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Ion Channels and their Modulation

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ABSTRACT

Ion channels are pore-forming proteins that help establish and control the small voltage gradient across the plasma membrane of cells by allowing the flow of ions down their electrochemical gradient. They may be classified by the nature of their gating, the species of ions passing through these gates and on the basis of number of gates (pores). There are a number of chemicals and genetic disorders, which disrupt normal functioning of ion channels and have disastrous consequences for the organism. Modulation of the properties of membrane ion channels is of fundamental importance for the regulation of neuronal electrical activity and of higher neural functions. The development of powerful new molecular biological and biophysical approaches has provided important new insights into the structure and function of ion channels and has revealed them as dynamic entities whose activity can be regulated.

INTRODUCTION

Ions are charged particles such as Na^+, H^+, K^+ and Cl^-. Ions have a significant effect on many cell processes and also influence the amount of water in the cell. Cells use inorganic ions for transmitting signals across the cell membrane or along the surface of the cell. Other cellular functions as diverse as secretion of hormones to fertilization of egg cells require ion transport across the cell membrane (Hille, 1992).

Ion channels are pore-forming proteins that help establish and control the small voltage gradient across the plasma membrane of cells by allowing the flow of ions down their electrochemical gradient (Hille, 1992). They are present in the membranes that surround all biological cells. There are over 300 types of ion channels in a living cell (Gabashvili et.al.,2007).

These channels regulate the flow of ions across the membrane in all cells. They are integral membrane proteins; or, more typically, an assembly of several proteins. Such "multi-subunit" assemblies usually involve a circular arrangement of identical or homologous proteins closely packed around a water-filled pore through the plane of the membrane or lipid bilayer (Camerino et.al 2007). For most voltage-gated ion channels, the pore-forming subunit(s) are called the α subunit, while the auxiliary subunits are denoted β, γ, and so on. Some channels permit the passage of ions based solely on their charge, positive (cation) or negative (anion). However, the archetypal channel pore is just one or two atoms wide at its narrowest point and is selective for specific species of ion, such as sodium or potassium. These ions move through the channel pore in a single file nearly as quickly as the ions move through free fluid. In some ion channels, passage through the pore is governed by a "gate," which may be opened or closed by chemical or electrical signals, temperature, or mechanical force, depending on the variety of channel.

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Classification

Ion channels are broadly classified into following groups:

- Nature of their gating,
- Species of ions passing through these gates, and
- Number of gates (pores).

1. Nature of Gating

On the basis of gating (what opens and closes the channels), ion channels are grouped into voltage-gated ion channels and ligand-gated ion channels (Harmar., 2009).

a. Voltage-gated ion channel

Voltage-gated ion channels are a class of trans-membrane ion channels that are activated by changes in electrical potential difference near the channel. These types of ion channels are especially critical in neurons, but are common in many types of cells. They have a crucial role in excitable neuronal and muscle tissues, allowing a rapid and coordinated depolarization in response to triggering voltage change. Found along the axon and at the synapse, voltage-gated ion channels directionally propagate electrical signals. They generally are composed of several subunits arranged in such a way that there is a central pore through which ions can travel down their electrochemical gradients. The channels tend to be ion-specific, although similarly sized and charged ions may sometimes travel through them (Harmar et.al., 2009).

Voltage-gated ion channels are further sub-classified as:

- Voltage-gated sodium channels
- Voltage-gated calcium channels
- Voltage-gated potassium channels (KV)
- Cation channels of sperm
- Voltage-gated proton channels
- Some transient receptor potential channels
- Hyperpolarization-activated cyclic nucleotide-gated channels

b. Ligand-gated ion channel (LGIC)

They are a group of transmembrane ion channels that are opened or closed in response to the binding of a chemical messenger (i.e., a ligand) (Hille, 2001) such as a neurotransmitter (Gabashvili et.al.,2007).

The binding sites of endogenous ligands on LGICs protein complexes are normally located on a different portion of the protein (an allosteric binding site) compared to where the ion conduction pore is located. The direct link between ligand binding and opening or closing of the ion channel, which is characteristic of ligand-gated ion channels, is contrasted with the indirect function of metabotropic receptors, which use second messengers. The ion channel is regulated by a ligand and is usually very selective to one or more ions like Na^+, K^+, Ca^{2+}, or Cl^-. Such receptors located at synapses, convert the chemical signal of presynaptically released neurotransmitter directly and very quickly into a postsynaptic electrical signal (Harmar et.al., 2009).

