Bidirectional Electrical Coupling Between Inspiratory Motoneurons in the Newborn Mouse Nucleus Ambiguus

JENS C. REKLING1 AND JACK L. FELDMAN1,2
1Department of Physiological Science and 2Department of Neurobiology, Systems Neurobiology Laboratory, University of California, Los Angeles, California 90095-1527

Rekling, Jens C. and Jack L. Feldman. Bidirectional electrical coupling between inspiratory motoneurons in the newborn mouse nucleus ambiguus. J. Neurophysiol. 78: 3508–3510, 1997. Some spinal and brain stem motoneurons are electrically coupled in the early postnatal period. To test whether respiratory motoneurons in the brain stem are electrically coupled, we performed single and dual whole cell patch recordings from presumptive motoneurons in the nucleus ambiguus in a rhythmically active brain stem slice from newborn mice. Two of eight (25%) biocytin-injected neurons showed dye-coupling and 4 of 11 (36%) of intracellularly recorded pairs of neurons showed evidence of bidirectional electrical coupling. Impulse activity in one cell elicited small spikelets in the other and hyperpolarization of one cell led to hyperpolarization of the other with a coupling ratio ($\Delta V_2: \Delta V_1$) of 0.03–0.14. We conclude that inspiratory ambiguous motoneurons in the newborn mouse brain stem are bidirectionally electrically coupled, which may serve to transmit or coordinate signals, chemical or electrical.

INTRODUCTION

Gap junctions between neurons can pass ions and small molecules, with the potential to synchronize their biochemical or electrical activity (Bruzzone and Ressot 1997). Here we present evidence for gap junctions between functionally identified brain stem neurons, showing that inspiratory-modulated motoneurons in the intermediate region of nucleus ambiguus are bidirectionally electrically coupled.

The nucleus ambiguus (excluding the external formation) contains motoneurons controlling muscles in the esophagus, pharynx, and larynx (Biegler and Hopkins 1987). The anatomic organization of the different motoneuron types follows a distinct pattern whereby 1) esophageal motoneurons are found at the rostral pole of the nucleus ambiguus, 2) pharyngeal and cricothyroid motoneurons in the intermediate nucleus ambiguus, and 3) laryngeal motoneurons in the caudal pole of nucleus ambiguus. We have recorded from neurons in the intermediate region, i.e., putative pharyngeal and cricothyroid motoneurons. The electrophysiological properties of the neurons matched that of inspiratory type 3 neurons, which show trains of EPSPs starting shortly before inspiratory activity on the XII nerve often followed by an afterdepolarization, and show evidence for the presence of a low-voltage activated Ca$^{2+}$ current (Rekling et al. 1996).

METHODS

In vitro preparation and solutions

Neonate (day 1–6) Balb C mice were deeply anesthetized by hypothermia, killed, and an 800 $\mu$m thick slice of the brain stem was cut on a Vibratome. The rostral cut was placed 150 $\mu$m caudal to the rostral boundary of the compact formation of nucleus ambiguus. The preparation was transferred to a 0.2-ml recording chamber and was superfused 3 ml/min with preheated (28.0°C) oxygenated (95% O$_2$-5% CO$_2$, pH 7.4) artificial cerebrospinal fluid (aCSF). The aCSF solution contained (in mM) 130 NaCl, 5.4 KCl, 0.8 KH$_2$PO$_4$, 26 NaHCO$_3$, 30 glucose, 1 MgCl$_2$, and 0.8 CaCl$_2$.

XII nerve recordings

Rhythmic respiratory-related motor output was recorded by a suction electrode placed on a hypoglossal XII rootlet. Nerve activity was amplified by a GRASS amplifier and filtered at 3 Hz to 1 kHz. Sixty hertz noise was eliminated by a HumBug (Quest Scientific) and rectified nerve activity was integrated (RC circuit, time constant 50 ms).

Single and dual whole cell patch recordings

Neurons in the nucleus ambiguus were visualized by using infrared video microscopy as previously described (Rekling and Feldman 1997). Glass micropipettes (resistances typically 2–3 MΩ) were filled with a solution containing (in mM) 115 HMeSO$_4$, 115 KOH, 5 NaCl, 1 MgCl$_2$, 0.01 CaCl$_2$, 0.1 1,2-bis(2-aminophenoxy)ethane-$N,N',N'',N'''$-tetraacetic acid, tetra K$^+$ salt (BAPTA), 10 $N$-2-hydroxyethylpiperazine-$N''$-2-ethanesulfonic acid (HEPES), and 3 2-ATP (Mg$^{2+}$), pH 7.3. In eight neurons, the pipette solution contained 1% biocytin (Molecular Probes) to label the neuron. Current-clamp recordings were performed by using one or two (dual recordings) Axoclamp-2A (Axon Instruments) amplifiers. The coupling ratio ($\Delta V_2: \Delta V_1$) between neurons was calculated by hyperpolarizing (1-s pulse, 200–300 pA) one neuron and measuring the resulting change in membrane potential in this ($\Delta V_1$) and a second coupled neuron ($\Delta V_2$).

Data acquisition, analysis, and statistics

Signals were recorded on videocassette (pulse code modulation, Vetter Instruments, 3000A), digitized at 1–20 kHz by using a Digidata 1200 A/D board (Axon Instruments), and analyzed on a personal computer with Axoscope software (Axon Instruments). Statistical values are given as means ± SD.

