A Short Guide to Electrophysiology and Ion Channels

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ABSTRACT: The birth and discovery of electrophysiological science took place in the 18th century laying the path for our understanding of nerve membrane ionic currents. The pore-forming proteins, ion channels, are involved and play critical roles in very important physiological and pathological processes, such as neuronal signaling and cardiac excitability, therefore, they serve as therapeutic drug targets. The study of physiological, pharmacological and biophysical properties of ion channels can be done by patch clamp, a gold standard and powerful electrophysiological technique. The current review, in addition to highlight and cover the history of electrophysiology, patch clamp (conventional and automated) technique, and different types of ion channels, will also discuss the importance of ion channels in different neurological diseases and disorders. As the field of neuroscience is growing, this manuscript is intended as a guide to help in understanding the importance of ion channels, particularly in neuroscience, and also in using the patch clamp technique for the study of molecular physiology, pathophysiology, and pharmacology of neuronal ion channels. Importantly, this review will spotlight on the therapeutic aspect of neuronal ion channels.

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INTRODUCTION

The science of electrophysiology dates back to the end of the 18th century in Italy when Luigi Aloisio Galvani discovered that dead frog leg muscles were able to twitch when an electric shock was applied to one of its nerves. His finding came in the 1780s during a frog preparation, in which its lower body half with exposed nerves and inserted wire across the vertebral canal began to contract vigorously. This occurred after one of Galvani's coworkers, presumably, his wife Lucia Galeazzi, touched the exposed frog's crural nerves with a lancet causing a spark to rise from a distant electric machine (1).

Galvani concluded that animal tissues possess an intrinsic electricity, termed animal electricity, which is involved in vital physiological processes, such as nerve conduction and muscle contraction (2). His colleague, Alessandro Volta at University of Pavia, was able to reproduce Galvani's results, however, he rejected his theory of intrinsic electricity. Volta stated that frogs lack such electricity instead the frog's contractions were due to an electricity that was already known and was triggered by metals used by Galvani to connect the species' nerves and muscles (3).

The two Italian scientists' contradicting theories led to years of discussions and numerous of experiments to prove and support their respective thoughts. These succeeding experiments led to Galvani laying the basis of electrophysiology and Volta inventing the electrical battery. Fellow scientists at the time gave their support either to Galvani or Volta, and some others even recognized and accepted both hypotheses, such as German pioneer Emil du Bois-Reymond, who is also known as the father of electrophysiology (4).

In the 1840s, du Bois-Reymond took both theories and solved the dispute regarding animal electricity and demonstrated the electrical nature of nerve signals (4). Among his inventions, the German scientist created neutral means of coupling instruments to tissue, such as "non-polarizable" electrodes made from an amalgam of zinc, zinc sulfate, and modeling clay. Du Bois-Reymond also invented the "magneo-electrometer" (AC generator), the "rhecord" (potentiometer), which produced graded shocks to his preparations, and the galvanometer, which was sensitive enough to record his results. With these devices, du Bois-Reymond was able to detect action currents in frog muscles in 1843 and four years later he invented a Wheatstone bridge circuit to the previous three-device set up to detect the same electrical signals in humans.

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Later, one of du Bois-Reymond's students, Julius Bernstein, invented a new instrument, a differential rheotome, also known as "current slicer," enabling him to study the amplitude, time course, and conduction velocity of the negative variation, or "action current," in the frog sciatic nerve in 1868. This allowed the recording of the exact time course of electrical activity in both nerve and muscle (5). The study was the first description of the action potential - a momentary change in electrical potential on the cell's surface, allowing a cell, such as a neuron, to transmit an electrical signal down the axon to other cells.

Over two decades later in 1902, Bernstein formulated his membrane theory on resting current in muscle and nerve fibers. The membrane theory was later developed by Howard Curtis and Kenneth Cole (6), who in the late 1930s were able to measure the full action potential of a squid giant axon by using an intracellular micropipette (6). This tissue, which controls part of the squid's water jet propulsion system, was used for technical purposes as it is large and therefore easier to handle than, for example, mammalian cells, which are much smaller in size. The obtained action potential by Curtis and Cole is a transient, regenerating change in the membrane potential that permits a wave of electrical excitation to pass along the plasma membrane of electrically excitable cells. It consists of two major phases; depolarization (a rapid change of the membrane potential from a negative to a positive value) and repolarization (return of the membrane potential from a positive to a negative, -40 to -95 mV, depending on the cell type) (6). In 1952, British biophysicists, Alan Hodgkin and Andrew Huxley, suggested the existence of ion channels (7), stating properties of some sodium channels in nerve and muscle cells were unique and these channels could be opened by depolarization. The existence of ion channels was confirmed in the late 1970s with an electrical recording technique, known as the "patch clamp", invented by Erwin Neher and Bert Sakmann (8), for which they were awarded the Nobel Prize in 1991.

PATCH CLAMP ELECTROPHYSIOLOGY

Electrophysiological studies permit the measurement of ionic currents across the cell membrane from single cells or tissue. This technique helps to understand the physiological and pathophysiological functions of excitable cells and tissue. Intracellular measurements on animal cells were first achieved in 1939 by Hodgkin and Huxley at Plymouth, UK, and by Curtis and Cole at Woods Hole Massachusetts, USA.

The Basis of the Voltage Clamp Technique (Ohm's Law)

Generally, voltage clamp measures an electric current (ion flow across a cell membrane) while the membrane voltage is held constant (clamp) with a feedback amplifier. The advantages of voltage clamp are that membrane ionic currents and capacitive currents are separated in this technique. Also, measuring the current when the voltage is controlled allows you to study the channel behavior much easier rather than having a freely changing voltage across the membrane. The voltage clamp is mainly convenient when investigating responses to membrane potential changes, particularly the activation of voltage-gated channels.

The theory behind voltage clamp technique can be described as to measure the total membrane current, I_m, two components are required, the ionic current, I_{ionic}, and the capacitance current, C_m along with dV/dt, the rate of change of voltage (volts per second increase or decrease) at a specific point in time.

$$I_m = I_{ionic} + C_m \, dV/dt \qquad (1)$$

The ionic current reflects the movements of ions through ion channels. The capacitance current is the accumulation of charge on one membrane side and its depletion on the other for a given voltage. Whenever, membrane potential is changed this charge stored on the membrane capacitor must be changed – giving rise to the capacitance current. With the voltage clamp techniques, once the voltage has been stepped to a new potential the membrane potential is held at a constant level, to create a zero value for dV/dt. This creates an initial capacitance transient that is brief (as voltage is changed) and then a current can be measured that is completely ionic (9).

To investigate ion channel properties, the voltage clamp technique is often performed with the help of native *Xenopus* oocytes (one of the many expression systems for recombinant ion channels). Two microelectrodes are placed within the oocyte. One is used to record the membrane voltage and the other passes a current to hold the cell membrane at a constant voltage. The data is used to calculate the voltage and time-dependent kinetics of the ionic currents. Extensions of the voltage clamp method have been developed in order to investigate a single channel activity, such

as fluctuation analysis, usage of artificial lipid bilayers and the patch clamp technique. The measurement of ion channel current is based on Ohm's law.

