Calcium-Dependent Plateau Potentials in Rostral Ambiguus Neurons in the Newborn Mouse Brain Stem In Vitro

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Rekling, Jens C. and Jack L. Feldman. Calcium-dependent plateau potentials in rostral ambiguus neurons in the newborn mouse brain stem in vitro. J. Neurophysiol. 78: 2483–2492, 1997. The nucleus ambiguus contains vagal and glossopharyngeal motoneurons and preganglionic neurons involved in respiration, swallowing, vocalization, and control of heart beat. Here we show that the rostral compact formation’s ambiguus neurons, which control the esophageal phase of swallowing, display calcium-dependent plateau potentials in response to tetanic orthodromic stimulation or current injection. Whole cell recordings were made from visualized neurons in the rostral nucleus ambiguus using a slice preparation from the newborn mouse. Bicucullin-labeling revealed dendritic trees with pronounced rostrocaudal orientations confined to the nucleus ambiguus, a morphological profile matching that of vagal motoneurons projecting to the esophagus. Single-stimulus orthodromic activation, using an electrode placed in the dorsomedial slice near the nucleus tractus solitarius, evoked single excitatory postsynaptic potentials (EPSPs) or short trains of EPSPs (500 ms to 1 s). However, tetanic stimulation (5 pulses, 10 Hz) induced voltage-dependent afterdepolarizations or long-lasting plateau potentials (>1 min) with a constant firing pattern. Depolarizing or hyperpolarizing current pulses elicited voltage-dependent afterdepolarizations or plateau potentials lasting a few seconds to several minutes. Constant spike activity accompanied the long-lasting plateau potentials, which ended spontaneously or could be terminated by weak hyperpolarizing current pulses. Current-induced afterdepolarizations and plateau potentials were dependent on extracellular and intracellular Ca$^{2+}$, as they were blocked completely by extracellular Co$^{2+}$, Cd$^{2+}$, or intracellular bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA). Orthodromically induced afterdepolarizations and plateau potentials were blocked by intracellular BAPTA. Afterdepolarizations and plateau potentials were completely blocked by substitution of extracellular Na$^+$ with choline. Afterdepolarizations persisted in tetrodotoxin. We conclude that rostral ambiguus neurons have a Ca$^{2+}$-activated inward current carried by Na$^+$. Synaptic activation of this conductance may generate prolonged spike activity in these neurons during the esophageal phase of swallowing.

INTRODUCTION

Motoneurons involved in such behaviors as locomotion, respiration, or swallowing transform central pattern generator input into spike activity appropriate for the necessary muscular contractions. Delineating the intrinsic properties of these motoneurons is essential to understand this transformation of synaptic current into membrane potential change and spike activity. Plateau potentials (relatively stable or slowly decaying membrane depolarizations that outlast synaptic input or current injection) are a particularly intriguing property of some motoneurons. Vertebrate spinal cord motor neurons exhibit Ca$^{2+}$-dependent plateau potentials in the presence of monoaminergic agonists, which may be important for the maintained motor output in postural hindlimb muscles (Conway et al. 1988; Houngaard and Kiehn 1989, 1993; Houngaard et al. 1988; Kiehn 1991; Kiehn et al. 1996). Other neurons express afterdepolarizations or long-lasting plateau potentials either as part of their normal electroresponsive repertoire (Hasuo et al. 1990; Llinas and Sugimori 1980; Nagatomo et al. 1993; Russo and Houngaard 1996; Schwindt et al. 1988) or after activation of permissive receptors (Caeser et al. 1993; Constanti et al. 1993; Fraser and MacVicar 1996; Haj-Dahmane and Andrade 1996; Nishimura et al. 1995). In this paper, we focus on the intrinsic properties of nucleus ambiguus motoneurons, which control muscles involved in respiration, swallowing, vocalization, or modulating heart rate (Altschuler et al. 1991; Bieger and Hopkins 1987; Standish et al. 1994). The compact formation forms the rostral and dorsal boundary of the nucleus ambiguus and extends from the caudal pole of the facial nucleus to the obex. The compact formation contains large motoneurons (20–30 μm diam), which project to striated and smooth muscle in the esophagus (Bieger 1993; Miller 1987). Synaptic input to these swallowing motoneurons comes mainly from the central subnucleus of the nucleus tractus solitarius (NTS), which is believed to contain esophagomotor reflex interneurons and command neurons driving ambiguus motoneurons in an organized rostrocaudal sequence during esophageal peristalsis (Bieger 1993; Miller 1987). We show that ambiguus neurons in the rostral compact formation display afterdepolarizations and long-lasting plateau potentials after synaptic activation or current injection due to activation of a Ca$^{2+}$-activated Na$^+$ current. These plateau potentials may underlie the production of prolonged spike activity essential for esophageal muscle contraction during swallowing.

