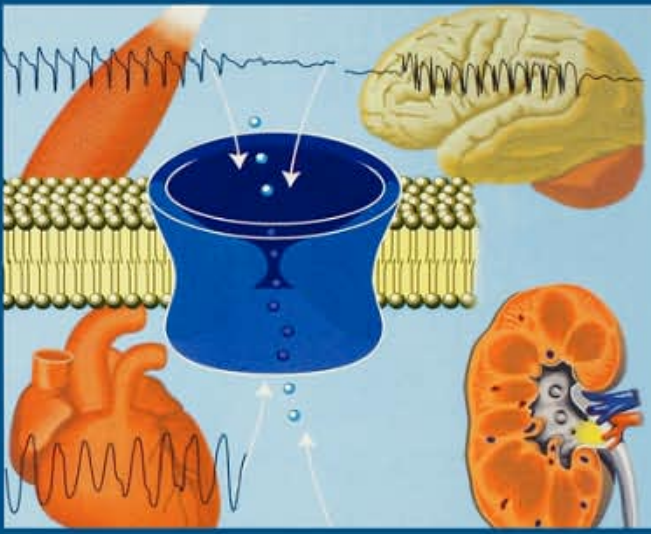

CHANNELOPATHIES

COMMON MECHANISMS IN AURA, ARRHYTHMIA AND ALKALOSIS

Edited by F. Lehmann-Horn
and K. Jurkat-Rott

ELSEVIER

Foreword

It was a simple question, that we tried to answer, when Bert Sakmann and myself made attempts to record single-channel currents in the early 1970s. What are the molecular mechanisms underlying the electrically and chemically induced permeability changes in excitable tissue: ion channels or other kinds of transporters? Our attention was focussed on the 'traditional' excitable cells: nerve, muscle neuroendocrine. We did not anticipate that ion channels are found in basically any cell type and that they mediate an incredible variety of regulatory functions. Even less would we have anticipated that mutations in channels are the basis of a whole range of disorders. It is rewarding to see that the tools, we created to answer this simple question posed above, in combination with modern molecular genetics would allow us to dissect and to better understand as many diseases as discussed in this volume. Even more, it is fascinating to realize, that the precision with which an ion channels' function can be studied, may teach lessons on pathogenetic mechanisms in general. The development from single channel recording to the detailed knowledge on diseases, presented in this book, is evidence of the credo of 'basic' researchers, that benefits for the public result in unpredictable ways from progress in basic knowledge and that any important advance in basic research sooner or later will contribute to solutions of very practical relevance.

Dr Erwin Neher
Professor of Biophysics
Göttingen, June 14th, 2000

Introduction

Ions channels – an exciting topic!

Editing a book on a topic indicates two things:
1. a considerable amount of knowledge has been accumulated; and
2. the nature of the material is well enough established to become teaching material.

Remarkably, this book is possible even though the field of ion channelopathies is only 10 years old – and the term channelopathies itself was coined only 8 years ago at a European Neuromuscular Center Workshop held in Ulm in 1992 (Neuromusc. Dis. 3:161–168).

But what predisposes ion channelopathies of so different medical specialties to be incorporated into one book? It is because the same basic pathomechanism underlies the clinical features, namely sustained membrane decharging (depolarization). Depending on the degree of this disturbance, affected tissue is rendered either in an hyperexcitable (i.e. myotonic muscle stiffness) or an unexcitable state (i.e. flaccid weakness) and ***this*** is capable of explaining the symptoms patients present with!

Of Becker and Bryant

The work of two pioneer scientists in this field is acknowledged:
- Peter E. Becker (born 1908), neurologist and geneticist; and
- Shirley H. Bryant (1924–1999), biophysicist

Both were interested in muscle stiffness, but each with a different approach. While Becker clinically described hereditary myotonia in man, recessive Becker myotonia, for the first time and on an incredibly large epidemiological scale, Bryant was the first to propose that a change of ion conductance, Cl^--ions, functionally causes the fear-evoked myotonic stiffness in the corresponding animal model, the fainting goats (see Chapter 2). That was 40 years ago, long before such thing as an ion channel was known to exist and when it was generally accepted that ions diffuse into the cells membrane through membrane 'holes'. It took another 30 years for the first causative gene encoding for an ion channel to be identified, the adult skeletal

muscle sodium channel gene, mutations in which are associated with dyskalemic paralysis, a disease of both man and, ironically, American race horses (see Chapter 1).

Patch clamp champs

Knowing the gene responsible for an hereditary disorder alone is not of much help without an understanding of the functional defect brought about by the disease-causing mutations therein. Unique among hereditary disorders is the availability of the technical possibilities to study exactly this by combining modern molecular biology with patch-clamp techniques (Erwin Neher and Bert Sakmann, Nobel Prize for medicine and physiology 1991). Current variants of this technique make the application of solution on the exterior and interior of whole cells and on membrane patches torn from the cell possible (outside-out or inside-out) – every thinkable configuration of solution and ion channel orientation that the heart of an ion channel researcher craves for.

Channel panel

Ion channels do not come alone, but rather in whole families of related proteins conducting each ion type with slightly modified function and varying tissue expression patterns. Structures of importance like pore, selectivity filter, voltage sensors, ligand binding sites, and opening and closing gates show conservation apparently for more than 600 million years. This is an evolutionary trick to on the one hand mediate many functions with the aid of one basic mechanism, but on the other hand, to compensate for an eventually disturbed function by closely related channel siblings. Therefore, the ion channelopathies known to date do not lead to death, not even to continuous disability, but rather require an out-of-the-normal situation, a so-called trigger, to present with recognizable symptoms. Watch out for similarities in clinical symptom patterns, triggers, channel structure with localization of disease-causing mutations, functional consequences of mutants, and acute and prophylactic therapy when reading this book. You will be able to identify new channelopathies in your own research field! Let us know!