LGICs are classified into three superfamilies:

Cys-loop receptors: $GABA_{A,}$ Glycine, Serotonin

Ionotropic glutamate receptors: AMPA, Kainate, NMDA

ATP-gated channels: P2X

2. Species of Ions passing through the gates (Harmar et.al., 2009)

- Chloride channels
- Potassium channels
- Calcium-activated potassium channels
- Inward-rectifier potassium channels
- Two-pore-domain potassium channels: This family of 15 members form what is known as leak channels, and they follow Goldman-Hodgkin-Katz (open) rectification.
- Sodium channels
- Voltage-gated sodium channels
- Epithelial sodium channels
- Calcium channels
- Proton channels
- Voltage-gated proton channels
- Non-selective cation channels: These let many types of cations, mainly Na^+, K^+ and Ca^{2+} through the channel.
- Most Transient receptor potential channels

3. Number of pores (Harmar et.al., 2009)

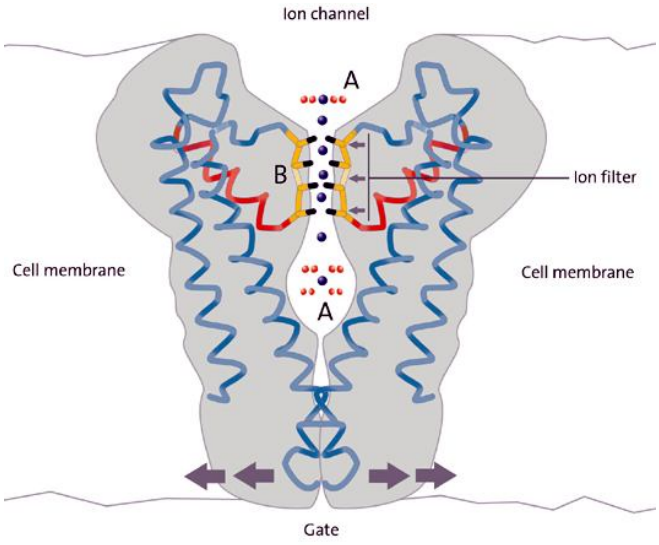
Single pore channels: almost all ion channels.

Two-pore channels: Catsper channels, TRP channels.

Detailed Structure of Ion Channels

An ion channel is usually equipped with four basic parts; a central conduction pathway (opening) for ions to pass through, an ion recognition site to allow passage of specific ions (selectively filter), one are more gates that may open or close, and a sensor that

senses the triggering signal and transmits it to the gate. Channels differ with respect to the ion they let pass (for example, Na^+, K^+, Cl^-), the ways in which they may be regulated, the number of subunits of which they are composed and other aspects of structure. Channels belonging to the largest class, which include the voltage-gated channels that underlie the nerve impulse, consist of four subunits with six transmembrane helices each. On activation, these helices move about and open the pore. Two of these six helices are separated by a loop that lines the pore and is the primary determinant of ion selectivity and conductance in this channel class. Clay Armstrong F first postulated the existence and mechanism for ion selectivity in the 1960s (Benzanilla, 1972). He suggested that the pore lining could efficiently replace the water molecules that normally shield potassium ions, but that sodium ions were too small to allow such shielding, and therefore could not pass through. This mechanism was finally confirmed when the structure of the channel was elucidated. The channel subunits of one class, for example, consist of just "P" loop and two transmembrane helices. The determination of their molecular structure by Roderick MacKinnon using X-ray crystallography won a share of the 2003 Nobel Prize in Chemistry.



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Because of their small size and the difficulty of crystallizing integral membrane proteins for X-ray analysis, it is only very recently that scientists have been able to directly examine what channels "look like." Particularly in cases where the crystallography required removing channels from their membranes with detergent, many researchers regard images that have been obtained as tentative. An example is the long-awaited crystal structure of a voltage-gated potassium channel, which was reported in May 2003 (Jiyang Y., 2003).

