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Review

Ion channels-related diseases★✪

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There are many diseases related to ion channels. Mutations in muscle voltage-gated sodium, potassium, calcium and chloride channels, and acetylcholine-gated channel may lead to such physiological disorders as hyper- and hypokalemic periodic paralysis, myotonias, long QT syndrome, Brugada syndrome, malignant hyperthermia and myasthenia. Neuronal disorders, e.g., epilepsy, episodic ataxia, familial hemiplegic migraine, Lambert-Eaton myasthenic syndrome, Alzheimer's disease, Parkinson's disease, schizophrenia, hyperekplexia may result from dysfunction of voltage-gated sodium, potassium and calcium channels, or acetylcholine- and glycine-gated channels. Some kidney disorders, e.g., Bartter's syndrome, policystic kidney disease and Dent's disease, secretion disorders, e.g., hyperinsulinemic hypoglycemia of infancy and cystic fibrosis, vision disorders, e.g., congenital stationary night blindness and total colour-blindness may also be linked to mutations in ion channels.

There are many diseases related to proteins embedded in cell membranes. Ion channels are one class of such molecules. Channel protein forms pores in cell membranes and allow particular ions to pass through them down the concentration gradient. A characteristic feature of ion channels is a gating mechanism that controls ion movement. There are three main types of channel gating. Voltage-gated channels are opened by a change in membrane potential. Molecules that bind to a specific site of the channel activate ligand-gated channels. The third group comprises channels activated by mechanical stimuli. Ion channels are essential to a wide range of physiological functions including neuronal signalling, mus-

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cle contraction, cardiac pacemaking, hormone secretion, cell volume regulation and cell proliferation. It is not surprising that ion channels are implicated in numerous diseases. Most of them are inherited disorders which result from mutations in genes encoding channel proteins. Some are autoimmune diseases in which the body produces antibodies to its own channel molecules.

In 1989 the first disorder, cystic fibrosis, was identified as an ion channel disorder (Tsui, 1992). From this moment the list of diseases is still growing. The study of ion channels diseases usually consists of two stages. First, the chromosome locus of the disease and the protein coded by that gene must be identified. Then the function of mutant channel expressed in special cells as HEK (human embryonic kidney cells) or *Xenopus* oocytes is studied with electrophysiological techniques. Gene mutations produce defective polypeptide chains that are not processed correctly and are not incorporated into the membrane or polypeptide chains that form channels but non-functional or with altered kinetics. Properties of channels may be studied by electrophysiological techniques. Whole-cell voltage clamp technique measures all channels of the cell in the same time. One can estimate maximum current flowing and its kinetics. The patch clamp technique is able to measure a single channel. Since the single channel fluctuates between an open and a closed state one can determine current amplitude, open channel probability or the duration of the closed and the open state.

This article describes several ion channel types that are postulated to cause a diversity of diseases, though detailed mechanisms of their contribution to observed symptoms is still unclear.

VOLTAGE-GATED SODIUM CHANNELS

Voltage-gated sodium channel is formed by the α subunit chain, which has 4 homologous, but not identical domains. There are 6 transmembrane segments in each domain. The β subunit is a smaller polypeptide, with a single transmembrane segment and a large extracellular domain. The β subunit plays a role in the gating of the channel, hastening the rates at which it opens and closes. The voltage-gated channels of nerves and muscles are crucial to nervous impulse propagation and muscle contraction. The channels are activated by depolarisation of the cell membrane. In the open state they are selectively permeable to sodium ions. The flow of ions into the cell produces strong local depolarisation called action potential that moves along the axon as new voltage-gated sodium channels opens due to the depolarisation. Potassium ion efflux through depolarisation-activated voltage-gated potassium channels and inactivation of the voltage-gated sodium channels curtails the action potential. The process continues until the resting potential is reset. Activation of muscle voltage-gated sodium channels triggers the flow of calcium ions into the cytoplasm from the sarcoplasmic reticulum and myofibril contraction.

Muscle disorders

Mutations in the α subunit of voltage-gated sodium channel (SCN4A) causes two types of autosomal dominant skeletal muscle disorders: periodic paralysis and myotonia. Hyperkalemic periodic paralysis is characterised by attacks of muscle weakness or paralysis between periods of normal muscle function. Attacks usually begin early in life. They tend to occur while resting after exercise and last 1–2 h. Weakness most commonly affects the muscles of the arms and legs. The level of potassium in the blood is normal or high. Myotonia is a general name for the clinical symptom of delayed relaxation of skeletal muscle following voluntary contraction. The symptoms may worsen after repeated exercise. Paramyotonia congenita is characterised by intermittent muscle stiffness or involun-

tary contraction that is often triggered by cold. Weakness may occur. Potassium-aggravated myotonia is muscle stiffness triggered by potassium-rich food such as bananas. It includes three syndromes: myotonia fluctuans, a mild myotonia exacerbated by potassium that varies in severity from day to day, myotonia permanens, a severe continuous myotonia, and acetazolamide-responsive myotonia congenita, a painful muscle stiffness.