Ohm's law: $I = V/R$ **or** $V = IR$ (2)

Here, the movement of charged ions across the membrane is the current (I) and measured in amperes (A). Voltage (V) is the driving force needed for the charged ions to move through the channel, measured in volts (V), and the resistance through the channel is represented as R, measured in ohms (Ω). From the equation, it can be seen that the current is inversely proportional to the resistance. Thus, when the voltage across a membrane is held constant, from Ohm's law, you can measure the current, the smaller the resistance, the larger the current and vice versa.

Patch Clamp Technique

Patch clamping is one of the most common electrophysiological techniques to study ion channel functions. As described, by clamping the voltage of an isolated excitable cell membrane, the currents that flow through ion channels may be measured. Also, it is worth mentioning that the membrane potential can be measured in current clamp experiment. In more detail, constant or time-varying current can be applied to measure the change in membrane potential, which mimics the current produced by a synaptic input.

Very small currents, 10-12 A (pA), through ion channels can be measured using the patch clamp technique. A glass micropipette, with a recording electrode inside, is placed on the cell surface. Suction is then applied to create a high resistance seal (gigaseal, derives from the range of the seal measured to at least one gigaohm) between the pipette tip and the cell membrane creating an isolated area (Fig. 1). This state is called the cell-attached configuration, which will be discussed further in the next section. There are two essential reasons for why the connection between the cell and pipette tip must be of a high seal resistance. If such a state is achieved then the membrane patch receives a more complete electrical isolation, meaning the current noise of the recording will be less. This allows single channel currents, with amplitude of 1 pA, to have a good time resolution. In other words, the gigaseal reduces the noise level of the recording extremely effectively and with this it increases the single channel currents' resolution.

There are three conditions which can help to achieve a gigaseal formation. These conditions are preferably: (1) The cell membrane surface must be clean and free of extracellular matrix. (2) Solutions should be filtered using 0.2 µm filters and cell cultures should be washed repeatedly to remove serum components in tissue culture media. (3) The tips of the electrode should be fire polished to promote gigaseal. This is particularly useful when there are difficulties of obtaining seal formation.

Patch Clamp Configurations

The patch clamp technique includes a number of different configurations enabling high-resolution recording of ionic currents flowing through the plasma membrane of a cell. Which configuration utilized for the study is determined by a number of factors, such as ion channel type, ion channel regulation, and modulation, as well as, research interest. The different configurations are (1) cell-attached, (2) inside-out, (3) whole-cell (4) perforated, and (5) outside-out.

Cell-Attached Configuration

When a gigaseal is achieved between the cell and the glass micropipette, the state is called cell-attached, the initial and simplest configuration (Fig. 1A). At this stage, the cell remains intact and single channel currents can be recorded within the membrane patch, inside the electrode. The cell-attached mode has though significant limitations e.g. the extracellular and intracellular ionic concentrations cannot be easily altered. Therefore, from the cell-attached mode, four other configurations can be obtained to study the electrical activity in the cell and cell membrane properties namely inside-out, whole-cell, perforated, and outside-out recordings. It is worth mentioning that with the cell-attached configuration, the cell membrane potential can be determined, but this will not be discussed in any details here.

Inside-Out Configuration

From an obtained gigaseal cell-attached mode, an inside-out recording of a single ion channel or a macroscopic current, can be made by abruptly pulling the membrane patch off the cell (Fig. 1C). This step maintains a gigaseal, while causing a rupture of the cell, the excised patch is defined as an inside-out patch, as the former cytosolic side of the patch now faces the bath solution, which is changed through perfusion. An inside-out patch recording enables the study of the effects of cytosolic factors on ion channels and is particularly favorable when studying ion channels regulated by intracellular ligands (10). A disadvantage with inside-out recording is that cytoplasmic

constituents are lost. These may be vital in modulating the behavior of ion channel proteins. An advantage of the loss of cytoplasmic constituents is that test factors can be added to the intracellular side of the ion channels.

Whole-Cell Configuration

The whole-cell mode is the most common configuration with the great majority of all electrophysiological recordings of neuronal cells, cardiac cells, and other cell types done through this procedure. From the cell-attached state, the membrane patch is disrupted by briefly applying a higher suction, in which direct contact with the cytoplasm is achieved establishing electrical and molecular access to the intracellular space (Fig. 1B). This recording mode measures the ion channels' currents across the entire plasma membrane of a single cell (11).

There are two main recording types within the whole-cell configuration namely, the voltage clamp mode, holding the voltage constant, enabling the study of ionic currents and the current-clamp mode, controlling the current and allowing the study of membrane potential changes. The advantage of using whole-cell mode is that there is a larger opening at the patch clamp electrode's tip generating lower resistance, and therefore, a better electrical access to the cell's inside. One of the disadvantages of this mode is that the electrode's volume is larger than the cell, causing the soluble contents of the inside of the cell to slowly be replaced by the electrode's contents, such as the wash-out of certain cytosolic factors important for cellular function. This is referred to as the electrode "dialyzing" the contents of the cell.

Perforated Configuration

To avoid the problem of dialyzing the cell contents, there is an alternative configuration, the perforated patch technique. This involves perforating the sealed membrane patch rather than rupturing it (12). The technique allows electrical access between the cell and the patch pipette using pore-performing antibiotics, such as amphotericin or nystatin. The antibiotic pores are permeable to small monovalent ions, such as Na^+, K^+, and Cl^-, but at the same time present a physical barrier to larger impermeable ions, e.g. Ca^{2+} and molecules, including tetraethylammonium and cytosolic signaling molecules, as well as, those with a molecular weight exceeding approximately 200, e.g. ATP and glucose. With this, the perforated configuration is suitable for studying ion channels while maintaining the integrity of the many cytoplasmic components. The advantages of this configuration are, among others, (1) allows minimal disruption of cytosolic components, (2) enables stable recording lasting for more than one hour, and (3) reduces rundown of current. This is while the disadvantages of perforated configuration include (1) longer time to achieve whole-cell configuration than conventional one, amid time spending waiting for perforation, (2) impossible to apply drugs through the patch pipette, and (3) no control of cytosolic Ca^{2+} (12).

Outside-Out Configuration

From an achieved conventional whole-cell mode, the pipette can be pulled slowly away from the whole-cell, allowing a vesicle to be formed from cell membrane resulting in a resealing of the membrane so that its outside now faces the bath solution (Fig. 1D). This mode is desirable when studying small channel populations or single channels and to manipulate the cell to various bathing solutions for rapid perfusion. In addition, this mode enables the study of extracellular ligand-gated ion channels (13). The main disadvantage is that an outside-out configuration is more difficult to achieve as several steps are involved in the patching process.

Technical Considerations

There are various expression systems for the functional characterization of ion channels. However, one of the most widely expression systems, which is used to study the electrophysiological, pharmacological, and biophysical properties of ion channels is oocytes from the *Xenopus laevis*. There are numerous advantages to use *Xenopus* oocytes as an expression system to study ion channels, such as maintaining *Xenopus laevis* is cost effective, their large size eases the injection of heterologous cRNA and the experiments (14, 15). Certainly, there are also several disadvantages to use oocytes as an expression system. Some of these disadvantages are as follow: A) Numerous pharmacological compounds are less potent in this system compared with native tissues or in mammalian cells. B) Oocytes express some endogenous channels and receptor. C) It is very difficult or impossible to express some channels in oocytes (15).

