Research Article

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Ion channels and neuronal excitability in polyglutamine neurodegenerative diseases

Abstract: Polyglutamine (polyQ) diseases are a family composed of nine neurodegenerative inherited disorders (NDDs) caused by pathological expansions of cytosine-adenine-guanine (CAG) trinucleotide repeats which encode a polyQ tract in the corresponding proteins. CAG polyQ repeat expansions produce neurodegeneration via multiple downstream mechanisms; among those the neuronal activity underlying the ion channels is affected directly by specific channelopathies or indirectly by secondary dysregulation. In both cases, the altered excitability underlies to gain- or loss-of-function pathological effects. Here we summarize the repertoire of ion channels in polyQ NDDs emphasizing the biophysical features of neuronal excitability and their pathogenic role. The aim of this review is to point out the value of a deeper understanding of those functional mechanisms and processes as crucial elements for the designing and targeting of novel therapeutic avenues.

Keywords: ion channels, neuronal activity, CAG repeats, neurodegeneration, biophysics

Introduction

Neurodegenerative diseases (NDDs) are irreversible and incurable disorders of the nervous system and the brain resulting in progressive degenerative processes, which ultimately lead to the death of the neurons; impaired or dead neurons cannot be structurally and functionally replaced, causing damages to muscular (i.e., ataxias) or mental (i.e., dementias) functioning. Those problems are typical of e.g., Alzheimer’s, Huntington’s, or Parkinson’s disease, spinal polyQ atrophies (spinocerebellar ataxias [SCA] and spinal and bulbar muscular atrophy [SBMA]), and spinal muscular atrophy (SMA) [1].

In NDDs, the system “nerve-muscle-synapse” is severely impaired and the analysis of the functional activity (i.e., how the cells work and interact by the nerve impulses) is crucial to track the neuronal illness. In particular, neuronal and neuro-muscular synapses are the major information-processing hubs for neural communication in the brain that contains trillions of synapses within a network of neurons [2]. Multiple synapses are extremely plastic; they can change their strength as a result of their own activity. Deficiency in synapse integrity contributes to cognitive impairment and motor disability [3,4]. Therefore, synaptic plasticity and synapse remodeling are essential for understanding the pathophysiological mechanisms of NDDs [5].

From a functional point of view, the basic process of synaptic transmission is the flow of ionic conductance across the neuronal plasmatic membrane which leads to membrane potential shift for the neuronal signal propagation. Neurons operate within defined activity limits, and feedback control mechanisms dynamically tune ionic flux to maintain this optimal range. This process is principally carried out by transmembrane proteins (ion channels) that came from an active translation route [6–8].

Accordingly, ion channels are key elements in the control of membrane physiology and neurotransmission [9]. Pathophysiology of ion channels may originate from either mutations of gene encoding components of the channel structure (channelopathy) or secondary dysfunctions:
both conditions affect intrinsic excitability of the cell and synaptic functions leading to pathophysiological signs of diseases [10].

In the majority of NDDs, the pathogenic role of ion channels has been widely demonstrated explored either for channelopathies or secondary dysfunction [11], on the contrary, in polyglutamine (polyQ) NDDs, the link between ion channels alteration of neuronal excitability and disease onset has been poorly considered.

PolyQ NDDs are produced by pathological expansions of cytosine-adenine-guanine (CAG) trinucleotide exonic repeats encoding a polyQ tract in the corresponding proteins. So far, polyQ NDDs include nine disorders, well identified and characterized: six types of spinocerebellar ataxias (SCA) (types 1, 2, 3, 6, 7, 17); Huntington’s disease (HD); spinal and bulbar muscular atrophy (SBMA); and dentatorubral pallidoluysian atrophy (DRPLA) [12–14]. The expanded polyQ tract triggers aggregation in the pathological neurons leading to dysfunction and progressive degeneration of specific neuronal populations [13].

In polyQ diseases, the role of the alteration of neuronal excitability, due to the dysregulation of ion channels activity and synaptic signaling in affected neurons, has been ascertained as a key factor in the pathophysiology and in the pathogenesis of the illness [11,15,16]. Therefore, in SCA, HD, and SBMA, the neuronal alteration has been clearly attributed to a pathogenetic role of several types of voltage-gated channels [11,15–20], nevertheless, in DRPLA, the direct or indirect pathophysiological effects of ion channels perturbation are still neglected.

Far to be comprehensive, this review will look specifically through biophysical mechanisms and processes underlying the pathophysiology of ion channels in polyQ NDDs. Particular attention will be paid to aspects/issues regarding drug screening and targeting in order to make a general survey of putative therapeutic avenues that can be developed to treat or alleviate these incurable diseases.

**Biophysics of ion channels: basic functional outlook**

In nervous cells, the action potential is a fast response (usually recorded and graphically represented as a millisecond long-lasting wave) generated by the presence of a stimulus which can be modulated by changing an electrical, mechanical, pharmacological, temperature, or light condition. With the advances in technology applied to the electrophysiology field, the origin of the action potential evidences the activity of certain proteins situated in the cell’s surface interchanging ions: Ion channels [9]. These proteins were first hypothesized as pores and then extensively studied during the golden analogical era of electrophysiology [21–30]. Decades after, with strong and convincing shreds of evidence, the pore-like function was confirmed by the patch-clamp technique in the 70–80’s decades [31–35]. From then on, the catalog of ion channels identities associated with biological functions has grown-up thanks to understanding their biophysical, pharmacological, genetic, and structural characterization, confirming their presence in all kinds of cells [9,36]. Depending on their properties and mainly by their ion/cation affinity, ion channels group in superfamilies as sodium (Na\(_\text{v}\)), potassium (K\(_\text{v}\)), calcium (Ca\(_\text{v}\)), and chloride (Cl\(_\text{v}\)) voltage-gated channels [9].

