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Characterization of ionic channels underlying the specific firing pattern of a novel neuronal subtype in the rat prepositus hypoglossi nucleus

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ABSTRACT

In our previous study on the prepositus hypoglossi nucleus (PHN), we found a neuronal subtype exhibiting a specific firing pattern in which the first interspike interval (ISI) was longer than that of the second, designated FIL (first interspike interval long) neurons. In the present study, we explored the ionic mechanisms underlying this firing pattern using whole-cell recordings of rat brainstem slice preparations. In addition to a longer first ISI, FIL neurons showed properties such as increased slow afterhyperpolarization (AHP) of the first spike relative to the second spike. The application of 4-aminopyridine (4-AP) shortened the longer first ISI and reduced the larger AHP of the first spike, but α-dendrotoxin affected neither the ISI nor the AHP. A voltage clamp study revealed that FIL neurons express transient outward currents with slow decay kinetics. When T-type Ca^{2+} currents alone or T-type Ca^{2+} plus persistent Na^{+} currents were blocked, the FIL firing pattern changed to one with transient hyperpolarization and delayed spike generation characteristic of late-spiking neurons. These findings indicate that A-type K^{+} currents showing slow decay, T-type Ca^{2+} currents, and persistent Na^{+} currents all contribute to the specific firing pattern of FIL neurons.

1. Introduction

Neurons in the central nervous system show specific firing patterns that can usually be verified from their responses to the injection of current pulses (McCormick et al., 1985; Connors and Gutnick, 1990). The various firing patterns are caused by the properties and distributions of different types of ionic conductances (Llinás, 1988; Baxter and Byrne, 1991; Hille, 2001). Because the temporal patterns of action potentials determine the output properties of individual neurons, characterizing the ionic conductances involved in neuronal firing patterns is essential for defining the functional properties of individual neurons.

The prepositus hypoglossi nucleus (PHN) is a brainstem structure that is involved in the control of horizontal gaze holding (Robinson, 1975, 1989; Fukushima et al., 1992; Fukushima and Kaneko, 1995; Moschovakis, 1997). In our previous study on the firing patterns of PHN neurons using whole-cell recordings of rat brainstem slice preparations (Shino et al., 2008), we found a characteristic firing pattern that had not been reported previously. This firing pattern is characterized by a first interspike interval (ISI) longer than the second ISI, and we thus designated neurons exhibiting this firing pattern as FIL (first interspike interval long) neurons. This firing pattern was observed in more than 20% of PHN neurons tested, and FIL neurons are considered to be one of the major neuronal types in the PHN together with two other types: continuous-spiking neurons that exhibit repetitive firing with relatively constant interspike intervals and late-spiking neuron that exhibit a delay in the generation of the first spike (Shino et al., 2008). Single-cell reverse transcription-polymerase chain reaction (RT-PCR) analyses following whole-cell recordings revealed that a large population of FIL neurons expressed glutamate decarboxylase (GAD) 65, GAD67, and/or glycine transporter 2 (GLYT2). Furthermore, our recent study using vesicular GABA transporter (VGAT)-Venus transgenic rats, in which inhibitory neurons express the fluorescent protein Venus (Uematsu et al., 2008), demonstrated that most inhibitory neurons exhibiting the FIL pattern express GAD65 and/or GAD67 [GAD(s)] and GlyT2 simultaneously (Shino et al., 2011). These findings suggest that FIL neurons form a unique subpopulation of inhibitory PHN neurons. Although the findings on the molecular characteristics of FIL neurons have accumulated, it remains unclear how the FIL pattern is generated. To further evaluate the functional significance of FIL neurons, it is necessary to clarify the ionic mechanisms responsible for the FIL pattern. In the present study, we therefore investigated the ionic channels underlying the FIL pattern using whole-cell patch clamp recordings in rat brainstem slice preparations.