More than 20 point mutations causing these disorders have been described. Generally, the mutant channels exhibit abnormal, long-lasting currents that prolong membrane depolarisation. The effects of mutations in channels expressed in HEK cells or *Xenopus* oocytes indicate that the slower decay of sodium current results from specific alterations of channel function as compared to the wild types:

- Val445Met, Thr704Met, Val1293Ile, Gly1306Glu, Ile1495Phe, Met1592Val: shift of steady-state activation to more negative potentials,
- Ser804Phe, Gly1306Glu, Gly1306Val: shift of steady-state fast inactivation to more positive potentials,
- Ser804Phe, Gly1306Glu, Gly1306Val, Thr1313Met, Arg1448Ser, Arg1448Cys, Arg1448Pro: slower rate of fast inactivation,
- Val445Met, Ile1495Phe: negative shift of steady-state slow inactivation,
- Thr704Met, Met1592Val: impaired slow inactivation,
- Ser804Phe, Val1293Ile, Met1360Val, Arg1441Pro, Arg1441Cys, (Arg1441 is rat equivalent of human Arg1448), Arg1448Ser: more rapid recovery from the fast inactivation state,
- Thr704Met: faster recovery from the slow inactivation state,
- Thr704Met, Gly1306Glu, Gly1306Val, Gly1306Ala, Arg1441Pro, Arg1441Cys, Arg1448Ser: slower deactivation

(Mitrovic *et al.,* 1995; Richmond *et al.,* 1997; Wagner *et al.,* 1997; Featherstone *et al.,* 1998; Green *et al.,* 1998; Bendahhou *et al.,* 1999a; 1999b; Mitrovic *et al.,* 1999; Rojas *et al.,* 1999; Takahashi & Cannon, 1999). Thr704Met and Met1592Val are the most common mutations in hyperkalemic periodic paralysis and Arg1448His, Arg1448Cys, Arg1448Pro in paramyotonia congenita.

Single channel recordings revealed that the mutations Met1360Val and Arg1448Cys, Arg1448Pro increase the frequency of channel reopening and prolong mean open times (Wagner *et al.,* 1997; Mitrovic *et al.,* 1999).

These reports indicate that the affected amino-acid residues are important for sodium channel inactivation. The lack of full inactivation and strong depolarisation cause a portion of sodium channels to enter a desensitised state leading to membrane inexcitability. This explains muscle weakness. Mild depolarisation leads to increased membrane excitability and repeated activation of the contractile apparatus that results in muscle stiffness. Some mutations presumably make channels sensitive to temperature, producing symptoms aggravated by cold (Featherstone *et al.,* 1998). The site of substitution, size and charge of the new amino acid is important for the kind and severity of symptoms.

Cardiac disorders

Long QT syndrome is an autosomal dominant (Romano-Ward syndrome) or a recessive (Jervell and Lange-Nielsen syndrome) cardiac disorder characterised by a very fast heart rhythm (arrhythmia) called “torsade de pointes” which leads to sudden loss of consciousness (syncope) and may cause sudden cardiac death, predominantly in young people. The QT interval on the electrocardiogram is the time from the onset of ventricular depolarisation (the Q wave) to the completion of repolarisation (the end of the T wave). Long QT is associated with two cardiac muscle ion channels: voltage-gated potassium channel and voltage-gated sodium channel. The altered ion channels function produces a pro-

longation of the ventricular action potential. Mutations in the α subunit of voltage-gated sodium channel SCN5A causes the channel to inactivate incompletely. Voltage-clamp studies revealed that:

- Lys1505, Pro1506, Gln1507 deletions, Arg1623Gln and Glu1784Lys: channels show a sustained inward current during membrane depolarisation,
- Asp1795 insertion: shift of steady-state activation of the channel to more positive potentials,
- Asp1790Gly and Asp1795 insertion: channels shift steady-state inactivation to more negative potentials,
- Arg1623Gln: channel has significantly slower macroscopic inactivation,

(An *et al.,* 1998; Bennet *et al.,* 1995; Kambouris *et al.,* 1998; Makita *et al.,* 1998; Bezzina *et al.,* 1999; Wei *et al.,* 1999).

Single-channel recordings showed that the Arg1623Gln mutant channel has prolonged open times with bursting behaviour and increased probability of long openings (Kambouris *et al.,* 1998; Makita *et al.,* 1998).

In effect the mutant channels reopen during prolonged depolarisation contributing to the persistent inward current carried by sodium ions that delays repolarisation. This produces prolonged action potential.

The Brugada syndrome (idiopathic ventricular fibrillation) is abnormal heart function that is represented in the electrocardiographic pattern by right bundle branch block and ST elevation in the right precordial leads (early repolarisation). This autosomal dominant disorder may cause sudden death of young people. Mutations in the SCN5A cardiac voltage-gated sodium channel can be responsible for the syndrome. Arg1512Trp, Thr1620Met and Ala1924Thr mutant channels expressed in *Xenopus* oocytes or a mammalian cell line resulted in faster current decay than in wild-type channels, prolonged recovery from inactivation and shift of steady-state activation and inactivation to more negative potentials (Dumaine *et al.,* 1999; Rook *et al.,* 1999). This can increase inward sodium current during the action potential upstroke.