A preliminary report of these results has been published in abstract form (Rekling and Feldman 1996).

METHODS

In vitro preparation

Neonate (day 1–6) Balb C mice were anaesthetized deeply with ether or hypothermia and killed with a cut across the thorax and removal of the lungs and heart. The entire brain stem and upper part of the medulla spinalis was removed from the animal as previously.
Solutions and bath-applied channel blockers

The recording chamber had a volume of 0.2 ml, a temperature of 28.0°C and was superfused constantly at a rate of 3 ml/min with preheated oxygenated (95% O₂-5% CO₂, pH = 7.4) artificial cerebrospinal fluid (ACSF). The ACSF solution contained (in mM) 130 NaCl, 5.4 KCl, 0.8 KH₂PO₄, 26 NaHCO₃, 30 glucose, 1 MgCl₂, and 0.8 CaCl₂. In some experiments, CoCl₂ was substituted for CaCl₂ with KH₂PO₄ replaced by KCl on an equimolar basis to avoid precipitation. CdSO₄ (Sigma) was used at a concentration of 100 μM. In some experiments, NaCl and NaHCO₃ were substituted with choline Cl⁻ and choline HCO₃⁻ on equimolar basis.

Orthodromic activation

A fine-tipped glass pipette containing 1 M NaCl or a bipolar platinum electrode (25 μm, FHC) was placed ipsilaterally in the dorsomedial slice (near the NTS) to activate synaptic inputs to the ambiguous neurons. Constant current or voltage pulses (100 μs to 4 ms, 1.5–3 mA, 3–7 volts) were given as a train of five pulses separated by 100 ms every 20 s or as single pulses every 20 s using an AMP1 Iso-Flex stimulator.

Whole cell patch recordings from visualized neurons

Neurons located in the compact formation of the rostral part of the nucleus ambiguous were visualized using infrared videomicroscopy (Dodd and Ziegglänsberger 1994). We used a Zeiss Axioskope (upright, fixed stage, mounted on an XY platform) with a ×2.5 and a ×63 objective. The ×63 objective was used in conjunction with DIC optics to visualize individual neurons and the recording pipette tip. The slice was illuminated from the caudal surface using a 50 W halogen lamp with a RG695 9505 filter (Omega Optical, 670 nm long-pass) placed in the light path. The image was acquired with a DAGE MTI CCD72 camera (normalized spectral sensitivity 0.2 at ~900 nm, i.e., the acquired image was a composite of light with a wavelength in the 670–700-nm range), contrast enhanced and displayed on an MTI 1000 video monitor. For printouts, the video image was digitized by a DT3155 frame grabber in a Pentium computer. Glass micropipettes were pulled from filamented glass tubes (1.5 mm OD, 0.86 mm ID) and filled with a solution containing (in mM) 115 NaMeSO₃, 115 KOH, 5 NaCl, 1 MgCl₂, 0.01 CaCl₂, 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, 3 2-ATP (Mg²⁺), and 0.1 leupeptin hemisulfate salt, pH = 7.3. In some experiments, the Ca²⁺ buffer strength was increased by increasing the BAPTA and CaCl₂ concentration to 10 and 1 mM, respectively. Leupeptin (a neutral protease inhibitor) was added to diminish the likelihood that neuronal structure would collapse during the whole cell recording (Kay and Wong 1987).