One of the other systems used to study ion channels is transient transfection of plasmid DNA into mammalian cells, typically human embryonic kidney cells 293 (HEK293), baby hamster kidney (BHK), or Chinese hamster ovary (CHO) cells line by using chemical reagents, such as Lipofectamine or Fugene. This method is efficient and cost-

effective to study, such as structure-function relationships of ion channels, as example to assess the effect of mutations or truncations. For this system, your cDNA should also contain the label gene, for example, enhanced green fluorescent protein (EGFP) and your microscope should be equipped with fluorescence to visualize the transfected cells for your experiment. Perhaps, for long term studies, it is preferred to construct stable cell lines. Contrary to transient transfection, constructing stable transfected cell lines is a time-consuming process, which typically takes numerous weeks, however, this system in long term is beneficial and preferable. (10, 16).

To assure the correct trafficking and normal surface expression of the channel of interest, an electrical fingerprint can be established. Meaning the current–voltage relationship or I-V curve, which is characteristic for that particular channel. For example, a typical seat like inflection of current-voltage relationship for the homomeric TRPC4 or TRPC5 compared with heteromeric channels (TRPC1/TRPC4 or TRPC1/TRPC5), which have smooth outward rectification. Also, to mimic the physiological environment, a temperature control device can be built into the rig. It is worth mentioning that the temperature should be adjusted to the type of pharmacological agents, which are used during the experiments, or the kind of experiment. The ideal temperature for some compounds is between 30-35 ºC, whereas for others compounds and experiments room temperature is suitable.

The micropipettes used for patch clamping have a length of 10-15 cm and an outer diameter of 1.2-1.5 cm and are in most cases made of a rod of glass, which typically contains a filament to enable the filling. Also, in term of inner diameter, there are two different micropipettes available one with thick-walled glass and other with thin-walled. The thin-walled glass is preferred in most experiments, however, when small pipette tips are required for particular experiments the thick-walled is better. There are two different glass types available; borosilicate and quartz. Most microelectrodes, which are used in the lab, are made from borosilicate glass because quartz is very expensive and needs a special pipette puller. The remaining important point to consider is that the micropipettes should be handled and kept clean from any dirt or dust.

In addition to practice and training, utilizing the patch clamp technology requires some methodological considerations. As mentioned, whole-cell recording is a useful technique for measuring a broad range of ionic currents. However, the series resistance (Rs) of the recording patch pipette limits this technique to study ion channels, particularly when measuring rapid ionic currents. To overcome this limitation, it is required to compensate for Rs by increasing the voltage clamp bandwidth.

The Rs generates two unwanted effects in whole-cell voltage clamp recordings. First, the series resistance initiates a voltage error (IR) that causes the cell membrane voltage (Vm) to deviate from the wanted clamping voltage at any time the ionic current flows. Secondly, the Rs causes lowering of the temporal resolution of the voltage clamp, usually to a level, which does not allow the accurate measurement of the rapid physiologic processes.

In regards to whole-cell patch clamp configuration, lowering of the temporal resolution of the voltage clamp by Rs makes the measurements very difficult. For that reason, the aim of Rs compensation is to decrease and preferably eliminate, Rs errors from whole-cell voltage clamp recordings. It is worth mentioning, the liquid-liquid junction potential (bath potential minus the potential inside the pipette) can cause voltage error as a result of current flow through the grounding electrode.

Data filtering frequencies and the signal conditioning are also very important subjects, which need to be considered when studying ion channels by using patch clamp technique. In other terms, one important question, which also needs to be answered is; why should signals be filtered? Selected frequencies can be removed from the signal by a filter, which is a circuit. To remove undesired signals and noise from the data, filtering is frequently performed. Low-pass filtering is generally used to perform the filtering, limiting the data bandwidth by removing signals and noise above the corner frequency of the filter. This is particularly important when studying single-ion channels. In addition, the acquisition rate is very important when measuring currents from single-ion channels. Since, if your system for recording is not fast enough, you will lose information about the channel gating. For that reason, acquisition rate can be vital for fast recordings.

Automated Patch Clamp Techniques

As standard patch clamping is a completely manual technique that requires experienced personnel and is a time-consuming process as well, automation has been the focus of biotech companies and researchers over the past 20 years. The developed automated systems use glass pipette-based or

planar-array based technique. The automation of the patch clamp technique is aimed at making the data acquisition simpler, more reliable, and time-efficient.

Glass Pipette-Based Patch Clamp Techniques

The glass pipette recording technique involves a total automation of the standard manual patch clamping with glass microelectrodes. This technique enables pipette control, production of true gigaseals, execution of compound application, and data acquisition, as well as, allows higher data production rates compared to the conventional manual method (17).

There are three approaches when using automated glass pipette-based technique. The first one involves a pipette coming into surface contact of a randomly chosen cell, which is suspended in one or more cell layers located in a density-gradient solution or at the air-liquid interface. A gigaseal can then be achieved and whole-cell recording or other configurations can be performed in the same manner as the manual recording technique. The second method is placing at the well bottom a tiny pipette tip, positioned vertically upwards in the center of a polyimide sheet. Suspension cells are then added and one of the cells attracts and attaches to the tip. This is achieved by applying negative pressure through the tip of the pipette. A gigaseal is then achieved and a recording can be made. The final approach suspended cells enters a glass pipette's inside, where a gigaseal is achieved and whole-cell patch recording can be made (18).

This automated system has its benefits, such as reducing the amount of labor and supervision needed, however, it also has some disadvantages, such as cells are selected randomly and parallel experiments of several cells for high throughput is challenging as precise control of multiple simultaneous pipettes is difficult to achieve.

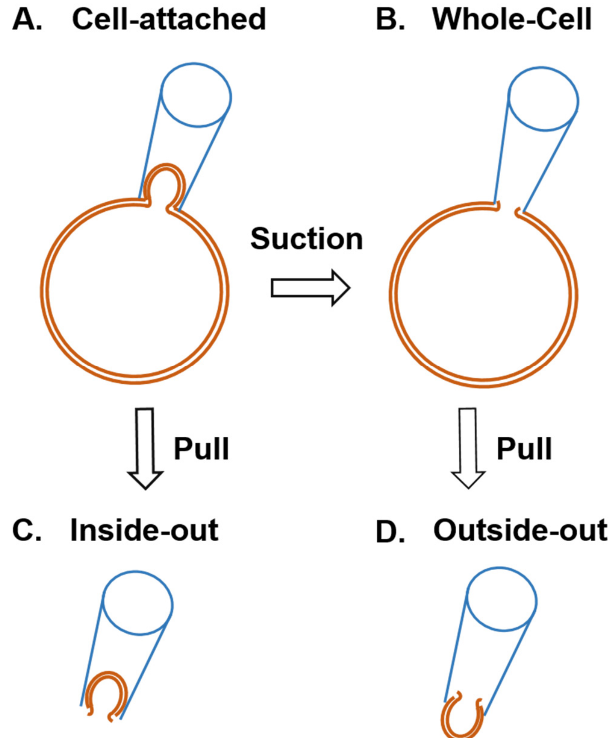

Figure 1. Schematic diagram of patch clamp configurations. The figure illustrates four recording methods for conventional patch clamp technique. A) Demonstrations of the tight gigaseal formation between the pipette and the cell membrane after application of mild suction. This state, which is the first step for other configurations, is called cell-attached configuration. B) Subsequently, to succeed the whole-cell configuration, the cell membrane can be ruptured by additional brief strong suction, allowing direct contact with the cytoplasm and this will establish electrical and molecular access to the intracellular environment. C) Depiction of the inside-out configuration, which can be obtained by abruptly pulling the membrane patch off the cell from an obtained gigaseal cell-attached. In this mode, the cytosolic surface of the membrane is exposed to the medium, allowing the alteration of the intracellular surface. If the pipette is pulled slowly away after whole-cell mode, the outside-out configuration will be accomplished, allowing a vesicle to be formed from cell membrane resulting in a resealing of the membrane so the cytosolic side faces the pipette solution (D).

