The Patch Clamp Technique in Ion Channel Research

K. Jurkat-Rott and F. Lehmann-Horn*

FINAL

Department of Physiology, Ulm University, D-89081 Ulm, Germany

Abstract: To understand the pathogenesis of a given ion channel disorder, knowledge of the mutation alone is insufficient, instead, the description of the associated functional defect is decisive. The patch clamp technique enables to achieve this both in native tissue as well as heterologous expression systems. By this technique, structure-function relationships of ion channels were elucidated that not only support the homology already suggested by amino acid alignments of different channel types, but that also pointed to regions important for gating, ion selectivity, or subunit interaction. Currently, effort is being made to develop automation of the technique which will result in a cost-effective, fast, and highly accurate method to test for drug actions on high throughput scales. This review contains an overview of channel structures, channel diseases, and methods to study channel function by the patch clamp technique.

Key Words: voltage clamp technique, channelopathies, channelomics, pharmacology, drugs, screening.

INTRODUCTION

Electrical excitability in the nervous system, heart, and skeletal muscle is conveyed by ion channels. Accordingly, mutations in ion channel genes cause disturbances of excitability leading either to pharmacogenetic predispositions or hereditary disorders. Elucidating the pathogenesis of these diseases by applying electrophysiological methods such as the patch clamp technique, has given rise to new approaches for basic research and has greatly contributed to knowledge of structure function relationships especially of voltage-gated ion channels. These techniques will be decisively involved in developing strategies for specific therapy in the future.

CHANNEL STRUCTURE

Ion channels are membrane-spanning proteins that can conduct ions through the membrane along or against their concentration gradient depending on the potential. Functional channels may consist of several different proteins, i.e. subunits, the α subunit of which contains the ion conducting pore and important binding sites for endogenous ligands or drugs. The pore may specific for a certain type of ion only or may conduct cations or anions in general. In the α subunits, the structures of the pore, its selectivity filter, and its activation and inactivation gates show high evolutionary conservation that allows one to make deductions on structure-function relationships from one channel type to the next (Fig. **1**).

VOLTAGE-GATED ION CHANNELS

In voltage-gated ion channels, the most extensively studied group of channels so far, special voltage-sensing transmembrane segments of the protein control the activation and inactivation processes, the voltage-dependent gating mechanisms (for review see Lehmann-Horn and Jurkat-Rott [1]). In cation channels, the basic motif of the α subunit, is a tetramic association of a series of 6 transmembrane α-helical segments, numbered S1-S6, connected by both intracellular and extracellular loops, the interlinkers (Fig. **1**). While for Ca^{2+} and Na^{+} channels the α subunit consists of a momomer, K^{+} channels form homo- or heterotetramers because each α subunit consists only of one domain with 6 transmembrane helices. Accessory subunits called β, γ, or δ do not share a common structure, some having one to several transmembrane segments and others being entirely intra- or extracellular. Functionally, they may influence channel expression, trafficking, and gating.

The voltage sensitivity of cation channels is conveyed by the S4 segments which are thought to move outward upon depolarization and channel opening [2, 3]. The ion conducting pore is thought to be lined by the S5-S6 interlinkers [4] which contain the selectivity filter [5]. While the localization of the activation gate may well be within the pore, the inactivation gate has been shown to be located in different regions in the Na^{+} and K^{+} channels [6, 7].

In contrast to the cation channels, not much about the structure/function relationship of Cl^{-} channels is known. They form homo- and heterodimers [8, 9]. Hydrophobicity blots have suggested 13 putative transmembrane helical segments [10], but by a combination of glycosylation and electrophysiological experiments on mutant proteins, it became clear that both the N- and C-term must be located intracellularly requiring an even number of transmembrane segments. A recent study confirmed the previously assumed two-pore structure with each pore to be formed by a separate subunit composed of 18 alpha helices not all of which cross the membrane fully [11].

CHANNEL STATES

Ion channels open (activate) in response to voltage changes or ligands, and they close (inactivate or desensitize) by an intrinsic process coupled to activation. The inactivated or desensitized channels require a time of recovery before they can be directly opened again implying that there are at least two closed stated exist: one from which the channel can

*Address correspondence to this author at the Abteilung Angewandte Physiologie, Universität Ulm, D-89081 Ulm, Germany; E-mail: frank.lehmann-horn@medizin.uni-ulm.de