When the perforated patch recording technique was used, nystatin (Sigma, 120–240 μg/ml) was included in the pipette solution, and membrane seals were achieved without backfilling the pipette with nystatin-free solution. QX-314 (15 mM; Astra) was included in the pipette solution. The micropipette was lowered toward the surface of the slice and the tip positioned just above a visualized ambiguous neuron at a typical depth of 10–80 μm. The neuron then was approached with positive pressure applied to the pipette (mouth controlled) until membrane dimpling was seen, at which point the pressure was reversed to obtain a gigaohm membrane seal. To obtain the whole cell configuration, a steady negative pressure was applied to the pipette until the membrane was punctured. Pipette resistance before seal formation was typically 2–3 MΩ, and after the whole cell configuration was achieved it was 3–10 MΩ. Current- and voltage-clamp recordings were performed using an Axoclamp-2A (Axon Instruments) amplifier. All recordings were corrected for a liquid junction potential of 13 mV (calculated using Axoscope software, Axon Instruments). Input resistance was measured by giving a small-amplitude (20 pA, 500 ms) hyperpolarizing current pulse from resting membrane potential. Criteria for suitable whole cell recording were stable input resistance for >10 min, no spontaneous action potentials at rest, and capability of repetitive spike discharges during current injection.

Orthodromic activation

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Processing for biocytin

In eight neurons, the pipette solution contained 1% biocytin (Molecular Probes) to label the neurons. Only one neuron was labeled in each experiment; there was no spillage of biocytin to other neurons or evidence of dye coupling. The tissue was fixed for 24–48 h at 4°C in 0.1 M phosphate buffer (PB, pH = 7.4) containing 4% paraformaldehyde and 0.25% glutaraldehyde. Sections (80 μm) were cut on a Vibratome and processed using an avidin/biotin/horseradish peroxidase kit (Vectastain ABC kit, Elite PK-6100 standard, Vector Laboratories). Sections were washed in PB and placed on gelatin-coated slides, air dried overnight and counterstained with 1% Neutral red in distilled water. After dehydration, sections were cleared in xylene and coverslipped with Pro-TeXX.

Infrared videomicroscopy and morphology of biocytin-labeled neurons in the rostral nucleus ambiguus

The compact formation of the rostral nucleus ambiguus was clearly visible in the slice using infrared videomicroscopy (Fig. 1A). The nucleus appeared as a circular group of large neurons in the ventrolateral slice, often circumscribed by blood vessels and more opaque than the adjacent tissue. Neuronal somas could be resolved to a depth of ~100 μm. Live neurons had a smooth surface (Fig. 1A, large arrow), whereas injured neurons (more positive resting membrane potential, no spike activity in response to current
injection) had a rough surface and were less opaque (Fig. 1A, small arrow). The smallest and largest somatic diameter of neurons in this video image was 11 ± 2 and 16 ± 2 μm (n = 9).

Eight neurons were labeled with biocytin, and their morphology reconstructed with the aid of a drawing tube (Fig. 1, B and C). All labeled neurons were in the compact formation of the rostral nucleus ambiguus (Fig. 1D, left), had three to five stem dendrites and a dendritic tree with a pronounced rostrocaudal orientation confined to nucleus ambiguus (Fig. 1, B and C). The smallest and largest somatic diameters were 13 ± 3 and 20 ± 4 μm (n = 7), i.e., somewhat larger than the somatic diameters in the live tissue. The axon only could be followed for short distances in seven neurons, leaving the slice at the cut rostral surface. In one neuron, the axon was retained within the slice and passed dorsomedial, turning sharply toward the lateral surface of the slice along the central tract of the vagal nerve (Fig. 1D, left).

