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ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE M-CURRENT IN RAT HYPOGLOSSAL MOTONEURONS

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Abstract—The M-current (I_M) is a voltage-dependent, persistent K^+ current so termed because it is strongly inhibited by the cholinergic agonist muscarine. The I_M main function is to limit neuronal excitability by contrasting action potential firing. Although motoneurons are sensitive to acetylcholine, the role of I_M in modulating their excitability is still controversial. The aim of the present report was to examine the presence of I_M in hypoglossal motoneurons (HMs) and its role in the modulation of firing properties using an *in vitro* model of rat brainstem slice. For this purpose, we employed the whole-cell patch-clamp technique to record HM responses upon stimulation with either a standard I_M deactivation voltage protocol or depolarizing current steps. Voltage commands from depolarized potential induced inward relaxations with the common characteristics of I_M, comprising inhibition by either muscarine (10 μM) or the selective I_M inhibitor linopirdine (30 μM). I_M was pharmacologically distinguished from the hyperpolarization-activated inward-rectifying current and, within the −20 to −50 mV range, deactivated with >100-ms time constant. Current-clamp experiments demonstrated that I_M strongly regulated HM action potential firing, since both muscarine and linopirdine increased spike frequency whereas the M-channel opener retigabine (20 μM) reduced it. Conversely, I_M seemed uninvolved in the generation of the medium afterhyperpolarizing potential. Our results suggest that HMs possess I_M, whose pharmacological modulation is an important tool to up- or down-regulate excitability, to be explored in experimental models of neurodegeneration.

Key words: muscarine, linopirdine, retigabine, spike firing, brainstem, KCNQ.

INTRODUCTION

The M-current (I_M) is a slow, non-inactivating voltage-dependent K^+ current first discovered in frog sympathetic neurons (Brown and Adams, 1980; Adams et al., 1982a). It was so termed because it is strongly blocked by the cholinergic agonist muscarine (Adams et al., 1982b) since muscarinic acetylcholine receptor (mAChR) activation triggers the degradation of cell membrane phosphatidylinositol 4,5-bisphosphate critical for M-channel opening (Suh and Hille, 2002). One of the most important functions of I_M is to limit neuron firing frequency by inducing membrane hyperpolarization due to K^+ efflux which reduces the rate of depolarization near spike threshold (Brown and Adams, 1980). The I_M ability to regulate action potential (AP) discharges has been demonstrated in several neuronal systems such as hippocampal (Yue and Yaari, 2004; Hu et al., 2007) and cortical (Guan et al., 2011; Gigout et al., 2012) pyramidal cells, cholinergic neurons in the peduncolopontine nucleus (Bordas et al., 2015), ventral tegmental area dopaminergic neurons (Koyama and Appel, 2006), vestibular primary afferent neurons (Pérez et al., 2009), and also spinal sensory and motor neurons (Alaburda et al., 2002; Rivera-Arconada and Lopez-Garcia, 2005; Lombardo and Harrington, 2016). Thus, selective pharmacological tools are employed to reveal the presence of I_M by studying how they impact neuronal firing pattern. I_M inhibitors, such as linopirdine and XE-991 (Aiken et al., 1995; Schnee and Brown, 1998), increase firing frequency by directly binding to M-channels and promoting their closure (Lamas et al., 1997). Conversely, the anticonvulsant drug retigabine potentiates I_M by increasing channel open probability (Tatulian and Brown, 2011), and stabilizing it in the open conformation so that its activation range is shifted toward more hyperpolarized membrane potential (Wickenden et al., 2000; Tatulian et al., 2001). I_M may also modulate resting membrane potential (Wladyka and Kunze, 2006), synaptic integration (Shah et al., 2011; Hönigsperger et al., 2015), theta frequency resonance oscillation (Hu et al., 2002), medium afterhyperpolarizing potential (mAHP) generation (Storm, 1989; Hu et al., 2007) and neurotransmitter release (Vervaeke et al., 2006; Peretz et al., 2007). Neuronal M-channels are mainly formed by KCNQ2, KCNQ3 (Wang et al., 1998), and KCNQ5 (Schroeder et al., 2000) potassium channel subunits which are targeted by several endogenous signaling molecules such as acetylcholine (ACh), bradykinin, angiotensin, and somatostatin (reviewed by Delmas and Brown, 2005).

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Abbreviations: ACh, acetylcholine; AP, action potential; APV, (2R)-amino-5-phosphonovaleric acid; DHβE, dihydro-β-erythroidine; DNQX, 6,7-dinitroquinoxaline-2,3-dione; E_K, potassium equilibrium potential; GABA, γ-aminobutyric acid; HM, hypoglossal motoneuron; I_h, hyperpolarization-activated inward-rectifying current; I_{in}, instantaneous current; I_M, M-current; I_{ss}, steady-state current; $KMeSO_4$, K-methyl-sulfate; mAChR, muscarinic acetylcholine receptor; mAHP, medium afterhyperpolarizing potential; MLA, methyllycaconitine; R_{in}, input resistance; R_s, series resistance; V_h, holding potential.