1389-2010/03 $41.00+.00

Original figure will be pasted here

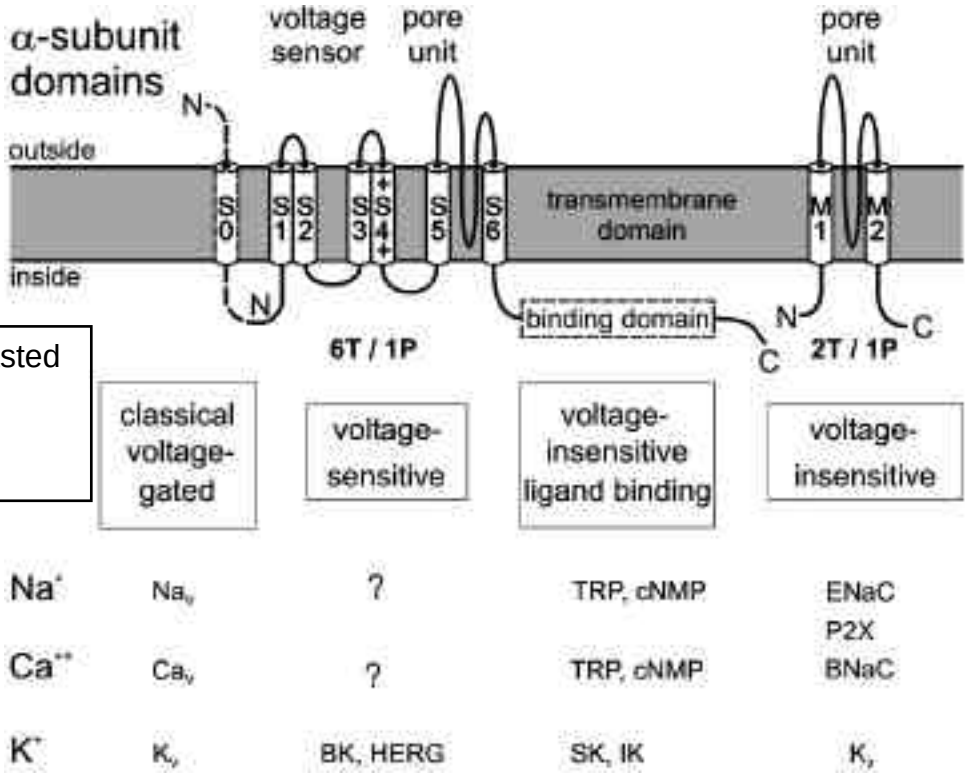


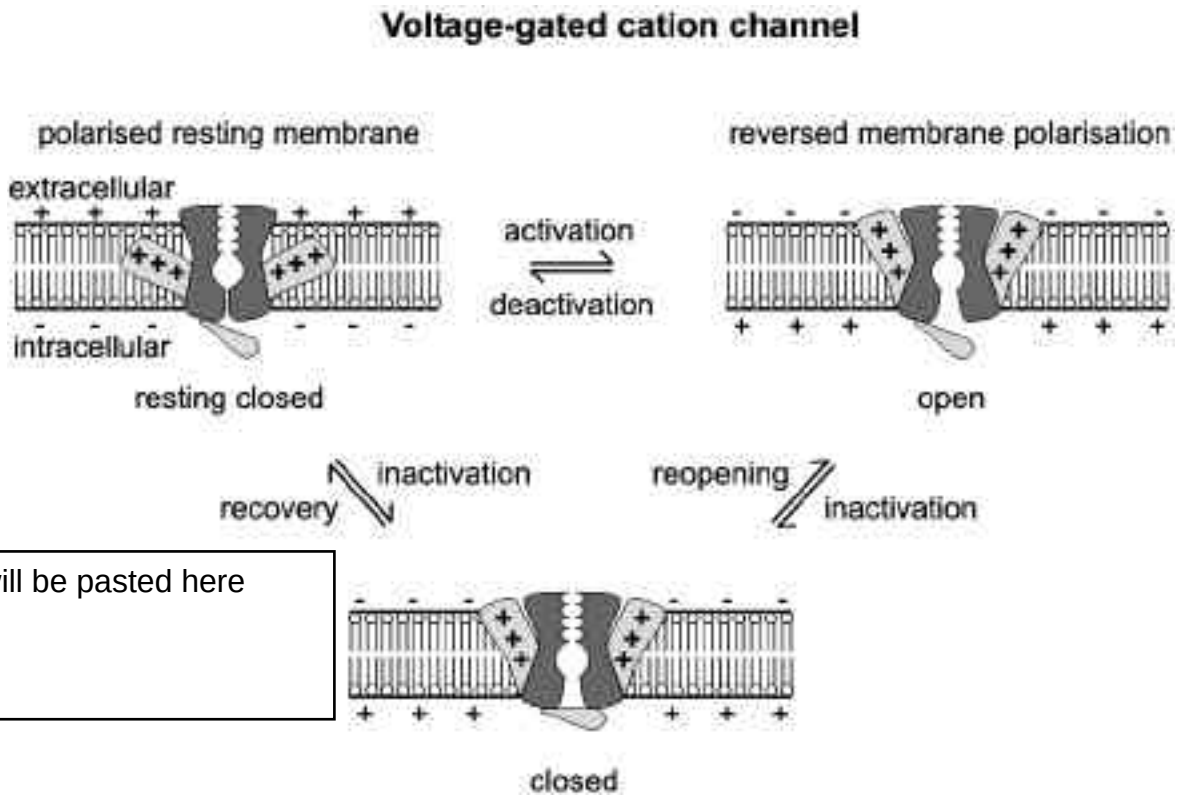
Fig. (1). Diversity of domains forming cation channel alpha subunits. The most simple domain, typically used for inward going rectifier K^+ channel alpha subunits, is a pore unit (2T/1P) that consists of 2 transmembrane segments M1 and M2, an extracellular loop dipping into the membrane and lining the pore, and intracellular N- und C-terminals. The transmembrane segments are thought to be alpha helices. All voltage-gated alpha subunit domains are 6T/1P domains since they contain a 4 transmembrane segment unit S1 to S4 acting as voltage sensor and the 2 transmembrane pore unit. S4 is the particular voltage-sensing segment that contains positive charges at each third amino acid residue. Ligand-gated cation channel alpha subunit domains usually possess a C-terminal binding site in addition to the 6T/1P domain. Although some ligand-gated channels, e.g., the Ca^{2+}-activated SK K^+ channel, contain a positively charged S4 segment they are not voltage-sensitive at all, maybe due to uncoupling of sensor and activation gate. BK (=*b*ig conductance K^+) K^+ channels possess an additional S0 segment. The following channels complete the classification of the alpha subunit domains: HERG, a K^+ channel encoded by the human ether-a-go-go related gene that is similar to the *drosophila* ether-a-go-go gene (eag); IK, a Ca^{2+}-activated K^+ channel with intermediate conductance; cNMP or CNG, Na^+ or Ca^{2+} channels that are *c*yclo-*n*ucleotide monophosphates *g*ated such as cGMP; and voltage-insensitive Na^+ channels of epithelial cells (ENaC) and in free nerve terminals of the brain (BNaC). BNaC was later identified to conduct Ca^{2+}. Not shown is another group of alpha subunit domains, 4T/2P, which contain 4 transmembrane segments and 2 pore units.

