the buccopharyngeal stage of swallowing before resuming immediately afterwards, while it was transiently enhanced (Asoh and Goyal 1978; Car and Roman 1970b; Elidan et al. 1990; Hellemans and Vantrappen 1974; Shipp et al. 1970). Andrew (1956), using the single-fiber recording technique on rats, observed, moreover, that the vagal fibers innervating the SES exhibited a spontaneous spike discharge, which was inhibited during swallowing and transiently reinforced afterward. The discharge inhibition lasted for 100–200 ms, which was similar to the duration of hyperpolarization occurring in the HD2-Mns, so that both events might be responsible for SES opening. The subsequent depolarization accompanied by accelerated spiking might correspond to the SES closure because of a phasic contraction that marks the beginning of esophageal peristalsis. A respiratory modulation (increased tone during inspiration) of SES activity has been commonly reported to occur in several species (Andrew 1956; Car and Roman 1970b; Hellemans and Vantrappen 1974), which might explain the inspiratory spiking of some HD2-Mns \((N = 6)\) observed in this study. In addition, Roman and Car (1970) have reported that single-shock SLN stimulation triggered brief, short-latency EMG responses in the SES area and over the first 4 or 5 cm of the CE \((8–10 \text{ ms in the case of SES and } 11–12 \text{ ms in that of the upper CE})\) that disappeared after section of the ipsilateral PET. These elementary reflexes (see Doty 1968) recorded in the cricopharyngeal muscle, corresponding to a reflex closure of SES, may be linked to the EPSPs induced by SLN stimulation in spontaneously active HD2-Mns (Fig. 8, 2). IPSPs were also induced, however, in the same population of Mns by peripheral afferent stimulation (especially in SLN): they may have corresponded to the SES reflex opening that is thought to happen, for example, after upper esophageal dis-tension (Freiman et al. 1981). A large number \((N = 16)\) of HD2-Mns were “silent” at rest, however, and these were probably involved in CE motor control. The fact that these neurons showed a powerful chloride-dependent hyperpolarization during the GH EMG activity indicates that the esophageal Mns were strongly inhibited during the buccopharyngeal stage of swallowing (see Roman 1986, 1991). Some of them \((N = 3)\) produced short-latency EPSPs, whereas others \((N = 13)\) responded with short-latency IPSPs to peripheral afferent stimulation (see Table 1). The former may project to the upper CE, because elementary reflexes were observed in this area by Roman and Car (1970). The latter
probably correspond to Mns of the lower CE. In response to SLN stimulation, Roman and Car (1970) did not in fact record any elementary reflexes in esophageal areas >8 cm distal to the SES. These authors suggested that the lack of response may have been due to the fact that Mns innervating the distal CE were inhibited when SLN afferents corresponding to the proximal CE were activated. Furthermore, the latency of the activation (depolarization with or without spikes) of the non-spontaneously spiking HD2-Mns on swallowing was longer than that of the spontaneously spiking HD2-Mns.

The HD3-Mns had their axons within PEB (N = 4) or cervical X (N = 8). During swallowing, they showed an identical hyperpolarization to that of the HD2-Mns, except that the latency of the subsequent depolarization was even longer (see Fig. 10, A1 and B1). The HD3-Mns therefore probably projected to the inferior CE or thoracic esophagus, which contains only striated muscle in sheep. The exact projection area of an Mn was identified by performing esophageal distention with a balloon that was sufficiently inflated to elicit reflex excitation of the neuron (depolarization wave); this area corresponded to the beginning of thoracic esophagus (Fig. 10B3). In addition, the depolarization wave produced by the HD3-Mns was often preceded by a slight chloride-dependent hyperpolarization, which occurred immediately after the strong buccopharyngeal hyperpolarization and coincided in time with the CE EMG activity (Fig. 10A1). It therefore seems likely that during primary peristalsis, the Mns of the inferior CE and thoracic esophagus are inhibited when those of the superior CE discharge. A similar result was obtained by Roman (1967, 1991) on recording the efferent vagal activity in awake sheep (with the use of a nerve suture technique) during swallowing. Roman reported that the firing of distal esophageal Mns during either primary or secondary peristalsis was abolished when proximal Mns were again called into play by distension of proximal esophagus. Lastly, the fact that application of stimulation to peripheral afferents (in particular those of SLN inducing reflex contractions in the proximal esophagus) (see Roman and Car 1970) triggered mainly IPSPs in HD3-MNs was another strong indication pointing to the existence of a proximodistal inhibition.