Responses to orthodromic activation

Rostral ambiguus neurons receive excitatory input from the central subnucleus of the NTS (Bieger 1993). Single-stimulus orthodromic activation, at constant stimulus strength using an electrode placed in the dorsomedial slice in the region of the NTS (Fig. 1D), evoked single excitatory postsynaptic potentials (EPSPs) or multiple EPSPs (500 ms-1 s) in ambiguous neurons (Fig. 2A, n = 4). Hyperpolarizing or depolarizing the membrane, using bias current, increased or decreased the amplitude of the single-stimuli EPSPs, respectively, but did not give rise to long-lasting phenomena (not shown). However, tetanic stimulation (5 pulses, 10 Hz) produced long-lasting plateau potentials in neurons with more depolarized potentials (Fig. 2, B and C). Given the variation in synaptic responses over consecutive trials (Fig. 2A), dual recordings were performed to compare responses after tetanic stimulation in two neurons simultaneously (n = 4). At resting membrane potential, both neurons responded to tetanic stimulation with a train of summating EPSPs (Fig. 2B, inset). When tetanic stimulation was repeated, depolarizing one or the other neuron using bias current, a long-lasting plateau potential with a tonic firing pattern ensued in the depolarized neuron with no such activity in the neuron remaining at resting membrane potential (Fig. 2B). Figure 2C shows the response to tetanic stimulation in a different neuron polarized to various membrane potentials. At hyperpolarized potentials, tetanic stimuli did not elicit any obvious change in membrane potential, but at more depolarized potentials, afterdepolarizations of increasing amplitude and duration and (with sufficient depolarizing bias current) long-lasting plateau potentials with a constant firing pattern were evoked (n = 7). These synaptically evoked responses could be due to intrinsic properties of ambiguous neurons. A series of experiments was designed to test this possibility.

Afterdepolarizations and plateau potentials after depolarizing and hyperpolarizing current pulses

Ambiguous neurons displayed active membrane responses when depolarizing and hyperpolarizing current pulses were injected (Fig. 3–5). The response to long depolarizing pulses (5 s) of increasing amplitude from resting membrane potential was delayed depolarization without spikes or delayed appearance of spikes followed by a small afterdepolarization (Fig. 3A). Long hyperpolarizing pulses produced a small sag and pronounced rebound depolarization, which lasted several seconds (Fig. 3B). After short depolarizing or hyperpolarizing current pulses (400–500 ms) injected from resting membrane potential, ambiguous neurons displayed prominent afterdepolarizations (Fig. 3C). The afterdepolarization had a fast rising phase (~400 ms), followed by a slow repolarizing phase (~s–s), and was preceded...
by a short afterhyperpolarization. With increasing current strength, spikes appeared during the initial part of the afterdepolarization (Fig. 3C, middle). Hyperpolarization from resting membrane potential produced a rebound afterdepolarization with an augmenting firing pattern (Fig. 3C, right). The amplitude and duration of the afterdepolarizations increased when short depolarizing or hyperpolarizing pulses, reaching the same final level, were given from increasingly more depolarized holding potentials, eventually evoking a ∼4-s plateau potential with a constant firing pattern (Fig. 3D).

When neurons were depolarized further with bias current, long-lasting plateau potentials (>1 min) followed depolarizing or hyperpolarizing current pulses (Fig. 4). The neuron shown in Fig. 4A was depolarized to −62 mV, and a 400-ms depolarizing current injection elicited a postpulse spike train lasting ∼70 s, at which point the membrane repolarized spontaneously. Hyperpolarizing pulses given from a depolarized membrane potential also were followed by long-lasting plateau potentials (Fig. 4C). The duration of these plateau potentials was dependent on the holding potential and lasted from a few seconds to >2 min, at which point the positive bias current was relieved to avoid overexciting the neuron. Plateau potentials could be terminated by small amplitude hyperpolarizing pulses (∼10 pA, >5 s, Fig. 4, B and C).

These results suggested that the afterdepolarizations and plateau potentials were voltage dependent, a notion we examined further using a prepulse stimulation paradigm (Fig. 5). In Fig. 5A, a 10-s subthreshold depolarizing pulse was given from resting membrane potential. When the same pulse was preceded by a 500-ms suprathreshold prepulse, an ∼8 s plateau potential was elicited. If the plateau potential was voltage independent, then the same prepulse should be succeeded by a depolarizing potential even if the postpulse membrane potential was more negative. This was not the case; when the same prepulse was followed by a 10-s hyperpolarizing pulse, there was no plateau potential (Fig. 5C, n = 7). Plateau potentials elicited by hyperpolarizing pulses showed the same voltage dependency (Fig. 5, D–F).

Afterdepolarizations and plateau potentials are dependent on extracellular and intracellular Ca2+

Afterdepolarizations and plateau potentials were abolished completely after substitution of extracellular Ca2+ with Co2+ or when 100 μM Cd2+ was added to the ACSF (n = 4 in each case; Fig. 6).