directly be activated (the resting state) and one from which it cannot (the inactivated or desensitized state). This suggests that there are at least two gates regulating the opening of the pore, an activation and an inactivation gate, both of which are usually mediated by the α subunit (Fig. **2**).

CHANNELOPATHIES

The ion channel diseases or channelopathies known to date do not lead to death, not even to continuous disability, but rather require an out-of-the-normal situation, so-called trigger, to elicit recognizable symptoms. Typically, the symptoms occur as episodic attacks lasting from minutes to days that show spontaneous and complete remission. Usually, onset is in the first or second decade of life with a decrease of frequency and severity at the age of 40 or 50 years. Channelopathies are found in very different tissues as brain, muscle, and epithelial tissue (for overview of known channelopathies, see Table **1**).

The first ion channelopathies to be recognized were the non-dystrophic myotonias and periodic paralyses of skeletal muscle. These are therefore best understood. Clinically, these appear as recurring episodes of muscle stiffness or weakness triggered by typical circumstances such as cold, exercise, oral K^+ load, or drugs. Muscle stiffness, termed myotonia, ameliorates by exercise and can be associated with transient weakness during quick movements lasting only for seconds. On the contrary, paradoxical myotonia or paramyotonia worsens with exercise and cold and is followed by long spells of limb weakness lasting from hours to days. According to mode of transmission and K^+ sensitivity, four forms of myotonia and paramyotonia may be distinguished (Table **1**). Therefore, myotonia is the clinical phenotype brought about by uncontrolled repetitive firing of action potentials leading to involuntary muscle contraction. The contrary, lack of action potentials or inexcitability results in muscle weakness. Two dominant episodic types of weakness with or without myotonia are distinguished by the



Original figure will be pasted here

Fig. (2). Scheme of three potential states of voltage-gated cation channels such as those for Na^+ , Ca^{2+} and K^+ ions. The resting closed channel state (upper left panel) is activated by membrane depolarization that causes a fast transition to the open state (upper right). Due to an intrinsic inactivation as present in Na^+ and Ca^{2+} channels and also in some K^+ channels, the channel closes (lower panel) from which it reopens very rarely. Repolarization of the membrane leads to recovery from the inactivated (refractory) state back to the resting state (upper left) from activation is again possible. There are probably more than one open and at least two inactivated (fast and slow) states (not shown). Note that transition from the resting to the inactivated state is also possible without channel opening, particularly during slow depolarization (socalled accommodation). Note also that the amphipathic voltage sensor helices move within the lipid bilayer when the membrane polarity changes. The voltage sensors are formed by the S3/S4 segments (see Fig. 5) and act as "paddle" in the membrane. Modified after Jiang *et al*. [12, 13].

serum K^+ level during the attacks of tetraplegia: hyper- and hypokalemic periodic paralysis.

For most muscle Na^+ channel channelopathies, the underlying pathogenesis mechanism is the same: a gating defect of the Na^+ channel destabilizing the inactivated state, i.e. channel inactivation may be slowed or incomplete. The enhanced inward Na^+ current results in an increased tendency of the muscle fibers to depolarize activating more Na^+ channels and generating additional action potentials and, thereby, myotonia. If the Na^+ channel inactivation defect produces stronger depolarization, Na^+ channels are increasingly inactivated leading to weakness. Because the mutant channels exert an effect on cell excitability, the mutations produce a dominant change or gain of function. In contrast, Cl^- channel mutations bring about loss of function changes leading to reduced Cl^- conductance. This results in lack of buffering of the after potentials and instability of the membrane resting potential which may generate repetitive action potentials under triggering circumstances and, thus, myotonia.

Corresponding to the phenotype and the type of defect, the Na^+ channel gain-of-function mutations are located mainly in the voltage sensing S4 segment of domain IV suggested to couple the inactivation to the activation process, in the III-IV interlinker known to contain the inactivation gate, and at several disseminated intracellularly faced positions potentially involved in generating an acceptor for the inactivation particle or steric hindrance of the binding of the two. In the Cl^- channel, the loss-of-function mutations are distributed all over the channel protein resulting in, most frequently, non-sense proteins or, less frequently, proteins with single amino acid exchanges of residues decisive for voltage-dependent gating and ion selectivity (for review of channelopathies, see Lehmann-Horn and Jurkat-Rott [1]).