Planar-Array Based Patch Clamp Techniques

The majority of automated patch clamping systems are planar-array based, in which glass pipette electrodes have been replaced with multi-well configurations in a plate-based or chip-based format. There are numerous planar-array based systems available on the market, however, the first widely available plate-based electrophysiology measurement platform was IonWorks HT, now owned by Molecular Devices, which comprises of computer-controlled fluid handling, recording electronics, and processing tools, which allows voltage clamp recordings of up to 384 individual cells per experiment (19, 20). The cells are placed in a single hole on the planar chip and the system generates some 100 megaohm seal. An electrical access is then attained by applying permeabilizing agent in the intracellular solution to perform a perforated patch clamp (21, 22).

The first chip-based system was the PatchXpress, which enables the study of voltage-gated and ligand-gated ion channels. The system uses glass substrates for parallel patch clamp recordings from 16 wells and allows continuous recordings during ligand applications. The electrical access is achieved by rupturing the membrane of the cell underneath the aperture (20, 23).

WHY STUDY ION CHANNELS

Ion channels are essential for life since they play a fundamental role in physiological processes, such as neuronal signaling, muscle contraction, and nutrient transport. Ion channels are pore-forming proteins, located in the plasma membrane of virtually all living cells. Ion channels also exist in membranes of intracellular organelles, such as the endoplasmic reticulum, endosomes, lysosomes, and mitochondria (24). They form aqueous pores across the lipid bilayer and often highly selectively allow the flow of particular inorganic ions, primarily sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), or chloride (Cl^-).

The movement across the cell membrane can be defined either as active, which transports ions from a lower concentration area to a higher, or passive, which uses kinetic energy and natural entropy transporting down a concentration gradient, i.e. from a higher concentration to a lower one. Conductance, selectivity, and gating are key properties for the passive system. These three aspects are essential for normal ion channel function.

Conductance is defined by the number of ions that flow thru the channel and is measured in siemens (S). The rate of single-channel conductance for typical ion channels varies from 0.1 to 100 pS (picosiemens). The first discoveries of actual value of ion conductance of a single pore were done with the help of single patch clamp recording. There is a wide range of single-channel conductance varying among different types of ion channels. Numerous channels may have the same selectivity but differ in single-channel conductance (25).

Selectivity involves a channel's ability to allow certain ions flow through the channel. Only ions of appropriate size and charge can pass in a single file through the narrow, water-filled channel, a process called selective permeability. Ion channels have the characteristics of being highly permeable to some and not to others. An example of this is sodium channels that are very permeable to sodium ions but less to potassium ions. Also, voltage-gated ion channels located in nerve and muscles cells are highly selective, with calcium channels as the most, then potassium and sodium. The permeability of calcium is more than 1,000-fold greater than sodium in calcium channel and in delayed rectifier potassium channels, potassium permeability is around 100-fold greater than sodium. This is while sodium permeability is just 12-fold greater than potassium in voltage-gated sodium channels (26). Selective permeability is an important process as if ion channels did not have some degree of selectivity they would not have the ability to generate electromotive forces, which is required for electrical signaling (6).

Gating of ion channels involves the process of opening and closing of the pore. This is determined by various factors, including external or internal ligands, mechanosensitivity, voltage, thermosensitivity etc., permitting ions either to pass through or block the ion flow. In one single second, an open channel can pass up to ten million ions (6) in and out of a cell. The flow creates an electrical current, which enables cells to communicate quickly. These types of signals play a fundamental role, allowing vital functions, such as, the brain to receive and process information, the heart to beat, and muscles to work. In other words, ion channels serve as communication pathways across cell membranes by movement of ions inside and outside of the cells. Therefore, these protein complexes are crucial for the normal function of the cells, and they are involved and play critical roles in very important physiological and pathological processes. A multitude of diseases result from the disruption of normal ion channel function. Disorders resulting from a mutation in the gene encoding an ion channel or its regulatory

proteins are called channelopathies. Common channelopathies include cystic fibrosis and long QT syndrome. Among the inherited mutations of ion channels are neurological disorders, e.g. neuromuscular and movement, migraine, pain, depression, and epilepsy, as well as, cardiovascular diseases and other human disorders.

In addition, ion channels are involved in non-genetic diseases, e.g. diarrhea, caused by toxicological effects on the function of ion channels. Hence, they have been drug targets (Table 1) for vital diseases, including arrhythmia and diabetes (16). To understand their regulation and dysregulation, which lead to these diseases and disorders, ion channels are among the most well-studied proteins (27).

ION CHANNELS GROUPS

Scientists have identified hundreds types of different ion channels, accounting for about 1.5% of the human genome, and still more to be discovered. These channels can be sorted into various groups based on ion type, ion selectivity, and molecular structure or by gating, which will be discussed in this review. The gating classification consists of the following subgroups: voltage-gated channels, ligand-gated channels (extracellular ligands), and others. Since, the cardiovascular and other ion channels are well established in term of their role in health and diseases, this review aims to highlight and focus on the therapeutic aspect of neuronal ion channels.

Voltage-Gated Channels

Voltage-gated ion channels (VGIC) were among the first channels Hodgkin and Huxley discovered more than five decades ago. They consist of six transmembrane domains, termed S1-S6, with cytoplasmic N- and C-terminal regions. VGICs open and close by changes in transmembrane voltage (28). These channels are common in many types of cells, particularly in neurons, where they hold a critical role in excitable neuronal and muscle tissue. There are four different types of voltage-gated ion channels: potassium, sodium, calcium, and chloride (29).

Voltage-Gated Potassium Channel

The voltage-gated potassium channel (VGKC) family is the largest and most diverse of the four VGICs and are found in all excitable cells (30). There are 40 VGKCs in the human genome and they are divided into 12 subfamilies labeled Kvα1-12. VGKCs are involved in various physiological processes, including repolarization neuronal and cardiac action potentials, regulating Ca^{2+} signaling, cell volume and driving cellular proliferation and migration (31). VGKCs are tetramers consisting of four separate polypeptide subunits in contrast to most voltage-gated sodium and calcium ion channels, which are formed by a single large polypeptide chain containing four homologous domains (32). Defects in VGKCs can lead to epilepsies due to its vital role in regulating neuronal excitability. Genetic mutations of this channel cause seizures and other conditions associated with neuronal hyperexcitability (33).

In the ion channel family, the VGKCs which are the most diverse group can be generally divided into two subgroups: the practically non-inactivating 'delayed' and the rapidly inactivation 'transient'. Single-channel recordings suggest that both delayed and transient potassium currents are arbitrated by numerous subtype of channels that have some common functional properties (6, 34).