Epilepsy

Epilepsy is characterised by bursts of synchronised discharges that cause seizures. Generalised epilepsy with febrile seizures is epilepsy provoked by the acute febrile illness. It affects nerve cells of the whole brain. Febrile seizures afflict approximately 3% of all children under six years of age and are by far the most common seizure disorder. A small proportion of children with febrile seizures later develop ongoing epilepsy with afebrile seizures. It is an autosomal dominant disorder. There are two candidates for the disorder: a gene encoding the β1 subunit of voltage-gated sodium channel (SCN1B) and a gene encoding the α subunit of voltage-gated sodium channel (SCN1A). Single amino-acid changes in both chains were found (Wallace *et al.,* 1998; Escayg *et al.,* 2000b). Co-expression of the Cys121Trp mutant β1 subunit with the brain sodium channel α subunit in *Xenopus laevis* oocytes demonstrated that the mutation interferes with the ability of the subunit to modulate channel-gating kinetics.

Malignant hyperthermia

Malignant hyperthermia is an inherited condition that causes severe uncontrollable fever during anaesthesia or while using muscle relaxants. It is inherited as an autosomal dominant trait. The anaesthetised patient rapidly develops high fever and muscle rigidity. During these episodes, muscle tissue is destroyed and released muscle pigments (myoglobin) may damage the kidneys and cause acute renal failure. Malignant hyperthermia can be fatal if not treated immediately. There was one family reported by Moslehi *et al.* (1998) that appeared to support the suggestion that a form of malignant hyperthermia is caused by mutations in the SCN4A gene. Ryanodine re-

ceptor and voltage-dependent calcium channel are more involved in malignant hyperthermia.

VOLTAGE-GATED AND INWARDLY RECTIFYING POTASSIUM CHANNELS

Voltage-gated potassium channel is built from 4 α protein chains surrounding a central pore. Each subunit forms 6 transmembrane regions. The β chain is a regulatory subunit that can co-assemble with the α subunit to modulate the gating kinetics and enhance the stability of the multimeric complex. β is a small polypeptide forming one transmembrane domain. The channels are important for proper repolarisation after action potential. They are activated by depolarisation after sodium channels activation. The flow of potassium ions out of the cell contributes to the re-establishing of resting potential.

Another class of potassium channels is inward rectifiers. The channel is made from 4 identical subunits. They have 2 membrane-spanning segments and 1 pore-lining segment. HERG is atypical with 6 transmembrane segments. Inward rectifiers open at membrane potentials near to or more negative than the resting potential and allow potassium ions to flow inwards but not outwards. In the heart, these channels also have small outward conductance that regulate resting potential and contribute to the terminal phase of repolarisation. At positive voltages, these channels close and thus help maintain the level of resting potential.

Episodic ataxia with myokymia syndrome

Episodic ataxia with myokymia syndrome is a dominantly inherited disorder. It is characterised by brief episodes of incoordination (ataxia) with continuous muscle movement (myokymia) at other times. Abrupt postural change, emotion and vestibular stimulation provoke it. Attacks last minutes. Brunt & van Weerden (1990) concluded that the myokymic activity results from multiple impulse generation in peripheral nerves. A number of different heterozygous missense point mutations of the neural voltage-gated potassium channel α subunit (KCNA1/Kv1.1) have been identified. D'Adamo *et al.* (1999) and Adelman *et al.* (1995) studied the function of the Val408Cys mutant channel. They demonstrated that Kv1.1 bearing this mutation produces channels with threefold reduction of the mean open duration compared with the wild type, faster kinetics and increased inactivation. The Phe184Cys mutation shifts the voltage-dependence to more positive potentials. The results suggest that affected nerve cells cannot repolarise efficiently. It lowers the amount of excitation needed to produce action potentials. This leads to uncontrolled muscle contractions.

Long QT syndrome and congenital hearing loss

Long QT, as described in the previous chapter, is a disorder of cardiac repolarisation. Mutations in 4 cardiac voltage-gated potassium channels are associated with long QT syndrome: KCNA8 (KCNQ1, KVLQT1) thought to be the one most commonly responsible, KCNE1, KCNE2 (minK) and KCNH (HERG). KCNQ1 encodes the α subunit of potassium channel. There are about 30 mutations in the KCNQ1 gene that could cause either Romano-Ward syndrome or Jervell and Lange-Nielsen syndrome. The mutations have two different effects: failing to produce functional channels (Arg174Cys, Glu261Lys) or altering channel kinetics. The mutations that produce functional channels:

- reduce macroscopic conductance (Leu272Phe, Ala300Thr),
- shift the voltage-dependence of activation strongly to more positive potential (Arg243His, Trp258Arg, Arg533Trp, Arg539Trp, Arg555Cys),

- slow the rate of activation (Arg243Cys, Trp248Arg),
- accelerate deactivation (Arg243His, Trp248Arg, Arg555Cys)

(Chouabe *et al.,* 1997; Shalaby *et al.,* 1997; Priori *et al.,* 1998; Franqueza *et al.,* 1999; Chouabe *et al.,* 2000; Schmitt *et al.,* 2000). The mutations generally diminish outward repolarising potassium current, which prolongs cardiac action potential. This can cause sudden arrhythmias.