VOLTAGE CLAMP AND PATCH CLAMP TECHNIQUES

Modern research into the properties of ionic channels was initiated by the pioneering work of Hodgkin and Huxley [14], who utilized the voltage-clamp technique using intracellular microelectrodes to provide the first detailed description of the ionic basis of the action potential in nerve axons. For the following 50 years, the voltage clamp became the principal tool for the study of ion channels. It is performed with 2 intracellular "sharp" microelectrodes (tip

Table 1. Hereditary Channelopathies

Gene	Locus	Channel protein	Disease	Inheritance	Change
Cardiac muscle					
KCNQ1	11p15.5	potassium channel α subunit, KCNQ1, KVLQT1	Long QT syndrome 1	dominant	loss
			Jervell and Lange-Nielsen	recessive	
HERG	7q35-36	potassium channel α subunit, HERG, eag related, Ikr	Long QT syndrome 2	dominant	loss
SCN5A	3p21	sodium channel α subunit	Long QT syndrome 3	dominant	gain
			Brugada syndrome		loss
KCNE1	21q22	β subunit of KVLQT1, MinK	Long QT syndrome 5	dominant	loss
KCNE2		β subunit of HE RG, MiRP1	Long QT syndrome inducible	dominant	loss
RYR2	1q42-43	ryanodine receptor 1, calcium release channel type 2	Catecholaminergic ventricular fibrillation	dominant	gain
CASQ2	1p13-11	Calsequestrin type 2, fast-twitch	Catecholaminergic ventricular fibrillation	recessive	gain
Skeletal muscle					
SCN4A	17q23.1-25.3	voltage-gated sodium channel Nav1.4 α subunit	Hyperkalemic periodic paralysis	dominant	gain
			Paramyotonia congenita		
			Potassium-aggravated myotonia		
			Hypokalemic periodic paralysis 2	dominant	loss
CACNA1S	1q31-32	voltage-gated L-type calcium channel α1 subunit, dihydro-pyridine (DHP) receptor	Hypokalemic periodic paralysis type 1	dominant	uncl ear
			Malignant hyperthermia type 5	dominant	uncl ear
RYR1	19q13.1	ryanodine receptor 1, calcium release channel	Malignant hyperthermia type 1	dominant	gain
			Central core disease		
KCNJ2	17q23-24	voltage-insensitive potassium channel α subunit Kir2.1	Andersen´s syndrome, Long QT syndrome 7	dominant	loss
CLCN1	7q32-qter	voltage-gated chloride channel ClC1	Thomsen myotonia	dominant	loss
			Becker myotonia	recessive	loss
		altered splicing	Myotonic dystrophy type 1	dominant	loss
			Myotonic dystrophy type 2	dominant	loss
ATP2A1	16p12	SERCA, Ca-ATPase, fast-twitch 1, ATP2A1	Brody´s syndrome	dominant, recessive	loss
Endplate					
CHRNA1	2q24-32	nAChR α1 subunit	Congenital myasthenic syndrome (nAChR = nicotinic acetylcholine receptor)	dominant and recessive	gain and loss of function
CHRNB1	17p12-11	nAChR β1 subunit			
CHRND	2q33-34	nAChR δ subunit			
CHRNE	17	nAChR ε1 subunit			
RAPSN	11p11	rapsyn, AChR-associated protein	Congenital myasthenic syndrome	recessive	loss

(Table 1) contd….

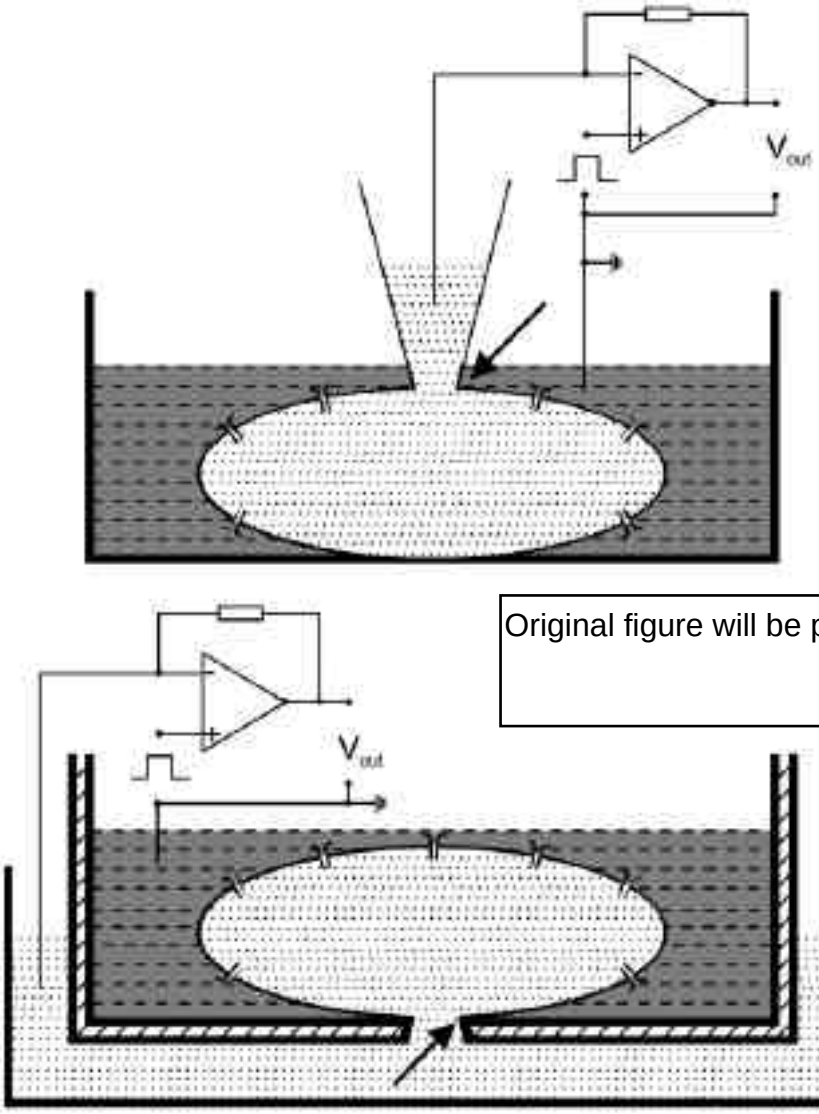
Gene	Locus	Channel protein	Disease	Inheritance	Change
Central nervous system					
SCN1A	2q24	Nav1.1 sodium ch α-subunit 1	Generalized epilepsy with febrile seizures plus (GEFS+2)	dominant	?
SCN1B	19q13.1	β1 subunit			
SCN2A	2q23	Nav1.2 sodium ch α-subunit 2			
SCN2A	2q23	Nav1.2 sodium ch α-subunit 2	Benign familial neonatal / infantile convulsions	dominant	?
KCNA1	12p13	potassium ch α1 subunit, A-type, Kv1.1	episodic ataxia type 1, partial epilepsy (?)	dominant	loss
KCNQ2	20q13.3	voltage-gated potassium channel α subunit Kv7.2	benign familial neonatal convulsions	dominant	loss
			Neuromyotonia		
KCNQ3	8q??	voltage-gated potassium channel α subunit Kv7.3	benign familial neonatal convulsions	dominant	loss
CACNA1A	19p13.1	calcium ch P/Q-type α1 subunit	episodic ataxia type 2	dominant	loss
			familial hemiplegic migraine 1		
			spinocerebellar ataxia type 6		
			absence epilepsy		
CACNA1F	Xp11.23	calcium ch retinal L-type α1 subunit	Congenital stationary night blindness (CSNB2)	recessive	loss
CLCN2	3q26	voltage-gated chloride channel ClC-2	idiopathic generalized epilepsies	dominant	loss or gain
CACNB4	2q22-23	calcium ch L-type β4 subunit	generalized epilepsy	dominant	gain
			episodic ataxia 3		
CHRNA4	20q13.3	nicotinic acetylcholine receptor α4 subunit	nocturnal frontal lobe, ADNFLE	dominant	loss
GLRA1	5q31.2	glycine receptor α1 subunit	Hyperekplexia = startle disease = stiff baby syndrome (STHE)	dominant	loss