Inwardly rectifying potassium (Kir) channels, which is a large family of potassium channels, having diverse physiological functions (35). These channels permit large inward currents and minor outward currents. So far, seven Kir channels subfamilies (Kir1.x – Kir7.x) have been reported which can be categorized into four functional groups; A) The classical Kir (Kir2.x) which are constitutively active. B) The G protein-gated Kir (Kir3.x) which are regulated by G protein-coupled receptors. C) The ATP-sensitive potassium channels (Kir6.x). D) The potassium transport channels (Kir1.x, Kir4.x, Kir5.x, and Kir7.x).

Numerous of VGKC channels are gated by neurotransmitters and membrane potential, therefore, they are essential for excitatory inputs and electrical conduction (36). In more detail, opening and closing of these channels which caused by changes in membrane potential permit passive flow of potassium ion from the cell to restore the membrane potential, therefore, they are the key components in the nervous system in propagation of electrical impulses. Having in mind that the majority of VGKCs are expressed in the central nervous system and importantly, they are involved in the excitability of neurons, make them potential drug targets in epilepsy, psychosis, and multiple sclerosis (37-42).

As mentioned earlier, voltage-gated potassium channels have tetrameric structure, however, an ion conduction pore and voltage-senor are the two independent domains, which make the tetrameric structure of Kv channels. A discrete member of the Kv subfamily is the Kv7 subunits due to the outward current, lacking inactivation and slow gating kinetics. These channels' characteristic

properties in the nervous system make them function as a footbrake to receive persistent excitatory input (36, 42).

In response to membrane potential changes, the voltage-sensor mainly controls the opening of the pore by switching from resting to active conformations. In general, in Kv channels the opening of the pore needs four voltage-sensors to rearrange while voltage-gated sodium channel only needs three voltage-sensors. This different property between the two channels (Nav and Kv) can possibly explain why Nav channels have to some extent faster pore opening (7, 43-46).

The Kv auxiliary subunits are not crucial for the ion permeation, however, they regulate the trafficking and gating of the channel, and possibly these accessory subunits are required for signaling pathways, such as protein kinases, which modulate the channels (47). Recently, Vacher and Trimmer reviewed in detail the regulation of dendritic Kv4 channels by the accessory subunits and phosphorylation (48).

Voltage-Gated Sodium Channel

The voltage-gated sodium channel (VGSC) is a large, multimeric transmembrane complex. VGSCs play a vital role in the initiation and propagation of action potentials in various cells including nerve, muscle, and other excitable ones (6). The channel consists of one α subunit and one or more smaller accessory β subunits (29). There are nine homologous members of the VGSC family: SCN1A to SCN11A, which encode the sodium selective ion channels NaV1.1 to NaV1.9 (49).

These associated β subunits are crucial for regulation and modulation of the channel function. To date, β1, β1B, β2, β3, and β4 have been identified as mammalian beta subunits and these are encoded by four genes: *SCN1B, SCN2B, SCN3B,* and *SCN4B*. Also, the β subunits are expressed in various cell/tissue types, including heart, skeletal muscle, PNS, CNS, adrenal gland and kidney (50). It has been reported that mutations in β subunits are associated with several diseases, such as epilepsy, cardiac arrhythmia, and sudden death syndromes (51). The VGSC β subunits serve as therapeutic targets, therefore, development of selective drugs targeting these accessory subunits would be beneficial.

It is well established that deficiency of VGSC function causes epilepsy and pain, however, growing evidence of the channel's role have shown in other conditions too, including autism, neurodegeneration, multiple sclerosis, muscle, and immune disorders, as well as, cardiovascular complications (52-56). Hopefully, in the near future, we can design subtype-specific blockers of these or improve the existing agents in term of subtype-selectivity.

Voltage-Gated Calcium Channel

Voltage-gated calcium channels (VGCC) are located in the membrane of excitable cells, such as in neurons, muscle, glial cells, etc. This group of ion channels is unique as VGCCs regulate, besides cellular excitability, a range of additional cellular functions, including coupling of electrical excitation to gene expression. Furthermore, VGCCs modulate numerous intracellular signaling pathways, which regulate functions, such as neurotransmitter and hormone release, neurite outgrowth, plasticity, synaptogenesis, and muscle contraction (28, 29). These channels are multi-subunit complexes consisting of a pore-forming and of α1, β, and α2δ subunits, and in some cases γ, identified in skeletal muscle and also in brain, although it remains unclear the exact interaction it has with other channel subunits (30). There are 10 α1 subunits, which are grouped into three subfamilies (1) Cav1.1−Cav1.4 (L-type); (2) Cav2.1 (P/Q-type), Cav2.2 (N-type), Cav2.3 (R-type); (3) Cav3.1−Cav3.3 (T-type). The three subfamilies are all involved in neuronal signaling (57).

Within the β class, there are four identified subfamilies (β1–β4), which are encoded by four distinct genes and each of them with splice variants (58). There are four genes encoding α2δ subfamilies labeled α2δ-1 to α2δ-4 and this group also has a number of splice variants (59). The γ subgroup consists of at least eight members Cacng1-Cacng8 and this subgroup remains still poorly understood (60).

Voltage-gated calcium channels are vital to brain function and in cases of dysfunction or inappropriate expression this leads to a number of neurological disorders, such as pain, epilepsy, ataxia, and migraine (61). VGCCs have been targeted in the cardiovascular system for a long time. Also, as a drug target in neurological disorders to treat pain by drugs named Gabapentin and Ziconotide. Nonetheless, VGCCs have a potential to serve as a drug target for the treatment of Parkinson's disease and absence epilepsy (62, 63).

Voltage-Gated Chloride Channels

Voltage-gated chloride channels (CLC) are found in the surface membrane of epithelial and excitable cells. CLCs are involved in the regulation of membrane excitability, cell volume, and the

transport of electrolytes, nutrients, and water (35,36). The CLC family consists of nine members in mammals, namely CLC-1, -2 –Ka and –Kb, which are plasma membrane chloride channels and CLC-3 to -7 are Cl^-/H^+ exchangers, which are located predominantly in intracellular compartments of the endosomal/lysosomal pathway (35,37). Mutations of voltage-gated chloride channels in humans have been linked to a number of disorders, including idiopathic epilepsy, osteopetrosis, and kidney disease with or without deafness (35).

The existence of accessory subunits for VGKC, VGSC, and VGCC are well known and well established. These auxiliary subunits which are not part of the channel pore are involved in the regulation of intracellular trafficking and modulation of the channels. So far, just three members of CLC family, ClC-Ka/b, ClC-7, and ClC-2, have been suggested to be linked with accessory subunits. To date, Barttin, Ostm1, GlialCAM are the three regulatory–auxiliary subunits of CLC chloride channel that have been identified (64). As an example, for comparison between these regulatory-auxiliary subunits of above mentioned CLC subfamily, Barttin and Ostm1 are required for endoplasmic reticulum exit, while GlialCAM is not necessary (64). Furthermore, recently it has been reported that palmitoylation of Barttin changes CLC-K channels into an active state (65).