KCNE1 codes for the β subunit that coassembles with the KCNQ1 α subunit to form the slowly activating cardiac potassium channel. The outward current of this channel has an increased amplitude and reaches its steady state only after 50 s. The $\alpha\beta$ channels make the contribution of potassium current to the cardiomyocyte repolarisation strongly dependent on the heart rate and on its regulatory state. Co-expression of mutant KCNE1 with KCNQ1 produces reduced currents through channels with altered gating, lowered amplitudes (Val47Phe, Trp87Arg) and accelerated deactivation (Ser74Leu, Asp76Asn) as compared with wild-type channels (Splawski *et al.,* 1997; Bianchi *et al.,* 1999).

HERG encodes an inwardly rectifying potassium channel that mediates repolarisation of ventricular action potentials. HERG channels show gating properties consistent with many of the inwardly rectifying potassium channels, but they also have an inactivation mechanism that attenuates efflux during depolarisation. The channels inactivate much more rapidly than they activate. The result is that most HERG channels are closed during the plateau phase of cardiac action potential. Rapid recovery from inactivation during repolarisation combined with a very slow subsequent transition to the closed state (deactivation) result in increased inwardly rectifying potassium current during the terminal phase of cardiac repolarisation. The increased incidence of cardiac sudden death has been observed in patients that lack HERG currents because they carry a genetic defect. Such mutants (Ile593Arg, Tyr611His, Gly628Ser, Val822Met) do not functionally express (Zhou *et al.,* 1998) or alter channel kinetics:

- Thr474Ile, Asn470Asp, Arg534Cys activate at more negative voltages,
- Arg534Cys accelerates activation,
- Arg56Gln, Asn33Thr activate more slowly and at more positive voltages,
- Phe29Leu, Asn33Thr, Gly53Arg, Arg56Gln, Cys66Gly, His70Arg, Ala78Pro, Leu86Arg, Arg534Cys accelerate deactivation,
- Asn629Asp: channel is nonselective for monovalent cations and replaces the outward repolarising current with the inward depolarising sodium current

(Zhou *et al.,* 1998; Chen *et al.,* 1999a; Nakajima *et al.,* 1999; Lees-Miller *et al.,* 2000).

The mutations are predicted to result in a diminished magnitude of potassium inwardly rectifying current consistent with the prolonged LQ interval observed in affected individuals.

KCNE1 can also assemble with HERG to modulate the rapid delayed rectifier current. Then mutations in KCNE1 may lead to the syndrome. Such mutations are observed and effect in suppressed HERG current (Bianchi *et al.,* 1999).

KCNE2 is the gene encoding the MinK-related peptide, a small integral membrane subunit that assembles with the HERG channel to alter its function. Three missense mutations associated with long QT syndrome and ventricular fibrillation (Gln9Glu, Met54Thr, Ile57Thr) were identified in the KCNE2 gene (Abbott *et al.,* 1999). Mutants form channels that open slowly and close rapidly, thereby diminishing potassium currents.

Congenital hearing loss is associated with Jervell and Lange-Nielsen syndrome and mutations in the KCNE1, KCNQ1 (Neyroud *et al.,* 1997) and the KCNQ4 (Kubisch *et al.,* 1999) gene of voltage-gated potassium channels. They are expressed in the inner ear. KCNQ1 is expressed as a complex with KCNE1 in the cells of a specialised endothelium, *stria*

vascularis. The stria is responsible for secretion of endolymph, the potassium-rich fluid that fills the middle chamber of the cochlea and bathes the sound-receptive hair cells. In Jervell and Lange-Nielsen syndrome endolymph secretion does not occur normally, the middle chamber of cochlea collapses, and hair cells degenerate. KCNQ4 is expressed in the outer hair cells. Their function is to increase the sensitivity of sound perception by mechanically amplifying sound vibrations within the cochlea. Mutations abolish the potassium current and probably exert an effect on endolymph homeostasis.

Bartter's syndrome

Bartter's syndrome is an autosomal recessive form of often severe intravascular volume depletion due to renal salt-wasting associated with alkalosis (a condition of excess base in body fluids) with reduced potassium (hypokalemic alkalosis), hypercalciuria (the presence of excess calcium in the urine) and increased production of the hormone aldosterone. Patients with Bartter's syndrome are often critically ill from birth onwards leading to renal failure. Mutations in the KCNJ1 gene coding for inwardly rectifying potassium channel (Kir 1.1) leading to loss of function are associated with the disease. Potassium channel in the kidney probably plays a major role in K^+ homeostasis. Disorder of channel function disrupts potassium recycling back to the tubule lumen and inhibits thereby the sodium-potassium 2 chloride cotransporter activity (Karolyi *et al.,* 1998). Classic Bartter's syndrome results from defective chloride transport.

Epilepsy

Benign neonatal epilepsy is an autosomal dominant form of epilepsy characterised by recurrent, brief, generalised seizures that begin on about the fourth day of life and cease after 1–3 months. Affected persons carry a 10–16% risk of developing epilepsy again later in life. Two α subunits of brain voltage-gated potassium channels are associated with the disorder: KCNQ2 and KCNQ3. Mutations have been found in these subunits which are responsible for epileptic seizures. Expression of the mutant channel in *Xenopus* oocytes did not yield measurable currents (Biervert *et al.,* 1998) or significantly reduced the potassium current (Lerche *et al.,* 1999). The KCNQ2 and KCNQ3 channel subunits can coassemble to form the M-channel. It opens occasionally at the resting potential and is slowly activated by depolarisation. M-channel activation causes a delayed membrane hyperpolarisation after a cell receives an excitatory input. Dysfunction of M-Channels causes neurones to become slightly depolarised and to fire multiple action potentials rhythmically after receiving excitatory inputs.