diameter <0.1μm), one for clamping the membrane potential to values according to various pulse protocols for activation, inactivation, etc., the other measuring the current conducted during the voltage steps. The advantage of this technique that is still used, particularly for expression systems based on Xenopus oocytes, is its simplicity. The disadvantage is that the membrane of larger cells and oocytes may not be homogenously clamped to the desired voltage which is especially problematic if rapidly-activating ionic currents are studied.

In the mean time, a second, this time Nobel Prize-winning development has revolutionized the field: the patch clamp technique, developed by Neher and colleagues [15], which allows to record the transmembrane current of single channel molecule. The special feature of patch clamp technique is that it allows the direct electrical measurement of ion channel currents while simultaneously controlling the cell's membrane potential. This technique is now the gold-standard measurement for characterizing and studying ion channels and one of the most important methods applied to muscular or neurophysiology.

WHOLE-CELL CONFIGURATION

Whole-cell recordings are performed with a glas pipette of ~1 μm diameter tip connected with a patch clamp amlifier (Fig. **3**). Once the pipette is in contact with the cell membrane (seal), suction on the pipette is applied rupture the patch of membrane under the pipette tip. This generates a low-resistance pathway for current and diffusional flow between the pipette electrode and the cell interior. The cell membrane is voltage-clamped at the pipette potential by virtue of this low-resistance pathway, and the electrode monitors the current flowing across the entire cell surface. The whole cell configuration allows measurements of the current of all channels in the cell membrane superimposed with noise. This "macroscopic" current corresponds to the average of many simultaneously conducting channels and therefore resembles intracellular recordings. Because of the



Original figure will be pasted here

Fig. (3). Patch clamp set-ups. Upper panel: Classical whole-cell configuration and circuit using a glas micoelectrode. Lower panel: Patch clamp chip and circuit. This arrangement can be multiplied and automated for high-throughput screening. In addition, the arrangement allows the scientists to install a second technique, e.g. fluorescence or force microscopy, from the upside of the set-up. The arrows indicate the site of the gigaohm seal.

simple and fast analysis, it is the configuration that is most frequently used.

Although whole-cell recording can produce highly accurate current recordings, two important limitations are associated with the technique. The first of these is correlated with the resistance in series with the membrane (R_s), which corresponds to the access resistance between the interior of the pipette and the cell cytoplasm (for details see Armstrong and Gilly [16]). Another limitation of the whole-cell configuration is that important intracellular regulatory molecules, like cAMP, Ca^{2+}, or GTP, can diffuse out of the cell through the patch electrode. Thus, the physiological regulation of these important second messenger substances is disrupted during whole-cell recording. The perforated-patch technique [17] provides a solution to this problem by a membrane-intact recording configuration which is obtained by including a pore-forming antibiotic, i.e. nystatin or amphotericin B, in the pipette solution. After a seal is formed on the cell, the antibiotic channels insert into the membrane patch under the pipette tip, thereby providing electrical continuity between the pipette solution and the cell interior.

CELL-ATTACHED CONFIGURATION

In contrast to the whole-cell mode achieved by rupturing the membrane patch, the cell-attached mode is already given as soon as a GigaOhm seal between the patch electrode and the cell membrane is established. The background noise can be sufficiently attenuated so that the current flowing through a single ion channel can be resolved (single-channel recordings) [15]. A special form is the bleb-attached mode

that enables the measurement of adult native cells without enzymatic treatment. Blebs can be formed, e.g. by stretching of skeletal muscle fibers in a high-Ca^{2+} bath solution [18, 19]. The advantage is that the plasma membrane is absolutely clean so that a GigaOhm seal can be relatively easily established.