Ligand-Gated Ion Channels

The second group, ligand-gated ion channels (LGICs) are oligomeric protein assemblies, which convert a chemical signal into an ion flux through the post-synaptic membrane (12). This function is vital for chemical synapses, as LGICs mediate the chemical to electrical signal transformation (38). This is while, LGICs can also be found in the pre-synaptic membrane, where they regulate transmitter release. The characteristic property that is required to be a member of the LGIC group is the ligand must bind in a different manner to the open and closed states. If it binds stronger to the open conformation, the ligand will be an activator and if it binds stronger to the closed one, it will be an inhibitor (12). LGICs are cell surface integral proteins, which are involved in the mediation of the fast neurotransmission in the nervous system. These channels cannot function alone instead they need auxiliary subunits for their assembly, trafficking, and pharmacological modulation. LGICs are used as pharmacological targets for a number of prescribed drugs such as quit smoking aids, anxiolytics, muscle relaxants, anticonvulsants, anti-emetics, and hypnotics (38). There are also numerous disorders of the central nervous system linked to dysfunction of LGICs including epilepsy, hyperekplexia, myasthenia, irritable bowel syndrome, Parkinson’s disease, schizophrenia, Alzheimer’s disease, smoking addiction, and alcohol dependence (38). Members of LGICs can been divided into four families: (1) cys-loop receptors, (2) ionotropic glutamate receptors, (3) ATP-gated P2X receptors, and (4) cyclic nucleotide-gated channels.

Cys-Loop Receptors

The Cys-loop receptors (CLRs), also referred as pentameric ligand-gated ion channels, all have a characteristic highly conserved 13 amino acid loop formed by a disulfide bond between two cysteine residues (66). Each CLR subunit consists of a large N-terminal extracellular ligand binding domain and four transmembrane segments (TM1-4). The TM2 is involved in pore formation and TM3 and TM4 are connected by one large intracellular loop that is involved in various intracellular modulations (67, 68). The CLR superfamily consists of nicotinic acetylcholine receptors (nAChR), γ-amino butyric acid A ($GABA_A$), $GABA_{A\text{-}\rho}$, glutamate-gated chloride receptor (GluCl), serotonin or 5-hydroxytryptamine (5 HT3), and glycine (GlyR) receptors.

Among the functions of CLRs in the human nervous system include membrane depolarization by passage of sodium and calcium through nACh and 5 HT3 receptors, and decrease of cellular excitability by hyperpolarization of the membrane potential caused by chloride permeability through inhibitory $GABA_A$ and Glycine receptors. Also, acetylcholine-activated nAChRs mediate essential neuromuscular and autonomic signals (69). Furthermore, $GABA_A$ receptors are the main mediator of inhibitory brain signals, while Gly-Rs appear to dominate in the brain stem and the spinal cord in the regulation of various motor and sensory functions, such as pain. Glycine receptors also have a role in processing visual and auditory signals (69). There are a number of known malfunctions of CLRs including mutations of muscle nAChRs, which lead to congenital myasthenic syndromes, mutations of $GABA_A$ and nAChRs cause epilepsy (70), and glycine receptor mutations lead to hyperekplexia (71, 72).

Ionotropic Glutamate Receptors

Ionotropic glutamate receptors (iGluRs) are tetrameric cation channels activated by neurotransmitter glutamate. The receptors consist

of four domains: (1) an intracellular C-terminal domain (CTD), (2) a large extracellular amino-terminal domain (NTD), (3) a membrane-proximal ligand binding domain (LBD labelled S1 and S2) and finally (4) three transmembrane domains (M1, M3, and M4) together with a re-entrant ion pore-lining loop (M2) region (73). iGluRs are essential for the central nervous system and play a key role in synaptic plasticity, which is vital for learning and memory. They also regulate processes in the spinal cord, peripheral nervous system, and retina. This group is divided into four subtypes based on their ligand binding properties or pharmacology and sequence similarity: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs; GluA1–4), kainate (KARs; GluK1–5), N-methyl-d-aspartate receptors (NMDARs; GluN1, GluN2A–D, and GluN3A-B), and delta (GluD1 and GluD2) receptors. As stated earlier, iGluRs are vital for the function of the central nervous system, however, these receptors are also involved in a number of diseases and disorders. Excessive activation of NMDARs followed by Ca^{2+}-load leads to cells death in cerebral insult in conditions, such as stroke or neurodegenerative conditions, including Alzheimer's disease or Huntington's disease (74, 75). This is while AMPARs have been linked to several neurodevelopmental disorders, including schizophrenia, Alzheimer's and Parkinson's diseases, and a contributing factor to the proliferation of glioblastoma tumors. It is also believed that AMPARs play a role in depression, seizure spread, and neuronal damage linked with epilepsy (76).

ATP-Gated P2X Receptors

The ATP-gated P2X receptors are nonselective cation channels that are activated by extracellular adenosine 5 triphosphate (ATP) permeable to Na^{+}, K^{+}, and Ca^{2+}. These receptors alter the conformation from closed to open state in response to ATP binding, enabling ions to flow quickly through the membrane (83). There are seven known P2X subunits (P2X1-7) in nearly all mammals and in various eukaryotic organisms. All subunits can assemble together to form homomeric or heteromeric functional ion channels, with the exception of P2X6, which seems to function only as a part of a heteromeric complex. All seven subunits do share a similar structure consisting of an extracellular loop where the ATP-binding site is located, and intracellular C- and N-termini linked by two transmembrane segments (TM1 and TM2) (84).

ATP-activated P2X receptors are found among others in the nervous system, where they are expressed in neurons, glia, and vascular cells (85). P2X receptors are expressed in the peripheral and central nervous system and have distinct properties as ligand-gated ion channels due to their activation by extracellular ATP, which is an excitatory neurotransmitter released from sympathetic, sensory and enteric nerves (86). It is suggested that abnormal pain signaling through P2X3 receptors contribute to neuronal sensitization and chronic pain (87). P2X4 together with P2X7 are expressed in glia cells and seem to contribute to neuroinflammation and chronic pain (88), with P2X7 subtype being a potential drug target in neurological diseases and disorders, such as neuropathic pain and epilepsy (89).

Other Classes of Ion Channels

There are a number of other ion channels that do not fit into the two mentioned classes; voltage-gated and ligand-gated channels.

Transient receptor potential (TRP) channels

The Transient Receptor Potential (TRP) channels are structurally similar to VGKCs and some members of the TRP superfamily have voltage-dependent gating. The TRP group consists of five families, namely TRPA (TRPA1), TRPC (TRPC1-7), TRPM (TRPM1-8), TRPN and TRPV (TRPV1-6), as well as, two subfamilies TRPP and TRPML. All TRP channels are tetrameric, consisting of six putative transmembrane (TM) domains and a pore-forming loop between TM5 and TM6, while intracellular C- and N-termini regulate channel assembly and function. Functional TRP channels are composed of four pore-forming TRP protein subunits, which can assemble as both homo-tetramers and hetero-tetramers (90, 91).

Also, recently the cation superfamily TRP channels became more attractive as a potential target to treat pain, epilepsy, anxiety and cancer due to their function (involvement in calcium signaling, sensors for the outside and inside environment of the cell) (92-96). Mutations of TRP channels cause various inherited diseases in the cardiovascular, renal, skeletal, and nervous system. Among them include chronic pain and overactive bladder (TRPV1), diabetes (TRPV1, TRPM4), Alzheimer's disease (TRPM7), and cancer (TRPC4/C1, TRPC6, TRPV2, and TRPM8) (14, 97).

Table 1. Therapeutic drugs targeting ion channels in neurological diseases and disorders.