Inhibitory mechanisms during swallowing

From previous studies in sheep (Jean 1972, 1990; Roman 1967, 1986, 1991), it was assumed that the successive excitation of Mns responsible for the swallowing motor sequence depended on an interneuronal network (see Fig. 1) located in the nucleus tractus solitarius and the surrounding reticular formation. This network also called a central pattern generator (see INTRODUCTION) was seen as programming the sequence through a special arrangement of excitatory and inhibitory connections between interneurons. According to this view, it was believed that inhibitory phenomena such as relaxation of sphincters or interruption of esophageal peristalsis by a new swallow (see below) resulted solely from a dysfacilitation of Mns. The present study provides the first evidence that a true inhibition is also visible at the level of Mn membrane. That is, apart from a few Mns that are depolarized at the onset of swallowing, all the others are strongly hyperpolarized during the buccopharyngeal stage and even later on. The functional significance of inhibitory inputs that would impinge on both the interneurons and Mns is not clear. It must be noted that inhibition at the membrane level of interneurons was inferred from indirect data, but to date it has not been demonstrated by intracellular recordings. Nevertheless, the initial inhibition exerted on HD-Mns during swallowing, by delaying their excitation, should play a prominent part in the temporal organization of the motor sequence, with Mns controlling more distal parts of the swallowing tract having longer periods of inhibition than those controlling more proximal parts.

In humans, inhibitory effects during swallowing have been seen in both the proximal and distal esophagus. Thus a second swallow occurring in short intervals (3–4 s) after a first swallow results in complete inhibition of the first peristaltic wave (Vane and Diamant 1987). Likewise, water injection into the pharynx inhibits the progression of a previous esophageal peristaltic contraction (Trifan et al. 1996). Moreover, Sifrim et al. (1994) have shown that swallowing induces inhibition or a reduction of excitation that occurs simultaneously throughout the length of the esophagus, and precedes the peristaltic contraction.

All these inhibitory effects could result from the mechanism demonstrated in this study, namely the swallowing-induced hyperpolarization of Mns. In the sheep, all the muscles active in swallowing are striated, including the muscular coats of the distal esophagus. On the other hand, in humans and other species such as the cat and monkey, the muscle coats of the mid- and distal esophagus are composed of smooth muscle with a myenteric intramural plexus and two important effector neurons: an excitatory cholinergic neuron and a nonadrenergic, noncholinergic inhibitory neuron (see Diamant 1993). Both neurons are under the control of vagal inputs. Therefore in these species inhibitory phenomena could result from a central inhibition of vagal Mns connected to cholinergic intramural neurons and/or a vagal activation of nonadrenergic, noncholinergic intramural neurons, the excitation of which is known to cause relaxation of smooth muscle (see Diamant 1993). Whatever the mechanism, it is clear that lack of inhibition, either central or peripheral (or even both), could result in esophageal motor disorders such as diffuse esophageal spasm, aperistaltic contractions, and achalasia (see Sifrim et al. 1994). In addition, dysphagia affecting the buccopharyngeal stage of swallowing (failure of SES opening, for example) could also result from dysfunction of central inhibitory mechanisms.

We are grateful to D. Catalin for technical assistance and for preparing the illustration.

This study was supported by CNRS (URA 1832).

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Received 1 May 1996; accepted in final form 29 October 1996.

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