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2. Materials and methods

2.1. Slice preparation and whole-cell recording

All experimental procedures were approved by the Animal Care and Experimentation Committee of Gunma University (approval number; 07-028, 10-003). Every effort was made to minimize the number of animals used and their suffering. The procedures for slice preparation and whole-cell patch clamp recording were similar to those described previously (Shino et al., 2008, 2011; Saito and Yanagawa, 2010). Young Wistar rats (aged 16–20 postnatal days) were deeply anesthetized with isoflurane (dose adequacy was judged by the absence of reflex movements to toe pinches) and decapitated. The brain was quickly removed, and frontal slices of the brainstem (250 μm in thickness) were cut with a Microslicer (Pro 7, Dosaka EM, Kyoto, Japan) in oxygenated ice-cold sucrose solution containing (in mM): 234 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 26 $NaHCO_3$, and 11 glucose and subsequently incubated in an extracellular solution containing (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 25 glucose oxygenated with 95% O_2 and 5% CO_2. After incubation for more than 1 h at room temperature, each slice was placed in a submersion-type recording chamber on an upright microscope (Leica DM LFS, Leica, Wetzlar, Germany) and continuously superfused with the extracellular solution at a rate of 4 ml/min. The bath temperature was maintained at 30–32 °C with an in-line heater (SH-27A, Warner Instruments, Hamden, CT). The PHN was defined using the rat brain atlas (Paxinos and Watson, 2007), and neurons visualized with Nomarski optics were selected for recording. Patch pipettes were filled with an internal solution containing (in mM): 120 K-methylsulfate, 20 KCl, 0.2 EGTA, 2 MgATP, 0.3 NaGTP, 10 HEPES, and 0.1 spermine that had been pH-adjusted to 7.3 with KOH. The osmolarity of the internal solution was 280–290 mOsm/l, and the resistance of the electrodes in the bath solution was 3–7 MΩ. The measured liquid junction potential (−5 mV) was corrected. Whole-cell recordings were performed using an EPC-8 patch clamp amplifier (HEKA, Darmstadt, Germany). To clarify the firing pattern of individual PHN neurons, we injected depolarizing current pulses (to 200–400 pA in 20–40 pA steps, 400 ms in duration) into the recorded neurons. The membrane potentials of the neurons before the injection of current pulses were −85 to −75 mV that were maintained by the injection of constant currents. Voltage-clamp recordings of transient outward currents (TOCs) were performed in a Ca^{2+}-free extracellular solution containing (in mM): 125 NaCl, 2.5 KCl, 3 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, and 0.25 μM tetrodotoxin (TTX). Series resistance was routinely compensated up to 70%. Voltage-gated total outward currents were evoked by the application of a series of voltage steps from −90 mV to −10 mV with 10 mV intervals for 1 s (test-steps) following a voltage step to −90 mV for 500 ms. Non-inactivating outward currents were evoked by the application of the same test-steps following a 500-ms voltage step to −30 mV. Because the total outward currents consist of both TOCs and non-inactivating outward currents, TOCs were isolated by subtracting the non-inactivating outward currents from the total outward currents. Steady-state inactivation was investigated with 1-s voltage steps to −20 mV following a series of 500-ms voltage steps from −90 mV to 0 mV at 10 mV intervals (pre-steps). Voltage and current signals were low-pass filtered at 3 kHz and digitized at 10 kHz. The data were acquired using a pClamp9 system (Molecular Devices, Foster City, CA). The drugs were purchased from Wako Pure Chemical (Osaka, Japan) except for 4-aminopyridine (Sigma–Aldrich Japan, Tokyo, Japan), α-dendrotoxin (Alomone Labs, Jerusalem, Israel), and riluzole hydrochloride (Tocris Bioscience, Bristol, UK).

2.2. Data analysis

Neurons displaying a membrane potential below −50 mV immediately after patch membrane rupture and a peak action potential higher than 0 mV were used for further analyses. Off-line analysis was performed with the Axograph software (Molecular Devices). The interspike interval (ISI) was defined as the time between successive pairs of spikes. The ratio of the first ISI to the second ISI was analyzed from spike trains with 10 action potentials or more during 400-ms pulses. The amplitude of the AHP was estimated as the difference between the most negative membrane potential of the AHP and the inflection point between the gradual depolarizing phase preceding the action potential and the rapidly rising phase of the action potential. To analyze the voltage dependence of the activation and inactivation of TOCs, we plotted the normalized chord conductance as a function of the membrane potential held by the test- or pre-steps. These plots were fit with the Boltzmann function: $G(V_m)/G_{max} = 1/\{1 + \exp[\pm(V_m - V_{1/2})/k]\}$, where $G(V_m)$ is the chord conductance at the membrane potential V_m, G_{max} is the maximal chord conductance, $V_{1/2}$ is the membrane potential of half-maximal conductance, k is a slope factor, and ± indicates the function that is used for the plots of steady-state inactivation (+) and activation (−). The decay phase of the TOCs was fit with the sum of two exponential functions: $I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$, where A_1 and A_2 are amplitudes obtained from the beginning of the fit range, and τ_1 and τ_2 are decay time constants ($\tau_1 < \tau_2$). The exponential fit was adapted to the decay phase from the peak of the TOC to the end of the applied pulse. All values are shown as the mean ± standard deviation (SD), and error bars in the figures represent the SD. Statistical significance was examined using the t-test (paired or unpaired) and one-way ANOVA with a post hoc Scheffe's test.

3. Results

3.1. Characteristics of the firing pattern of FIL neurons

Fig. 1 shows the three major firing patterns of PHN neurons in response to depolarizing current pulses, namely continuous spiking, FIL, and late spiking. Although both continuous-spiking neurons and FIL neurons show repetitive firing in response to current pulses, the first ISI is shorter than or equal to the second ISI in continuous-spiking neurons (Fig. 1A), whereas the first ISI is longer than the second ISI in FIL neurons (Fig. 1B). Late-spiking neurons also show a firing pattern with a longer first ISI in response to strong depolarizing current pulses (Fig. 1C2, lower trace). However, they show transient hyperpolarization following the onset of membrane depolarization (arrow, Fig. 1C1, upper trace) and a long delay in the generation of the first spike when weak or moderate current pulses are applied. In response to weak or moderate current pulses, FIL neurons never show transient hyperpolarization or delayed spike generation.

In our previous study (Shino et al., 2008), the FIL pattern was established as the ratio of the first ISI to the second (ISI ratio) as larger than 1. This ratio was routinely estimated from a spike train with 10 spikes or more induced by the application of 400-ms depolarizing current pulses from the membrane potential of −75 to −85 mV. To validate these criteria for the FIL pattern, we investigated the dependence of the ISI ratio on (1) the firing rate and (2) the baseline membrane potential prior to injection of current pulses. We first investigated the dependence of the ISI ratio on the firing rate. Fig. 2A shows spike trains of an FIL neuron that fired different numbers of spikes, which were induced by injecting current pulses of different amplitude. The membrane potential prior to the injection of current pulses was maintained at −75 to