Preface

In the last 10 years the combination of electrophysiological and molecular genetic investigations led to the exploration of the growing family of diseases caused by mutations in genes encoding voltage- and ligand-gated ion channels, the so-called channelopathies.

Although the underlying mutations are rare and restricted to single genes expressed in a specific tissue, channelopathies may be important models for much more frequent disorders of non-monogenic etiology. Most channelopathies have a certain clinical pattern in common. Typically the symptoms occur as episodic attacks lasting from minutes to days that show spontaneous and complete remission, onset in the first or second decade of life, and – for some unknown reason – show amelioration at the age of 40 or 50. Frequently the attacks can be provoked by rest following physical activity or exercise itself, hormones, stress and certain types of food. Surprisingly, many patients with channelopathies respond to acetazolamide, a carbonic anhydrase inhibitor. Most channelopathies show no chronic progression, however, there are a few exceptions which unfortunately do not respond to acetazolamide treatment. Examples are cerebellar degeneration in episodic ataxias or proliferation of the transverse tubular system in periodic paralysis both of which are probably associated with an altered gene expression in the affected cells triggered directly by the mutant proteins or the overall cell dysfunction.

This book describes human hereditary ion channel diseases of voltage- and ligand-gated ion channels covering the diverse fields of medicine myology, neurology, cardiology, and nephrology requiring a wide and interdisciplinary readership. Interesting parallels in pathogenetic mechanisms of disease are especially emphasized to interest even highly specialized readers in entities outside of their fields. Each author has written an objective overview of his or her particular subject in a way that should allow the reader within a short period of time to obtain a comprehensive picture of the present state of the art. The authors and editor anticipate that many more channelopathies will be identified after this book has gone to press. We believe that we know only the tip of the iceberg.

Frank Lehmann-Horn
Guest Editor

Karin Jurkat-Rott
Co-editor

Channelopathies – Common Mechanisms in Aura, Arrhythmia and Alkalosis
F. Lehmann-Horn and K. Jurkat-Rott, editors

Chapter 1

Sodium and calcium channelopathies of sarcolemma: periodic paralyses, paramyotonia congenita and potassium-aggravated myotonia

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Abstract

Periodic paralyses, paramyotonia congenita and potassium-aggravated myotonia are dominantly inherited muscle disorders caused by mutations in genes encoding voltage-gated sodium or calcium channels. Three so-called sodium channel diseases, hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PC) and potassium-aggravated myotonia (PAM) are caused by mutations in the α-subunit of the human skeletal muscle sodium channel, whereas in hypokalemic periodic paralysis (HypoPP) mutations in the α-subunit of the L-type human skeletal muscle calcium channel are found. The clinical phenotype of HyperPP are transient episodes of muscle weakness or paralysis often provoked by rest after exercise or by ingestion of potassium-rich food. HypoPP is also characterized by episodes of generalized paralysis occurring usually in the morning with on average longer duration than in HyperPP. Attacks may be provoked by carbohydrate-rich food or heavy exercise the preceding day. Decisive for classification is the level of serum potassium during a paralytic attack, which may fall below 2 mmol/l in HypoPP, whereas in the hyperkalemic form, it may rise above 5.5 mmol/l. PC is characterized by paradoxical myotonia, which is muscle stiffness increasing with repeated activity, and weakness triggered by exposure to cold. The clinical phenotype of PAM is muscle stiffness induced or aggravated by depolarizing agents such as potassium, typically without weakness or cold-sensitivity. This article provides an overview of clinical features, genetics, pathogenesis and therapy of the sodium and calcium channelopathies of sarcolemma.

Introduction

In the last 10–15 years, the combination of electrophysiological and molecular genetic investigations led to the identification of the growing family of the so-called

'channelopathies', diseases caused by mutations in different voltage-gated or ligand-gated ion channels. Since ion channels provide the basis for the regulation of excitability in nerve and muscle cells, it is not surprising that mutations in channel encoding genes, leading to a dysfunction of these highly specific, membrane spanning proteins, result in hyper- or hypoexcitability of the corresponding cells. Meanwhile, channelopathies involving tissues such as skeletal muscle, brain and heart are known. These are myotonias and periodic paralyses, inherited cardiac arrhythmias (the long-QT syndromes), episodic ataxias, familial hemiplegic migraine and some familial epileptic syndromes.

The first diseases in which the underlying pathophysiology has been identified to be due to an ion channel defect were skeletal muscle diseases, the non-dystrophic-myotonias and periodic paralyses (Lehmann-Horn et al., 1994). Predominant symptoms of the myotonias and of the periodic paralyses are transiently occurring muscle stiffness and episodes of muscle weakness, respectively. Myotonia is caused by an increased excitability of the sarcolemma, the episodes of weakness by a reduced excitability. Both symptoms are caused by a depolarization of the muscle fibre membrane. Whereas a slight depolarization will induce hyperexcitability, i.e. myotonia, a strong and sustained depolarization causes hypoexcitability and muscle weakness. The relation is so close that diseases exist that exhibit both symptoms.

Non-dystrophic myotonias may be divided into chloride and sodium channel diseases. The chloride channel myotonias Thomsen and Becker are caused by mutations in the gene encoding the skeletal muscle chloride channel ClC-1 and will be described in the next chapter. Sodium channel myotonias are caused by mutations in the gene encoding the adult skeletal muscle sodium channel α-subunit (SCN4A). These are paramyotonia congenita and potassium-aggravated myotonia.

Periodic paralysis is the common name for a number of rare diseases characterized by episodes of flaccid weakness. There are primary and secondary forms. The primary forms show an autosomal dominant mode of inheritance and are commonly classified upon the change in serum potassium concentration during attacks of weakness as hypokalemic, normokalemic and hyperkalemic. Secondary types of periodic paralyses occur in association with hyperthyroidism or with wastage or retention of body potassium.