Single-channel recordings in the cell-attached mode have shown that many channels, e.g., the voltage-gated Na^+ channel, possess only two conductance levels: zero when the channel is closed and a constant conductance when the channel is open (Fig. **4**). Following depolarization, there is a brief delay before channels open. The intervals are not identical during each depolarization; in fact, the opening and closing of a given single channel is a random process even though the open probability depends on the voltage and is more sensitive to the voltage than an electronic device such as a transistor. After a subsequent short interval, the open time, the current jumps back to zero as the channels close. Usually, a normal Na^+ channel does not reopen even though the depolarization may be pertained by the voltage clamp step for a certain time. To attain the average behavior of a single channel, a macroscopic whole-cell Na^+ current.

OUTSIDE-OUT AND INSIDE-OUT CONFIGURATIONS

Variants of the patch clamp technique enable the application of solution on the exterior and interior of membrane patches not only attached but also torn from the cell in different configurations with respect to the membrane orientation: outside-out or inside-out (Fig. **5**). If the recording electrode is withdrawn from the cell after a cell-attached patch is formed, the patch of membrane can be excised from the cell with the inside surface of the membrane facing the bath solution; this is called the inside-out configuration. An outside-out patch can be formed by removing the electrode after entering the whole-cell configuration. Both of these modes allow the measurement of a few channel proteins or even a single channel depending on the channels density in the membrane patch. An advantage of these cell-free patch recordings is that they provide more accurate control of the membrane potential. In the cell-attached mode, the membrane potential of the patch is equal to the resting potential of the cell minus the pipette potential. Since the resting potential may not be known with certainty or might vary during an experiment, there will be uncertainty in the value of the patch membrane potential. However, in the isolated patch configurations, the magnitude of the patch potential is equal to the pipette potential and is therefore known with precision. A second advantage is that the solution bathing one surface of the patch can rapidly be changed simply by changing the bath solution.

DISCUSSION AND PERSPECTIVE FOR DRUG SCREENING

Although very powerful, the patch clamp technique is extremely labor-intensive and thereby limited to a

Original figure will be pasted here

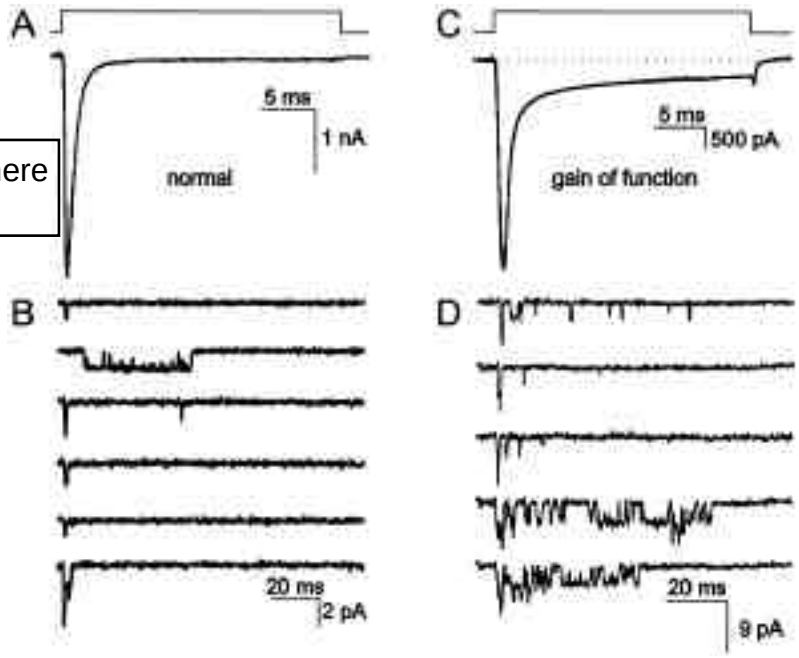
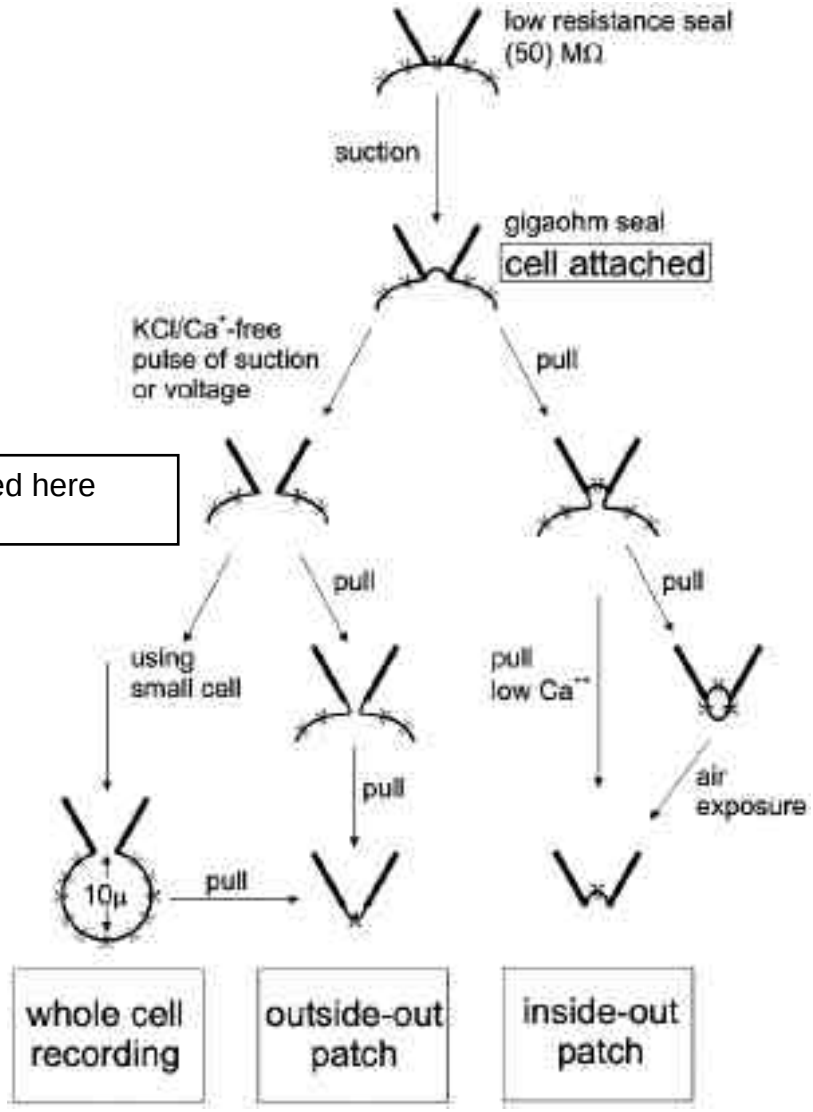


