REVIEW ARTICLE

ION CHANNELS AND CHANNELOPATHIES

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Since the discovery of ion channels in 1970s, the aetiology of many diseases has been traced back to channelopathies. Many toxins produced by snakes, fish, spiders and other insects paralyse the ion channels. Many physiological mechanisms have been studied at molecular level and have confirmed the involvement of ion channels. The ion channels are the recent target sites for pharmaceutical biosynthesis of new drugs. Ion channels have been classified according to the type of gating, number of gates and species of ions passing through them.

The hypothetical existence of ion channels was described for the first time in 1952 by British biophysicists; Alan Hodgkin and Andrew Huxley to support their noble winning theory of nerve impulse. In 1970s, the existence of ion channels was confirmed by the invention of 'patch clamp' technique by Erwin Neher and Bert Sakmann who won a Nobel Prize for it. In 2003, the Nobel Prize was awarded to American scientists, Roderick MacKinon and Peter Agre for their x-ray crystallographic structure studies on ion channels and Aquaporins respectively.

Ion channels are integral proteins of cell membrane of all cells. They usually have a central pore made up of alpha units. The supporting sub units are named as beta, gamma, delta, and epsilon. Some channels have structural configuration in such a manner that some proteins act as a 'gate'. The gate can be opened or closed by different stimuli such as chemical ligand or electrical signal, temperature or mechanical force depending upon type of channel. Some channels are 'ungated' and are called Leak channels.

CLASSIFICATION OF ION CHANNELS

Based on type of ions passing through them, ion channels are classified as sodium channels, potassium channels, calcium channels, chloride channels, proton channels and aquaporins

SODIUM CHANNELS

Sodium channels are further classified as ungated sodium (Leak) channels, voltage gated sodium channels, ligand gated sodium channels and stretch activated sodium channels.

Ungated sodium (leak) channels[2] are leak channels present in the cell membrane of all excitable cells. They play little role in resting membrane potential (RMP). The equilibrium Nernst potential for these channels is +60 mV. They have no role in the action potential of excitable cells. Voltage gated sodium channels[2] channels are present in the cell membrane of all excitable cells. They are responsible for depolarization phase of action potential in most of the excitable tissues. Sodium channel have two gates; 'm' activation and 'h' inactivation gates. At onset of action potential, these channels are responsible for depolarization phase.

Ligand gated sodium channels[3] include nicotinic acetylcholine gated channels, epithelial sodium channels (ENaCs) also called Amiloride inhibitable channel and cyclic nucleotide gated sodium channels (CNG channels). Nicotinic acetylcholine gated sodium channels are present at neuromuscular junction, in autonomic ganglions and in brain. Two molecules of acetylcholine (Ach) bind to each alpha subunit causing influx of sodium and thus depolarization. Opened channels have diameter of 0.65 nm and are negatively charged inside repelling chloride and other negative molecules.

ENaCs (Epithelial Sodium Channels) also known as Amiloride Inhibitable Channels are found in Principle cells of renal distal convoluted tubules and cortical collecting tubules, colon, lungs and brain. These channels are made up of 3 subunits alpha, beta and gamma encoded by 3 different genes. Mineralocorticoids increase the number of active ENaCs in the apical membrane of the renal cell. Cystic Fibrosis Transmembrane Regulator (CFTR) down-regulates sodium absorption via ENaCs. The diuretic Amiloride inhibits these channels.

Cyclic nucleotide gated sodium channels (CNG channels) are activated by intracellular binding with cAMP or cGMP. These are hyperpolarizing – activated CNG channels. Channels in SA node of heart and in rods and cones of the eye are such examples. Stretch activated sodium channels (BNC 1)[3] are closely associated with touch receptors. This channel protein when hyper-expressed causes neurons they are in to degenerate. It is not yet clear that whether BNC 1 is part of touch receptor complex or the initial part of neural fibre where initiation of spike potential occurs. The receptor may be opened mechanically by pressure on skin.

