Cell, Vol. 80, 681–686, March 10, 1995, Copyright © 1995 by Cell Press

Overexcited or Inactive: Ion Channels in Muscle Disease

Review

Eric P. Hoffman,* Frank Lehmann-Horn,† and Reinhardt Rüdel†
*Departments of Molecular Genetics and Biochemistry, Human Genetics, and Pediatrics
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15261
†Department of Physiology
University of Ulm
D-89069 Ulm
Federal Republic of Germany

Overview

All animals are equipped with the capacity for rapid motor response that excitable cells—nerve and muscle—mediate. Voltage-sensitive ion channels on the surface membranes allow the cells to generate brief and reversible alterations of the voltage (action potentials) along the surface of these cellular cables. Ion channels, notably those conducting Na^+, Ca^{2+}, and K^+, are large proteins with membrane-spanning pores that are regulated by both voltage sensors and gates in the same polypeptide. The critical role of ion channels in all excitable cells, and the complex interplay of activation and inactivation of the different ion currents underlying the action potential, has led many to suggest that inherited defects of voltage-sensitive channels could be incompatible with life.

This view dramatically changed four years ago when the gene coding for the major α subunit of the human muscle sodium channel was isolated, localized to chromosome 17q, and found to show genetic linkage to hyperkalemic periodic paralysis (hyperPP) (Fontaine et al., 1990). Patients with this hereditary disorder show episodic loss of excitability of skeletal muscle. Soon after, two additional muscle disorders, paramyotonia congenita (PC) and potassium-aggravated myotonia (PAM), were linked to this same locus (Koch et al., 1991; Ptáček et al., 1991a; Ebers et al., 1991). The most common inherited disorder of horses was then found to be linked to the equine homolog of the same gene (Rudolph et al., 1992).

Over the last two years, two additional human hereditary muscle diseases showing membrane excitation abnormalities, myotonia congenita (MC) and hypokalemic periodic paralysis (hypoPP), were linked to genes encoding the chloride and calcium ion channels (Koch et al., 1992; Fontaine et al., 1994). As the amino acid changes underlying these disorders have been identified and characterized, this disease-based research has complemented ongoing in vitro mutagenesis and electrophysiological studies in the search for structure–function relationships in the channel molecules. Thus far, accumulated knowledge has resulted in a greater understanding of most facets of these disorders, from basic molecular pathophysiology to better patient diagnosis and management.

The Muscle Sodium Channel Diseases

The three hereditary muscle diseases in this group (hyperPP, PC, and PAM; see Rüdel et al., 1993, for review) are all inherited as dominant traits. Interestingly, the pathophysiology includes both hyper- and hypoexcitability, frequently in the same patient at different times. Fortunately for the patients, the presenting symptoms of muscle stiffness (myotonia) and muscle weakness (paralysis) are intermittent. The attacks are typically triggered by exposure to cold (PC), ingestion of potassium-rich food (PAM), or rest after a heavy work load (hyperPP). The symptoms usually disappear spontaneously within an hour or so, and attacks can be prophylactically treated with medication or avoided by behavior modification (i.e., avoidance of high potassium foods or vigorous exercise). Nonetheless, symptoms can pose an unwelcome burden on the activites of daily living for these patients.

Prior to molecular studies, electrophysiological experiments on excised muscle specimens from patients revealed the presence of a noninactivating Na^+ current (Lehmann-Horn et al., 1981, 1987). If these results reflected the primary defect, then the key symptoms of stiffness and weakness could be caused by the same mechanism: a sustained but variable depolarization of the muscle fiber membranes. To determine whether the Na^+ changes were the site of the primary biochemical defect, genetic linkage analyses were done using the cloned human sodium channel α subunit gene as a candidate. Both hyperPP and PC were found to be tightly genetically linked to the sodium channel gene on 17q (Fontaine et al., 1990; Ptáček et al., 1991a; Koch et al., 1991; Ebers et al., 1991). These reports were followed by a series of publications documenting single base changes in the sodium channel gene. The mutant genes express abnormal channel proteins with single amino acid changes that exert a dominant effect on the muscle action potential in both human and horse patient muscle (change-of-function) (Rojas et al., 1991; Ptáček et al., 1991b; Rudolph et al., 1992; McClatchey et al., 1992; Lerche et al., 1993; see Rüdel et al., 1993) (Figure 1, top).