Neurological Diseases & Disorders	Target Ion Channel	Drug	Chemical Structure	Ref
Epilepsy	Voltage-gated sodium channel blocker	Phenytoin	O, NH, HN, O	(77)
		Carbamazepine	N, O, NH_2	(53)
		Topiramate	O, NH_2, S, O, O, O, O, O, O, O	(78)
		Lamotrigine	Cl, Cl, N, N, H_2N, N, NH_2	(79)
Local Anesthesia	Voltage-gated sodium channel blocker	Lignocaine	H, N, N, O	(80)
Pain	Voltage-gated calcium channel blocker	Gabapentin	O, OH, NH_2	(81)
	Voltage-gated calcium channel blocker	Ziconotide	O, NH, O, O, O, H, N, O, O, O	(82)

The transient receptor potential (TRP) with the TRPV, TRPA, and TRPM subfamilies play a vital role in temperature sensation, detecting temperatures from a range between 4 °C to 52 °C. The TRPC4 and TRPC5 channels assemble as homomers or heteromerise with TRPC1 to allow Ca^{2+} and Na^{+} entry into cells. These homomeric or heteromeric channels can be activated by the natural product (-)-Englerin A, the metal ion Gadolinium (III) or the physiological agonist sphingosine-1-phosphate (21). The homomeric TRPC4 and TRPC5 channels can be inhibited by ML204 and by the anti-histamine Clemizole hydrochloride (98, 99). In this TRP subfamily, the

TRPC3 TRPC6 channels can be activated by the diacylglycerol analogue OAG (1,2-oleoylacetyl-glycerol). Other TRP subfamilies, such as TRPM2, TRPM8, TRPV1, and TRPV4 channels can be activated by hydrogen peroxide (H2O2), menthol, capsaicin, and 4α-PPD, respectively (100).

Cyclic Nucleotide-Gated Ion Channels
The family of cyclic nucleotide-gated (CNG) ion channels are tetramers consisting of four subunits with a central pore. Each subunit contains six transmembrane (TM) domains, a pore loop domain between TM5 and TM6, and intracellular N- and C-termini regions. These channels are activated by the direct binding of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) to large C-terminal cyclic nucleotide-binding domain and a depolarization or a hyperpolarization event (101).

CNG channels are activated directly by intracellular cyclic nucleotides. These channels consist of nonselective cation channels found in the membranes of various tissue and cell types, such as in the heart and the central and peripheral nervous systems. CNGs differ from other members of the ligand-gated family (extracellular ligands) as their binding site is located on the cytoplasmic side of the cell membrane. In addition, CNGs do not desensitize or inactivate, like other ligand-gated channels, following a long period of exposure to cyclic nucleotides (102).

These channels play a vital role in the function of numerous sensory pathways, such as vision and olfaction i.e. sense of smell, and also in other essential cellular functions, including hormone release and chemotaxis, movement of cells or organisms in response to chemical signals. There are six CNG channel genes that have been identified in mammals, namely homologous A subunits (CNGA1–CNGA4) and B subunits (CNGB1 and CNGB3) (103).

Mutations in CNG subunit genes have shown to cause visual disorders in humans, such as retinitis pigmentosa, an inherited, degenerative eye disease, affecting night vision and peripheral vision, causing severe vision impairment and possibly blindness (104). Another visual disorder caused by mutations in CNG genes is achromatopsia, a hereditary visual disorder, which is characterized by decreased vision, light sensitivity, and the absence of color vision (105).

ATP-Sensitive Potassium Channels
ATP-sensitive potassium (K_{ATP}) channels are voltage insensitive inward rectifier potassium channel, which are regulated by nucleotides. These channels are ubiquitously expressed and link metabolic state to electrical excitability. In heart, in response to ischaemic stress, they play a protective role and in vascular smooth muscle regulation of vascular tone (vasorelaxation) (16). K_{ATP} channels are crucial in the regulation of glucose-induced insulin secretion. In pancreatic β-cells, an increase in ATP/ADP ratio, which is generated by glucose uptake and metabolism, closes the K_{ATP} channels to elicit membrane depolarization, calcium influx, and a secretion of insulin, the primary hormone of glucose homeostasis (16).

K_{ATP} channels have been shown to be expressed in several regions of the brain, including the substantia nigra and in the hypothalamus (106-109). It has been proposed that K_{ATP} channels may play a role in the suppression of seizures in ATP-depleted conditions (110). K_{ATP} channels are the major drug target (diabetes, angina, severe hypertension, and baldness) among potassium channels (111).

Mechanosensitive Ion Channels
Mechanosensitive ion channels are found in nearly all types of cells and they can respond to a number of physical forces, including vibration, stretch, and sound waves (112). There are two mammalian mechanosensitive ion channels, namely Piezo1 and Piezo2, which consist of some 2,500 to 2,800 amino acids. It is believed they hold between 26 to 40 transmembrane domains (113). Piezo1 and 2 are expressed in the lungs and bladder, two organs that both hold vital mechanosensory functions. In addition, Piezo1 can be found in the skin, red blood cells, and recently it has been suggested that this channel acts as pivotal integrators in vascular biology (114). Mutations in the function of Piezo1 cause hereditary xerocytosis, an autosomal dominant hemolytic anemia denoted by primary erythrocyte dehydration. This is while, Piezo2 is expressed in sensory neurons of the dorsal root and trigeminal ganglia, suggesting it plays a role in the sensation of touch. Mutations of this channel are linked to distal arthrogryposis, a disorder characterized by deformities in joints (contractures) restricting hand and feet movement (115).

Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channels
Another ion channel is cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels, which transport chloride ions in and out of cells, controlling water movement in tissues, a necessity for the production of mucus that

lubricates and protects the lining of the airways, digestive and reproductive systems and other organs. Dysfunction of the CFTR leads to a number of diseases, including cystic fibrosis and bronchiectasis (116, 117).

Temperature-Gated Channels

Temperature-gated ion channels are a group of membrane proteins, which enable ions to flow across the membrane through changes in temperature. The main subtype in this group is the transient receptor potential (TRP) with the TRPV, TRPA, and TRPM subfamilies as mentioned earlier. A recently discovered member is the calcium-activated chloride channel belonging to the TMEM 16 family of ion channels that is involved in heat sensitivity and possibly nociception, the sensory nervous system's response to a harmful or possibly harmful stimuli (118).

Other ion channels, including voltage-gated proton (HV) channels, human ether-a-go-go-related gene (hERG) potassium channels, two-pore-domain potassium (K2P namely TREK-1 and TRAAK) channels, and voltage-gated chloride channels are strongly modulated by changes in temperature as well as a number of pathological mutations in the voltage-gated sodium channels (NaV) that boost the temperature dependence of the NaV channel activity (119-121).

Acid-Sensing Ion Channels

Likewise, growing evidence showing that acid-sensing ion channels, which are ubiquitous expressed in the mammalian nervous system, are involved in important neurological diseases and conditions, such as pain, anxiety, and epilepsy. Therefore, these channels can serve as potential pharmacological targets (122-128).

