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COMMENTARY

A role for external Ca^{2+} in maintaining muscle contractility in periodic paralysis

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Periodic paralysis is an ion channel disorder of skeletal muscle wherein recurrent episodes of severe weakness are caused by anomalous depolarization of the resting potential, V_{rest}, that persists for minutes to hours, with associated inactivation of voltage-gated sodium channels and loss of fiber excitability (Cannon, 2015). Clinical management of periodic paralysis is symptomatic; that is to say, it minimizes provocative maneuvers that trigger attacks of weakness or use interventions that may reduce attack frequency and severity (Statland et al., 2018). Administration of calcium gluconate has been used empirically in an attempt to hasten recovery from an ongoing attack of weakness (Lehmann-Horn et al., 2004), and in this issue of the *Journal of General Physiology*, Uwera et al (2020) use a mouse model of hyperkalemic periodic paralysis (HyperKPP; Hayward et al., 2008) to systematically assess the efficacy of Ca^{2+} in reducing the susceptibility to high-K^+ induced loss of force and explore the mechanistic basis for protection.

The empirical use of calcium gluconate as abortive therapy for an episode of HyperKPP dates back to the 1950s (Gamstorp, 1956), before it was known that this dominantly inherited disorder is caused by gain-of-function missense mutations in the skeletal muscle isoform of the α subunit of the voltage-gated sodium channel, $Na_V1.4$ (Cannon, 2015; Lehmann-Horn et al., 2004). Controlled trials on the effectiveness of Ca^{2+} in HyperKPP have never been performed, and anecdotal reports describe mixed results (reviewed in Samaha, 1965), although there is one convincing example wherein low serum total Ca^{2+} (<2.1 mM; normal 2.1–2.6) and Mg^{2+} (<0.5 mM, normal 0.6–1.1 mM) secondary to chemotherapy dramatically worsened the symptoms of HyperKPP (Mankodi et al., 2015). To address the question of a role for extracellular Ca^{2+} in modulating susceptibility to weakness in HyperKPP, Uwera et al (2020) performed ex vivo contraction studies and microelectrode measurements of V_m in an established mouse model for HyperKPP ($Na_V1.4$-M1592V knock-in; Hayward et al., 2008).

This study convincingly demonstrates that reducing Ca^{2+} aggravates the susceptibility to high-K^+ induced loss of force in HyperKPP muscle. The force–K_e^+ relation has a midpoint (50% loss) of ∼11–12 mM for HyperKPP muscle in 2.4 mM Ca^{2+}, and this shifted leftward to ∼8 mM in 1.3 mM Ca^{2+}. Moreover, the tetanic force decreased to 20–30% of baseline in 0.3 mM Ca^{2+}, even while K^+ remained at a control level of 4.7 mM (e.g., Fig. 1 in Uwera et al., 2020). In contrast, WT muscle tolerates a 12 mM K^+ challenge in 2.4 mM Ca^{2+} (∼75% of baseline force). At the lowest concentration of Ca^{2+} tested (0.3 mM), WT muscle also had a pronounced loss of force during a high-K challenge (e.g., 50% reduction in 10 mM K^+). These observations led the authors to propose several mechanisms that may contribute to enhanced K^+-sensitivity in low Ca^{2+}: (1) the gating of voltage-dependent channels will have an apparent left (hyperpolarized) shift caused by the reduced screening of negative surface charge on the external face of the plasma membrane in low divalent cation solutions (Hille, 1968); (2) impaired Ca^{2+} release, which is an intrinsic dependence of excitation–contraction on extracellular Ca^{2+} that is not alleviated by substitution with Mg^{2+} (Brum et al., 1988); and (3) enhanced depolarization of V_{rest}. The latter is more complex than appears at first glance because it includes possible contributions from (i) a depolarized shift of the equilibrium potential for K^+; (ii) a hyperpolarized shift of $Na_V1.4$ activation in low divalent cation solutions; and (iii) for HyperKPP fibers gain-of-function defects manifest as impaired inactivation and a hyperpolarized shift of activation. Taken together, it is proposed these effects cause a depolarization-dependent loss of force in low Ca^{2+} that occurs in WT fibers only in when K^+ is increased (e.g., 10 mM), but happens in HyperKPP fibers even in normal K^+ because the $Na_V1.4$ gain-of-function defect increases the propensity for depolarization.