Before genetic studies were possible, an extensive electrophysiological survey, carried out with excised muscle specimens of all kinds of myotonia patients was performed (Lipicky et al., 1971; Rüdel and Lehmann-Horn, 1985; Lehmann-Horn et al., 1987a,b). Normal excitability of muscle fibres requires a high resting potential and short-lasting action potentials. Both requirements are not fulfilled in periodic paralyses and myotonias. Voltage-clamp studies on single fibres have revealed that inactivation of Na^+ currents is incomplete for hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PC) and potassium-aggravated myotonia (PAM) causing a depolarization of the sarcolemma (Lehmann-Horn et al.,

1987a,b; Lerche et al., 1993). A depolarization is also the underlying pathomechnism for HypoPP (Rüdel et al., 1984), however, up to now no abnormal membrane conductance has been observed.

According to the electrophysiological abnormalities, subsequent genetic studies revealed indeed the gene encoding the α-subunit of the adult human skeletal muscle sodium channel as the site of the defect for HyperPP, PC and PAM. In contrast, HypoPP was linked to the gene encoding the α1-subunit of the skeletal muscle L-type calcium channel, the dihydropyridine (DHP) receptor. Several disease-causing point mutations in both channel genes were identified up to now.

In order to examine the functional consequences of these mutations on a molecular level, extensive studies using heterologous expression of the mutant genes and the patch clamp technique were carried out. These studies identified different gating defects of the sodium channel affecting mainly the inactivation which can nicely explain the pathophysiology of HyperPP, PC and PAM. The link between the calcium channel mutations and the pathomechanism of HypoPP remains to be elucidated.

Sodium channel diseases

Voltage-gated sodium channels are responsible for the initiation and propagation of action potentials in excitable cells. There are three main conformational states of the channel protein, a closed or resting state at hyperpolarized potentials, an open state occurring upon depolarization and an inactivated state that follows the opening at maintained depolarization or can be directly reached from the closed state (Fig. 1). In order to regulate the action potential properly, the depolarizing sodium current needs to be quickly activated and inactivated. If inactivation is too slow or incomplete, the repolarizing phase of the action potential is delayed and a stable resting potential can not be maintained which is the common underlying pathomechanism of the sodium channel disorders.

Mutations in *SCN4A*, the gene encoding the adult skeletal muscle sodium channel α-subunit, can cause three clinically distinct syndromes: *paramyotonia congenita, potassium-aggravated myotonia* and *hyperkalemic periodic paralysis*. Although many patients show the typical clinical phenotype of only one of these diseases, overlap syndromes do occur.

The *SCN4A* gene contains 24 exons distributed over about 30 kb. The intron–exon boundaries are known and primer sets consisting of intron sequences for amplification of all 24 exons by use of PCR are available (George et al., 1993). *SCN4A* is only expressed in skeletal muscle and its product is the only sodium channel detectable in the fully differentiated tissue.

The adult human muscle sodium channel is a 260 kDa glycoprotein of about 2000 amino acids consisting of four highly homologous domains (I–IV) with six trans-

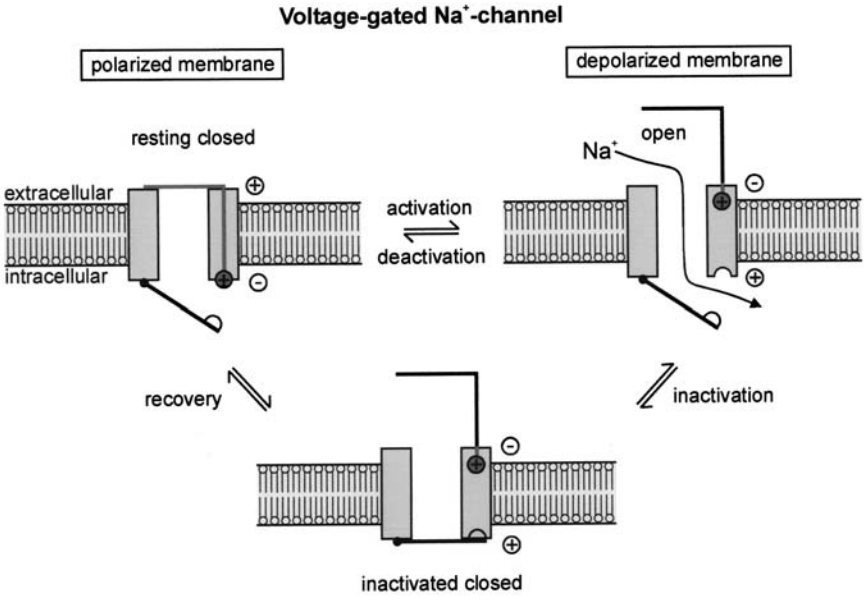

Fig. 1. A model of the sodium channel in resting, open and inactivated state. The sodium channel opens from a resting closed state upon depolarization and than closes spontaneously into the inactivated state. In order to open again the sodium channel has to recover from inactivation. Two channel gates are indicated, the activation gate (in red), coupled with the voltage sensor which opens the channel and the inactivation gate (in black) which closes the channel pore from the intracellular side.

membrane segments each (S1–S6, Fig. 2). The four interlinkers between segments S5 and S6 dip into the membrane and form the lining of the channel pore (Catterall, 1995). The S4 helices contain four to eight positively charged amino acids at every third position which serve as voltage sensors (Stühmer et al., 1989; Yang et al., 1996; Horn, 1998, Fig. 3). The third functional important structure is the interlinker connecting repeats III and IV, which is indispensable for fast inactivation of the channel. Most likely, this part of the protein acts as an inactivation gate or particle in a way that has been compared with a tethered ball or a hinged-lid (Armstrong and Bezanilla, 1977; West et al., 1992, Fig. 4). Yet unknown parts of the intracellular orifice of the pore or its surroundings may act as acceptor for the particle.

The reader who is interested in naturally occurring animal models of sodium and calcium muscle channelopathies should refer to the overview table of known channelopathies and a recent review (Lehmann-Horn and Jurkat-Rott, 1999).