Hyperinsulinemic hypoglycemia of infancy

Hyperinsulinemic hypoglycemia of infancy (HHI) is an autosomal recessive disorder of glucose metabolism characterised by unregulated secretion of insulin and profound hypoglycemia. Thomas *et al.* (1996) discovered a Kir6.2 (inwardly rectifying potassium channel) gene mutation in an affected individual with severe HHI. The ATP-sensitive potassium current of the affected channel plays a role in secretion and muscle contraction by coupling metabolic activity to membrane potential. In pancreatic beta cells, ATP-potassium channels are crucial for the regulation of glucose-induced insulin secretion (Sharma *et al.,* 2000).

VOLTAGE-GATED CALCIUM CHANNELS

Voltage-gated calcium channels consist of the large principal subunit called $\alpha 1$, which contains the channel pore, together with regulatory $\alpha 2$, β, χ and δ subunit. The $\alpha 1$ subunit

has 4 homologous domains with 6 transmembrane segments each. Calcium channels are found in brain, muscle, and a wide variety of other tissues. They are entirely responsible for voltage-gated depolarisation in vertebrate smooth muscle, and serve to maintain depolarisation during the plateau of action potential in vertebrate heart muscle. They are also crucially important in controlling the internal calcium ion concentration in many muscle cells, secretory cells and at nerve terminals. Calcium ions act also as intracellular messengers and calcium channels are intimately involved in regulation of cell functions.

Hypokalaemic periodic paralysis

Hypokalaemic periodic paralysis is an autosomal dominant skeletal muscle disorder in which episodes of muscle weakness occur. Affected people have first attacks of limb weakness as teenagers. Patients have low serum potassium levels. The disorder is associated with mutations in the skeletal muscle voltage-gated calcium channel $\alpha 1$ subunit (CACNA1S, CACNL1A3), especially in the voltage sensor regions of the channel. They lead to calcium currents with reduced amplitude and voltage-dependence shifted to more negative potentials (Arg528His) or to more positive potentials (Arg1239His, Arg1239Gly) (Morrill & Cannon, 1999). Voltage-gated calcium channels in skeletal muscle are involved as voltage sensors in excitation-contraction coupling, a process whereby electrical signals generated by action potentials at the muscle cell surface are transduced into intracellular release of calcium and ultimately muscle fibre contraction. Rapid inactivation seems to interfere with this process and leads to the observed symptoms.

Episodic ataxia

As already mentioned, episodic ataxia is a lack of coordination. It can result from mutations in the CACNA1A (CACNL1A4) gene, which codes for the pore forming $\alpha 1$A subunit of voltage-gated calcium channel (Ophoff *et al.,* 1996). The channels are present in the presynaptic terminal of motor axon. They are involved in the control of membrane excitability and neurotransmitter release. The mutations probably produce non-functioning channels.

The Ca^{2+} channel β subunits regulate voltage-dependent calcium currents through direct interaction with the $\alpha 1$ subunits. The β and $\alpha 1$-binding motifs are conserved, and all β subunits can stimulate current amplitude, voltage dependence, and kinetics. Inactivation of the $\beta 4$ subunit of calcium channel in the lethargic mouse neurological mutant results in a complex neurological disorder that includes ataxia. Escayg *et al.* (2000a) found a premature termination mutation in members of an affected family.

Familial hemiplegic migraine

Familial hemiplegic migraine, a rare autosomal dominant disorder, is characterised by attacks of hemicranial pain. There are reports that a dysfunction of the CACNL1A4 channel $\alpha 1$A subunit may be involved (Ophoff *et al.,* 1996; Spranger *et al.,* 1999). Expression experiments show that mutations in the calcium channel alter inactivation gating and can lead to both gain and loss of function of the channel (Kraus *et al.,* 1998; Hans *et al.,* 1999).

Lambert-Eaton myasthenic syndrome

Lambert-Eaton myasthenic syndrome is an autoimmune disease, caused by antibodies to calcium channels at the nerve terminal on which acetylcholine release depends. The targets of antibodies are the $\alpha 1$ subunits of P/Q-type voltage-gated calcium channel (Takamori *et al.,* 2000). This results in a decrease in the amount of acetylcholine released by the nerve impulse. Patients typically have weakness of legs and arms.

Epilepsy

To determine the role of the CACNB4 gene encoding the $\beta 4$ subunit in this human disorder, Escayg *et al.* (2000a) screened for mutations in small pedigrees with familial epilepsy and ataxia. A premature-termination mutation was identified in a patient with juvenile myoclonic epilepsy (brief spells of loss of consciousness and repetitive bilateral myoclonic jerks in the shoulders and arms after awakening, without loss of consciousness). It lacks the 38 C-terminal amino acids containing part of an interaction domain for the $\alpha 1$ subunit. The missense mutation Cys104Phe was identified in a German family with generalised epilepsy. The Ca^{2+} channel β subunit regulates voltage-dependent calcium currents through direct interaction with the $\alpha 1$ subunit. The results of functional tests of the truncated protein in *Xenopus laevis* oocytes demonstrated a small decrease in the fast time constant for the inactivation of the cotransfected $\alpha 1$ subunit. Further studies will be required to evaluate the *in vivo* consequences of these mutations.