POTASSIUM CHANNELS

Potassium channel are classified as ungated (Leak) K^+ channels, voltage gated K^+ channels and ligand gated K+ channels. Ungated (Leak) K^+ channels[2] are present in all excitable tissues and at resting state remains open all the time. These are mainly

responsible for RMP. Voltage Gated K^+ channels[2] are tetramers with each of the subunit forming part of the single pore through which K^+ ions passes. During resting state, gate of K^+ channel is closed. They are responsible for repolarization phase of action potential. Types of Voltage Gated K^+ channels are present in cardiac muscle[4] are Inward rectifying K^+ channel (IKr), delayed rectifying K^+ channel (Kv or IKs) and transient outward K^+ channel (IK_{To}). IKr channels protein crosses membrane six times combined with a small protein called min-K (because of its size) that has only single membrane-spanning domain. It opens at very negative potential i.e. less than – 40 mV and shows a reduced K^+ efflux at positive membrane potential. This effect is opposite to the normal outward rectification seen in Kv channels.[5] K^+-ATP and K^+-Ach channels show some inward rectification. At plateau phase of cardiac muscle action potential, these channels allow K^+ influx, but oppose the K^+ efflux. Kv channels are activated by membrane depolarization and cause K^+ efflux. These channels are responsible for the repolarization of cardiac action potential in phase three in ventricular muscle and purkunje fibers.[6]

IK_{To} channels cause transient K^+ efflux after depolarization and produce phase one of cardiac muscle action potential. These produce an early incomplete repolarization that occurs before the plateau phase.

Ligand gated K^+ channels[7] include Ca^{++}-activated K^+ channel also called BK channels, ATP-sensitive K^+ channel, Acetylcholine (Ach)-activated K^+ channel, oxygen-sensitive K^+ channel and RomK channels. Ca^{++} Activated K^+ channels (BK channels) are present in heart and vascular smooth muscles. The activity of Ca^{++} activated K^+ channel is increased by Ca^{++} sparks caused by high local Ca^{++} concentration inside the cell. K^+ efflux causes increased the membrane potential, thus closes the voltage-gated Ca^{++} channel resulting in relaxation of cardiac muscle. In vascular smooth muscles, these channels are called 'Big K' or 'BK channels' as K^+ flows through them at a high rate. In ATP sensitive K^+ channels, ATP keeps these K^+ channels in closed position. Lack of ATP, e.g., in ischemia or hypoxia opens these channels resulting in increased K^+ efflux causing hyperpolarization. These channels are found in smooth muscle of systemic vessels but not in pulmonary vessels. The opening of these channels results in relaxation of smooth muscle of blood vessels in contrast to pulmonary vessels which undergo vasoconstriction by hypoxia. B Islets of Langerhans of pancreas also has ATP sensitive K^+ channels. High blood glucose enters B cells of pancreas through glucose transporter-2 (GLUT-2), which is not insulin-sensitive. Increased blood glucose increases ATP level which closes ATP gated K^+ channel causing membrane potential to reach threshold. Action potential (AP) results which cause the Ca^{++} channel to open. Exocytosis occurs and insulin is released. These channels accelerate repolarization and shorten the cardiac AP. Prostacyclins, Vasoactive intestinal peptide (VIP), Nitrous oxide (NO) and adenosine act in part via K^+-ATP opening. Acetylcholine (Ach) Activated K^+ channels open by vagally secreted Ach. The channels are present in SA node of the heart causing decreases spontaneous depolarization which results in bradycardia, and slower AV nodal conduction. Oxygen sensitive K^+ channels are present in smooth muscles of pulmonary vessels and also in carotid bodies. Lack of O_2 inhibits these channels; decreased K^+ efflux leads to membrane potential approaching threshold. AP occurs and Ca^{++} influx through 'L' type channels. Contraction occurs and hypoxia results in vasoconstriction in pulmonary vasculature in contrast to hypoxia induced vasodilatation caused by ATP sensitive K^+ channels in systemic vasculature. RomK K^+ Channels are present in thick ascending limb of Loop of Henle both at basolateral and luminal membrane of kidney. It moves K^+ from the cells to the interstitium and tubular lumen. Stretch Activated Potassium Channels are activated by mechanical stretch. These are present on the hair cells of inner ear.

CALCIUM CHANNELS

Calcium channels are classified into voltage gated calcium channels, ligand gated calcium channels and stretch-activated calcium channels.