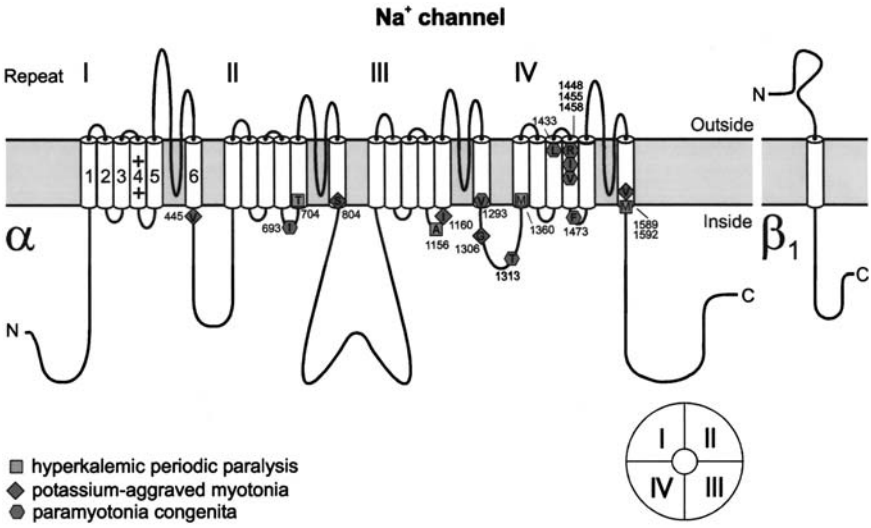

Fig. 2. Mutations predicted in the skeletal muscle sodium channel α-subunit, hSkm-1. Conventional one-letter abbreviations are used for wild-type amino acids (aa). Each aa position is given by the respective number. In two positions (G1306, R1448) three different natural mutations have been detected. The different symbols used for the point mutations indicate the resulting diseases as explained at the bottom of the left-hand side. Included are the positions of mutations causing HyperPP, PC and PAM (modified after Lehmann-Horn and Rüdel, 1996).

Paramyotonia congenita

Clinical features

The hallmarks of this disease as first described by Eulenburg (Eulenburg, 1886) and later confirmed in many families by Becker (Becker, 1970) are: (i) paradoxical myotonia, defined as muscle stiffness increasing with continued activity; (ii) severe worsening of the myotonia by cold; (iii) weakness after longer exposure to cold in most cases. In some families patients have spontaneous attacks of weakness like those occurring in HyperPP. The condition is transmitted as a dominant with complete penetrance.

Paramyotonic symptoms are present at birth and remain often unchanged for the entire lifetime. In the cold, the face may appear mask-like, and the eyes cannot be opened for several seconds. Working in the cold makes the fingers so stiff that the patient becomes unable to move them within minutes. The stiffness then gives way to weakness. After warming, the hands may not regain strength for several hours.

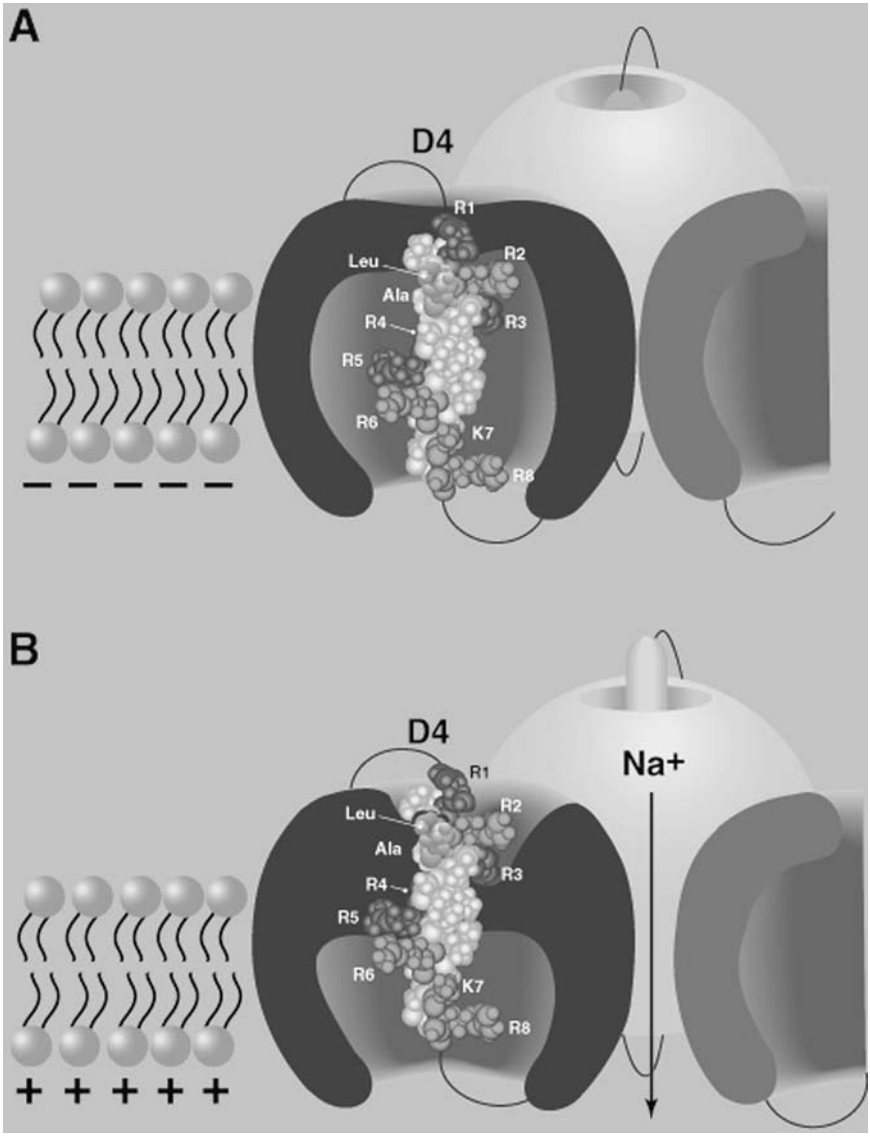

Fig. 3. Model for the movement of the voltage sensor. The voltage sensor S4 in domain IV contains eight positively charged residues, either arginines (R) or lysines (K). Two conformational states of IV/S4 are shown, the inward state at very negative potentials and the outward state at depolarized potentials. For details see Section 5 (taken from Yang et al., 1997 with kind permission).