When an electrophysiologist faces an unknown channel identity, the best way to start is finding the key to the subject’s voltage activation, which provides important information and permits to define its ion affinity. Chemistry and biophysics play the main role here applying concepts of Nernst equilibrium potential, chemical solutions, and application of electrical stimuli, which conduct to the association of the channel to a superfamily. Then, another stimulus-factors permit to define and complete the subject’s profile, for example, the use of pharmacology helps to know a way to enhance, block, deactivate, and inactivate the electrical properties of the subject applying venoms or toxins of natural or chemically synthetic origins, resulting in a concrete definition of families, types, and subtypes of channels in the same superfamily [9,36].

Despite the importance of knowing how the channels respond to a stimulus and concluding their affinity, the association of the function with protein’s structure is nowadays another mandatory issue in order to complete the profile of each studied subject. The structure–function relationship started to develop during the boom of genetics and molecular biology from 1980s. In fact, the combination of molecular biology and electrophysiology is the most efficient formula to have a complete view of each membrane channel type and then is possible to go beyond associating them to pathologies and many other biological functions of life [37,38]. As a coarse analogy of the roles of windows and doors in a house, now it is possible to know the ion channels assembling parts and their roles, e.g., selectivity pores, electronic flux sites,
agonist or antagonist binding sites, intracellular and extracellular accessories, etc. It is even possible to predict the behavior of the structural modifications, which can be the origin of any of the emerging and numerous “channelopathies,” i.e., ion channel-induced pathologies cataloged [37]. According to recent literature, some members of each superfamily can be associated with polyQ NDDs [13].

**Voltage-gated sodium channels (Na<sub>v</sub>)**

Hodgkin and Huxley understood the sodium channels (Na<sup>+</sup> channels) at the very beginning of an era in which it was not possible to study their molecular structure as they were studied only by voltage-current amplification, physics equations, and saline solutions [23,25,26]. Nowadays, with a common profile completed, we know that Na<sup>+</sup> channels serve as activators, inhibitors, or allosteric modulators of their voltage-dependent gating processes. The three main behaviors of a sodium channel along the duration of an action potential are fast voltage-dependent activation, rapid and selective ion conductance, and fast inactivation on the millisecond time scale [39]. As was once expected, modifications in the molecular structure by conformational mutations, deletions, or amino acid sequence repetitions produce important clues to understand them and they were first associated by this structure–function studies [39]. Sodium channels trigger in response to cell’s depolarization and then inactivate within 1–2 ms producing a fast characteristic peak with considerable magnitudes (in a whole-cell recording), at the unitary level, each channel opens briefly and remains closed after the cell readjusts its ion internal conditions, so that, they are ready to open again.

**Voltage-gated potassium channels (K<sub>a</sub>)**

Potassium channels (K<sup>+</sup> channels) are responsible to re-equilibrate the resting potential of the cell after the depolarization caused by sodium or calcium at the beginning of the action potential [23,24,26,40–42]. Their structure is smaller and less complicated with respect to other types of channels and this superfamily is found in bacteria kingdom to the higher vertebrates [42]. Their natural role is to drive the potassium ions out of the cytoplasm when the cell is depolarized due to the excess of potassium in the cell when responding to an input, although some types of potassium ion channels require calcium to goal their activity [40,41]. Thus, their opening and activation could be fast, but the closing and deactivation is slow unlike sodium channels, and depending on the subtype, they have that peculiarity to be inactivated in a window of dozen milliseconds to even seconds which means thousand times scaled. Evolution improved their selectivity filter, achieving efficacy to conduct large quantities of potassium ions through them in a highly selective manner. This fast rate of flux is required to repolarize the cell as fast as possible [42]. They are present in almost all human tissues and their structure is the simplest in comparison with other superfamilies and actually with this premise one can imagine their participation in physiological processes not necessarily related to the generation of action potentials. New subfamilies classification has been formally established including e.g., new voltage-gated K<sup>+</sup> channels (K<sub>v</sub>), Ca<sup>2+</sup>-activated K<sup>+</sup> channels (K<sub>Ca</sub>), two-pore K<sup>+</sup> channels (K<sub>2P</sub>), and inwardly rectifying K<sup>+</sup> channels (K<sub>ir</sub>); however, in the near future new groups could be added [43].

**Voltage-gated calcium channels (Ca<sub>v</sub>)**

At least ten families are part of the voltage-gated calcium channels (Ca<sup>2+</sup>). The members are key transducers of membrane potential changes into intracellular Ca<sup>2+</sup> transients that initiate many physiological events [44]. The pharmacological blockade has been the key to identifying types and subtypes. In the nervous system, they are associated with the mechanisms of neurotransmitter release and they are the key to signal transducers of electrical excitability, converting the electrical signal of the action potential in the cell surface membrane to an intracellular Ca<sup>2+</sup> transient [45,46]. The basic library of calcium channels divides them into L, N, P, Q/R, and T types. With all these different subtypes, a new way to identify ion channels by the use of pharmacology drugs was set [44,45,47]. L-Type calcium channels are represented by the cloned subunits Ca<sub>1.1</sub>, Ca<sub>1.2</sub>, Ca<sub>1.3</sub>, and Ca<sub>1.4</sub>, and all of them are related to muscle and cardiovascular problems; while N, P, and Q/R types are considered an essential part of the mechanism of neurotransmitter release, their cloned subunits are Ca<sub>2.1</sub>, Ca<sub>2.2</sub>, and Ca<sub>2.3</sub>. For the T-type channels, there are studies on the pacemaker repetitive fire of neurons determined by their main subunits Ca<sub>3.1</sub>, Ca<sub>3.2</sub>, and Ca<sub>3.3</sub> [44]. So, this catalog expanded more and more based on the knowledge of drugs binding to any of the conformational subunits specifically affined to
any type of calcium channel; actually, there are important applications in industrial, medical, and even nutraceutical research applying this knowledge.