Two experimental designs were used to test for a possible link between a rise in intracellular Ca2+ and the generation of afterdepolarizations and plateau potentials. First, cell-attached patch recordings were established using pipettes filled with a high concentration (10 mM) of the Ca2+ chelating agent BAPTA. Immediately after going from cell-attached to whole cell condition, a depolarizing pulse, repeated every ∼20 s, was given to elicit an afterdepolarization (Fig. 7A). Within 1 min there was a complete disappearance of the afterdepolarization (n = 7; Fig. 7B). In none of these cells could long-lasting plateau potentials be elicited at more positive potentials. Second, a cell-attached perforated patch recording configuration was established using a pipette filled with nystatin and a high concentration (10 mM) of BAPTA. After 5 min of successful perforated patch recording, with depolarizing pulses every ∼20 s, the neuron continued to produce plateau potentials (Fig. 7C). On breaking the patch, the plateau potential decreased in duration and disappeared after ∼1 min of whole cell recording (n = 3, Fig. 7D).

Afterdepolarizations and plateau potentials after hyperpolarizing pulses also were blocked by intracellular diffusion of
FIG. 3. Delayed excitation and sag/rebound potentials in response to long current pulses and afterdepolarizations and plateau potentials in response to short current pulses. A: long depolarizing pulses (5 s) given from resting membrane potential, sub- and suprathreshold for spiking. Note the delayed excitation and afterdepolarizations after the stronger pulses. B: hyperpolarizing pulses of increasing amplitude given from resting membrane potential. Note the small sag and the pronounced rebound depolarization to larger pulses. C: short depolarizing and hyperpolarizing pulses (500 ms) given from resting membrane potential. Note the short-lasting afterdepolarization with or without spikes. D: depolarizing pulses given from different membrane potentials using bias current but reaching same final level (···). Note the increase in duration and amplitude of the afterdepolarization at more depolarized potentials, eventually giving rise to a ~4-s plateau potential with a constant firing pattern.

FIG. 4. Plateau potentials elicited by depolarizing and hyperpolarizing pulses from more positive membrane potentials. A: depolarizing pulse given from a depolarized membrane potential using bias current. Note the long-lasting plateau potential with a spontaneous membrane repolarization after ~70 s. B and C: plateau potentials elicited by depolarizing or hyperpolarizing pulses and terminated by hyperpolarizing pulses (4.7-s duration) ~70 s into the plateau response.
FIG. 5. Voltage dependency of the plateau potentials. A: subthreshold 10-s long depolarizing pulse. B: same pulse as in A preceded by a 500-ms depolarizing prepulse. C: same prepulse as in B succeeded by a 10-s long hyperpolarizing pulse. D: subthreshold 10-s long depolarizing pulse. E: same pulse as in D preceded by a 500-ms hyperpolarizing prepulse. F: same prepulse as in E succeeded by a 10-s long hyperpolarizing pulse. Note that plateau potentials are only present when the depolarizing/hyperpolarizing prepulses are succeeded by a depolarizing pulse, which does not elicit any spikes without the prepulses.

BAPTA (not shown). Intracellular nystatin was not the cause of the reduction of the plateau potentials because neurons recorded with a low-BAPTA patch solution and nystatin showed a plateau potential undiminished after 5 min of whole cell recording (n = 3; not shown). The afterdepolarizations and plateau potentials elicited by tetanic stimulation of the dorsomedial slice also were blocked by intracellular diffusion of a high concentration of BAPTA (Fig. 7E, n = 4). All neurons tested showed no afterdepolarization or plateau potentials when depolarized to a potential just subthreshold to spontaneous firing (using bias current) at stimulus strengths that in other neurons (with low BAPTA), and in the same preparation, were effective.

Afterdepolarizations persist in TTX and afterdepolarizations and plateau potentials are abolished by choline substituted for extracellular Na⁺.