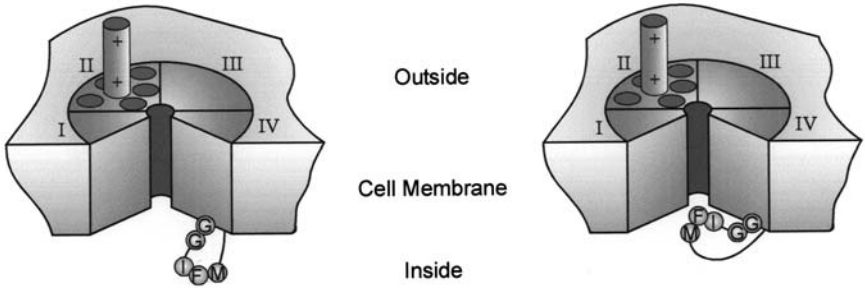

Fig. 4. Hinged-lid model for fast inactivation of the sodium channel. When inserted in the membrane, the four repeats of the sodium channel fold to generate a pore as schematically indicated. The outward movement of the voltage sensor that opens the channel pore is indicated. Three hydrophobic aa (isoleucine, fenylalanine, methionine) were proposed to form an inactivation particle closing the channel pore from the cytoplasmic side in a hinged-lid fashion (modified after West et al., 1992).The hinge could be a pair of glycines, one of which is mutated in PAM (G1306A/V/E, Lerche et al., 1993; Mitrovic et al., 1995, see text).

Under warm conditions many patients have no complaints. Muscle pain or muscle atrophy are not typical for the disease.

Diagnosis

The diagnosis of PC is suggested by the clinical picture described above and a positive family history. The EMG shows generalized spontaneous activity in the form of fibrillation-like potentials and myotonic discharges, often also at a normal muscle temperature. The serum CK may be elevated, up to 10 times above normal.

The diagnosis can be verified by cooling hand and forearm in a water bath at about 15°C for 15–30 min. Cooling induces muscle stiffness and later weakness which can be verified by a reduction of the amplitude of the evoked compound muscle action potential (Subramony et al., 1983; Gutmann et al., 1986; Jackson et al., 1994). A more precise measure of myotonia and weakness can be obtained determining the isometric force and relaxation time of the long finger flexor muscles before and after cooling (Ricker et al., 1986). Relaxation time can be prolonged from 0.5 s up to 50 s and contraction force reduced by more than 50%. A muscle biopsy is NOT necessary to diagnose PC. The diagnosis may be confirmed by a mutation within the SCN4A gene.

Therapy

Antiarrhythmic drugs, such as mexiletine, are effective in preventing muscle stiffness and weakness induced by physical activity or exposure to cold (Ricker et al., 1980; Streib, 1987). The majority of PC patients, however, require no treatment

and know best how to deal with their symptoms. In severe cases 360 mg mexiletine (Mexitil Depot®), once to twice a day is recommended. A cardial check-up should precede the antimyotonic mexiletine therapy and serum concentration should be carefully checked because of the small therapeutic breadth. Some patients wish to abolish their symptoms to be able to participate in special events like swimming or winter sports. In these cases 360 mg mexiletine should be taken 1 h before the event.

In paramyotonic HyperPP, the combined use of mexiletine and hydrochlorothiazide can prevent stiffness and weakness induced by cold, and the spontaneous attacks of HyperPP (Ricker et al., 1986).

Genetics

After electrophysiological measurements had revealed the TTX-sensitive sodium channel to be a strong candidate for the site of the defect in PC and HyperPP (see below, Lehmann-Horn et al., 1987a,b), a candidate gene approach demonstrated linkage of HyperPP to SCN4A on chromosome 17q23 (Fontaine et al., 1990). This was the first evidence for the existence of a human sodium channel disease. Three groups showed independently that also PC is linked to the *SCN4A* locus (Ebers et al., 1991; Koch et al., 1991; Ptacek et al., 1991a). The first mutation in an ion channel was found in HyperPP (Rojas et al., 1991).

Up to date, about 20 point mutations have been detected in different parts of the alpha subunit of the human skeletal muscle sodium channel (Fig. 2). Ten of them lead to PC and most of these are located within the voltage sensor IV/S4 (Ptácek et al., 1992a; Wang et al., 1995). One mutation situated in the III–IV interlinker is supposed to be a part of the inactivation gate of the channel (T1313M; McClatchey et al., 1992).

Pathogenesis

In contrast to myotonia congenita Becker and Thomsen, electrophysiology on excised muscle specimens of PC patients revealed a normal chloride conductance. Instead, the specific abnormality found was a non-inactivating component of the sodium current (Lehmann-Horn et al., 1987a,b). Studies on PC muscle fibres have shown that upon cooling the resting membrane potential depolarizes to values around –40 mV. This depolarization can be prevented by TTX and the weakness of muscle bundles in vitro can be antagonized by potassium channel openers which hyperpolarize the muscle membrane (Lehmann-Horn et al., 1987b; Lerche et al., 1996b). These results proved that weakness is caused by membrane depolarization due to an increased sodium influx through voltage-gated sodium channels.

