Leaky channels make weak muscles

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Mutations in the skeletal muscle voltage-gated calcium channel (CaV1.1) have been associated with hypokalemic periodic paralysis, but how the pathogenesis of this disorder relates to the functional consequences of mutations was unclear. In this issue of the JCI, Wu and colleagues recapitulate the disease by generating a novel knock-in CaV1.1 mutant mouse and use this model to investigate the cellular and molecular features of pathogenesis. They demonstrated an aberrant muscle cell current conducted through the CaV1.1 voltage-sensor domain (gating pore current) that explains an aberrant muscle cell current conducted through the CaV1.1 voltage-sensor domain (gating pore current) that explains an aberrant muscle cell current conducted through the CaV1.1 voltage-sensor domain (gating pore current) that explains an aberrant muscle cell current conducted through the CaV1.1 voltage-sensor domain (gating pore current) that explains an aberrant muscle cell current conducted through the CaV1.1 voltage-sensor domain (gating pore current). A feedback mechanism maintains low CaV1.1-mediated channel activity in the presence of extracellular Ca2+. The authors also examined the molecular and cellular mechanisms underlying the channelopathy.

Ion channels are ubiquitous membrane proteins that confer selective ionic permeability to the plasma membrane or intracellular membranes and enable a variety of important physiological processes, including membrane excitability, synaptic transmission, signal transduction, cell volume regulation, and transcellular ion transport. The vital nature of ion channels is reflected by the existence of inherited disorders caused by mutations in genes that encode these proteins (1–5). These “channelopathies” represent more than 50 human genetic diseases, including several affecting skeletal muscle contraction, such as the periodic paralyses and non-dystrophic myotonia (see “Muscle channelopathies”).

Hypokalemic periodic paralysis

Plasma membrane channels in skeletal muscle are essential for the generation and propagation of action potentials, leading to release of intracellular calcium through the process of excitation–contraction coupling. Ion channel dysfunction can hinder contraction by impairing action potential firing along the membrane. A characteristic symptom of this phenomenon is known as “periodic paralysis,” a form of paroxysmal weakness that occurs in the absence of neuromuscular junction or motor neuron disease. Periodic paralysis is most often thought of as an inherited disease, but certain acquired conditions can produce a similar phenotype. Disturbances in plasma potassium ion concentration often accompany bouts of weakness, and the direction of change has been used to classify the condition as hypokalemic, hyperkalemic, or normokalemic periodic paralysis (6). These conditions are not lethal, because respiratory muscles are spared.

Although the clinical features and inheritance pattern have been known since the first half of the twentieth century, the pathophysiology of periodic paralysis remained mysterious until approximately 30 years ago, when investigators in Germany published their electrophysiological observations on explanted intercostal muscle fibers from subjects with periodic paralysis (7). These studies revealed that resting membrane potential was less nega-
Muscle channelopathies

Voltage-gated sodium channel (NaV1.4; SCN4A)
  - Hyperkalemic periodic paralysis
  - Normokalemic periodic paralysis
  - Hypokalemic periodic paralysis, type 2
  - Paramyotonia congenita
  - Potassium-aggravated myotonia
  - Congenital myasthenic syndrome

Muscle chloride channel (CIC-1; CLCN1)
  - Dominant myotonia congenita (Thomsen’s disease)
  - Recessive generalized myotonia (Becker’s myotonia)

Dihydropyridine receptor/voltage-gated Ca2+ channel (CaV1.1; CACNA1S)
  - Hypokalemic periodic paralysis, type 1
  - Malignant hyperthermia susceptibility
  - Andersen syndrome
  - Ryanodine receptor/Ca2+ release channel (RYR1; RYR1)
  - Malignant hyperthermia susceptibility
  - Central core disease
  - Acetylcholine receptor, α, β, δ, and ε subunits
  - Congenital myasthenic syndromes

Voltage-gated channel structure and function

The sodium and calcium channels expressed in nerve, heart, and muscle belong to a superfamily of ion channels that are gated (opened and closed) by changes in membrane potential (9). The main pore-forming subunit of an L-type voltage-gated calcium channel (CaV1.1), and less commonly by SCN4A mutations (2). Although both syndromes are associated with notable allelic heterogeneity, CACNA1S and SCN4A mutations associated with HypoPP are clustered within voltage-sensor domains. A brief digression on the topic of voltage-gated channel structure and function will help clarify the pathophysiological significance of this mutation clustering.

Formation of the pore domain (14). The current flowing through the voltage-sensor pore (also known as the gating pore) was termed the “omega” or “gating pore” current (Figure 1B).