The sodium channel α subunit, a 260 kDa glycoprotein with about 1,800 amino acids, has four homologous domains, each containing 225–325 amino acids (Figure 1, top). Each of the four domains has at least six hydrophobic segments (S1–S6) that are thought to traverse the plasma membrane (see Catterall, 1988, for review). Between segments S5 and S6 of each domain, the extracellular loop is thought to dip into the plasma membrane and participate in the formation of the pore, as has been shown more directly for the analogous region in potassium channels. The S4 hydrophobic helices have a high density of charged amino acids, and these charges are thought to participate in sensing voltage changes across the plasma membrane (voltage sensor). Another part of the protein to which a certain function has been assigned is the intra-

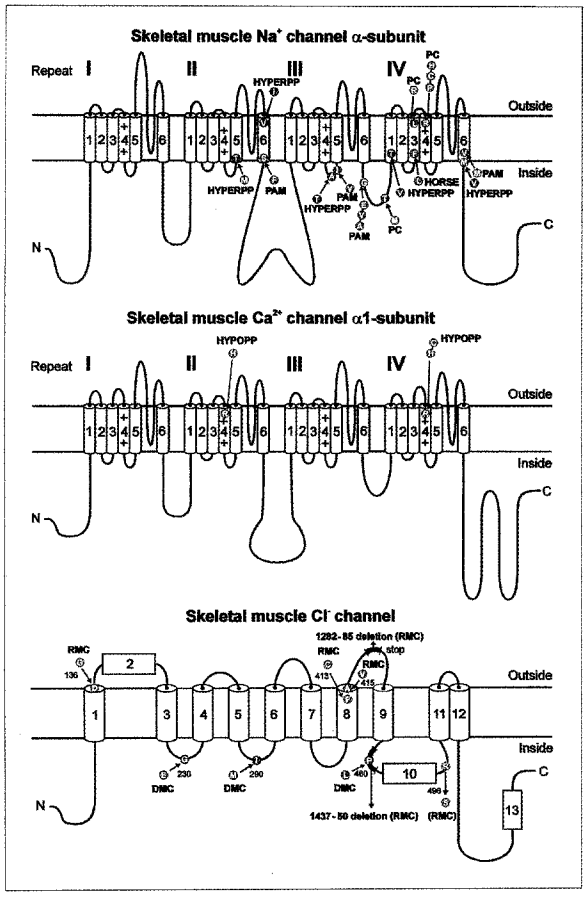

Figure 1. Mutations Identified in Muscle Ion Channels

The chloride channel model is as proposed by Mitrovic et al. (1994), as modified by Middleton et al. (1994).

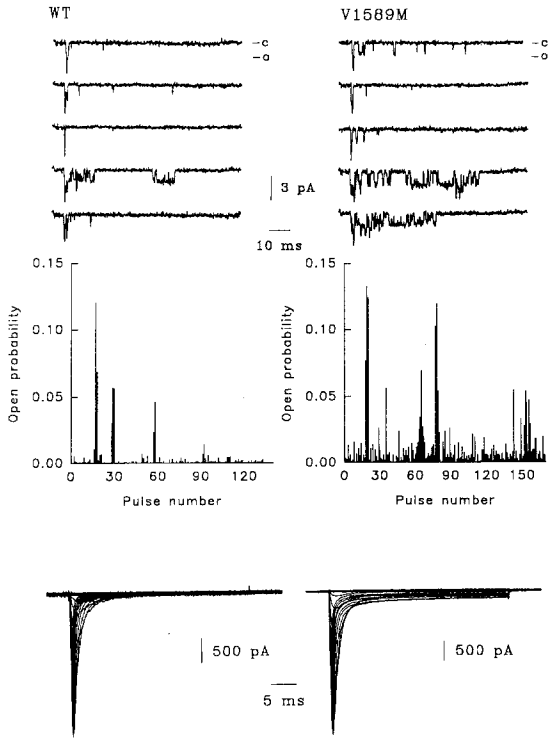

Figure 2. Faulty Sodium Channel Inactivation in a Potassium-Aggravated Myotonia Patient Mutation Expressed In Vitro

Shown are patch-clamp data from normal (wild-type) and PAM (V1589M) sodium channels expressed in HEK293 cells. Both single-channel recordings during five depolarization pulses each (top) and channel open probabilities for late reopenings as a function of pulse number (center) show the tendency for late reopenings of the mutant channel. Current-versus-time plots (bottom) recorded in the whole-cell mode show a persistent inward Na^+ current with the mutant channel.

cellular loop connecting domain III-S6 with domain IV-S1. Most likely, this part of the protein acts as the inactivation gate of the channel by swinging in and out of the intracellular opening of the pore to regulate ion flow through the channel (Armstrong et al., 1973).