Electrophysiological recordings on heterologously expressed mutant sodium channels causing PC typically show a pronounced slowing of the current decay and a small persistent current due to incomplete inactivation (Fig. 5), a shift of

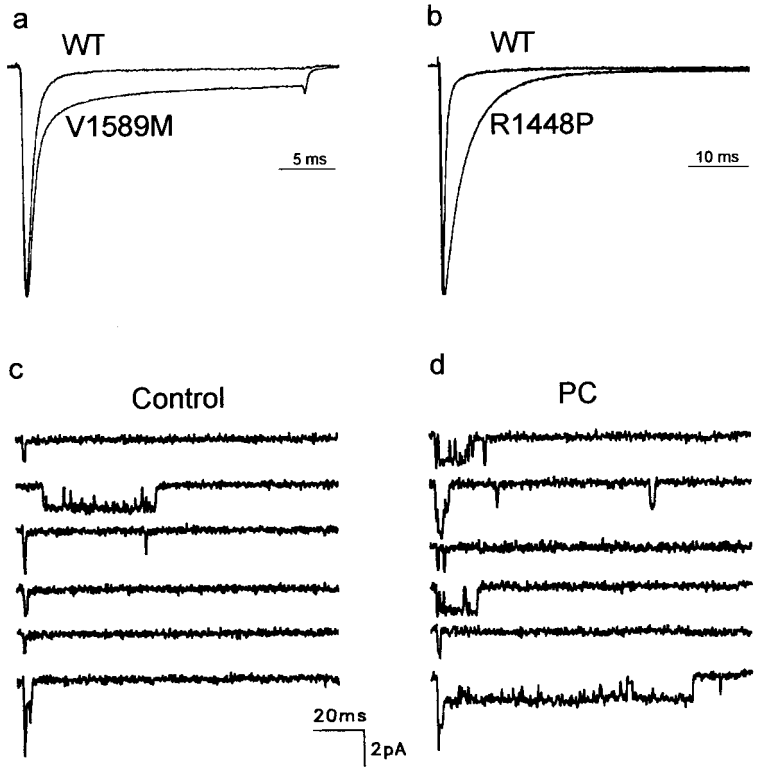

Fig. 5. Impaired inactivation of mutant sodium channels. Sodium currents conducted by WT and mutant channels expressed in a heterologous system (human embryonic kidney cells). Whole cell currents were elicited by a membrane depolarization from a holding potential of 100–0 mV. The recording in (A) shows V1589M channels (Mitrovic et al., 1994) and in (B) R1448P channels (Mitrovic et al., 1999), in comparison with the wild type (WT). (A) Shows a persistent current as a typical finding for mutations causing HyperPP or PAM. (B) Shows a strongly slowed inactivation as an example for PC. Both, the persistent current and the slowing of inactivation are caused by more frequent reopenings of sodium channels as shown in single channel recordings in (C). Recordings from native muscle specimens are shown for a normal control (left panel) and for a PC patient carrying the R1448P mutation (right panel, Lerche et al., 1996a).

the steady-state inactivation curve in the hyperpolarizing direction and a faster recovery from inactivation (Chahine et al., 1994; Yang et al., 1994; Lerche et al., 1996b; Mitrovic et al., 1996; Mitrovic et al., 1999; Hayward et al., 1996). Cooling enhanced the slowing of inactivation and the persistent sodium current, however, this effect was not specific for mutant channels (Lerche et al., 1996b; Fleischhauer et al., 1998; Mitrovic et al., 1999). The electrophysiological findings may explain the

clinical symptoms as follows: the slowed current decay provides a plausible explanation for paradoxical myotonia, since the abnormal sodium influx mainly occurs during an action potential. Hence, only continued exercise results in sufficient accumulation of intracellular sodium to cause muscle depolarization and myotonia. Weakness upon cooling is most probably explained by a combination of an increased persistent current, exceeding a certain threshold in the cold, and the left-shift of steady-state inactivation decreasing the number of sodium channels available for an action potential (Lerche et al., 1996b; Wagner et al., 1997; Mitrovic et al., 1999). The faster recovery from inactivation also contributes to the pathophysiology of PC promoting the development of myotonia by shortening the refractory period after an action potential (Hayward et al., 1996).

An important question in the pathophysiology of dominantly inherited diseases, such as channelopathies, is the level of expression of the mutant protein. Using a quantitative kinetic analysis of Na^+ currents measured in muscle specimens biopsied from two PC patients, we were recently able to estimate the percentage of functional mutant protein in the native muscle membrane, a so far unknown parameter in the pathophysiology of channelopathies and other dominantly inherited diseases (Mitrovic et al., 1999). In contrast to mRNA measurements, western blots, antibody staining or other molecular biological or biochemical approaches, which determine the level of mutant RNA or protein without regard to its function, the electrophysiogical evaluation allowed us to determine the ratio of the mutant protein that is pathophysiologically relevant. Our analysis suggests that no more than 38% of the sodium channels in the PC muscle specimen were of the mutant type, which is significantly less than the 50% suggested by the autosomal dominant mode of inheritance. Thus, the clinical severity of the phenotype seems to depend not only on the extent of the electrophysiological dysfunction of the mutant channels but also on their fraction in the muscle membrane. For another Na^+ channelopathy, the equine periodic paralysis in Quarter horses, the severity of the clinical phenotype has been shown to correspond to mRNA levels in native muscle probes (Zhou et al., 1994).

Potassium-aggravated myotonia

This disease has been newly defined when long-known clinical knowledge could be combined with recent genetic and molecular biologic information (Lerche et al., 1993; Heine et al., 1993; Ptácek et al., 1992b, 1994a). Becker (1977) investigated more than 100 families with non-dystrophic dominant myotonia and proposed several subtypes of what he thought was myotonia congenita. Molecular biology revealed that many of these conditions were in fact caused by mutations in the gene encoding the muscle sodium channel. A few forms could be classified as special

types of paramyotonia, as they did show cold- and exercise-induced stiffness, albeit no cold-induced weakness. Other conditions, however, were too inconsistent with the definition of PC.