RESULTS

Single and dual recordings were made from inspiratory type 3 neurons (Rekling et al. 1996) in slices displaying rhythmic respiratory-related activity on the XII nerve ($n = 28$). Most neurons were spontaneously active at rest ($n = 20$), the remaining had a resting membrane potential of

RAPID COMMUNICATION


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ELECTRICAL COUPLING BETWEEN AMBIGUOUS NEURONS


−61 ± 23 mV (n = 8). Input resistance was 227 ± 97 MΩ (n = 28).

Anatomic location and morphology of type 3 neurons

Figure 1A shows a composite video image of the ventrolateral surface of the slice where type 3 neurons were found (~150 μm caudal to the rostralmost part of nucleus ambiguus). The large type 3 neurons seen in the middle of the image form a nucleus-like structure (†) with a fuzzy boundary. Type 3 neurons injected with biocytin had somas in or just ventral to the nucleus ambiguus (n = 8, Fig. 1B). Dye coupling was observed in two biocytin injection experiments, with a weakly stained neuron close to the injected neuron (Fig. 1B, inset). The smallest and largest somatic diameters of the biocytin-filled neurons were 17 ± 3 and 23 ± 4 μm, the number of stem dendrites was 5 ± 1, and putative axons (fibers with constant diameter) passed along the initial part of the central tract of the vagal nerve, but disappeared at the rostral cut of the slice after a few hundred microns (n = 8).

Dual recordings from type 3 neurons

Dual whole cell patch recordings were made from 11 pairs of type 3 neurons. These inspiratory neurons (Rekling et al. 1996) show trains of EPSPs (with or without spikes, Fig. 1C) starting shortly before inspiratory activity on the XII nerve (169 ± 65 ms, n = 20, Fig. 1D). The inspiratory burst is often followed by an afterdepolarization (Fig. 1C).
FIG. 3. Impulses in a type 3 neuron can elicit spikes or changes in firing frequency in a coupled neuron. A: depolarizing pulse in cell 2 elicits spikes in cell 1, which was kept at a depolarized membrane potential. B: depolarizing pulse in cell 2 increases firing frequency in cell 1, which was kept above threshold for firing (instantaneous firing frequency: \(8.9 \pm 0.9\) spikes/s before pulse, \(11.0 \pm 1.6\) spikes/s during pulse, and \(9.36 \pm 1.0\) spikes/s after pulse. Frequency increase was statistically significant, Student’s \(t\)-test, \(P < 0.05\)). C: depolarizing current pulse in postinspiratory phase in cell 2 elicits spikes on top of burst afterdepolarization in cell 1.

inset). After a hyperpolarizing pulse the membrane shows a marked rebound depolarization (most likely because of the activation of a low-voltage activated Ca\(^{2+}\) current, Fig. 1E).

Four of 11 pairs of type 3 neurons showed evidence of electrical coupling. Bidirectional electrotonic spread of current between these four pairs was evident when impulses were elicited in one neuron by depolarizing current pulses, which gave rise to small spikelike potentials in the other neuron (Fig. 2A). Figure 2B shows a spike-triggered average, where one cell was kept suprathreshold for firing by a steady depolarizing bias current. The impulses (62-mV spike height) in the active neuron were attenuated to 1.1 mV in the paired neuron, with spike afterhyperpolarization also evident, but attenuated less in the paired neuron. Hyperpolarizing current pulses in one neuron elicited attenuated hyperpolarizations in the paired neuron (Fig. 2C). The coupling ratio \(\Delta V_2/\Delta V_1\) ranged between 0.03 and 0.14 and the variation of coupling ratios in the two directions between a coupled pair ranged from 10 to 40% \((n = 4)\). In the most strongly coupled of the four pairs (coupling ratio 0.14) spike activity in one neuron was able to elicit spikes or change the firing frequency of the other neuron when spikes were induced between inspiratory bursts (Fig. 3, A and B) or in association with the afterdepolarization after an inspiratory burst (Fig. 4C).

DISCUSSION

Gap junctions between embryonic neurons are believed to be used primarily for exchange of molecules, whereas postnatal and adult neurons may utilize gap junctions to coordinate both biochemical and electrical events (Kandler and Katz 1995; Walton and Navarrete 1991). By using paired recordings, we have shown that inspiratory motoneurons in the nucleus ambiguus are electrically coupled in the postnatal period and that the coupling ratio in some pairs is so strong that impulse activity in one neuron can elicit spikes or change the firing frequency in a coupled neuron.

We cannot completely rule out the possibility that chemical synaptic transmission between neurons may contribute to some of the coupling potentials, because we did not perform experiments under pharmacological blockade of synaptic transmission. The relatively low percentage of dye-coupled neurons and electrically coupled pairs of neurons may be a result of perturbations of gap junction function by the recording pipette solution or aCSF, because electrical coupling is strongly influenced by changes in intracellular pH (Rörig et al. 1996). Thus we hypothesize that gap junctions between ambiguous neurons synchronize electrical activity in addition to allowing exchange of small molecules important for motoneuronal maturation. The fact that ambiguous neurons (functionally unidentified) show dye-coupling in adult animals (Lewis 1994) suggests that gap junctions and synchronization of electrical activity may persist in adult animals. The neurons we recorded from are most likely pharyngeal or cricothyroid motoneurons and the synchronization of electrical activity of these motoneurons may be important for maintaining upper airway patency during inspiration as suggested for electrically coupled genioglossal motoneurons in newborn rats (Mazza et al. 1992).

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Address for reprint requests: J. C. Rekling, Department of Physiological Science, UCLA, Box 951527, Los Angeles, CA 90095-1527.

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REFERENCES