Modulation of Ion Channels

Modulation of the properties of membrane ion channels is of fundamental importance for the regulation of neuronal electrical activity and of higher neural functions. Among the many potential molecular mechanisms for modulating the activity of membrane proteins such as ion channels, protein phosphorylation has been chosen by cells to play a particularly prominent part. Regulation by phosphorylation is not restricted to one or another class of ion channel; rather, many, and perhaps all, ion channels are subject to modulation by phosphorylation. Similarly, a number of different protein kinase signaling pathways can participate in the regulation of ion channel properties, and it is not unusual to find that a particular channel is modulated by several different protein kinases, each influencing channel activity in a unique way. Finally, the biophysical mechanisms of modulation also exhibit a striking diversity that ranges from changes in desensitization rates to shifts in the voltage dependence and kinetics of channel activation and inactivation.

Modulation of ion channel activity is complex. Site-specific phosphorylation, by multiple protein kinases under the control of several intracellular second messenger systems, may increase or decrease conductance. Ion channels are essential for the function of excitable cells by mediating electrical currents and controlling specific ion concentrations; however, they are also widely expressed in non- excitable cells. G-Protein coupled receptors (GPCR) can modulate ion channel activity through two pathways:

-an indirect pathway that involves a common second messenger leading to the phosphorylation of the channel.

-a direct pathway, involving binding of Gβγ directly to the channel also known as membrane delimited modulation (Dascal 2001). GPCRs modulate ion channel activity. Acetylcholine (muscarinic), P2Y, somatostatin, cannabinoid, $GABA_B$, adrenaline, serotonin, glutamate, dopamine and opioid receptors have all been shown to modulate Ca^{2+} and K^+ channel activity (Magoski et.al.,1998).

The Muscarinic Receptors:

Muscarinic receptors modulate Ca^{2+} channel inhibition through two mechanisms, the voltage independent and the voltage dependent. Accumulated data from pharmacological experiments have demonstrated that in rat, as well as in mouse, the M_1 receptor is involved in the voltage independent mechanism of inhibition, depressing several high voltage dependent Ca^{2+} currents. The M_1 receptor modulates both N- type and L-type channels through the voltage independent mechanism involving a cytoplasmic second messenger. This mechanism is related to muscarinic receptors M_1, M_3 and M_5.

On the other hand, muscarinic receptors M_2 and M_4 are considered to mediate Ca^{2+} channel inhibition through the voltage dependent mechanism, by direct binding of the Gβγ subunit to the α1 subunit of the channel. Although both M_2 and M_4 are

considered to modulate calcium channels by the same mechanism, a recent paper describes an experiment done with knockout mice which revealed that in M_4 deficient mice the voltage dependent mechanism was not affected, while M_2 knockout mice lacked the voltage dependent pathway. Muscarinic receptors also modulate K^+ channels. Stimulation of muscarinic receptor M_2 by acetylcholine in the heart results in activation of the K^+ channels through the membrane delimited mechanism (Shapiro et.al .,1999).

The P2Y Receptors:

Eight mammalian P2Y receptors are known: the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$. Two subfamilies are defined within the P2Y family, which are distinct from each other on the basis of sequence identities and signaling properties:

All P2Y receptor subtypes are expressed in brain tissue and all are capable of modulating ion channels (with the exception of $P2Y_{14}$ that has not been characterized).

In primary sympathetic neurons, $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ have been reported of inhibiting both Ca^{2+} and K^+ currents. Ca^{2+} channel (N-type) inhibition is achieved by both mechanisms: the voltage dependent and the voltage independent. Inhibition of K^+ channels (Kv7.2, 3 and 5) by those receptors is mediated by phospholipase-C (PLC) and IP3-dependent increases in intracellular Ca^{2+} (Filippov et.al., 1998). Although $P2Y_{1,2}$ and $P2Y_6$ receptors were shown to equally inhibit both Ca^{2+} and K^+ current, coupling of the P2Y4 receptor to Kv7 channels was much more efficient than to Ca^{2+} channels. The $P2Y_{12}$ receptor, in contrast, inhibits only Ca^{2+} channels through the voltage dependent mechanism (Filippov et.al., 1998; Boehm S 2003).

The Somatostatin Receptors:

These receptors modulate several ion channels. Among them are the Kir channels, Kv channels, Ca^{2+} activated K^+ channel (KCa) and the high voltage activated L- and N-type Calcium channels (Krantic et.al.,2004)[12] In pituitary cells, somatostatin receptor have been found to activate K^+ channels and to inhibit voltage dependent Ca^{2+} channels. In the pancreatic β-cell line MIN-6, SSTRs activated two types of inward rectifier K^+ channels; the K ATP and Kir3 channels. These receptors were also shown to modulate the transient receptor potential vanilloid 1 channel (TRPV1) (Carlton et.al., 2004).