Clinical features

As a characteristic finding, PAM patients never experience muscle weakness and are not substantially sensitive to cold. Four clinical phenotypes can be distinguished with regard to the severity of myotonia and response to therapy. One group of affected persons experiences muscle stiffness that tends to fluctuate from day to day, hence the name 'myotonia fluctuans' (Ricker et al., 1990, 1994; Lennox et al., 1992). Their muscle stiffness is provoked by exercise, and often it occurs with some delay during rest after heavy exercise. The stiffness may then last for 0.5–2 h. On many days or even for weeks, afflicted persons experience no symptoms at all. More severely affected are persons with a generalized moderate myotonia, that may also show a kind of delayed warm-up phenomenon. The most severe form of PAM and of myotonia in general was called 'myotonia permanens' (Lerche et al., 1993). It is characterized by very severe and persisting myotonia. When the myotonia is aggravated, e.g. by intake of potassium-rich food, ventilation might be impaired by stiffness of the thoracic muscles. In particular, children can suffer from acute hypoventilation and this may lead to cyanosis and unconsciousness, so that such episodes were occasionally mistaken for epileptic seizures. In spite of the misdiagnosis, antiepileptic medication, e.g. administration of carbamazepine, was useful in these cases because of its antimyotonic effects. Such patients would probably not survive without continuous treatment.

The fourth related subtype is associated with acetazolamide-responsiveness of myotonia (Trudell et al., 1987), also described as atypical myotonia congenita (Ptácek et al., 1992b). In addition to stiffness, patients also report of muscle pain. Both the stiffness and pain are alleviated by acetazolamide.

In PAM, depolarizing agents such as potassium or suxamethonium may aggravate the myotonia, but do not induce weakness. It is well known for myotonic disorders that the risk of depolarizing relaxants inducing anaesthesia-related events is increased. The incidence of such events seems to be highest in myotonia fluctuans families (Ricker et al., 1994; Vita et al., 1995). There seems to be no other biological reason for this other than the frequent absence of clinical myotonia in these patients making the anaesthesiologists unaware of the condition.

Diagnosis.

The diagnosis of PAM is suggested by a generalized myotonia with dominant inheritance, by the absence of weakness and cold sensitivity and possibly with some of the special features described above. However, in many cases PAM cannot be

clinically differentiated from myotonia congenita (MC) Thomsen. In this case oral potassium loading which induces a myotonic attack in PAM but not in MC Thomsen might be useful. This test is contraindicated in patient with myotonia permanens since it may provoke severe attacks. The EMG shows generalized myotonic discharges, in myotonia fluctuans patients often in the absence of clinical myotonia.

Therapy

As in PC, mexiletine is the drug of choice in preventing muscle stiffness. However, treatment is usually only necessary in severe cases (360 mg mexiletine (Mexitil Depot®) once to twice daily).

Genetics and pathogenesis

Six point mutations at four different positions are responsible for PAM. Four of the substitutions are located in the supposed inactivation gate (Fig. 2). Three of them affect the same nucleotide, resulting in three different amino acid substitutes for one (G1306) of a pair of glycines (1306/7) supposed to be essential for proper inactivation. The more the substitutes differ from glycine by having side-chains of variable length and charge and/or ramification, the greater is the degree of the electrophysiological defect in vitro and the more severe are the clinical symptoms (McClatchey et al., 1992; Lerche et al., 1993; Mitrovic et al., 1995). Glutamic acid, having a long side-chain, causes myotonia permanens, the most severe form of myotonia known. Valine, an amino acid with a side-chain of intermediate size, is the substitute in patients with moderate myotonia and alanine, distinguished by a short side-chain, results in the benign myotonia fluctuans. Electrophysiological experiments with some of the PAM-mutants expressed in human embryonic kidney cells have shown slowed inactivation, increased persistent current (Fig. 5) and a shift of the steady-state inactivation curve in the depolarizing direction (Mitrovic et al., 1994, 1995; Hayward et al., 1996). As mentioned above (see PC), both a slowed inactivation and an increased persistent sodium current cause membrane depolarization and muscle membrane hyperexcitability. The right shift of the steady-state inactivation curve extends the availability of sodium channels at more positive potentials and may, in contrast to PC-causing mutants which show a left shift of the curve, prevent the development of paralysis. Mutations at the position G1306, additionally show slowed deactivation (Mitrovic et al., 1995; Hayward et al., 1996). This defect could increase the influx of sodium ions during repolarization which might also contribute to myotonia. However, compared to the defects of fast inactivation, the contribution of impaired deactivation to clinical myotonia should be rather small. Deactivation defects were also described for two other mutations causing either PAM or PC (Richmond et al., 1997; Featherstone et al., 1998), however for the PC-causing

mutation (R1448P), deactivation is not impaired in the voltage range where no inactivation occurs (Mitrovic et al., 1999).

Hyperkalemic periodic paralysis

The disease was first described by Tyler et al. (1951); Helweg-Larsen et al. (1955) and was extensively investigated by Gamstorp (1956) who clearly differentiated it from 'paroxysmal familial paralysis' and named it 'adynamia episodica hereditaria'. Clinically, the most striking difference of the two diseases is that, during the paralytic episodes, serum potassium decreases in the former and increases in the latter. To stress this distinction, the names hypokalemic periodic paralysis and hyperkalemic periodic paralysis, respectively, are now preferred.

Hyperkalemic periodic paralysis is transmitted as an autosomal dominant trait with complete penetrance, although incomplete penetrance was reported for families with rare mutations (McClatchey et al., 1992; Wagner et al., 1997). The disease has three clinically distinct variants. It can occur (i) without myotonia, (ii) with clinical or electromyographic myotonia, or seldom (iii) with paramyotonia. In some patients, a chronic progressive myopathy may develop which seems to be genetically determined (mutation T704M) (Bradley et al., 1989; Ptácek et al., 1991b; Lehmann-Horn et al., 1993).