Of the 16 disease-causing amino acid changes identified to date, five result in PC, five in hyperPP, and six in PAM. At first glance, no obvious correlations can be made between the location or type of amino acid change and the clinical phenotype (Figure 1, top). However, none of the mutations are located in domain I, and only domains II and IV contain changes in transmembrane segments. The transmembrane mutations are never located in the center of the membrane, but instead are near the extracellular or intracellular face. Four amino acid changes have been detected in the part of the protein thought to act as the inactivation gate (III-S6 to IV-S1 linker) (Figure 1, top). Three of these mutations generate different amino acid changes for one of a pair of glycines known to be essential for proper channel inactivation (the so-called hinge site; West et al., 1992). The more the substitution differs from the normal glycine (longer side chains, greater charge, or both), the greater the membrane hyperexcitability and the more severe the clinical symptoms of PAM (Lerche et al., 1993). A change from glycine to glutamic acid, an amino acid with a long side chain, causes permanent myotonia (the most severe form of PAM), and valine, an amino acid with a side chain of intermediate size, causes moderate myotonia, while alanine, with a short side chain, results in a benign, often subclinical form of myotonia. Similar associations of the severity of amino acid change with severity of clinical symptoms have been seen in cold-sensitive PC patients in whom a charged arginine in domain IV-S4 is changed to a histidine, cysteine, or proline (Ptáček et al., 1991b; Wang et al., 1995). A patient having the proline change is a clinically severe PC patient who shows toe walking and marked cold sensitivity of both her myotonia and her subsequent weakness (Wang et al., 1995).

Expression of normal and mutant sodium channels in heterologous systems has shown that all the mutant channels studied to date have impaired inactivation of the Na^+ current (Figure 2) (Cannon and Strittmatter, 1993; Chahine et al., 1994; Mitrovic et al., 1994). Surprisingly, mutations leading to the different clinical phenotypes showed similar electrophysiological results: mutations present in PC, PAM, or hyperPP patients all showed an increased steady-state current, owing to a shift in the equilibrium between two gating modes of inactivation. The twelve amino acid

substitutions on the cytoplasmic surface of the channel probably alter N-type (ball and chain) inactivation of the channel; however, it is not clear why the five substitutions on the extracellular surface also affect fast inactivation. The extracellular surface mutations may have uncovered a region of the sodium channel that is important for inactivation that was not previously identified via in vitro mutagenesis studies. The preponderance of amino acid changes affecting inactivation may simply be a consequence of the dominant inheritance pattern seen in all sodium channel diseases: faulty inactivation would be expected to have a dominant effect on the myofiber. The mutant channels have change-of-function alterations that lead to a persistent inward Na^+ current, which in turn depolarizes the cell, first activating and then inactivating the normal sodium channels coexpressed in the myofibers of a heterozygous patient. On the other hand, mutations reducing activation or conductance would be expected to show a recessive inheritance pattern more consistent with loss-of-function of channel activity. Patients with recessively inherited sodium channelopathies have not been identified; however, it is likely that such complete loss-of-function of the muscle sodium channel is not compatible with life.

Most hyperPP, PC, and PAM patients are quite sensitive to elevations of systemic potassium or to cold temperatures. Surprisingly, the mutant channels do not seem to show a strong response to high extracellular potassium or low temperature in voltage-clamped membrane patch experiments. Perhaps factors that trigger attacks of myotonia or paralysis in vivo exert their effect indirectly, either by causing a persistent Na^+ current via K^+-induced membrane depolarization in myofiber t tubules (hyperPP, PAM) (Cannon et al., 1993) or via cold-induced depolarization due to reduced sodium pump activity (PC). Alterations of the interaction of mutant channels with their environment (membrane or associated proteins) are also hypothesized. These effects may be seen only in mature myofibers and not in channels expressed in heterologous cell systems. Also puzzling is the apparent fine line between attacks of hyperexcitability (myotonia) and hypoexcitability (paralysis) in patients. The same amino acid change can alternately induce attacks of severe myotonia or severe paralysis, often in the same patient (the horse disease is a particularly dramatic example [Spier et al., 1990]). Current pathophysiological models suggest that mild depolarizations of the myofiber membrane (10–20 mV) from impaired inactivation of mutant channels serve to make the membrane hyperexcitable, with excessive contractions induced by voluntary muscle activity, experienced by the patient as muscle stiffness. On the other hand, when the depolarizations caused by the abnormal channels are more severe (20–30 mV), both the normal and mutant sodium channels become fixed in a state of inactivation, causing weakness or paralysis in patients. Thus, the fine line between myotonia and paralysis seen clinically may simply reflect subtle differences in the severity of membrane depolarization (10–20 mV versus 20–30 mV). Fortunately in humans, the heart muscle (which uses a different sodium channel gene) and the diaphragm (which uses the same gene, but seems refractory to attacks) are spared; hence, attacks are not life-threatening in humans. Some affected horses, however, die from attacks as a result of shock or ischemia.