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Motoneurons are known to be sensitive to ACh (Rekling et al., 2000) that can activate nicotinic as well as muscarinic receptors. Under physiological conditions, while the main action by nicotinic receptors is to modulate transmitter release at premotoneuron level (Quitadamo et al., 2005; Corsini et al., 2016; reviewed by Dani and Bertrand, 2007), activation of muscarinic receptors expressed by motoneurons is associated with persistent excitation (Kurihara et al., 1993; Haj-Dahmane and Andrade, 1996; Hornby et al., 2002; Miles et al., 2007) which might include I_M block. An interesting model to explore the role of I_M in motoneuron activity is the nucleus hypoglossus as its motoneurons are the sole voluntary motor supply to tongue muscle (Lowe, 1980) and represent also a rhythmic output of the brainstem respiratory network in concert with the pre-Bötzinger nucleus inspiratory rhythmicity (Ballanyi et al., 1999).

Previous studies have provided dissimilar data on the role of the I_M in controlling firing of spinal motoneurons (Miles et al., 2005; Rivera-Arconada and Lopez-Garcia, 2005; Lombardo and Harrington, 2016); however, there is no report of its presence in HMs. The present study, therefore, was initiated as a first approach to examine the presence and function of the I_M in rat HMs, to characterize its basic properties and to find out its contribution to repeated firing, alongside other formerly characterized K^+ currents (Viana et al., 1993; Töpert et al., 1998; Lape and Nistri, 1999). For this purpose, we employed a combination of electrophysiological (voltage- and current-clamp) techniques and pharmacological tools to isolate and detail I_M in HMs from rat brainstem "silent" slices, which did not allow studying the impact of I_M modulation on inspiratory-related HM bursting.

Our data indicate that I_M is present in HMs and that it is a potent regulator of neuronal AP burst, especially in the membrane potential range above the threshold when repetitive firing is generated. Because of these characteristics, I_M would be a suitable candidate to down-regulate motoneuron excitability.

EXPERIMENTAL PROCEDURES

Animals and slice preparation

All experiments and treatment were performed in accordance to the protocol approved by the ethics committee (prot. 3599, 28th May 2012) of the Scuola Internazionale Superiore di Studi Avanzati (SISSA) and were carried out following the Italian legislation and European Communities Council Directive of 24 November 1986 (86/609/EEC) for animal experimentation. All efforts were made to minimize the number of animals and their suffering during the present experiments. Brainstem slices containing the nucleus hypoglossus were prepared as recently described (Corsini et al., 2016). Briefly, the whole brainstem was isolated from neonatal Wistar rat (postnatal day 2–6; P2–P6), rapidly decapitated under i.p. urethane-anesthesia (10% solution, 0.1 mL injection). An average of two slices (270-μm-thick) per animal, obtained with a vibrating tissue slicer (VT1000S, Leica, Wetzlar, Germany), were incubated for 20 min at 32 °C and then maintained for 20 min at room temperature before electrophysiological recording in continuously carbogenated (95% O_2 and 5% CO_2) Krebs solution containing (in mM): 130 NaCl, 3 KCl, 1.5 NaH_2PO_4, 1 $CaCl_2$, 5 $MgCl_2$, 25 $NaHCO_3$ and 11 glucose (pH 7.4; 310–330 mOsm).

Electrophysiological recording

Single slices were placed in a recording chamber and superfused (2–3 mL/min) with carbogenated Krebs solution containing (in mM): 130 NaCl, 3 KCl, 1.5 NaH_2PO_4, 1.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$ and 12 glucose (pH 7.4; 300–320 mOsm) at room temperature. Whole-cell patch-clamp recordings were performed on HMs, visually identified with infrared microscopy based on their large soma diameter (~25 μm) and compact location within the nucleus hypoglossus (Cifra et al., 2012).

Voltage-clamp experiments were carried out with an L/M EPC-7 amplifier. Patch pipettes were routinely filled with a K-gluconate based solution (in mM: 110 K-gluconate, 20 KCl, 5 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 EGTA, 2 ATP-Mg, 1 GTP-Na and 10 sucrose; pH 7.2 with KOH, 290–310 mOsm; 5–6 MΩ pipette resistance). In a few experiments, K-methylsulfate ($KMeSO_4$) or CsCl replaced K-gluconate in the pipette solution. In accordance with standard protocols to investigate the properties of the I_M (Adams et al., 1982a), cells were clamped at depolarized holding potential (V_h = −10 mV) while series resistance (R_s; 5–20 MΩ) was routinely monitored without applying compensation; data were discarded when R_s exceeded 20% of the initial value. All recorded currents were filtered at 3 kHz and sampled at 5–10 kHz.

Current-clamp experiments were carried out using an Axoclamp-2B amplifier. Patch pipettes were filled with the same K-gluconate-based intracellular solution described above to obtain a pipette resistance of 10–15 MΩ. After seal rupture, cells were kept at their resting membrane potential and current step protocols were applied. The bridge balance was continuously monitored and reset whenever necessary.

All potential values were corrected off-line subtracting the values of liquid junction potential (11, 7, and 3 mV for K-gluconate-, $KMeSO_4$-, and CsCl-based solutions, respectively). Voltage and current steps generation and data acquisition were performed with Clampex 9.2 software (Molecular Devices, Sunnyvale, CA, USA). Cell input resistance (R_{in}) was calculated measuring the current or voltage response to 10 mV or 50-pA hyperpolarizing steps from V_h or resting membrane potential. In all experiments where drugs were applied, their superfusion started 3 min after gaining access to the cell interior. Data were collected as a routine after 8 min to allow for recording stabilization.

Electrophysiological data analysis

All electrophysiological data were analyzed using Clampfit 10.0 (Molecular Devices). In order to study and characterize the I_M a standard I_M deactivation protocol (Adams et al., 1982a) was applied in voltage-clamp