Because these S4 segment histidine substitutions created unnatural channels, astute researchers investigating the functional consequences of channelopathy-associated mutations recognized that this mechanism might explain the pathophysiology of HypoPP. Specifically, sodium channel mutations associated with HypoPP that replace S4 segment arginine residues create channels that conduct an anomalous inward current at resting membrane potentials (15–18). The in vivo relevance of this mechanism was demonstrated subsequently using a mouse model of the disease (NaV1.4 R669H knock-in), in which an anomalous inward current was detected in muscle cells at hyperpolarized potentials (19). These investigations offered a molecular explanation for HypoPP caused by sodium channel mutations, but did not address what happens with the more common calcium channel mutations. Furthermore, prior studies of mutations engineered in human CaV1.1 did not reveal a consistent and compelling pattern of channel dysfunction that would explain the phenotype, in part because of the difficulty of expressing this channel in heterologous cell systems.

Calcium channel mutant mice

To address the pathogenesis of HypoPP caused by CACNA1S mutations, Wu and colleagues in the laboratory of Stephen Cannon report in this issue of the JCI the inves-
These findings offered a mechanistic link between calcium channel mutation and attacks of muscle weakness. Anomalous gating pore current creates a precarious balance between the inward and outward currents that maintain the membrane potential. Factors such as hypokalemia that transiently depress potassium currents, which are required to maintain a normal resting membrane potential, can render the muscle severely depolarized and, consequently, inexcitable owing to inactivation of sodium channels.

Now that a common mechanism of evoking gating pore current has been established in HypoPP, several new and intriguing questions emerge. Can aberrant gating pore current be selectively blocked, and would this prevent attacks of paralysis? Do carbonic anhydrase inhibitors such as acetazolamide and dichlorphenamide, which are effective treatments for HypoPP regardless of genotype, affect gating pore current directly or indirectly? Are there triggering factors that act specifically by potentiating gating pore current? Finally, what is the basis for vacuolar myopathy, and why is there a sex-biased penetrance of mutations? These and other important questions can now be addressed using the mouse model developed by Wu and colleagues.

Although HypoPP was the first channelopathy for which aberrant gating pore current was implicated in disease pathogenesis, many other S4 mutations in voltage-gated sodium, calcium, and potassium channels have been found in other inherited disorders of membrane excitability, including epilepsy and cardiac arrhythmia syndromes (21). Are some of these conditions also “gating poreopathies”? A recent study demonstrating that an S4 segment mutation in the cardiac sodium channel (NaV1.5 R219H) associated with dilated cardiomyopathy evokes an aberrant proton current (22) suggests that this may be a more widespread phenomenon.
Low levels of phosphate can disrupt bone ossification and predispose to fractures. FGF23 is one of the major determinants of phosphate homeostasis, acting to increase urinary phosphate excretion. However, the regulation of FGF23 is incompletely understood. In this issue of the JCI, Smith et al. show that the cleaved form of αKlotho, the membrane-bound form of which is an FGF23 coreceptor, serves as a novel endocrine regulator of phosphate homeostasis, capable of inducing FGF23 production in osteocytes.

Phosphate is a critical component of bone, and it serves numerous biological functions in the synthesis of DNA and membrane lipids, protein modifications, energy metabolism, and second messenger formation. FGF23 and parathyroid hormone (PTH) are the major regulators of phosphate homeostasis (1, 2). Both hormones increase urinary phosphate excretion by reducing proximal tubular expression of the sodium-dependent phosphate cotransporters, NPT2a and NPT2c, thereby lowering serum phosphate levels. However, in contrast to FGF23, which decreases serum levels of 1,25-dihydroxyvitamin D (1,25D) by inhibiting renal 1α-hydroxylase and stimulating 24-hydroxylase, PTH increases renal production of 1,25D, which consequently inhibiting renal 1α-hydroxylase, PTH increases renal production of 1,25D, which consequently enhances the absorption of phosphate (and calcium) from the intestinal tract (3).

FGF23 belongs to the subfamily of endocrine FGFs and mediates its phosphate-regulating actions in the kidney through FGF receptors (FGFRs), most prominently FGFR1. These actions require the transmembrane form of αKlotho (mKL), which acts as the coreceptor that enhances the binding affinity of FGF23 to different FGFRs (4, 5). Ablation of αKlotho in mice leads to severe hyperphosphatemia, elevated levels of 1,25D, hypercalcemia, diffuse soft tissue calcifications, accelerated aging, and premature death (6). These findings are largely indistinguishable from those observed in FGF23 loss-of-function models (7, 8).

Osteocytes are the primary cellular source of FGF23 after the fetal period (9), but little is known about their regulation and secretion. Although discovery of the molecular causes of monogenic hypophosphatemic rickets disorders has helped to identify a set of bone-derived proteins that decrease FGF23 production, the mechanisms of how these inhibitors suppress FGF23 remain largely unknown. Furthermore, despite our knowledge that changes in dietary phosphate intake can alter FGF23 levels in healthy individuals (10, 11), how serum phosphate or phosphate balance is sensed and how this signal is converted to the osteocyte to alter FGF23 expression remains completely unknown.

A novel regulator of FGF23 synthesis In this issue of the JCI, Smith et al. provide intriguing new findings about the