Voltage-dependent afterdepolarizations could be elicited when TTX (2 μM) was added to the ACSF (Fig. 8, A and B, n = 4). Even at depolarized potentials, afterdepolarizations were short lasting (e.g., ~9 s after a depolarizing pulse and ~18 s after a hyperpolarizing pulse in Fig. 8, A and B); no long-lasting plateau-like depolarizations (>30 s) were seen in TTX. In normal ACSF, neurons continued to show afterdepolarizations with QX-314 in the pipette solution to block fast Na⁺ currents (n = 2, not shown). Thus the afterdepolarizations were not carried by Na⁺ ions flowing through inactivating Na⁺ channels. However, afterdepolarizations and plateau potentials were dependent on influx of extracellular Na⁺ (Fig. 8, C–F). When extracellular Na⁺ was replaced with choline, there was a complete disappearance of both depolarization- and hyperpolarization-elicited plateau potentials (n = 3). In none of these neurons could an afterdepolarization or plateau potential be elicited even with stronger depolarizing pulses (Fig. 8D) or after depolarization from a more positive membrane potential.

FIG. 6. Plateau potentials were blocked by extracellular Co²⁺ or Cd²⁺. A and B: depolarizing pulse before and after substitution of CaCl₂ with CoCl₂. C and D: hyperpolarizing pulse before and after CoCl₂. E: depolarizing pulse before and after 100 μM Cd²⁺. Note the complete disappearance of the plateau potentials in Co²⁺ and Cd²⁺. Neurons were depolarized slightly using bias current.

DISCUSSION

Motoneurons actively transform their synaptic input into appropriate firing patterns. Their intrinsic membrane proper-
Chelation of intracellular Ca\(^{2+}\) with bis(o-aminophenoxy)-N,N',N'-tetraacetic acid (BAPTA) blocks the afterdepolarizations and plateau potentials. **A:** depolarizing pulse given from resting membrane potential 10 s after the membrane seal was broken and the whole cell recording configuration established with a high BAPTA pipette solution. **B:** depolarizing pulse 67 s after the membrane seal was broken. Note the complete disappearance of the afterdepolarization elicited by the depolarizing pulse after \(\sim 1\) min recording with high BAPTA. **C:** depolarizing pulse given from resting membrane potential 5 min after a cell-attached nystatin recording was established with a high BAPTA pipette solution. **D:** depolarizing pulse given from resting membrane potential 60 s after the membrane seal was broken and the whole cell recording configuration established with the high BAPTA nystatin pipette solution. Note the persistence of the plateau potential in the nystatin configuration and disappearance of the plateau potential when BAPTA is introduced into the neuron. **E:** tetanic orthodromic stimulation 2 min after penetration with a high BAPTA pipette solution. Note that a few spikes are evoked during the stimulus, but no plateau potential is present at the more depolarized potential.

**Identity of recorded neurons**

The nucleus ambiguus has a distinct viscerotopic organization with two major divisions (Altschuler et al. 1991; Bieger and Hopkins 1987; Hopkins 1995): the classical nucleus ambiguus, which innervates striated muscles in the larynx, pharynx, and esophagus, and the external formation, which innervates parasympathetic ganglia in the heart. The classical nucleus ambiguus has three subdivisions. 1) The compact formation, which forms its dorsal boundary, contains large motoneurons with dendritic fields restricted to the longitudinal axis of the compact formation. These neurons project to all levels of the esophagus and control the esophageal phase of swallowing (Cunningham et al. 1990). 2) The semicom pact formation, which lies ventral to the compact formation and contains motoneurons with dendrites having extensive extranuclear arborizations that innervate the pharyngeal constrictors and the cricothyroid muscle. 3) The loose formation, which extends from the level of the obex to the pyramidal decussation, containing motoneurons innervating the intrinsic larynx muscles (except the cricothyroid muscle). Infrared videomicroscopy of the live slice and anatomic reconstruction of biocytin labeled neurons showed that somas of the neurons in this study were in the compact formation. These neurons had dendritic trees confined to the nucleus ambiguus with a pronounced rostrocaudal orientation and an axon that followed the central tract of the vagal nerve. Thus we surmise that we recorded from esophageal motoneurons, but we cannot exclude that some neurons were laryngeal or pharyngeal motoneurons because there is a partial overlap of the different motoneuronal groups in the compact formation (Altschuler et al. 1991).