The cellular and physiological factors dictating the onset and severity of attacks in hyperPP are other important areas of continued investigation. The relationships among exercise, systemic potassium, catecholamines, and other factors influencing muscle metabolism are currently being studied in horses with hyperPP. The horse has also been used to show the first correlation of levels of mutant mRNA relative to normal RNA as a likely determinant of clinical severity in dominantly inherited disease (Zhou et al., 1994). Another interesting hypothesis concerns the possible key role of K^+ diffusion in the extensive t tubule system of myofibers in the development of paralytic attacks (Cannon et al., 1993). Finally, molecular mutation and pharmacology studies are underway in human patients to determine whether drug efficacy can be correlated with specific amino acid changes of the channel.

Muscle Calcium Channel Disease

When one considers calcium channels in skeletal muscle fibers, one initially thinks of excitation–contraction coupling, not the action potential itself. In hypokalemic periodic paralysis (hypoPP), clinical electrophysiological studies had established that the problem resided in the failure of membrane excitation. Thus, it was surprising when this disorder was genetically linked to a muscle calcium channel, specifically the $\alpha 1$ subunit of the skeletal muscle dihydropyridine (DHP) receptor (voltage-sensitive calcium channel conducting the slow L-type Ca^{2+} current), a channel involved much more in excitation–contraction coupling than action potentials (Fontaine et al., 1994).

HypoPP is clinically very similar to hyperPP, with serum K^+ levels as the major distinguishing laboratory finding: during an episode of paralysis, hypoPP patients have low levels of serum K^+ (sometimes as low as 1.8 mM; normal levels are 3.5–5.0 mM), but levels in hyperPP patients are high (up to 7.0 mM). Additionally, hypoPP patients never show signs of myotonia, and episodes of paralysis tend to last longer and can be triggered by hormones such as insulin and adrenaline and by carbohydrate intake.

The skeletal muscle DHP receptor is located primarily in the membrane of the transverse tubular system and consists of five subunits: $\alpha 1$, $\alpha 2/\delta$, β, and γ (Catterall, 1988). The $\alpha 1$ subunit (Figure 1, middle) contains the receptor for dihydropyridines and other calcium channel antagonists as well as the ion-conducting pore. It is assumed to form physical associations with the calcium release channel of the sarcoplasmic reticulum (the ryanodine receptor) and thus translate membrane potential changes to intracellular calcium release (excitation–contraction coupling). Although structurally very similar to the sodium channel α subunit, several substructures have functions that are thought to be different.

Soon after the demonstration of linkage of hypoPP to the gene encoding DHP receptor $\alpha 1$, point mutations were detected that predict substitutions of histidine for arginine in II-S4 and IV-S4 (Jurkat-Rott et al., 1994; Ptáček et al., 1994) (Figure 1, middle). The functional consequences of

these mutations are just now being explored. Whole-cell recordings from myotubes cultured from the muscles of patients with the mutation in IV-S4 revealed a reduction of the DHP-sensitive L-type current to 30% of the normal amplitude (Lehmann-Horn et al., 1995). Myotubes with the mutation in II-S4 show a dramatic shift in the inactivation curve to more negative potentials by 40 mV, driving channels to the inactivated state at low membrane potentials (Sipos et al., 1995). Although the mutations reduce the number of positive charges in voltage sensors, they do not affect channel activation. There is a striking parallel in the sodium channel diseases, in that mutations of the analogous domain IV-S4 region of the sodium channel α subunit modify channel inactivation but not voltage-dependent activation (Chahine et al., 1994). However, electrophysiological studies of the mutant sodium channels have clearly documented the expected change of function (persistent inward Na^+ current), consistent with the dominant inheritance pattern of the disorder. The preliminary electrophysiology studies of the calcium channel mutations suggest instead a loss of function (decreased Ca^{2+} current): this biochemical defect does not match the dominant inheritance pattern of hypoPP. As has occurred with the chloride channel (see below), the disease inheritance patterns may help define the larger order structure of the muscle calcium channel: the dominant inheritance may indicate that the functional channel could be composed of multiple α subunits, with amino acid changes in hypoPP causing dominant negative alterations of the channel.

How the inactivation of the L-type Ca^{2+} current is related to hypokalemia-induced attacks of muscle weakness is still a mystery. The hypokalemia-induced membrane depolarization observed in excised muscle fibers might reduce Ca^{2+} release indirectly by inactivating sodium channels, or directly by affecting the voltage control of the channel. The triggering of attacks by insulin and adrenaline may similarly be direct or indirect: both insulin and adrenaline have been shown to hyperpolarize muscle membranes (Li and Sperelakis, 1993). The markedly decreased phenotypic expression of the mutations in women is not understood and is likely complex; however, this observation may point to an effect of hormones on the muscle calcium channel.

