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Extracellular magnesium and calcium reduce myotonia in ClC-1 inhibited rat muscle

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Abstract

Loss-of-function mutations in the ClC-1 Cl^- channel trigger skeletal muscle hyperexcitability in myotonia congenita. For reasons that remain unclear, the severity of the myotonic symptoms can vary markedly even among patients with identical ClC-1 mutations, and may become exacerbated during pregnancy and with diuretic treatment. Since both these conditions are associated with hypomagnesemia and hypocalcemia, we explored whether extracellular Mg^{2+} and Ca^{2+} ($[Mg^{2+}]_o$ and $[Ca^{2+}]_o$) can affect myotonia. Experimental myotonia was induced in isolated rat muscles by ClC-1 inhibition and effects of $[Mg^{2+}]_o$ or $[Ca^{2+}]_o$ on myotonic contractions were determined. Both cations dampened myotonia within their physiological concentration ranges. Thus, myotonic contractile activity was 6-fold larger at 0.3 than at 1.2 mM $[Mg^{2+}]_o$ and 82-fold larger at 0.3 than at 1.27 mM $[Ca^{2+}]_o$. In intracellular recordings of action potentials, the threshold for action potential excitation was raised by 4–6 mV when $[Mg^{2+}]_o$ was elevated from 0.6 to 3 mM, compatible with an increase in the depolarization of the membrane potential necessary to activate the Na^+ channels. Supporting this notion, mathematical simulations showed that myotonia went from appearing with normal Cl^- channel function to disappearing in the absence of Cl^- channel function when Na^+ channel activation was depolarized by 6 mV. In conclusion, variation in serum Mg^{2+} and Ca^{2+} may contribute to phenotypic variation in myotonia congenita patients.

Keywords: Myotonia congenita; ClC-1 channels; Extracellular magnesium; Extracellular calcium; Phenotypic variation

1. Introduction

ClC-1 Cl^- channels generate about 80% of the total membrane conductance in resting skeletal muscle fibers [1–3]. The physiological effect of this Cl^- membrane conductance is twofold: Firstly, the current required to trigger an action potential is markedly reduced in the absence of Cl^- membrane conductance [4], demonstrating that the Cl^- conductance acts to dampen muscle fiber excitability. Secondly, by contributing to repolarizing currents during action potential firing in muscle, the ClC-1 Cl^- channels play an important role in preventing re-excitation processes and myotonia in the skeletal muscle fibers themselves [4,5].

In myotonia congenita [6], loss-of-function mutations in the ClC-1 channel result in myotonic symptoms of spontaneous muscle excitations and delayed relaxations [7]. Currently, about 130 different ClC-1 channel mutations have been reported from myotonia congenita patients [8] and the different degrees of ClC-1 dysfunction induced by such mutations may explain part of the marked variation in symptoms in this group of patients [9]. However, variation in symptoms amongst these patients is not fully explained by the different ClC-1

Abbreviations: 9-AC, 9-anthracenecarboxylic acid; AUC, area under the curve; $[Ca^{2+}]_o$, extracellular Ca^{2+} concentration; EDL, extensor digitorum longus; $[Mg^{2+}]_o$, extracellular Mg^{2+} concentration; NKR, normal Krebs-Ringer solution; V_{rest}, Vth, membrane potential threshold for action potential excitation

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mutations since family members with identical ClC-1 mutations can have markedly different degrees of myotonic symptoms [9]. Also, certain conditions are known to be associated with worsening of myotonic symptoms. Thus, during pregnancy [10] and with use of diuretics [11], myotonic symptoms are known to worsen. Both of these conditions are associated with increased risk of hypomagnesemia and hypocalcemia [12,13]. However, whether variation in extracellular Mg^{2+} and Ca^{2+} concentration ($[Mg^{2+}]_o$, $[Ca^{2+}]_o$) can introduce variation in myotonic symptoms and contribute to worsening of symptoms is not known.