**Afterdepolarizations and plateau potentials: underlying ionic mechanism**

Rostral ambiguous neurons displayed voltage-dependent afterdepolarizations and plateau potentials after tetanic orthodromic stimulation or injected current (depolarizing or hyperpolarizing). Two previous studies have focused on the electroresponsive properties of ambiguous neurons in adult mammals (guinea pigs). Johnson and Getting (1991) did not report any afterdepolarizations in vagal and respiratory-related neurons in and around the nucleus ambiguus. In contrast, Nishimura et al. (1995) found subthreshold depolarizations, sensitive to extracellular Co\(^{2+}\), in rostral ambiguous neurons; this phenomenon was not studied further but bears resemblance to the subthreshold depolarizations in response to the long depolarizing pulses we observed. The fact that only \(\sim 30\%\) of the ambiguous neurons showed subthreshold depolarizations and no plateau potentials were reported in the study by Nishimura et al. (1995) suggests that the balance between currents underlying plateau potentials and other...
voltage-activated currents may shift during postnatal development. Thus long-lasting plateau potentials may be an intrinsic property of immature ambiguous neurons and may tend to disappear with neuronal development. Alternatively, the generation of plateau potentials in adult ambiguous motoneurons may be controlled by neuromodulators as described in spinal motoneurons, where plateau potentials are a latent property unveiled by monoamines (Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988).

Current-induced afterdepolarizations and plateau potentials in ambiguous neurons were blocked completely by extracellular Ca\(^{2+}\) and Cd\(^{2+}\), or intracellular BAPTA; synaptic activation was blocked by intracellular BAPTA. Thus influx of Ca\(^{2+}\) and Ca\(^{2+}\)-activated processes within these neurons are necessary for these potentials. Ca\(^{2+}\) influx during synaptic activation could be through \(N\)-methyl-D-aspartate (NMDA) channels, which show a distinct voltage dependence, pass Ca\(^{2+}\), and are involved in the synaptic transmission between the NTS and the nucleus ambiguus (Wang et al. 1991). However, because current-induced and synaptic potentials were indistinguishable, this suggests activation of voltage-sensitive Ca\(^{2+}\) channels as the common mechanism. Furthermore, with intracellular Ca\(^{2+}\) chelated, synaptic activation did not produce any long-lasting depolarizing potentials even at more depolarized membrane potentials (Fig. 7E), suggesting that cation influx, including Ca\(^{2+}\), through NMDA receptors was not producing the afterdepolarization and plateau potentials. Because substitution of extracellular Na\(^+\) with choline blocked the afterdepolarizations and plateau potentials, we propose that these potentials in ambiguous neurons are the result of influx of Ca\(^{2+}\) through voltage-sensitive Ca\(^{2+}\) channels; this activates voltage-sensitive channels that pass Na\(^+\). This would explain why afterdepolarizations and plateau potentials only were seen after the tetanic synaptic activation. Single-stimulus orthodromic activation produced single EPSPs or multiple EPSPs that were insufficient to depolarize the membrane to the level for activation of the Ca\(^{2+}\)-activated inward current. Tetanic orthodromic activation induced summing EPSPs, leading to a sufficient membrane depolarization to activate voltage-sensitive Ca\(^{2+}\) channels.

Activation of the Ca\(^{2+}\)-activated inward current was evident in response to subthreshold as well as suprathreshold depolarizing pulses, giving rise to delayed excitation and postpulse afterdepolarizations respectively. Hyperpolarizing pulses also were followed by similar afterdepolarizations and plateau potentials. Several mechanisms could contribute to this phenomenon. The ambiguous neurons in this study showed a slow sag in response to hyperpolarizing pulses, resembling the response seen in adult ambiguous neurons expressing an \(I\), (Johnson and Getting 1991). After hyperpolarization, the activation of \(I\) causes a postinhibitory rebound depolarization, which could drive the membrane to a depolarized level sufficient to activate voltage-sensitive Ca\(^{2+}\) channels eliciting afterdepolarizations or plateau potentials. A low-voltage–activated Ca\(^{2+}\) current (\(T\)-channel), deactivates by hyperpolarization, also may be involved in the induction of the postinhibitory afterdepolarizations and plateau potentials because these channels have been described several types of brain stem motoneurons in newborn animals (Euastuche and Guertaud 1995; Viana et al. 1993). Long-lasting plateau potentials (>30 s) were not seen in TTX, suggesting that a continuous activation of the Ca\(^{2+}\)-activated inward current may require interaction with other ionic currents at depolarized potentials (e.g., a persistent Na\(^+\) current) or that Ca\(^{2+}\) influx during spiking may sustain activation of the Ca\(^{2+}\)-activated inward current.