**Voltage-gated chloride channels (ClCs)**

ClC is a small but predictable growing-up superfamily, which is nowadays investigated generating important results in the achieved role in the cell as is the case of the other superfamilies. The first described chloride channel belonging to the ClC gene family was ClC-0 [48] which was found more than a decade before and was identified by biochemical isolation from *Torpedo californica* using the lipid bilayers technique and some of their electrical properties were tackled [49]. Then, it was also found in the cells of *Torpedo electroplax*, suggesting a hypothetical double pore-like structure [50], which was confirmed in subsequent years. Homo and dimeric forms of the ClC channels are present in mammals and plants. Their identification is associated with the occurrence of important human pathologies including neurodegeneration, leukodystrophy, mental retardation, deafness, blindness, myotonia, hyperaldosteronism, renal salt loss, proteinuria, kidney stones, male infertility, and osteoporosis [51]. In physiological terms, the ClC channels (ClC-0 to ClC-7) can be present in excitable and non-excitable cells, and they permit the balance of both protons and anions between the extracellular space and cytoplasm, which defines the level of neutrality of cells by sensing cell’s electrochemical H+ gradient more than Cl− [52]. Dimeric forms of the channels (2 pores) demonstrate that pores act in an independent manner and depending on the type they conduct protons or chloride anions. ClC-1 works differently and even contrarily to K channels as they maintain their activity (inward chloride) at very negative potentials, while they exhibit low outward activity during depolarization [51,53]. This phenomenon is important in muscles, as myotonia is provoked by the alteration of this channel when its conductance is reduced, as the level of chloride in muscle is necessary for the physiology of contraction [54]. In a series of experiments in which the ClC-1 was modified by its RNA, the conclusion was clear: the aberrant splicing of the CLCN1 mRNA provoked myotonia in HD [53], which reveals the importance of ClC in polyQ diseases. In addition, a pathogenic role of ClC-2-like chloride channels has been identified and functionally characterized in cells modeling SBMA [19] (Section 3.3 below).

**Ion channels in polyQ NDDs**

**HD**

HD is a progressive brain disorder characterized by unwanted choreatic movements, behavioral and psychiatric symptoms, and dementia [55]. The disease is caused by an expanded CAG trinucleotide repeat (of variable length) in *Htt*, the gene that encodes the protein huntingtin. HD is caused by a polyQ expansion in the N-terminus of huntingtin, a 350 kDa cytoplasmic protein [55].

The transgenic mouse model (R6 lines) is one of the most employed systems in the electrophysiological characterization of HD pathophysiology. There are two variants that express approximately 115 (R6/1) and 150 (R6/2) CAG repeats within the huntingtin gene exon 1 [56]. Through the use of intracellular recordings in brain slices, a variety of variations in the passive and active membrane as well as synaptic properties of striatal medium-sized spiny neurons (MSNs) was noted in the R6/2 transgenic mice. Neurons from R6/2 mice exhibited numerous electrophysiological alterations, including a shift to positive values of resting membrane potential [57], increased input resistances, and decreased membrane time constants, suggesting an active modification of ion channel activities. Moreover, it was found that in neurons of the symptomatic R6/2 transgenic model, the stimulus intensities required to evoke excitatory postsynaptic potentials (EPSPs) were increased as compared with controls. These EPSPs showed slower rise times and did not decay back to baseline by 45 ms, suggesting a substantial component mediated by glutamatergic transmission [58].

In R6/2 transgenic and a CAG knock-in models, differential responses to N-methyl-D-aspartate (NMDA) stimulation using a cell swelling assay were observed [57]. Furthermore, spontaneous excitatory postsynaptic currents were detected to be reduced in R6/2 mice [59]. Nonetheless, the participation of NMDA channels in the physiopathology of HD was elegantly demonstrated by the measurement of NMDA-evoked current density in neurons from striatal brain slices and acutely dissociated medium-sized striatal neurons of R6/2 mice. Neurons from R6/2 transgenic mice showed an increase in NMDA-evoked current density as compared with their littermate controls [59]. Interestingly, they also found that this phenomenon was present in striatal neurons from the YAC72 mice model, a construct that contains human genomic DNA spanning the full-length gene and all its
regulatory elements with 72 CAG repeats [60]. Within glutamatergic transmission, postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) or kainate channels showed a minor contribution [57,59]. However, AMPA or NMDA-mediated currents were smaller in cortical pyramidal neurons from R6/2 mice as compared with the control ones [61]. Regarding GABAergic synaptic transmission, it was noted that spontaneous synaptic currents and inhibitory postsynaptic currents were increased in R6/2 MSNs [62,63].