Voltage-Gated Ca^{++} Channel[8] are of following types: 'L' (long lasting), 'T' (transient), 'N', 'P', 'Q' and 'R' types. 'L' (Long Lasting) Type Ca^{++} channels[3,8] are also called DHP (Dihydropyridine) receptors as these are blocked by DHP. These are high voltage activated channel present in vascular and cardiac tissues. Influx of Ca^{++} via these channels from ECF occurs which triggers the release of Ca^{++} stored in sarcoplasmic reticulum. In skeletal muscles, Ca^{++} influx do not occur through DHP receptors, instead they serve as the 'voltage sensors' and 'triggers' that unlock release of Ca^{++} from nearby sarcoplasmic reticulum via Ryanodine receptors. 'T' (Transient) Type Ca^{++} channels low voltage activated and are rapidly inactivated. These are present in SA Node of the heart and causes phase 4 of action potential that is slow depolarization of pace maker potential. 'N', 'P', 'Q' and 'R' Type[4] calcium channels are found in neuronal cells. The functions of these channels are not yet known.

Ligand Gated Ca^{++} Channels[3] includes Ryanodine receptors, Inosine triphosphate (IP3) receptors, SOCC (store-operated Ca^{++} channels) and CatSper protein (cAMP mediated Ca^{++} channel).

Ryanodine Receptors are channels present at sarcoplasmic reticulum of skeletal and cardiac muscle. In skeletal muscle, DHP receptors at T-tubules when stimulated by AP, act as voltage sensor and trigger and unlocks ryanodine receptors and cause influx of Ca^{++} into cell from sarcoplasmic reticulum (SR). In cardiac muscle, Ca^{++} influx through DHP receptors from ECF triggers release of Ca^{++} from SR through ryanodine receptors. These channels are called ryanodine receptors because these are locked in open position by the plant alkaloid ryanodine. IP3 receptors are present at sarcoplasmic reticulum of skeletal muscles, cardiac and other cells as well. IP3 act as second messenger and cause release of Ca^{++} from endoplasmic reticular by binding to IP3 receptors. SOCC are present in cell membrane and open up when transient release of Ca^{++} influx stores in cytoplasm. Ca^{++} influx through these channels replenishes the intracellular Ca^{++} supply and refills the endoplasmic reticulum. IP3 act as the chemical mediator which causes internal release of Ca^{++} from the ER and the activation of the SOCC. CatSper Protein is a Ca^{++} ion channel localized in the principle piece of sperm tail that permits cAMP generated Ca^{++} influx. The ability to move forward (progressive motility) that is acquired by the sperm in the epidydmus involves activation of this unique protein. Stretch-Activated Ca^{++} channel is a direct link between mechanical stimulus and ion channel. These channels are present in touch receptors.

CHLORIDE CHANNEL[3,9]

Chloride channels are classified into CLC Dimeric channels, GABA and Glycine receptors (Pentameric chloride channel) and CFTR (Cystic Fibrosis Transmembrane Regulator)

CLC Dimeric Channel channels are dimers consisting of two sub-units and are voltage-gated channel. Two types of CLC chloride channels are found in stria vascularis in the inner ear. These are CIC-Ka chloride channel and CIC-Kb chloride channel. An integral membrane protein; Bartin is found to be essential for CIC-Kb chloride channel and CIC-Ka chloride channel. This stria vascularis in the inner ear is responsible for maintaining the high K^+ concentration in the scala media that is essential for normal hearing. Thick ascending limb of Loop of Henle also contains CIC-Kb chloride channel. It is responsible for reabsorption of Na^+, K^+ and Cl^- along with other channels including Na^+-K^+-$2Cl^-$ co-transporter, RomK K^+ channel and Baritin.

GABA and Glycine receptors are chloride channels with five subunits with a single pore in the centre. GABAa and GABAb receptors are mostly found in CNS. GABAc receptors are found in almost exclusively in retina. 'A' cells of Islets of Langerhans of pancreas contain GABAa receptors. 'B' cell of Islets of Langerhans of pancreas also releases GABA which acts on GABAa receptors on 'A' cells. Insulin thus inhibits glucagon secretion by 'A' cells by producing increased chloride conductance and hyperpolarization.

Glycine receptors are pentamers made up of two subunits Glycine receptors in brainstem and spinal cord exert direct inhibitory effect by increased chloride conductance through these channels. CFTR is a cAMP regulated chloride channel found in the apical membrane of epithelial cells of kidneys, respiratory system, liver and female reproductive tract. CFTR also down-regulates Na absorption via ENaC. In cystic fibrosis, CFTR gene is defective.