Clinical features

The attacks usually begin in the first decade of life. Initially they are rare but then increase in frequency and, in severe cases, may recur daily. The attack commonly starts in the morning before breakfast and lasts 15 min to an hour, and then spontaneously disappears. Often rest provokes the attack, and prior strenuous work usually aggravates it. Potassium loading, cold environment, emotional stress, glucocorticoids, and pregnancy provoke or worsen the attacks. After strenuous exercise, weakness can follow within a few minutes of rest. Sustained mild exercise after a period of strenuous exercise may postpone or prevent the weakness in the exercising muscle groups while the resting muscles become weak. The generalized weakness is usually accompanied by a significant increase of serum potassium (more than 5.5 mM).

The course of the paralytic attacks is the same in all three forms of HyperPP. Cooling can induce weakness, but not stiffness, and reheating restores contractile force quickly (except for the paramyotonic form). EMG studies are required to determine the presence or absence of myotonia which is usually very mild. In the non-myotonic form, clinical and electrical myotonia are both absent. Cooling may provoke weakness but does not cause substantial myotonia. Paramyotonic HyperPP is characterized by attacks of generalized muscle weakness associated with hyper-

kalemia and by paradoxical myotonia (for details see previous section on paramyotonia congenita).

Normokalemic periodic paralysis, a variant of the hyperkalemic form

This rare disorder resembles HyperPP in many respects but differs from it in that the serum potassium does not increase even during serious attacks. The existence of normokalemic periodic paralysis as a nosological entity has been questioned because some patients with this condition are sensitive to oral potassium salts (Poskanzer and Kerr, 1961). The disorder is transmitted as an autosomal dominant trait with high penetrance in both sexes. The attacks begin in the first decade of life and are provoked or worsened by rest after exercise, exposure to cold and by potassium loading. A urinary potassium retention, the lack of a beneficial effect of glucose, and failure of the serum potassium to increase in attacks are differences to primary HyperPP. However, in at least one such family, the condition is caused by the common T704M mutation in *SCN4A* normally associated with HyperPP (F. Lehmann-Horn, pers. commun.).

Diagnosis

The diagnosis of HyperPP is based on the presence of typical attacks of weakness or paralysis combined with an increased serum potassium during the attacks, the positive family history, and the myotonic or paramyotonic phenomena, if present. Except for some older patients with progressive myopathy, the muscles are well developed. The serum CK is sometimes elevated up to 200–300 U/l. When the diagnosis is unclear, a provocative test can be performed.

An elegant test consists of exercise on a bicycle ergometer for 30 min so that the pulse increases to 120–160 beats/min followed by absolute rest in bed (Ricker et al., 1989). It should be preferably performed in the morning in the fasting state. The serum potassium rises during exercise and then declines to almost the pre-exercise level, as in healthy individuals. Ten to 20 min after the onset of rest, a second hyperkalemic period occurs in the patients in contrast to normal subjects, and during this period the patients become paralyzed. If a paralytic attack is not induced, the test can be combined with the administration of 40–80 mmol oral potassium chloride. This test should be performed in anesthesiologic stand-by and is contraindicated in subjects already hyperkalemic and in those who do not have adequate renal or adrenal reserve. Recordings of the evoked compound muscle action potential during rest and exercise are also helpful in confirming the diagnosis of periodic paralysis (McManis et al., 1986). An abnormally high serum potassium level between attacks suggests secondary rather than primary HyperPP.

Therapy

Preventive therapy consists of frequent meals rich in carbohydrates, a low-potassium diet, and avoidance of fasting, strenuous work, and exposure to cold. Many patients are able to prevent or abort attacks by continuing slight exercise and/or by oral ingestion of carbohydrates at the onset of weakness (e.g. 2 g glucose per kg body weight). However, severe attacks may fail to respond to these measures (Gamstorp, 1956). Interestingly, attacks occur more frequently on holidays and weekends when patients rest in bed longer than usual. Thus, patients are advised to rise early and have a full breakfast.

Some patients can abort or attenuate attacks by the prompt oral intake of a thiazide diuretic or acetazolamide, or by inhalation of a β-adrenergic agent. The beneficial effect of the diuretics is probably due to their capacity to lower the serum potassium level. The effects of the β-adrenergic agents is probably mediated via stimulation of the sodium–potassium pump (Clausen, 1986). Calcium gluconate, 0.5–2 g given intravenously, has also terminated attacks in some patients.

It is often advisable to prevent attacks by the continuous use of a thiazide diuretic (Gamstorp, 1956) or acetazolamide (McArdle, 1962; Riggs and Griggs, 1979). Diuretics in modest dosages at intervals from twice daily to twice weekly are very effective in mild cases. The drug should not lower the serum potassium below 3.3 mM or the serum sodium below 135 mM (McArdle, 1962). In severe cases, 50 or 75 mg of hydrochlorothiazide should be taken daily early in the morning. For the dosage of acetazolamide see HypoPP therapy.

Genetics

Four mutations in *SCN4A* were found to cause HyperPP (Fig. 2). T704M is the most frequent *SCN4A* mutation and, in addition to HyperPP with or without myotonia, often causes chronic progressive myopathy (Ptácek et al., 1991b). All other mutations cause myotonic HyperPP without permanent weakness. M1592V, the first of all detected sodium channel mutations (Rojas et al., 1991), always causes HyperPP associated with myotonia. Two rare mutations (A1156T, M1360V) are of interest since they were discovered in families with overlap syndromes of HyperPP and PC showing incomplete penetrance in females (McClatchey et al., 1992; Wagner et al., 1997; F. Lehmann-Horn, pers. commun.).

Pathogenesis

In vitro electrophysiological studies on muscle specimens from patients with HyperPP revealed a large persistent sodium current due to incomplete inactivation of the sarcolemmal sodium channels depolarizing the muscle membrane to values of about −40 mV (Lehmann-Horn et al., 1987a,b, 1991; Cannon et al., 1991). At this