The Cannabinoid Receptors:

Inhibition of pre-synaptic N-type and P/Q-type Ca^{2+} channels have been demonstrated by several cannabinoids (endocannabinoids as well as exogenously applied) in heterologously expressed mammalian neurons, cultured hippocampal neurons, neuroblastoma-glioma cells (N-type only) and in exogenously expressed CB_1 receptor in pituitary tumor cells (P/Q-type). Endocannabinoid stimulation of the CB_1 receptor has also shown to activate the Kir3 channels both in pituitary tumor cells and in heterologously expressed mammalian neurons. It has been suggested that different agonists of CB_1 receptor might affect the selectivity of the interaction between the receptor and the G-proteins by inducing different conformational states of receptor (Glass and Northup, 1999).

The $GABA_B$ Receptors:

$GABA_B$ receptors mediate slow synaptic inhibition in the brain and spinal cord and are activated by gamma aminobutyric acid (GABA), which is a major inhibitory neurotransmitter. $GABA_B$ receptors are coupled to the pertussis toxin-sensitive G-proteins, the Gαi/o. The functional $GABA_B$ receptor is a heterodimer, which consist of two subunits, $GABA_BR1$ and $GABA_BR2$. Cell surface expression is also dependent on the receptor being a heterodimer. $GABA_BR2$ was shown to be essential for trafficking of the receptor to the cell surface as well as for activation of effector systems, while $GABA_BR1$ was found to be important for the ligand binding along with $GABA_BR2$ (Couve et.al.,1998). Inhibition of Ca^{2+} (N-type and P/Q- type) channels on one hand and on the other hand activation of Kir3 channels by $GABA_B$ receptors has been demonstrated, probably by the same voltage dependent mechanism involving direct binding of the Gβγ subunits (Wang & Lambert, 2008).

Adrenoceptors:

β-Adrenergic receptors (β-ARs) mediate the effects of epinephrine and norepinephrine. In the human heart, three subtypes of β-ARs, β_1, β_2 and β_3, have been identified and modulate cardiac function. It has been reported that the L-type calcium current ($I_{Ca,L}$) are modulated by β_3-AR activation(Cheng et.al., 2001). Adrenaline has been reported of directly inhibiting Ca^{2+} currents and delayed rectifier K^+ currents and activating sustained Na^+ current. It has also been seen to inhibit both Ca^{2+} activated non-selective cationic currents and Ca^{2+} activated K^+ currents, the latter via inhibition of the underlying activating Ca^{2+} current (Bouryi and Lewis 2001).

Serotonin (5HT) receptors:

With the exception of $5\text{-}HT_3$ receptor that is a ligand-gated ion channel, all other serotonin receptors are G protein-coupled receptors that activate an intracellular second messenger cascade to produce an excitatory or inhibitory response. 5HT has been seen to decrease high voltage-activated calcium channel currents in a dose-dependent and reversible manner in acutely dissociated neocortical pyramidal neurons(Gincel& Shoshan., 2004).

Glutamate Receptors:

The amino acid glutamate, synthesized in the mitochondria, serves multiple functions, including acting as a neurotransmitter and participating in degradative and synthetic pathways. All ionotropic glutamate receptors (NMDA, Kainate, and AMPA) are ligand-gated nonselective cation channels which allow the flow of K^+, Na^+ and sometimes Ca^{2+} in response to glutamate binding. Glutamate has been seen to modulate the

channel activity of bilayer-reconstituted voltage-dependent anion channel (VDAC). It has also been reported to modulate the opening of the mitochondrial permeability transition pore (PTP), of which VDAC is an essential component (Foehring, 1996).

Dopamine receptors:

Activation of D_1-receptor has been reported to reduce peak Na^+ current in an acutely isolated hippocampal neurons via a modulatory mechanism involving phosphorylation of the Na^+ channel subunit by cAMP-dependent protein kinase (PKA) (Cantrell,1999) .

Opioid Receptors:

Opioid peptides, acting via a mu-type opioid receptor, have been reported to strongly potentiate large conductance Ca^{2+}-dependent K^+ (BK) channel current. Opioids have also been seen to inhibit voltage-activated Ca^{2+} currents (Twitchell & Rane., 1993).