Fig. (4). Single channel recordings of Na^+ currents on two membrane patches, one from an adult muscle fibre of a healthy control (left), the other from that of a patient with a skeletal muscle Na^+ channelopathy. Both patches contained 4 Na^+ channels, performed in the bleb-attached mode. A, C: Superposition of 500 depolarization steps from –100 to –30 mV, as indicated by the upper rectangular pulse, leads to a "macroscopic" Na^+ current peak. B, D: Six subsequent of the 500 original current traces were selected to show the downward deflections corresponding to channel openings. Note that channel bursts and late reopenings in the original traces occur much more frequently in the mutant channels than in the normal and lead to a persistent current in the averaged curve as long as the membrane is depolarized by the artificial voltage step or, e.g. *in vivo*, by increased extracellular K^+ (hence the name of the myotonia, namely K^+-aggravated myotonia). Modified after Lerche *et al*. [20] and Mitrovic *et al.* [21].



Original figure will be pasted here

Fig. (5). Schematic representation of the procedures leading to recording configurations. A fine-tipped (about 0.5 to 5 micrometer in tip diameter) glass patch electrode is used as a current monitor and the voltage in the pipette is held at a desired level. The first step in applying the technique is the formation of a high-resistance seal between the patch electrode and the surface of the cell. Once the seal is established, several recording configurations are available to the investigator, and these fall into two broad categories. On one hand, current flow through the patch of membrane under the electrode tip can be monitored, in which case single channel currents are usually recorded. Alternatively, for whole-cell recording, the patch of membrane can be disrupted so that the electrode monitors current flow through the entire cell surface. Theùéis inserted for easier recognition of the orientation of the membrane and the channels. Modified after Sakmann and Neher [22].

throughput of 10-20 individual cell measurements per day. An alternative would be to develop technologies for high throughput screening. Achieving this goal requires more than just the automation of existing patch-clamp techniques; it requires the development of an entirely new paradigm for making electrophysiological measurements based on positioning a cell on a small pore separating two isolated fluid chambers in a manner that requires no manual intervention or micro-manipulation (Fig. **3** bottom). In order to perform whole-cell electrophysiological measurements within this geometry, two criteria must be met. First, a high-resistance seal must form between the cell membrane and peripheral region of the substrate pore to insure that the current measured between the two electrodes passes through the cell membrane. Second, in order to be able to control the cell's membrane potential, a low-resistance electrical pathway must form through the cell wall that covers the pore. Basically, three strategies fulfilling these criteria are being developed for automation of the technique: one is to simulate human behavior in manipulating the glass pipettes into contact with the cell to form the seal in the two-electrode voltage clamp configuration for oocytes [23, 24], a second is a patch clamp method with arrays of pipettes into which the cells are placed and by suction the seal is produced [25], and the last is based on replacement of the pipette altogether by a planar glass or silicon chip with micro apertures to form the seal [26, 27].

The electrophysiological study of mutant channels expressed in cell systems allows not only to characterize the functional alterations, but also to develop new, more specific strategies for the therapy of ion channelopathies, e.g. by testing drugs specifically designed either to block mutant channels that reveal a gain-of-function, or to activate non-mutant channels that could compensate for channels functionally lost by a mutation. The hitherto limitations in throughput are being overcome by the automation of the patch clamp technique by which it will become both cost-effective and fast and, at the same time, enable the highest sensitivity and most accurate description of drug effect compared to any other ion channel drug screening method [28, 29].

Already now, ion channel modulator drugs account for several billion US$ worldwide sales, i.e. Cl^- channel activators (benzodiazepine anxiolytics and anti-epileptics), K^+ channel blockers (sulphonylurea anti-diabetics, amiodarone-type anti-arrhythmics), Ca^{2+} channel blockers (verapamil-type anti-arrhythmics), and Na^+ channel blockers (lidocaine-type anesthetics, mexiletine-type anti-arrhythmics, and anti-epileptics like lamotrigine and carbamazepine). As membrane-localized proteins, ion channels are easily accessible to drugs and, because of the great variety of expression patterns and tissue-specific splicing, most-likely have a restricted adverse-effect profile. This makes them ideal drug targets especially given the possibility of such precise observation of the drug effect on channel function by the patch clamp technique.