Ariano and collaborators analyzed the expression of K+ channels subtypes in MSNs and large spiny striatal interneurons (LANs) of R6/2 and R6/1 HD mouse models [64]. They found, through immunostaining and immunoblotting assays, a substantial reduction in inwardly rectifying K+ channel (Kir2.1 and Kir2.3) and Kir2.1 protein expression in MSNs but not in LANs of R6/2 mice. Additionally, the authors reported an increased cyclic AMP-mediated phosphorylation of NMDA channels that in turn promotes the channel open time and elevates neuronal glutamate responsiveness, contributing to excitotoxicity [64]. Later, it was identified (in striatal MSNs from symptomatic transgenic mice) an increased input resistance, depolarized resting membrane potentials, and reductions in both inward and outward rectifying K+ currents that strongly correlate with the observed decreases in the expression of Kir2.1, Kir2.3, and Kir2.1 K+ channels [65]. Altogether, those works demonstrated that the selectively vulnerable MSN neurons of the striatum in HD [66] are depolarized by up to +12 mV and displayed lower membrane conductance, as well K+ current [64,65].

Besides the neuronal ion channels in HD, the CIC-1 and Kir conductances were evaluated in dissociated ex vivo adult skeletal muscle fibers from R6/2 transgenic HD mice. A decrease in the resting chloride and potassium conductances was recorded as well correlated with a reduction in the total mRNA of CIC-1 and Kir2.1, respectively, from HD mice muscle [53]. Moreover, Tong and collaborators [67] first reported that Kir4.1 current was robustly decreased in striatal astrocytes with huntingtin nuclear inclusions in R6/2 as well as Q175 HD mice models. To note, the viral delivery of Kir4.1 channels to striatal astrocytes restored Kir4.1 conductance, ameliorated aspects of MSN dysfunction, prolonged lifespan, and attenuated some motor phenotypes in R6/2 mice. Thus, in HD, glutamate uptake activity critically depends on Kir4.1 conductance in striatal astrocytes [68]. The voltage-gated potassium channel subfamily 7 or KCNQ channels were also proved to have a role in HD by a reduction in KCNQ2/3-mediated M currents that caused impairment of the fast activity-dependent homeostatic control of excitability in striatal output neurons of R6/2 mice [69]. The SK3 channels belonging to small conductance calcium-activated K+ channel or SKCa [70] and KATP potassium channels [71] have been indirectly demonstrated to play a role in the regulation of the neuronal excitability in HD.

It was found that the store-operated calcium (Ca2+) entry (SOCE) pathway activity is enhanced in neuronal MSN expressing mutant Huntingtin. Two main protein families contributing to SOCE: Transient receptor potential canonical (TRPC) channels and Orai. The Orai1 protein forms highly selective Ca2+-permeable CRAC channels with a strong inward rectification. The pathologically increased SOCE in MSNs from HD cell models is maintained by both TRPC1- and Orai-containing channels and required stomatal interaction molecule 1 (STIM1) for its activation [72–74]. In an extended work, whole-cell recordings from induced pluripotent stem cell (iPSC)-based model of HD identified that both ICRAC (CRAC channels) and ISOC (TRPC channels) amplitudes are pathologically enhanced in HD GABAergic MSNs [75,76]. Electrophysiological analyses confirm that L-type Ca2+ channels, in particular Ca1.2 subtype, are affected in a bacterial artificial chromosome (BAC)-mediated transgenic mouse model of HD. It was shown that Huntingtin protein might disrupt calcium homeostasis via upregulation of L-type Ca2+ channels by triggering an increase in L-type Ca2+ currents and total Ca2+ current density in cortical neurons from BACHD mice [77].

As a special mention, the patch-sequencing (Patch-sec) approach has been recently carried out in an in vitro HD model to investigate the effects of mutant Huntingtin on synaptic transmission and gene transcription in single striatal neurons [78]. This technology could be applied both to better understand the molecular mechanisms underlying a complex neurological disease at the single-cell level and to provide a platform for screening the ion channel targeting for therapeutics of NDD.

**Pharmacology of ion channels in HD**

Although gene silencing therapies offer a possible solution to suppress the effects of HD [79], it is necessary to mention the contribution to ion channel research and even to continue to hypothesize as 20 years ago [80], that ion channels could be involved in HD pathogenesis despite the few information available. There is a lack of information in channels’ pharmacology applied to investigate the HD but some works throw up important evidence. With respect to calcium channels, taking a
complete panoramic of the so called SOCE [81], nowadays it is clear that huntingtin (Htt) and inositol-3 phosphate (IP3) bind IP3 receptors (IP3R), intracellular cation channels that mediate Ca\(^{2+}\) release from the Endoplasmic Reticulum (ER) [82]. The binding effect of the mutant form mHtt is crucial for the orchestration of SOCE mechanism, which belongs to non-excitatory cells, but it is an important clue for a possible pharmacological treatment tackling HD [82].

In order to discover other types of calcium channels participating in the SOCE affected during HD, the results of research in pharmacology applied to primary culture neurons pre-incubated with glutamate originated from BACHD mice (expressing the mutant mHtt) indicated the potentiated blockage of an L-type calcium channel Ca\(_{v}\)1.2 with Nifedipine when compared to WT [77]. This work is similar to the results obtained when comparing BACHD and WT mice in which the potentiated blockage influenced the Ca\(_{v}\)2.2 (a N-type calcium channel) when the conotoxin ω-CTx-GVIA was applied [83]. Among calcium channels studied by their pharmacology, SOCE mechanism must be considered, although the possibility to obtain a treatment for HD by their pharmacological mechanism is still far. Another example is the Ryanodine Receptor (RyR) which is a calcium channel that transports Ca\(^{2+}\) from reservoirs to the cytoplasm and results altered by mHtt in HD neurons [84]. Presenilin (PS1), although it is not a typical calcium channel, is considered a nine-subunit cationic channel with the main role to deliver calcium to ER, its mutations are linked to Alzheimer disease [85] and still not verified in HD. Finally, the Orai Channels, known as Ca\(^{2+}\) release-activated Ca\(^{2+}\) channels (CRACs), are expressed in the brain or in cancer cells [82].