An indirect method was used to assess whether the variations of extracellular Ca^{2+} used in the contractility studies caused a shift in the voltage-dependence of sodium channel availability. The peak amplitude of the Na^+ current was estimated from the maximum dV_m/dt during the upstroke of the action potential (AP; Hodgkin and Katz, 1949). The limitations of using this approach to determine the voltage-dependence of availability are well known: (i) dV_m/dt is proportional to the total sum of ionic currents and therefore is representative of I_{Na} only when the

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relative contribution of other currents is much smaller, as normally occurs during the maximum rate of rise for normal APs, but less so for attenuated APs; and (ii) changes in $[K_e^+]$ are used to vary V_m, but precise control is not possible and so binning of data over a range of V_m is required for the analysis. Even with these caveats, the authors show for WT muscle that reducing extracellular Ca^{2+} from 2.4 to 0.3 mM (with constant Mg^{2+} of 3.1 mM) caused a 5 mV leftward shift of Na^+ channel availability (visually estimated from Fig. 14 A in Uwera et al. (2020)). This shift was prevented by maintaining a constant divalent concentration (2.4 mM Ca^{2+} + 3.1 mM Mg^{2+} → 0.3 mM Ca^{2+} + 5.2 mM Mg^{2+}), consistent with the expectation of a negative surface charge effect. For HyperKPP muscle (Na_V1.4-M1592V), the dV_m/dt technique revealed the previously reported impairment of slow inactivation (Hayward et al., 1997), such that in 2.4 mM Ca^{2+} availability was barely reduced even for the largest test depolarization of −62 mV. Again, in support of a surface charge effect, a large decrease in availability was observed in 0.3 mM Ca^{2+}, consistent with a left shift of gating, and which was also reversed by increased Mg^{2+}.

The relation between low Ca^{2+} and depolarization of V_{rest} follows the same trend observed for low Ca^{2+} and the loss of contractility. Namely, for WT fibers in 4.7 mM K^+, a reduction of Ca^{2+} from 2.4 to 0.3 mM did not cause depolarization or a loss of force. Only when external K^+ was increased to 10 mM did WT fibers have an additional loss of force and depolarization in response to reducing Ca^{2+}. Conversely, HyperKPP muscle always depolarized and had lower tetanic force in response to a reduction of Ca^{2+} (2.4 mM → 0.3 mM), regardless of whether external K^+ was 4.7 or 10 mM. This pattern is qualitatively consistent with their proposed mechanism for loss of contractility in low Ca^{2+}, wherein depolarization (from high K^+ or from the HyperKPP mutation) is necessary to exhibit the Ca^{2+} sensitivity. It would be interesting to test whether the depolarization induced by low Ca^{2+} would be prevented if the total divalent concentration were held constant. These data might provide additional insight on whether the left shift of gating contributes to depolarization, perhaps by enhancing a small subthreshold Na^+ current.

The major finding of this study, that low Ca^{2+} clearly exacerbates the K^+-induced loss of force in HyperKPP, has important translational value to the management of this muscle channelopathy. The robust demonstration of the deleterious effect of low Ca^{2+} would have been impractical to establish in clinical studies or with human biopsy material, which demonstrates the power of high-fidelity mouse models of human disease. Another important point is that both WT and HyperKPP muscle show this Ca^{2+} sensitivity in the proper context. As the authors point out, this implies the exacerbation of weakness for HyperKPP in low Ca^{2+} is not because of a specific mechanism imparted by the Na_V1.4 mutation. Instead, the loss of force in low Ca^{2+} is a fundamental property of skeletal muscle under conditions where V_{rest} is depolarized.

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