Muscle Chloride Channel Diseases

Patients who show pure myotonia, with little sensitivity to cold or potassium levels, are usually given the diagnosis of MC. Both dominant and recessive inheritance patterns have been frequently found, with the dominant form taking the name of Thomsen (a physician noting the disorder in his own family), while the recessive form is called Becker myotonia. It was not previously clear whether these disorders represented different mutations of the same gene or different genes. This issue was settled with the finding of genetic linkage of both disorders to the gene coding for the adult human muscle chloride channel (Koch et al., 1992).

In both dominant and recessive MC, the predominant symptom is muscle stiffness due to uncontrolled bursts of spontaneous action potentials. The myotonia is most severe during voluntary muscle activity following a period of rest. Pharmacological experiments on muscles from an animal model, the myotonic goat, showed a reduction of the Cl^- conductance of the muscle fiber membranes (Bryant, 1969). A similar Cl^- conductance defect was later found in both dominant and recessive MC in humans (Lipicky et al., 1971; Rüdel et al., 1988). The genetic linkage analysis of MC pedigrees was made possible by the expression cloning of the chloride channel from the Torpedo marmorata electric organ (ClC-0), a channel that shows very little homology to other ion channels (see Steinmeyer et al., 1994).

The ClC-1 protein is responsible for the high resting membrane conductance of skeletal muscle cells. The functional channel is thought to be a homotetramer, with each subunit having approximately 1000 amino acids (Lorenz et al., 1994). To date, nine point mutations in exons, a base change affecting a splice consensus site, and two deletions have been described in the human chloride channel gene (Figure 1, bottom); however, mutations have been found in only about 15% of unrelated MC patients studied to date. Three of the point mutations cause dominantly inherited Thomsen myotonia (George et al., 1993), with one of them occurring in Thomsen's own family (Steinmeyer et al., 1994). The remainder of the mutations appear to cause the recessive Becker form. It is important to note, however, that only 19 of 117 unrelated Becker patients are mutation positive, and only 5 of the 19 (25%) have had mutations identified on both *ClC-1* alleles (Koch et al., 1992; Heine et al., 1994). Of these 5, 3 were homozygous for a single mutation, while the remaining 2 have been shown to be compound heterozygotes. All other patients with the recessive form have had only one putative mutation identified and are presumed to have an unidentified mutation on their other gene. The very large number of presumed compound heterozygotes is worrisome and requires further investigation. Moreover, some presumed disease-causing amino acid substitutions have been found in normal individuals, which leads one to ask whether these are harmless polymorphisms or actual mutations that cause recessive or dominant disease when combined with other base changes either in *cis* or in *trans*. Clearly, genotype–phenotype correlations in this disease are at an early stage, and these studies are complicated by both the double inheritance patterns and the relatively mild nature of the disease.

ClC-1 forms a functional channel when it is expressed in Xenopus oocytes. Voltage-clamp analyses have shown that it conducts over the whole physiological voltage range, with inward-going rectification in the negative potential domain. Its single-channel conductance, estimated from noise analysis, is very low, near 1 pS (Pusch et al., 1994). The large macroscopic Cl^- conductance, therefore, must result from a high channel density in the membrane. When ClC-1 channels containing amino acid substitutions leading to MC were expressed in oocytes, the Cl^- currents were completely missing (Steinmeyer et al., 1994). These data clearly suggest that many of the changes lead to a loss of function of the chloride channel.

Coexpression of wild-type ClC-1 with different mutant loss-of-function ClC-1 channels yielded interesting results that may explain why MC can be alternatively transmitted as either a dominant or a recessive trait (Steinmeyer et al., 1994). The functional chloride channel is most likely a homo-oligomer of ClC-1 subunits (Middleton et al., 1994), and the ClC-1 protein harboring the dominant amino acid change may oligomerize with the normal ClC-1 coexpressed in the same cell. The mutant subunit may then destroy the function of the entire complex; such dominantly inherited dominant negative mutations are common in proteins that form functional oligomers. Thus, both the recessive and dominant forms of the disease probably lead to a complete or near-complete loss of function of the Cl^- conductance. This conclusion is supported by experiments with myotonia-generating drugs, which have shown that blockage of 50% of the physiological Cl^- current is not sufficient to produce myotonic activity (Palade and Barchi, 1977).