VOLTAGE GATED PROTON CHANNELS[10]

These channels are highly *p*H sensitive open in depolarization when electrochemical gradient is outward. They allow protons to move outwards from the cell causing an increase in cellular pH. These channels play important role in phagocytes during the 'respiratory burst'. When the phagocytes engulf the bacteria or other microbes, reactive oxygen species (ROS) are produced to kill micro organisms by the enzyme NADPH oxidase in the membrane of the phagocytes. This enzyme is electrogenic, causing electron movement across the cell membrane. Proton efflux occurs through proton channels to balance the electron movement electrically.

AQUAPORINS[3]

Movement of water molecules across the cell membrane by simple diffusion is assisted by the movement through water channel called aquaporins. Aquaporin-1, 2 and 3 are found in kidneys, Aquaporins 4 are found in brain, Aquaporins 5 are found in salivary and lacrimal glands and in lungs. Aquaporins 9 are present in liver, spleen, lungs and leucocytes.

These channels are stored in the endosdomes inside the renal cells. Anti-diuretic hormone (ADH) act on aquaporin 2 channels in collecting ducts of kidneys and causes rapid translocation of these channels to the luminal membrane of the renal tubule.

CHANNELOPATHIES [11–13]

Extensive research has been done and is continued on ion channels. Ion channels are a favourite site for invention of new drugs. Moreover many genetic disorders are found to be caused by defective channel proteins. In addition to that, many toxins and venoms produced by spiders, snakes, scorpion, bees, fish, snails and others act by incapacitating ion channels.

TOXINS

Following are a few toxins resulting in channelopathies:

Tetrodotoxin: It is used by puffer fish for defence and causes sodium channel blockade. It is used in research studies on ion channels by scientists.
Saxitoxin: It is produced by dino flagellates. It blocks the voltage gated sodium channels.
Conotoxin: It is used by cone snails to hunt prey.
Dendrotoxin: It is produced by the mamba snakes, and blocks potassium channels.
Ciguatoxin: It is produced by marine fish. It is a potent sodium channel blocker and causes intense numbness, paresthesia and muscle weakness.

GENETIC

Channelopathies are caused by defects in genes. Inheritance pattern can be recessive or dominant.
Myesthenia Gravis: It is caused by development of antibodies against Ach gated sodium channels at NMJ in skeletal muscles. There is gradual paralysis of skeletal muscles. Involvement of respiratory muscles may prove fatal
Periodic paralysis: Two forms of periodic paralysis exist; hyperkalaemic and hypokalemia and are caused by different genetic defects in voltage gated potassium channels. There is sudden periodic onset of weakness triggered by hyperkalaemic or hypokalemia. In both forms there is dominant inheritance. Dietary restriction can reduce the frequency of attacks.
Malignant Hyperthermia: There is defect in Rynidine calcium channel.
Cystic fibrosis is caused by mutations in the CFTR gene, which forms chloride channel. These channels are situated at the apex of epithelial membrane. The hindrance of chloride efflux into lumen and defect in reabsorption of sodium results in thick desiccated mucus.
Paramyotonia Congenita (PC): There is congenital gene mutation in alpha-1 subunit of sodium channel
Generalized epilepsy with febrile seizures plus (GEFS+)
Episodic Ataxia (EA), characterized by sporadic bouts of severe discoordination and can be provoked by stress, startle, or heavy exertion such as exercise.
Congenital hyperinsulism: There is defect in inward rectifier K^+ channel.
Long QT syndrome is a ventricular arrhythmia syndrome caused by mutations in one or more of presently ten different genes, most of which are potassium channels and all of which affect cardiac repolarization.
Brugada syndrome is another ventricular arrhythmia caused by voltage-gated sodium channel gene mutations.
Eaton Lamberd Syndrome: It is caused by defect in voltage gated Ca^{++} channels in pre synaptic membrane at NMJ in skeletal muscles.
Liddles Syndrome: It is a ENaC channel disorder.
Beckers Myotonia: Skeletal muscle chloride channel disorder
Diabetes Incipidus Type 2: There is autosomal gene mutation. The defect is in Aquaporin channel protein.
Bartter Syndrome: It is autosomal recessive disorder. One of 04 channels protein mutation occurs; CLC-Ka, CLC-Kb, Barttin protein or ROMK. Features are hypotension, hypokalemia, alkalosis+ deafness.

With advanced research techniques, the pathology of different diseases is now explored at the molecular level. Exploring the mechanisms operative at ion channels level, help to understand not only the physiological mechanisms operative in human body, to comprehend the underlying pathology of the diseases but also help in finding the target sites for pharmaceutical drugs.

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