Based on this, the present study explored the effect of small perturbations within the physiological ranges of $[Mg^{2+}]_o$ and $[Ca^{2+}]_o$ on myotonic behavior in isolated rat muscles exposed to saturating concentrations of the ClC-1 channel inhibitor, 9-AC, whereby experimental myotonia can be induced [14]. This use of chemically-induced models of myotonia rather than use of genetic models of congenital myotonia allowed comparison of experimental observations with simulated myotonia using an established mathematical model of rat muscle [15]. In particular, simulation were employed to explore for a role of modulation of the function of voltage gated Na^+ channels in experimental observations of clear dampening of myotonia by $[Mg^{2+}]_o$ and $[Ca^{2+}]_o$.

2. Materials and methods

2.1. Animal handling

No experiments were performed on live animals, and all handling and killing of animals complied with Danish animal welfare regulations and the local guidelines for animal handling at Aarhus University. Contractile force was measured in soleus or EDL muscles after dissection from either young 4-week-old male/female wistar rats weighing 70–75 g or adult 12–15-week-old female rats weighing ~220 g. Cellular electrical properties were determined in surface fibers of soleus or EDL muscles dissected from 12–15-week-old animals. All animals were fed *ad libitum* and kept at a constant temperature (21 °C) and day length (12 h).

2.2. Solutions and compounds

After dissection, all muscles were initially incubated for at least 30 min in normal Krebs–Ringer bicarbonate solution (NKR) containing (mM): 122 NaCl, 25 $NaHCO_3$, 2.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.27 $CaCl_2$ and 5.0 D-glucose. To induce experimental myotonia, 100 μM of the ClC-1-channel inhibitor 9-AC (Sigma Aldrich, DK) was added to NKR. To prepare a solution containing a low $[Mg^{2+}]$, NKR was mixed with a Mg^{2+} free solution that was prepared as NKR except that $MgSO_4$ was replaced by Na_2SO_4. Similarly, a solution with reduced $[Ca^{2+}]$ was obtained by mixing NKR with a solution with low $CaCl_2$ (0.3 mM). To raise $[Mg^{2+}]$ or $[Ca^{2+}]$ above those in NKR, $MgCl_2$ or $CaCl_2$ were added to NKR from concentrated stock solutions of 1000 and 550 mM, respectively. In all experiments, the contractile response or electrophysiological properties were determined at least 30 min after $[Mg^{2+}]_o$ or $[Ca^{2+}]_o$ had been changed. For experiments where the extracellular K^+ was manipulated, solutions with low K^+ (3 mM) and high K^+ (5 mM) were prepared by reducing or increasing the KCl included in NKR, respectively. The effect of extracellular acidosis on myotonia was determined in muscles that were incubated in either a Mg^{2+} free solution containing normal Ca^{2+} or a low Ca^{2+} solution (0.3 mM) containing normal Mg^{2+}. pH was reduced by partly replacing $NaHCO_3$ with NaCl. All solutions were maintained at 30 °C and equilibrated with a mixture of 95% O_2 and 5% CO_2 throughout the experiments (pH ≈ 7.4 with 25 mM $NaHCO_3$).

2.3. Measurement of contractile force

Muscles were dissected out with a piece of bone (proximal end of tibia) still attached to the tendon. At this end of the muscles, the tendon was placed between two metal pins embedded perpendicularly in a vertically oriented plexi-glass plate that also contained the wire electrodes for stimulation. The gap between the pins was too narrow for the piece of bone to pass through them. At the other end of the muscle, a metal hook, connected to a force transducer (Grass FT03), was pushed through the tendon. The transducer could be moved vertically to stretch the muscle. The muscles were lengthened until isometric twitch force production was maximal. Contractions were evoked through field stimulation using constant voltage pulses applied via two platinum wire electrodes passing current across the central part of the muscle. Muscles were activated to contract using pulses of 0.2 ms duration and supra-maximal voltage (24–30 V cm^{-1}). For twitch contractions, muscles were stimulated for 2 s at 5 Hz while tetanic contractions were elicited using 2 s at 30 (soleus) or 1 s at 90 Hz (EDL and soleus). Data was sampled at 1 kHz using an interface and software from Cambridge Electronic Design (Power1401, Signal 4.0, Cambridge, UK). Depending on stimulation frequency, myotonic contractile activity can increase both peak force and cause markedly prolonged relaxation. To encompass both of these changes with myotonia, the area below the force responses, the area under curve (AUC), was determined. For every recording the following was done: The resting force was initially determined and then subtracted from the entire trace yielding only the active force response to stimulation. AUC was then obtained from area below this active force response.