Congenital stationary night blindness

Congenital stationary night blindness is a recessive, nonprogressive retinal disorder characterised by decreased visual acuity and loss of night vision. Mutation analysis of the CACNA1F gene coding for the $\alpha 1$F subunit expressed in retina in 20 families with the syndrome revealed 6 different mutations, all of which predicted premature protein termination (Bech-Hansen *et al.,* 1998).

Malignant hyperthermia

As it was already mentioned this is a disorder provoked by general anaesthesia. Mutations in the sarcoplasmic calcium release channel (ryanodine receptor, RYR1) are the most frequent cause of malignant hyperthermia (McCarthy *et al.,* 2000). The channels mediate calcium release in skeletal muscle during excitation-contraction coupling. Mutations alter the channel kinetics for calcium inactivation and make the channel hyper- and hyposensitive to activating and inactivating ligands, respectively. Calcium release channels open persistently, causing sustained muscle contraction, fever and muscle injury. Mutations in other channels are less common causes of malignant hyperthermia e.g., mutation in the $\alpha 1$ subunit of the human skeletal muscle L-type voltage-dependent calcium channel (CACNL1A3) (Monnier *et al.,* 1997).

VOLTAGE-GATED CHLORIDE CHANNELS

Voltage-gated chloride channels have several functions including the regulation of cell volume, membrane potential stabilisation, signal transduction and transepithelial transport. They are located in all tissues. The model of the channel structure assumes 4 subunits, each with 13 domains but not all of the segments actually cross the membrane. Muscle chloride channels regulate the electric excitability of the skeletal muscle membrane by holding membrane potential near its resting level. Kidney chloride channels are presumably important for Cl^- reabsorption.

Myotonia

Myotonia or muscle stiffness is a muscle abnormality in which relaxation after voluntary contraction is delayed. The stiffness lessens as muscles are used. Myotonia is based on the hyperexcitability of the muscle fibre membrane. There are two types of the syndrome. The recessive form, in which the stiffness starts in childhood in the leg muscles and spreads to the arms, neck and face, is known as Becker's disease or generalised myotonia. Less severe Thomsen's disease or myotonia congenita is a dominant form. Myotonias are associated with mutations in the muscle voltage-gated chloride channel CLC-1. Mutations

seem to have different effects on channel function:

- Val165Gly, Phe167Leu, Gly200Arg, Val236Leu, Gly285Glu, Val286Ala, Ile290Met, Phe307Ser, Ala313Thr, Arg317Gln, Ile329Thr, Arg338Gln, Phe413Cys, Pro480Leu, Gln552Arg, Ile556Asn: shift of channel activation toward positive voltages so the channel open probability is near 0 at resting potential,
- Gly230Glu: changes in anion and cation selectivity and the cation-to-anion permeability ratio; outward rectification instead of the wild-type inward rectification at positive potentials,
- Met485Val: reduction of single channel conductance

(Pusch *et al.,* 1995; Fahlke *et al.,* 1997; Wollnik *et al.,* 1997; Kubisch *et al.,* 1998; Zhang *et al.,* 2000).

The mutant channels cannot contribute significantly to the repolarisation of action potentials. Without such repolarisation, sodium channels have enough time to recover from inactivation leading to typical myotonic runs, which are a series of repetitive action potentials.

Kidney disorders

X-linked nephrolithiasis, or Dent's disease, encompasses several clinical syndromes of low molecular weight proteinuria (the presence of excess protein in the urine), hypercalciuria (the presence of excess calcium in the urine), nephrocalcinosis (deposition of calcium oxalate or phosphate in renal tubules and the areas between the tubules), nephrolithiasis (kidney stones), and renal failure. It is associated with mutations in the CLCN5 gene encoding the kidney-specific voltage-gated outwardly rectifying chloride channel (CLC-5). Expression in *Xenopus* oocytes of wild type and the mutants demonstrated that the mutations, Leu200Arg, Ser270Arg, Leu278Phe, Trp279termination, Gly506Glu, Ser520Pro, Arg648termination, Arg704termination, either abolish or markedly reduce chloride conductance (Lloyd *et al.,* 1996; Igarashi *et al.,* 1998). Gunther *et al.* (1998) and George (1998) showed that the CLCN5 gene is expressed in renal proximal tubule cells, which normally endocytose proteins passing the glomerular filter. Gunther *et al.* (1998) suggested that CLC-5 might be essential for proximal tubular endocytosis by providing an electrical shunt necessary for the efficient acidification of vesicles in the endocytotic pathway, explaining the proteinuria observed in Dent's disease.

Classic Bartter's syndrome has been demonstrated to result from defective chloride transport across the basolateral membrane in the distal nephron due to mutations in the kidney chloride channel gene. Examination of this gene reveals loss-of-function mutations that impair renal chloride reabsorption (Simon *et al.,* 1997).