The confirmation of the release of calcium from compartments in the presence of glutamate and the blockade of calcium currents [83], opened the possibility to study other receptors in HD: Glutamate and glutamine receptors. NMDA receptors were the target of investigation with the obtaining of KO mice as models of HD [86] and after the structural analysis of Htt [87]. Glutamate receptors named mGluR1 and mGluR5 were proposed to study in HD and other NDDs as they activate multiple pathways with functions at the cellular level [88]. The focusing on the electrophysiology of cells expressing the mutant mHtt throws the conclusion that the biophysical properties of the channel do not suffer modifications.

A series of enhancers and blockers of the cationic/ Ca\(^{2+}\) channel family TRPC tested in different systems during patch-clamp experiments can be related to HD as results concluded important effects in the channel opening [89]. One of the most involved in HD is the TRPC5 channel, in which its currents are elicited using Cs\(^{+}\) as a carrier. In experiments of patch-clamp in neuronal striatal cells STHdhQ111/111 (a Htt knock-in mutant), results evidenced that pre-treated cells with 2-bromopalmitate (2-BP) produced a negative ongoing in the current in comparison with no treatment condition, even if GTP\(_{y}\)S was used [90]. The magnitude of this result sustains the importance of this cationic channel in Huntington’s pathology.

In the case of K\(^{+}\) channels, the inward K\(^{+}\) currents and K\(_{ir}\) 2.1, 2.3, and K\(_{4.1}\) were under review using R6/2 transgenic mice and applying common patterns of stimulation, results indicated that the inward K\(^{+}\) currents (range –120 to –60 mV) is reduced in the mutant even if the carrier was Cs\(^{+}\) [91]. So, the involvement of K\(_{ir}\)4.1 in the development of HD pathology has been ascertained in mouse models, although these channels are attractive not by their functional properties for themselves, but by the fact that their expression is affected when they are expressed in astrocytes with mHtt [92]. Importantly, this similar phenomenon happens in the case of NMDA receptors as mentioned above.

**SCA**

The autosomal-dominant SCA, also known as Ataxia, is a superfamily including groups of neurological and neurodegenerative disorders in which diagnosed symptoms include impaired speech, lack of limbs’ movements coordination, and absence of body balance; with the advance of the disease, patients end in wheelchair confinement. The causes of motor dysfunction presented in Ataxia have their main origins in the incorrect function of neurons of the cerebellum and their associated pathways [17]. Ataxias divide clinically into three groups based on observations of the diagnosed patients and for the case of Group I, the main symptoms are visible in the body: tremors in superior limbs, dystonia, hearing loss, cognitive impairment; meanwhile, in Group II apart from the cerebellar ataxia, patients present retinal degeneration. Finally, in Group III the problems denote a kind of “pure” cerebellar ataxia, which is located specifically in cerebellum neurons [93]. At the cellular level, the problem is mostly located in cerebellar neurons which correspond to the most important motor area and in which the dominant cell present is the Purkinje-cell type. Depending on the neurological conditions and the ambient, ataxias appear in the cells’ nucleus when
genetic material presents alterations. Pair-based pointed or multiple mutations in nuclear DNA produce problems in different cellular pathways going from the nucleus transcriptional dysregulation, generation of RNA toxicity, loss of adequate proteostasis, and even protein aggregation; most of these problems lead to the alterations of the neuronal activity or loss of neuronal populations [94,95].

Recently, evidence in clinical reports and experimental works in animal models of SCA establish the existence and description of the different types of SCA, which are numbered from 1 to 35. These can be associated with clinical Groups I–III, and the research has provided important information about mutations in genes responsible for physiological changes and structural phenotypes altered from membrane proteins underlying their misfunction for each specific type of the Ataxias described [17,93]. The different types of proteins affected by genetic problems grouped in three main blocks: neurotransmitters’ receptors, signal receptors, and voltage-gated channels. There are at least 30 genetic alterations associated with ataxias, but focusing on the group of voltage-gated channels, a total of seven genes have been considered responsible for the alteration in the function of the members of the neuronal activity or loss of neuronal populations [94,95]. Evidence points to recognize that around a dozen of the 35 types of SCA reported end in a direct channel alteration although it is possible to predict that future studies will extend the list. Bushart and Shakkotai [17] reported nine channelopathies in SCA where sharply identified ion channels mutations – charged to different channel types – cause ataxic phenotypes. Interestingly, only one of those channelopathies is referred to polyQ SCA, i.e., the CACNA1A-induced SCA type 6 (SCA6) in charge of a Ca21 calcium channel.

Voltage-gated sodium channels (N_{Na}) related to ataxias

For the case of sodium superfamilies, two types of ataxias exist in which direct affectations of members of this superfamily occur: in Ataxia SCA27, where FGFI4 gene mutation produces the loss of the N_{Na}1.6 channel expression, which implies a negative effect on Ca21 and Ca22 function as they are channels that are sensitive to the entering of sodium into cell. These findings conclude a failure in the expression of channels in the cell’s membrane with consequences in sodium influx reduction and loss of excitation [96–98]. The other type is the SCA5 Ataxia, where alterations in the SPTBN2 gene, which encodes for sodium channel complexes mGluR1 and EAAT4 (glutamate transporters), provokes impaired long-term potentiation of the Purkinje cell and an excess of glutamate inside the cell. The cells present abnormal dendritic development originated from the RNA toxicity as minor affection, while in extreme conditions cells activate other pathways conducting to death [99–102].