Conclusion

The ion channel mutations clearly do not permanently and irreversibly alter the capability of a patient to conduct action potentials in muscle; indeed, the mutation causing periodic paralysis in quarter horses has been shown to impart a distinct competitive advantage in the show ring (Naylor, 1994)! These mutations probably reflect an ascertainment bias: there are probably many more mutations that occur in muscle ion channels, but these are probably incompatible with life. Importantly, these mutations permit proper cell function until extra- or intracellular conditions (shifts in pH, electrolyte concentrations, temperature) exacerbate the molecular pathology, leading to episodic and catastrophic failure of channel function. The ion channel abnormalities illuminate the critical nature of the gating of voltage-dependent channels in intact muscle, and the possible effect of myofiber mileau on gating.

Electrophysiological studies of the abnormal channels in excised muscle specimens or in heterologous expression systems suggest that our current knowledge of channel structure–function relationships is far from comprehensive. This is vividly illustrated by the finding of mutations in the important S4 transmembrane domain: this region has been clearly demonstrated in potassium and sodium channels to function as a voltage sensor for channel activation, yet disease-causing mutations in the analogous region of domain IV of both the sodium and the calcium channel indicate that this region is important for inactivation as well.

Important questions remain. What are the consequences of cold temperatures and systemic potassium levels on the mutant sodium channels containing single amino acid changes? The effects seen in vivo have not been well reproduced in in vitro expression studies, yet such effects define these clinical disorders. Why do all the mutations seem predominantly to alter inactivation of sodium channels, rather than other channel subfunctions? Is this purely a consequence of patient ascertainment (i.e., viable mutations)? The chloride and calcium channelopathies have been studied much less extensively, and even more questions exist. Particularly fascinating in the chloride channelopathies is the effect of exercise on membrane excitability, and the possible dominant negative effect of the mutant proteins on the normal channels in the same cell. The chloride channel diseases are unprecedented in human molecular genetics in the finding of only one mutant allele in the vast majority of recessive Becker myotonia cases. Do these patients truly represent complete loss of function of the chloride channels, and if so, where are the other mutations? Finally, the molecular basis for the majority (85%) of pure myotonia patients remains to be identified, an observation that suggests that the candidate gene approach may continue to be fertile ground for research in the ion channelopathies.

Despite the many questions, it is clear that human disease has served as an informative experimental system to study ion channel function. This system should continue to complement the ongoing methodical in vitro studies using more controllable and established heterologous assays.

References

Armstrong, C. M., Bezanilla, F., and Rojas, E. (1973). Destruction of sodium conductance in squid axons perfused with pronase. J. Gen. Physiol. *62*, 375–391.

Bryant, S. H. (1969). Cable properties of external intercostal muscle fibres from myotonic and nonmyotonic goats. J. Physiol. *204*, 539–550.

Cannon, S. C., and Strittmatter, S. M. (1993). Functional expression of sodium channel mutations identified in families with periodic paralysis. Neuron *10*, 317–326.

Cannon, S. C., Brown, R. H., Jr., and Corey, D. P. (1993). Theoretical reconstruction of myotonia and paralysis caused by incomplete inactivation of sodium channels. Biophys. J. *65*, 270–288.

Catterall, W. A. (1988). Structure and function of voltage–sensitive ion channels. Science *242*, 50–61.

Chahine, M., George, A. L., Jr., Zhou, M., Ji, S., Sun, W., Barchi, R. L., and Horn, R. (1994). Sodium channel mutations in paramyotonia congenita uncouple inactivation from activation. Neuron *12*, 281–294.

Ebers, G. C., George, A. L., Barchi, R. L., Ting-Passador, S. S., Kallen, R. G., Lathrop, G. M., Beckmann, J. S., Hahn, A. F., Brown, W. F., Campbell, R. D., et al. (1991). Paramyotonia congenita and hyperkalemic periodic paralysis are linked to the adult muscle sodium channel gene. Ann. Neurol. *30*, 810–816.

Fontaine, B., Khurana, T. S., Hoffman, E. P., Bruns, G. A. P., Haines, J. L., Trofatter, J. A., Hanson, M. P., Rich, J., McFarlane, H., Yasek, D. M., Romano, D., Gusella, J. F., and Brown, R. H. (1990). Hyperkalemic periodic paralysis and the adult muscle sodium channel α-subunit gene. Science *250*, 1000–1003.

Fontaine, B., Vale-Santos, J. M., Jurkat-Rott, K., Reboul, J., Plassart, E., Rime, C. S., Elbaz, A., Heine, R., Giumaraes, J., Weissenbach, J., et al. (1994). Mapping of the hypokalemic periodic paralysis (hypoPP) locus to chromosome 1q31–32 in three European families. Nature Genet. *6*, 267–272.