NICOTINIC ACETYLCHOLINE RECEPTOR CHANNELS

The nicotinic acetylcholine receptor (nAChR) channels are among the best known of channels. They consist of 5 subunits: two α chains and one β, one χ or one ε and one δ chain. The ε subunit occurs in adult mammals. It takes place of the χ subunit present in newly born. The nAChR channels are present in the postsynaptic membrane of vertebrate muscle and nervous cells. Electrical stimulation of the presynaptic axon releases acetylcholine into the synaptic gap. Acetylcholine combines with nAChRs. Their channels open and allow sodium and potassium ions to flow through. The resulting depolarisation acts as a trigger for the activation of voltage-gated channels and ultimately for muscle contraction or propagation of the nerve impulse along the neurone. Neuronal nAChRs are also found in presynaptic membranes. They are implicated in controlling and modulating the release of various neurotransmitters.

Congenital myasthenia

Disorders of the neuromuscular junction lead to several types of myasthenia. Slow channel congenital myasthenia is an inherited autosomal dominant syndrome, characterised by muscle weakness, rapid fatigue, progressive muscle atrophy, generation of repetitive muscle action potentials in response to a single nerve stimulus, and degeneration of the postsynaptic region of the muscle. It results from mutations in the muscle nAChR channel. There are many identified mutations in all adult subunits:

- αGly153Ser, αVal249Phe, βVal266Met, εThr264Pro, εLeu269Phe increase the receptor's affinity for ACh,
- αGly153Ser, αAsn217Lys, αVal249Phe allow multiple reopenings before ACh dissociation,
- αAsn217Lys, βVal266Met, εThr264Pro, εLeu269Phe slow the rate of channel closing,
- αVal249Phe, βVal266Met, εThr264Pro, εLeu269Phe increase spontaneous opening of the channel,
- αAsn217Lys, αVal249Phe, εLeu269Phe enhance desensitisation

(Ohno *et al.,* 1995; Sine *et al.,* 1995; Engel *et al.,* 1996; Gomez *et al.,* 1997; Milone *et al.,* 1997).

The increased duration of channel opening or bursts of openings produce a prolonged excitatory postsynaptic current, prolonged depolarisation of the muscle membrane, inactivation of voltage-gated Na^+ channels and failure of muscle excitability. The prolonged depolarisation also causes enhanced Ca^{2+} entry, which may account for the progressive destruction of the postsynaptic neuromuscular junction.

Fast channel syndrome resembles symptoms of slow channel syndrome, but mutation in the nAChR channel produces a reduction in ACh affinity and decreases the rate of channel openings. Ohno *et al.* (1996) reported a mutation, εPro121Leu that causes fast channel syndrome. It lies in the ε subunit close to the residue, which participates in agonist binding. The mutation reduces the affinity of nAChR for ACh and the rate of channel opening. Fewer receptors are activated, and those, which are, exhibit shorter burst duration. The excitatory postsynaptic current is reduced and depolarisation is too small to elicit the action potential.

Myasthenia gravis

Myasthenia gravis is an autoimmune disease caused by antibodies directed toward nAChR. It consists in muscle weakness that usually increases with continued activity but improves after periods of rest. Any muscle can be affected, however, the muscles that control eye movement, the eyelids, facial expression and swallowing are most frequently affected. The disease results from a reduction in the number of functional nAChR by lysis of the postsynaptic membrane following antibody binding, which leads to destruction of the endplate region of the muscle (Engel *et al.,* 1977). Blocking of the receptor by antibodies which reduces peak current amplitude has also been demonstrated (Bufler *et al.,*1998).

Epilepsy

Autosomal dominant nocturnal frontal lobe epilepsy is a rare form of partial epilepsy that causes brief, frequent and violent seizures almost exclusively during light sleep. A single mutation in the neuronal nAChR α4 subunit was detected in affected individuals: phenylalanine replaced serine as the 247th residue, a highly conserved amino acid in the second transmembrane domain (Steinlein *et al.,* 1995). It is thought to lie within the pore as it is in the binding site for chlorpromazine, which is an open channel blocker. Expression of the mutant channel in *Xenopus* oocytes resulted in faster desensitisation to ACh and slower recovery from desensitisation than that of the wild type channel (Weiland *et al.,* 1996; Kuryatov *et al.,* 1997). The effect of the

mutation is to decrease the channel open time, reduce single channel conductance and increase the rate of desensitisation. The possible connection of these activity changes with epilepsy is that α4-containing nAChRs might mediate the release of the inhibitory neurotransmitter GABA. The reduction in nAChR function would then result in the enhanced excitability of postsynaptic neurones and lower the seizure threshold.

Other human central nervous system disorders

Alzheimer's disease (AD), Parkinson's disease (PD) and schizophrenia can be also associated with the neuronal nAChR, though the mechanism is still unknown. AD and PD are neurological degenerative disorders. AD is characterised by loss of memory and difficulty in learning. Eventually all mental functions fail. Microscopic examination shows that cholinergic cells of the brain producing acetylcholine die and disappear. PD symptoms are tremor or trembling of hands, arms, legs, jaw and face, rigidity of the limbs and trunk, slowness of movement. The hallmark of the disease is the loss of brain cells producing dopamine. There is general consensus that the number of cortical neuronal nicotinic receptors is decreased in both diseases. It is confirmed in recent studies (Rinne *et al.,* 1991; Perry *et al.,* 1995). Loss of the nicotinic receptor may precede degeneration of cholinergic and dopaminergic neurones in affected regions.