Voltage-gated potassium channels (K_{pot}) directly related to ataxias

Four types of ataxias conduct to the direct influence of members of the VGPC superfamily, especially the family of K_{pot} or KCN channels. Most of these channels exhibit large ongoing outward currents during experimental voltage stimulation and under physiologically normal conditions, depending on the type of K_{pot} channel; they open between the moment depolarization – after Na+ entry to the end of the action potential – and repolarization of the cell’s membrane [103]. Affected members are K_{v}1.1, K_{v}3.1, K_{v}3.3, and K_{v}4.3 [104]. In SCA1, the KCNA1 is the only gene associated with SCA1 and encodes a K_{v}1.1 Shaker-like potassium channel. Loss-of-function (LoF) of this channel is also associated with spasmodic epilepsy in human patients and KO mice and sudden death in episodes of epilepsy in mice models. Pointed mutations of this gene can produce interesting phenomena in the treatment of patients, e.g., lack of response to acetazolamide, oxcarbamazepine or clonazepam, carbamazepine-resistant epilepsy, and contra negative effects in patients, such as kidney stones, muscle stiffening, fatigue, gastrointestinal disturbances, impaired concentration, and memory [104].

Other works on SCA1 and SCA2 conclude the relation of these ataxias to the KCNMA1 gene, which is expressed phenotypically as a BK channel, a Ca2+-activated potassium channel, which in other genetic alterations is identified as a cause of epilepsy without dyskinesia [93,105]. Research in point mutations directed to obtain gain-(GoF) or loss-(LoF) of-function of the channel in KO mice models concluded the LOF as the determinant modification of these SCAs 1 and 2 [106].

In SCA3, also known as Machado-Joseph disease (MJD), characterized by CAG repeat expansions in ataxin 3 (ATNX3) protein, a pathogenic role has been assigned to dysfunctions of K_{v} channels. In fact, hypereexcitability of Purkinje neurons is associated with the reduced transcripts of two voltage-gated K+ channels, Kcnal6 and Kcnc3, which cause a motor impairment phenotype. Recently Antisense Oligonucleotide (ASO) therapy has
been proved successful in rescuing transcript levels of \(Kcnq6\) and \(Kcnq3\) and restoring the normal excitability of SCA3 Purkinje neurons [107,108].

Moreover, evidence shows that in SCA13, the alteration of \(KCNC3\) gene produces a loss of gain in the potassium channel \(K_{v}3.3\) function maintaining the channel in a stochastically continuous open-state and reducing the closed-state. This event leads to the loss of excitability of the Purkinje cell [109,110]. The other type affected is the SCA19/22 Ataxia, in which the alteration of the \(KCND3\) gene provokes the loss of \(K_{v}4.3\) channel when it alters the function and even membrane location. In conclusion, the missing of the channel in neurotransmitter release zones produces long potentiation problems in cells [111–113].

**Voltage-gated cation channels (VGCCs) related to ataxias**

A series of works claim that the main subject of the study in this superfamily is the \(CACNA\) gene, which encodes a calcium channel, and which implies the modification of calcium concentrations in cytoplasm and neurotransmitter release zones. There are three types of ataxia associated with members of this channel’s superfamily.

First, in the polyQ SCA6 Ataxia, the altered \(CACNA1A\) gene (encodes the transcription factor \(\alpha1ACT\) of the \(\alpha\)-subunit of the calcium channel), produces 19–33 expansion repeats which compromise the development and survival of the Purkinje cell [114–117]. A second and more deep in evidence of the \(CACNA1A\) gene in SCA2 suggests that its expression modifies the channel \(Ca_{v}2.1\) and complementarily produces an error expression forming a poly-ubiquitin, which could be the origin of the errors of affected \(Ca_{v}2.1\) subunit [118]. Third direct evidence happens in the SCA42 ataxia, \(CACNA1G\) (encodes the gamma subunit of the channel) produces the loss of \(Ca_{v}3.1\) function which implies important alterations in Purkinje cell physiology as loss of bursting and excessive requirement of calcium influx to cell, driving it to death pathway [119].

**SBMA**

SBMA, also known as Kennedy disease, is a neuromuscular disease characterized by loss of lower motor neurons and skeletal muscle atrophy. In SBMA, a mutation has been identified within the q11-12 site of the X-chromosome. That alteration leads to an expanded trinucleotide repeat \((CAG > 37)\) in the first exon of the androgen receptor (AR) gene, encoding glutamine [120]. In normal conditions, the polyQ-AR tract ranges from 9 to 36 residues. However, a repeat number higher than 38 is considered pathogenic [120]. Under pathological conditions, the stimulation of the AR by the natural agonists testosterone or dihydrotestosterone (DHT) is required for the induction of polyQ-expanded AR-mediated toxicity [121]. After androgen binding, the activated AR translocates to the cell nucleus, where it interacts with specific sequences on the DNA, ultimately resulting in either the up or downregulation of the target genes involved in the control of several processes of the cell status. There are some pathogenic mechanisms involved in the development of disease including ligand-dependent nuclear translocation [122,123] protein misfolding and clearance [124] as well as endoplasmic reticulum stress [125]. Besides, there is a neuronal component that contributes to the motor dysfunction observed in mice, indicating the polyQ-expanded AR in motor neurons produces secondary pathology in muscle during the development of the full range of SBMA symptoms [126].

It has been demonstrated that variations in gene transcription have a major role in the development of SBMA [124,127]. Notably, a genome-wide transcriptome analysis found that motor neurons exhibit an increased expression of synapse-related gene sets involved in neuromuscular transmission, suggesting the neuromuscular synapse as a putative mechanism underlying SBMA alterations [128]. In accordance with the molecular observations, abnormal motor and sensory nerve conduction were found during the early clinical characterization of SBMA [129]. Consistent with motor neuron disease, nerve conduction analyses in genetically confirmed SBMA patients showed lower amplitudes of compound motor action potential or sensory nerve action potential as compared to control patients [130].