George, A. L., Crackover, M. A., Abdalla, J. A., Hudson, J. A., and Ebers, G. C. (1993). Molecular basis of Thomsen's disease (autosomal dominant myotonia congenita). Nature Genet. *3*, 305–310.

Heine, R., George, A. L., Pika, U., Deymeer, F., Rüdel, R., and Lehmann-Horn, F. (1994). Proof of a non-functional muscle chloride channel in recessive myotonia congenita (Becker) by detection of a 4 base pair deletion. Hum. Mol. Genet. *3*, 1123–1128.

Jurkat-Rott, K., Lehmann-Horn, F., Elbaz, A., Heine, R., Gregg, R. G., Hogan, K., Powers, P., Lapie, P., Vale-Santos, J. E., Weissenbach, J., and Fontaine, B. (1994). A calcium channel mutation causing hypokalemic periodic paralysis. Hum. Mol. Genet. *3*, 1415–1419.

Koch, M. C., Ricker, K., Otto, M., Grimm, T., Bender, K., Zoll, B., Lehmann-Horn, F., Rüdel, R., Harper, P. S., and Hoffman, E. P. (1991). Linkage data suggesting allelic heterogeneity for paramyotonia congenita and hyperkalemic periodic paralysis. Hum. Genet. *88*, 71–74.

Koch, M. C., Steinmeyer, K., Ricker, K., Wolf, F., Otto, M., Zoll, B.,

Lehmann-Horn, F., Grzeschik, K. H., and Jentsch, T. J. (1992). Tight linkage of recessive and dominant forms of human myotonia to skeletal muscle chloride channel gene. Science *257*, 797–800.

Lehmann-Horn, F., Rüdel, R., Dengler, R., Lorkovic, H., Haass, A., and Ricker, K. (1981). Membrane defects in paramyotonia congenita with and without myotonia in a warm environment. Muscle Nerve *4*, 396–406.

Lehmann-Horn, F., Kuther, G., Ricker, K., Grafe, P., Ballanyi, K., and Rüdel, R. (1987). Adynamia episodica hereditaria with myotonia: a non-inactivating sodium current and the effect of extracellular pH. Muscle Nerve *10*, 363–374.

Lehmann-Horn, F., Sipos, I., Jurkat-Rott, K., Heine, R., Brinkmeier, H., Fontaine, B., Kovacs, L., and Melzer, W. (1995). Altered calcium currents in human hypokalemic periodic paralysis myotubes expressing mutant L-type calcium channels. Proc. Soc. Gen. Physiol., in press.

Lerche, H., Heine, R., Pika, U., George, A. L., Mitrovic, N., Browatzki, M., Weiss, T., Bastide-Rivet, M., Franke, C., LoMonaco, M., Ricker, K., and Lehmann-Horn, F. (1993). Human sodium channel myotonia: slowed channel inactivation due to substitutions for a glycine within the III/IV linker. J. Physiol. *470*, 13–22.

Li, K. X., and Sperelakis, N. (1993). Isoproterenol- and insulin-induced hyperpolarization in rat skeletal muscle. J. Cell. Physiol. *157*, 631–636.

Lipicky, R. J., Bryant, S. H., and Salmon, J. H. (1971). Cable parameters, sodium, potassium, chloride, and water content and potassium efflux in isolated external intercostal muscle of normal volunteers and patients with myotonia congenita. J. Clin. Invest. *50*, 2091–2103.

Lorenz, C., Meyer-Kleine, C., Steinmeyer, K., Koch, M. C., and Jentsch, T. J. (1994). Genomic organization of the human muscle chloride channel ClC-1 and analysis of novel mutations leading to Becker-type myotonia. Hum. Mol. Genet. *3*, 941–946.

McClatchey, A. I., Van den Bergh, P., Pericak-Vance, M. A., Raskind, W., Verellen, C., McKenna-Yasek, D., Rao, K., Haines, J. L., Bird, T., Brown, R. H., and Gusella, J. F. (1992). Temperature-sensitive mutations in the III-IV cytoplasmic loop region of the skeletal muscle sodium channel gene in paramyotonia congenita. Cell *68*, 769–774.

Middleton, R. E., Pheasant, D. J., and Miller, C. (1994). Purification, reconstitution, and subunit composition of a voltage-gated chloride channel from Torpedo Electroplax. Biochemistry *33*, 13189–13198.

Mitrovic, N., George, A. L., Jr., Heine, R., Wagner, S., Pika, U., Hartlaub, U., Zhou, M., Lerche, H., Fahlke, C., and Lehmann-Horn, F. (1994). Potassium-aggravated myotonia: the V1589M mutation destabilizes the inactivated state of the human muscle sodium channel. J. Physiol. *478*, 395–402.