The Ca\(^{2+}\)-activated inward current described here shares some characteristics with the Ca\(^{2+}\)-activated non-specific cationic conductance (\(I_{\text{Ca,AN}}\), present in both vertebrate and invertebrate neurons and in many nonneuronal cells (Partridge and Swandulla 1988; Partridge et al. 1994). CAN channels are activated by a rise in intracellular Ca\(^{2+}\) and pass Na\(^+\) and K\(^+\), and, in some cells, also Ca\(^{2+}\). The afterdepolarizations and plateau potentials we observed were highly voltage sensitive, whereas most neuronal CAN channels are voltage insensitive (Partridge and Swandulla 1988). However, CAN channels in Schwann cells and cortical pyramidal neurons (activated by muscarine) are highly voltage dependent, channel activity increasing with membrane depolarization (Bevan et al. 1984; Haj-Dahmane and Andrade 1996).

![Fig. 8. Afterdepolarizations persist in tetrodotoxin (TTX), but afterdepolarizations and plateau potentials disappear when choline is substituted for extracellular sodium. A: short depolarizing pulse followed by an afterdepolarization in TTX. Membrane responses during the pulses in A and B are truncated. C and D: depolarizing pulses before and after substitution of choline for extracellular sodium. E and F: hyperpolarizing pulses before and after choline. Note that the afterdepolarizations persist in TTX and that the plateau potentials are abolished in choline.](image-url)
Functional significance

The esophageal phase of swallowing is controlled by neurons in the brain stem (Miller 1987). In rats, the esophageal swallowing central pattern generator is believed to be located in the central subnucleus of the NTS, which sends excitatory projections to neurons in the compact formation of the nucleus ambiguus (Barrett et al. 1994; Bieger 1993; Wang et al. 1991). In addition, esophageal motoneurons receive excitatory input from cholinergic neurons in the zona intermediales recticularis parvcellularis mediated by nicotinic receptors (Zhang et al. 1993). Activation of these pathways gives rise to the smooth peristaltic wave of striated muscular contraction in the esophagus (Miller 1987). During swallowing, induced by stimulation of the superior laryngeal nerve, neurons in the NTS fire short bursts (120–200 ms, 80–250 Hz) of action potentials preceding activity in ambiguous motoneurons (Kessler and Jean 1985). Our stimulation paradigm (400 ms, 10 Hz), using an electrode placed near H ASUO, H., P HELAN, K. D., T WERY, M. J., AND G ALLAGHER, J. P. A calcium-activated inward current in guinea-pig motoneurons (Kessler and Jean 1985). Our stimulation paradigm (400 ms, 10 Hz), using an electrode placed near the NTS, had a longer duration and slower rate than these bursts of activity recorded in vivo at body temperature but elicited clear afterdepolarizations and plateau potentials in the ambiguous neurons. In addition to excitatory synaptic input, some swallowing neurons in the rostral nucleus ambiguous display chloride-dependent hyperpolarizations during the buccopharyngeal phases of swallowing, followed by postinhibitory rebound potentials lasting ~1 s (Zoungrana et al. 1997). Thus we hypothesize that ambiguous neurons receive a short-lasting excitatory or inhibitory synaptic input from the swallowing pattern generator in the NTS that is transformed to a train of spikes due to an induced short plateau potential or rebound depolarization. The underlying depolarization is the result of a Ca2+-activated inward current carried by Na+ ions elicited by influx of Ca2+ through voltage-gated Ca2+ channels. This hypothesis can be tested in vivo, by injecting BAPTA into esophageal motoneurons during fictive swallowing.

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