SUMMARY

Since the discovery of animal electricity by Galvani over 200 years ago, the science of electrophysiology has developed into a practice widely used to study voltage change and ionic currents in biological cells and tissues. Interestingly, ion channels have been targeted even before they have been cloned as example; cocaine (VGSC blocker) was announced as a first local anesthetic in 18th century (129) and sulfonylureas (K_{ATP} channel blocker) as antidiabetic agents in 1950s (16). Also, phenytoin and carbamazepine (VGSC blockers), which target ion channels, have been used to treat neurological disorders for a long time. Moreover, ion channels have always been of interest as targets, particularly, in the central nervous system, the peripheral nervous system, the cardiovascular system, and diabetes in drug discovery for the pharmaceutical industry.

The patch clamp recording of ion channels is an important electrophysiological technique that has permitted the understanding of the role of ion channels (physiological, pharmacological and biophysical proprieties) in vital cell processes, namely action potentials and nerve activity as well as in cardiovascular and other systems. Also, it is worth mentioning that microelectrode array (MEA) is a decent tool for the neurotoxicological assessments (130). MEA, or microelectrode arrays, devices consist of multiple shanks or plates of which neuronal signals are recorded, connecting neurons to electronic circuitry. MEA devices are grouped into two classes namely implantable MEAs used in vivo experiments, and non-implantable used in vitro.

While ion channels are essential for the existence of human life, they also play a role in various diseases and disorders, including cardiovascular, muscular, and neurological. Therefore, ion channels are very attractive as therapeutic targets. However, with the hundreds of different types of ion channels having diverse functions and structures, drug discovery is a challenge due to the major logistical and technical differences. There are a number of problems, which occur when studying ion channels, including misfolding of transmembrane domains and misassembly when they are overexpressed. Also, when ion channels are overexpressed, they are often unstable and poorly tolerated. These are some of the issues that need to be resolved. Importantly, most of drugs are not selective to their target, in other words, currently, barely any agent that targets ion channels is specific to a single channel, this property of the drug causes toxicity and adverse effect. Therefore, the development of new ion channel drug agents remains a challenging task and is discouraging in drug discovery for the pharmaceutical industry. Nevertheless, what is encouraging is that new ion channels subtypes have been cloned and also some of them crystallized in recent years. This gives hope to target a single channel in the process of drug discovery to treat neurological diseases and disorders.

Taken together, our understanding of biology and physiology of ion channels have grown exceedingly during the past decades and the next important milestone in the field of ion channel will be to study potent and selective small molecules with the purpose to identify novel therapeutic targets for patient benefit.

PATCH CLAMP SET UP

A conventional patch clamp recording setup comprises of a number of components, including a microscope, a specialized table with a surrounded cage, and on the side an amplifier and a PC with pre-installed electrophysiology data acquisition and analysis software. Here, components of a patch clamp rig will be briefly described in general without detailing any brand names.

Anti-vibration table or vibration isolation table: consists usually of a heavy slab on pneumatic supports, which is set up in order to isolate the rig from external sources of vibrations, as small vibrations measuring even of picometer magnitude can disrupt the recording.

Faraday Cage: consists of wire mesh enclosure placed on top of the anti-vibration table, to shield the devices inside from external interferences. This prevents small sources of interferences, including radio waves, which can disrupt or obscure electrical signals while recording.

Microscope: a specialized optical magnification tool, such as a high-resolution inverted microscope or a fluorescence microscope, depending on the type of experiment.

Recording chamber, perfusion chamber, or bath chamber: where the cells for the experiment are placed for recording. The chamber is positioned onto the microscope stage underneath the microscope objective.

Microscope stage: where the experimental specimen is placed for observation/recording. The stage can usually be adjusted horizontally and vertically.

Micromanipulator: a device that gives accurate and reliable maneuvers of the micropipette with nanometer precision to the area of the cell membrane, a vital step for successful recordings.

Headstage: holds the patch-pipette or micropipettes with built-in circuitry to transmit acquired electrical signals onto the amplifier. The device also contains vital electric circuitry that decreases noise.

Patch Clamp Amplifier: an electronic device that measures an electrical current through the ion channel or voltage across the cell membrane. The amplifier is used to strengthen (amplify) the current signals before they are recorded.

Digital Audio Tape (DAT) Recorder: a device that stores the patch clamp recordings, providing a permanent record of the data.

Analog-to-Digital Conversion (ADC) interface or Digitizer: a data acquisition instrument, which is a part of the amplifier that converts the analog current signal acquired by the amplifier into digital signals, so as to perform data analysis and display on computer.

Computer: a PC with installed electrophysiology data acquisition and analysis software, which can process the obtained signal with various settings, such as filtering, curve fitting, noise removal and parameter determination.

PRACTICAL GLOSSARY FOR ION CHANNELS STUDY

Action Potential: a transient regenerating change in membrane potential that permits a wave of electrical excitation to pass along the plasma membrane of electrically excitable cells.

Gigaseal: achieved when a seal between a cell membrane and electrode has an electrical resistance of a gigaohm or greater. Such a connection is essential for patch clamp recording in electrophysiology, as it lowers current noise and influencing the quality of the recording.

Conductance: reflects the ease of charge flow of ions thru the channel, i.e. how many ions enter the cell during a specific period of time and this is measured in siemens (S). Conductance is the inverse of resistance, meaning if the conductance of a certain ion membrane is low then the resistance to movement of that ion across the membrane is high. Most ion channels' conductance lies in a range between 1 to 200 picosiemens (10^{-12}).

Gating: The process, in which ion channels open and close their pores due to a stimulus, such as a change in membrane potential, temperature, drugs, chemical transmitters or a mechanical deformation. Voltage-gated channels open or close their pores depending on the electrical potential across the plasma membrane. Ligand-gated channels open or close depending on their binding of ligands to the channel. This is while others depend on factors such as cell volume, intracellular

metabolic state (ATP concentration), intracellular ligand and/or second messenger presence (cyclic AMP due to light, calcium) and extracellular ligands (neurotransmitters e.g. acetylcholine, GABA).

Membrane Potential or **Transmembrane Potential** or **Membrane Voltage:** the difference in electric potential between the cells' interior and exterior, measured in millivolts (mV). The membrane potential is generated by the ion movement across the membrane where the ionic concentrations of the intracellular and extracellular fluid differ. At a resting state, the inside of the nerve cell membrane is negative at typically -70 mV with respect to the outside.

Open State Probability (Po): the amount of time that an ion channel is in open configuration.

Permeability: a measurement of how fast ions cross a cell membrane via an open channel. The permeability is directly proportional to the total number of open channels for a specific ion in the membrane.

Pore: a part of the ion channel that forms path ions use to move from one side of membrane to another. Pores are often lined with some hydrophilic amino acids and sometimes they are aqueous. Ions often move through the segments of the pore in a single file nearly as quickly as the ions move through free solution.

Rectification: A nonlinear current-voltage relation occurs when the channel gating or conductance is affected by voltage, which enables better flow of ions in one direction than another. This sometimes occurs when other ions "block" the pore.

Reversal Potential or **Nernst potential:** the membrane potential at which the net flow for that particular ion from one side of the membrane to other side is zero. This zero-current state is the potential at which the overall direction of the ion flux reverses.

Selective permeability: the process in which cell membranes allow only certain ions of a specific size in its hydrated form and/or charge to pass through.

Selectivity Filter: a specific part of an ion channel that is responsible for selecting which type of ion to let through its membrane. It is believed to be located in the narrowest part of the ion's pore, as it is the part of the channel where the ion and the protein would probably interact the most.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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