Naylor, J. M. (1994). Selection of quarter horses affected with hyperkalemic periodic paralysis by show judges. J. Am. Vet. Med. Assoc. *204*, 926–928.

Palade, P. T., and Barchi, R. L. (1977). Characteristics of the chloride conductance in muscle fibers of the rat diaphragm. J. Gen. Physiol. *69*, 325–335.

Ptáček, L. J., Trimmer, J. S., Agnew, W. S., Roberts, J. W., Petajan, J. H., and Leppert, M. (1991a). Paramyotonia congenita and hyperkalemic periodic paralysis map to the same sodium channel gene locus. Am. J. Hum. Genet. *49*, 851–854.

Ptáček, L. J., George, A. L., Griggs, R. C., Tawil, R., Kallen, R. G., Barchi, R. L., Robertson, M., and Leppert, M. F. (1991b). Identification of a mutation in the gene causing hyperkalemic periodic paralysis. Cell *67*, 1021–1027.

Ptáček, L., Tawil, R., Griggs, R. C., Engel, A., Layzer, R. B., Kwieciński, H., McManis, P. G., Santiago, L., Moore, M., Fouad, G., Bradley, P., and Leppert, M. F. (1994). Dihydropyridine receptor mutations cause hypokalemic periodic paralysis. Cell *77*, 863–868.

Pusch, M., Steinmeyer, K., and Jentsch, T. J. (1994). Low single channel conductance of the major skeletal muscle chloride channel, ClC-1. Biophys. J. *66*, 149–152.

Rojas, C. V., Wang, J., Schwartz, L., Hoffman, E. P., Powell, B. R., and Brown, R. H., Jr. (1991). A methionine to valine mutation in the skeletal muscle sodium channel α-subunit in human hyperkalemic periodic paralysis. Nature *354*, 387–389.

Rüdel, R., Ricker, K., and Lehmann-Horn, F. (1988). Transient weakness and altered membrane characteristic in recessive generalized myotonia (Becker). Muscle Nerve *11*, 202–211.

Rüdel, R., Ricker, K., and Lehmann-Horn, F. (1993). Genotype-phenotype correlations in human skeletal muscle sodium channel diseases. Arch. Neurol. *50*, 1241–1248.

Rudolph, J. A., Spier, S. J., Byrns, G., and Hoffman, E. P. (1992). Linkage of hyperkalemic periodic paralysis in quarter horses to the horse adult skeletal muscle sodium channel gene. Animal Genet. *23*, 241–250.

Rudolph, J. A., Spier, S. J., Byrns, G., Rojas, C. V., Bernoco, D., and Hoffman, E. P. (1992). Periodic paralysis in quarter horses: a sodium channel mutation disseminated by selective breeding. Nature Genet. *2*, 144–147.

Sipos, I., Jurkat-Rott, K., Harasztosi, C., Fontaine, B., Kovacs, L., Melzer, W., and Lehmann-Horn, F. (1995). Skeletal muscle DHP receptor mutations alter calcium currents in human hypokalemic periodic paralysis myotubes. J. Physiol. *483*, 299–306.

Spier, S. J., Carlson, G. P., Holliday, T. A., Cardinet, G. H., and Pickar, J. G. (1990). Hyperkalemic periodic paralysis in horses. J. Am. Vet. Med. Assoc. *197*, 1009–1017.

Steinmeyer, K., Lorenz, C., Pusch, M., Koch, M. C., and Jentsch, T. J. (1994). Multimeric structure of ClC-1 chloride channel revealed by mutations in dominant myotonia congenita (Thomsen). EMBO J. *13*, 737–743.

Wang, J. Z., Dubowitz, V., Lehmann-Horn, F., Ricker, K., Ptáček, L., and Hoffman, E. P. (1995). In vitro structure/function studies: consecutive Arg1448 changes to Cys, His, and Pro at the extracellular surface of IVS4. Proc. Soc. Gen. Physiol., in press.

West, J., Patton, D., Scheuer, T., Wang, Y., Goldin, A. L., and Catterall, W. A. (1992). A cluster of hydrophobic amino acid residues required for fast Na^+ channel inactivation. Proc. Natl. Acad. Sci. USA *89*, 10910–10914.

Zhou, J., Spier, S. J., Beech, J., and Hoffman, E. P. (1994). Pathophysiology of sodium channelopathies: correlation of normal/mutant mRNA ratios with clinical phenotype in dominantly-inherited periodic paralysis. Hum. Mol. Genet. *3*, 1599–1603.