Earlier, Lieberman’s lab found a decreased mRNA expression in skeletal muscle of \(CLCN1\) as well as muscle voltage-gated sodium channel type IV, \(\alpha\)-subunit \((Scn4a)\), in a mouse model of SBMA. In a strong correlation with previous observations, they also identified, by electromyography, evidence of the aberrant electrical activity indicative of both myopathic and neurogenic processes [131]. Soon after, axonal excitability techniques applied to seven SBMA patients discovered an axonal hyperexcitability associated with an increased nodal persistent sodium current in motor axons of the median nerve [132]. Afterward, it was found that a shift of around 16 mV of membrane potential at rest (measured by intracellular recording) of muscle fibers of mice expresses SBMA phenotype. The
authors associated the equilibrium potential drift with a reduction in chloride channel (CLCN1) mRNA expression, confirming the synaptic dysfunction and unveiling the role of ion channels in SBMA pathophysiology [133]. Later, it was shown that SBMA is accompanied by defects in neuromuscular synaptic transmission suggesting that toxic AR in SBMA impairs both presynaptic and postsynaptic mechanisms. Notably, presynaptic defects displayed deficits in quantal content, reduced size of the readily releasable pool, and impaired short-term facilitation. Whereas prolonged decay times in spontaneous miniature end-plate potentials as well as evoked end-plate potential marked resistance to μ-conotoxin, a sodium channel blocker, and enhanced membrane excitability were observed as postsynaptic defects. Within the molecular correlate, quantitative PCR revealed an upregulation of mRNAs encoding neonatal isoforms of the voltage-gated sodium channel (NaV1.5) and the nicotinic acetylcholine receptors (γ-subunit) in SBMA mice muscles [134]. Finally, it was recently demonstrated in SBMA patients that an alteration of compound muscle action potential is related to an increase in increased nodal persistent sodium and reduced potassium currents in axon-innervating muscular tissues [135].

Beyond molecular approaches demonstrating the role of ion channels in SBMA, the first electrophysiological analysis of ion channels activity was performed in NSC-34 neuroblastoma-motor neuron hybrid cell line [136]. Ruling out the possible contribution of Na+, K+, or Cl− channels, this study only focused on the pharmacological characterization of voltage-dependent calcium channels showing that the exposure to androgen (mibolerone) produced a negative shift in steady-state activation of T-type Ca2+ channels in cells transfected with polyQ-expanded AR (44 glutamines). No differences were observed for L-type, N-type, or P-type Ca2+ channels [137].

Based on the previous findings, we took advantage of an improved SBMA cell model to tackle the ion channel activity. This SBMA cell model employs a motor neuron cell line (MN-1), which are hybridoma cells derived from embryonic mouse spinal cord motor neurons and neuroblastoma cells. MN-1 cells were stably transduced with lentiviral vectors expressing a human AR transgene with either a non-pathological 24Q tract (AR24Q) or an expanded 100Q tract (AR100Q) [138,139].

We developed a systematic characterization of ionic alterations in the SBMA cell model in which it was clear that the expression of polyQ-expanded AR causes an androgen-dependent reduction in the macroscopic membrane ionic currents (Figure 1) [16,19].

These findings strongly correlate with the ion channels’ mRNA expression and nerve conduction

Figure 1: Membrane currents isolated in physiological (AR24Q) and pathological (AR100Q) conditions. The pathophysiological mechanisms associated with SBMA disrupt the ion channel activity in SBMA MN-1 cells exposed to DHT. The treatment with clenbuterol (a) or PACAP (b) reverts the dysregulation of Kv2-type or CIC-2-like ionic currents, respectively. The voltage stimulation protocol is depicted for potassium as well as chloride currents (modified and adapted from refs. [19,20]).
abnormalities [130,131,133,134]. Considering the observations of \( CLCN1 \) mRNA regulation in SBMA, its contribution to the ionic cellular activity was explored. We found chloride currents most likely belonging to the CIC-2 subfamily but not CIC-1. CIC-2-type currents showed significantly increased amplitudes in the AR100Q cells exposed to DHT [19]. Further, it was seen that \( \text{K}_2\text{v} \)-type delayed outward potassium currents are subjected to androgen modulation in AR100Q SBMA cells. The biophysical assessment showed decreased tail currents and conductances of delayed-rectifier \( K^+ \) currents sensitive to guanidino-glutamate-1E, a \( \text{K}_2\text{v}1.1 \) and \( \text{K}_2\text{v}2.2 \) channel blocker [20].

Even so, the major line of ion channels has been analyzed in SBMA. The specific subtype of ion channels to SBMA pathophysiology remains to be characterized, particularly in tissue obtained from animals or muscle/motor neuron-derived human induced pluripotent stem cells (hiPSCs) from SBMA patients.

Pharmacology of ion channels in SBMA

Since ligand-induced nuclear translocation of mutant AR protein has been revealed to be a critical stage in motor neuron degeneration in SBMA. Some therapeutic strategies available for SBMA are based on the native functions of AR through androgen deprivation by leuprolerin and dutasteride treatment [140]. Other than hormonal treatments, therapeutic strategies have been developed to target the AR toxicity into motor neuron function. In this sense, insulin growth factor-1 (IGF-1) and the pituitary adenyl cyclase activating polypeptide (PACAP), previously shown to protect MN-1 cells from the toxicity of mutant AR [141,142], were demonstrated to exhibit an effective role in the recovery of macroscopic ionic membrane currents of the pathological MN-1 cells expressing the mutant AR [16]. In further work, we reported that PACAP exerts part of its effectiveness by the modulation of CIC-2 channels [19]. The incubation with PACAP (100 nM) restored the increased CIC-2-type currents to normal conditions, recorded from AR100Q cells (Figure 1a).