The possible involvement of nicotinic receptors in schizophrenia has been suggested by the high prevalence of smoking in schizophrenic patients. A cluster of symptoms that typically include delusions, hallucinations, disordered thinking, and emotional unresponsiveness describes the disease. Preliminary experiments indicate that patients have fewer ligand binding sites in the hippocampus (Freedman *et al.,* 1995; Leonard *et al.,* 1996). It might lead to a failure of cholinergic activation of inhibitory interneurones manifested as decreased gating of response to sensory stimulation.

GLYCINE RECEPTOR

Glycine receptor is a neurotransmitter-gated ion channel. Binding of glycine to its receptor increases the chloride conductance and thus produces hyperpolarisation that inhibits neuronal firing. The channel is a pentamer composed of α and β subunits. The subunit has 4 transmembrane domains.

Hyperekplexia

Hyperekplexia (or startle disease) is an autosomal dominant neurologic disorder characterised by muscular rigidity of central nervous system origin, particularly in the neonatal period, and by an exaggerated startle response to unexpected acoustic or tactile stimuli. It results from mutations in the α1 subunit of glycine receptor chloride channels that disrupt inhibitory synaptic transition. The most frequently observed mutation is Arg271Leu or Arg271Gln. These and other mutations: Pro250Thr, Gln266His and Lys276Glu, lead to reduced chloride current due to abnormal channel kinetics: lower sensitivity to glycine, and reduced open times. Arg271Leu, Arg271Gln decreased single channel conductance (Rajendra *et al.,* 1994; Lewis *et al.,* 1998; Moorhouse *et al.,* 1999; Saul *et al.,* 1999).

CYSTIC FIBROSIS CHLORIDE CHANNELS

Cystic fibrosis transmembrane conductance regulator (CFTR) has 2 membrane-spanning domains, each with 6 putative transmembrane segments. It is activated by cAMP. The channel is localised to the apical membranes

of epithelial cells lining air tracts and secretory tubules.

Cystic fibrosis

Cystic fibrosis is a hereditary disease that afflicts about one in 2500 children. Sufferers have thick mucous secretion that blocks the smaller lung airways and the ducts of pancreas. This leads to inflammation and infection of the lung and pancreas, then progressive destruction of both organs and eventual death, usually before 30. There is also excessive salt loss in the sweat glands. Cystic fibrosis results from a failure of chloride transport that is associated with mutations of the gene coding for the CFTR channel. Normally water movement follows chloride efflux through the channels into the lumen of the tubules. Failure of chloride transport results in a reduction in the amount of fluid produced by epithelial cells, so they become blocked with thick secretions. About 500 mutations have been identified in the CFTR gene. The most common and severe mutation (occurs in 70% of all defective CFTR genes) is the deletion of phenylalanine at position 508. This mutation leads to the retention of channel protein within the cell and failure of channel trafficking to the plasma membrane (Cheng *et al.,* 1990). Many other mutations have the same effect (Vankeerberghen *et al.,* 1998). Milder forms of the disease result from such mutations as Arg117His, Glu193Lys, Arg334Trp and Arg347Pro which produce channels that are less likely to open or have reduced amplitude (Sheppard *et al.,* 1993; Seibert *et al.,* 1997).

cGMP-GATED CATION CHANNELS

Cyclic nucleotide-gated (CNG) cation channels are essential in visual and olfactory signal transduction. Cyclic guanosine monophosphate (cGMP) opens cation-selective channels in vertebrate photoreceptor cells. These channels are responsible for the flow of sodium and calcium ions into the rod outer segments in the dark. This depolarises the cell membrane. Light absorption by the visual pigment leads indirectly to the activation of cGMP phosphodiesterase which breaks down cGMP and so closes the channels. The retinal rod cGMP-gated cation channel is a hetero-oligomer composed of 2 homologous subunits, α and β, each with cytoplasmic N and C termini and 6 putative transmembrane domains.

Total colour-blindness

Total colour-blindness, also referred to as rod monochromacy (RM) or complete achromatopsia, is a rare, autosomal recessive congenial disorder characterised by photophobia, reduced visual acuity, nystagmus and a complete inability to discriminate between colours. Kohl *et al.* (1998) reported the identification of missense mutations in CNGA3 (α3 subunit) in 5 families with RM.

NONSELECTIVE CATION CHANNELS

Polycystic kidney disease

Polycystic kidney disease (PKD) comes in two hereditary forms: the autosomal dominant form is one of the most common life-threatening genetic diseases. In the syndrome, cysts develop in both kidneys. The cysts may range in size from a pinhead to the size of a grapefruit. When many cysts develop, the kidneys can grow to a football size or larger. The first documented case of PKD dates back to Stefan Batory, the King of Poland. There are reports that the gene mutated in most cases of the disease codes for pore forming subunits of a channel permeable to sodium, potassium and calcium ions (Nomura *et al.,* 1998; Chen *et al.,* 1999).

CONCLUSIONS

Hundreds of publications on ion channels-related diseases have appeared in recent years. In the review we selected the ones that show the convincing linkage between disease and ion channel dysfunction. There are also many interesting web sites. One can find a full list of diseases in which defects of ion channels play a role at http://www.neuro.wustl.edu/neuromuscular/mother/chan.html

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