ACKNOWLEDGEMENTS

We thank U. Richter for drawing the cartoons. This work was supported by the German Research Foundation (DFG) (JU 470/1), the Research Programme “Functional nanoscopy of pathologically altered biomembranes” of the Ministry for Science, Research and the Arts in the State of Baden-Württemberg and the network on *Excitation-contraction coupling and calcium signaling in health and disease* of the IHP Program funded by the European Community.

REFERENCES

[1] Lehmann-Horn, F. and Jurkat-Rott, K. (1999) *Physiol. Rev.*, **79**, 1317-1372.

[2] Mannuzzu, L.M.; Moronne, M.M. and Isacoff, E.Y. (1996) *Science*, **271**(5246), 213-216.

[3] Yang, N.; George, A.L. Jr. and Horn, R. (1996) *Neuron*, **16**(1), 113-122.

[4] MacKinnon, R. and Yellen, G. (1990) *Science*, **250**(4978), 276-279.

[5] Perez-Garcia, M.T.; Chiamvimonvat, N.; Ranjan, R.; Balser, J.R.; Tomaselli, G.F. and Marban, E. (1997) *Biophys. J.*, **72**(3), 989-996.

[6] Vassilev, P.M.; Scheuer, T. and Catterall, W.A. (1988) *Science*, **241**(4873), 1658-1661.

[7] Hoshi, T.; Zagotta, W.N. and Aldrich, R.W. (1990) *Science*, **250**(4980), 533-538.

[8] Middleton, R.E.; Pheasant, D.J. and Miller, C. (1996) *Nature*, **383**(6598), 337-340.

[9] Ludewig, U.; Pusch, M. and Jentsch, T.J. (1996) *Nature*, **383**(6598), 340-343.

[10] Jentsch, T.J.; Steinmeyer, K. and Schwarz, G. (1990) *Nature*, **348**(6301), 510-514.

[11] Dutzler, R.; Campbell, E.B.; Cadene, M.; Chait, B.T. and MacKinnon, R. (2002) *Nature*, **415**(6869), 287-294.

[12] Jiang, Y.; Lee, A.; Chen, J.; Ruta, V.; Cadene, M.; Chait, B.T. and MacKinnon, R. (2003) *Nature*, **423**(6935), 33-41.

[13] Jiang, Y.; Ruta, V.; Chen, J.; Lee, A. and MacKinnon, R. (2003) *Nature*, **423**(6935), 42-48.

[14] Hodgkin, A.L. and Huxley, A.F. (1952) *J. Physiol.*, **117**, 500.

[15] Hamill, O.P.; Marty, A.; Neher, E.; Sakmann, B. and Sigworth, F.J. (1981) *Pflügers Arch.*, **391**(2), 85-100.

[16] Armstrong, C.M. and Gilly, W.F. (1992) *Methods Enzymol.*, **207**, 100-122.

[17] Horn, R. and Korn, S.J. (1992) *Methods Enzymol.*, **207**, 149-155.

[18] Quasthoff, S.; Franke, C.; Hatt, H. and Richter-Turtur, M. (1990) *Neurosci. Lett.*, **119**(2), 191-194.

[19] Lerche, H.; Fahlke, C.; Iaizzo, P.A. and Lehmann-Horn, F. (1995) *Pflügers Arch.*, **429**(5), 738-747.

[20] Lerche, H.; Mitrovic, N.; Dubowitz, V. and Lehmann-Horn, F. (1996) *Ann. Neurol.*, **39**(5), 599-608.

[21] 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) *J. Physiol.*, **478**(Pt 3), 395-402.

[22] Sakmann, B. and Neher, E. (1985) *Single-Channel Recording*, Plenum Press, New York.

[23] Trumbull, J.D.; Maslana, E.S.; McKenna, D.G.; Nemcek, T.A.; Niforatos, W.; Pan, J.Y.; Parihar, A.S.; Shieh, C.C.; Wilkins, J.A.; Briggs, C.A. and Bertrand, D. (2003) *Receptors Channels*, **9**(1), 19-28.

[24] Schnizler, K.; Kuster, M.; Methfessel, C. and Fejtl, M. (2003) *Receptors Channels*, **9** (1), 41-48.

[25] Lepple-Wienhues, A.; Ferlinz, K.; Seeger, A. and Schafer, A. (2003) *Receptors Channels*, **9**(1), 13-17.

[26] Fertig, N.; George, M.; Klau, M.; Meyer, C.; Tilke, A.; Sobotta, C.; Blick, R.H. and Behrends, J.C. (2003) *Receptors Channels*, **9**(1), 29-40.

[27] Asmild, M.; Oswald, N.; Krzywkowski, K.M.; Friis, S.; Jacobsen, R.B.; Reuter, D.; Taboryski, R.; Kutchinsky, J.; Vestergaard, R.K.; Schroder, R.L.; Sorensen, C.B.; Bech, M.; Korsgaard, M.P. and Willumsen, N.J. (2003) *Receptors Channels*, **9**(1), 49-58.

[28] Mattheakis, L.C. and Savchenko, A. (2001) *Curr. Opin. Drug Discov. Devel.*, **4**(1), 124-134.

[29] Willumsen, N.J.; Bech, M.; Olesen, S.P.; Jensen, B.S.; Korsgaard, M.P. and Christophersen, P. (2003) *Receptors Channels*, **9**(1), 3-12.