Drugs acting on Ion channels

a) Calcium channel blockers:

Calcium channel blockers work by blocking voltage-gated calcium channels in cardiac muscle and blood vessels. This decreases intracellular calcium leading to a reduction in muscle contraction. In the heart, a decrease in calcium available for each beat results in a decrease in cardiac contractility. In blood vessels, a decrease in calcium results in less contraction of the vascular smooth muscle and therefore an increase in arterial diameter (CCB's do not work on venous smooth muscle), a phenomenon called vasodilation. Vasodilation decreases total peripheral resistance, while a decrease in cardiac contractility decreases cardiac output. Since cardiac output and peripheral resistance determines blood pressure, blood pressure drops. Calcium channel blockers are especially effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients (Nelson, 2010).

Examples: Amlodipine, Aranidipine, Azelnidipine, Barnidipine, Benidipine, Cilnidipine.

b) Potassium channel blockers:

Potassium channel blockers used in the treatment of cardiac arrhythmia are classified as class III antiarrhythmic agents. These agents predominantly block the potassium channels, thereby prolonging repolarization (Lenz & Hilleman, 2000). The prolongation of the action potential duration and refractory period, combined with the maintenance of normal conduction velocity, prevent re-entrant arrhythmias. Amiodarone is also safe to use in individuals with cardiomyopathy and atrial fibrillation, to maintain normal sinus rhythm. Examples: Azimilide, Bretylium, Clofilium, Tedisamil, Sematilide

c) Potassium channel openers:

A potassium channel opener facilitates ion transmission through potassium channels. Examples: Diazoxide, Minoxidil, Nicorandil, Pinacidil, Retigabine, Flupirtine

d) Sodium channel blockers:

Sodium channel blockers are agents that impair conduction of sodium ions (Na^+) through sodium channels. Voltage-gated sodium channels play an important role in action potentials. If enough channels open when there is a change in the cell's membrane potential, a small but significant number of Na^+ ions will move into the cell down their electrochemical gradient, further depolarizing the cell. Thus, the more Na^+ channels localized in a region of a cell's membrane, the faster the action potential will propagate, and the more excitable that area of the cell would be. The ability of these channels to assume a closed-inactivated state causes the refractory period and is critical for the propagation of action potentials down an axon channels. The family of sodium channels has nine known members. The proteins of these channels are named Nav1.1 through Nav1.9 (Jessell et.al., 2000).

The following naturally produced substances persistently activate (open) sodium channels:

i. Alkaloid based toxins: Aconitine, Batrachotoxin, Brevetoxin, Ciguatoxin, Delphinine, Grayanotoxin, Veratridine

ii. Gating modifiers: μ-conotoxin, δ-atracotoxin, Scorpion venom toxins, such as Birtoxin

e) Chloride channel openers:

Some members of this family are activated by voltage, while Ca2+, extracellular ligands and pH activate others.

Example: Selamectin

Selamectin is the active ingredient in Revolution, a topical insecticide and antihelminthic used on dogs and cats. Selamectin works by replacing glutamate which normally interacts with receptors that open chloride channels at muscle synapses found in parasites (Suzuki et.al., 2006).

Clinical Applications:

Drugs that act on cation channels are used in various clinical applications.

With sodium channels, these are:

- Cardiac excitation: Suppression of arrhythmia
- Neural conduction: Local anaesthesia
- Cerebral excitation and conduction: Suppression of epilepsy

Potassium channels are drug targets in the following areas:

- Cardiac excitation: Suppression of arrhythmia (experimental)

- Vascular smooth muscle tone: Reduction of blood pressure
- Pancreatic β-cells: Enhancement of insulin secretion

Drugs acting on calcium channels have a similar range of applications:

- Cardiac excitation: Suppression of arrhythmia
- Vascular smooth muscle tone: Reduction of blood pressure (Hille,1992)

CONCLUSION

Ion channels play an important role in numerous cell types. Several disease states are related to dysfunctional ion channels. Prominent among these are cardiac arrhythmias, diabetes, hypertension, angina pectoris and epilepsy. Drugs have been developed to target ion channels and to prevent the channels from conducting ions. They are widely used as local anaesthetics, anti-arrhythymics (to prevent irregular heartbeats), antihypertensives (to lower blood pressure) and anti-epileptics (to prevent seizures).

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