It was observed that the treatment of patient-derived myotubes with the \( \beta \)-agonist clenbuterol restored the trophic effect of DHT, which was predictive of improved muscle pathology and motor function in a mouse model of SBMA [143]. Following this observation, our group teased out the impact of clenbuterol in the neuron membrane ionic currents. Our combined electrophysiological and pharmacological approach allowed us to reveal that clenbuterol modifies delayed outward potassium currents. We found that treatment with DHT (10 nM) results in a notorious reduction in outward currents of the cells expressing AR100Q as compared to AR24Q control cells (Figure 1a). Treatment of AR100Q cells with 10 \( \mu \)M clenbuterol prevented the DHT-induced outward current amplitude diminution and restored the normal function through the modulation of \( \text{K}_2\text{v}2 \)-type potassium channels [20].

One interesting therapeutic approach was demonstrated by the administration of curcumin compounds to the mouse model of SBMA that resulted in significant improvement of motor function and increased lifespan [144,145]. Recent studies have revealed that many ion channels and a large number of molecular structures like proteins are modulated by curcumin [146]. Therefore, this natural compound, and/or other nutraceuticals, could exhibit its therapeutic effect in SBMA through the regulation of ion channel activity. Nonetheless, the molecular mechanism remains to be explored.

A graphical summary showing the ameliorative molecular mechanisms involved in the regulation of \( \text{K}_2\text{v}2 \)-type and CIC-2-like channels in SBMA MN-1 cells is reported in Figure 2.

**DRPLA**

DRPLA is a rare NDD caused by the expansion of unstable CAG trinucleotide repeat expansion in the \( ATN1 \) gene which makes the disease-causing protein named atrophin-1. The long CAG repeat changes the structure of the atrophin-1 protein and leads to accumulation of the protein in neurons, which interfere with normal cell functions and cause cell death [147]. DRPLA together with SCA13 and SCA17 belongs to the superfamily of Autosomal Dominant Cerebellar Ataxia (ADCA) Type I characterized by common clinical features like ataxia and dystarthis which are pathological signs of cerebellar dysfunctions. DRPLA affects mainly the dentate nucleus, globus pallidus, the cerebellar cortex, and the cortex regions. The progressive cerebellar ataxia comes with myoclonus, epilepsy, dementia, choreoathetosis, and psychiatric symptoms. However, the DRPLA phenotype characterized by nonprogressive myoclonus epilepsy (54–67 repeats) and onset at a later age (older than 21 years) is clinically similar to HD and SCAs, making difficult the differential diagnosis because the chorea component of the DRPLA phenotype can mask the presence of the ataxia [147].

As far as to our knowledge, apart from several papers dealing with excitability features of epileptic seizures – without any identification of the pathogenesis of
myoclonus – unfortunately, the current literature does not include any account on the role of ion channels in the DRPLA pathogenic neuronal activity. Nevertheless, just to mention, in the superfamily of ADCA Type IV ataxias (non polyQ), channelopathies have been identified causing hereditary episodic ataxias. In particular, episodic ataxia type 1 (EA1), due to LoF variants in KCNA1 gene, codes Kv1.1 potassium channels; and episodic ataxia type 2, due to mutations in the CACNA1A gene, encodes α1 subunit of the P/Q-type voltage-gated Ca\(^{2+}\) channel (CaV2.1)\[148\].

**Conclusion**

Considering the exception of the CACNIA-induced channelopathy in SCA6, identified and characterized so far only in polyQ NDDs, striking evidence speaks in favor of a wide decisive role of ion channel dysregulation, triggering the alteration of neuronal excitability, in the onset of pathological phenotype in polyQ NDDs (Table 1 summarizes the main findings reported above). In this regard, it could be hypothesized that the altered neuronal activity, and its structural and functional correlates, might be considered as a putative pathophysiological marker of the diseases. In support of this hypothesis pharmacological amelioration of the voltage-gated channel altered activity in *in vivo* and *in vitro* models of the diseases demonstrate that at least an attenuation of the disease outcome can be afforded to rescue the pathological neuronal activity to more functional levels.

Accordingly, the results reported above for SBMA models point hopefully in this direction, where the electrophysiological evidences on the amelioration role of PACAP and clenbuterol confirm these drugs, respectively, as a potential therapeutic drug for long-lasting treatment \[142\], and as candidate for pilot clinical trials on patients providing the first evidences for a positive effect on SBMA disease progression \[149\]. In addition, IGF-1 is under phase I/II clinical trials jointly with several international institutions.

As a consequence, the drug screening addressed to ion channel targets, nowadays is a well-established procedure which is opening to the discovery of combined drugs to design new clinical trials – e.g., the combination of chlorzoxazone-baclofen which improves motor impairment in SCA1 modifying the irregular spiking activity of Purkinje neurons in *Atxn1*\[^{154Q/20}\] mice \[150\] – that can be extended to other polyQ NDDs also. On the other hand, new therapeutic approaches are coming to the front of the stage involving the role of ion channel-mediated neuronal activity – e.g., the repair of iPSCs in SCA3 using CRISPR/Cas9 gene correction \[151\], and the ASO therapy...
which improves the physiology of Purkinje cells in a mouse model of SCA3 rescuing $K_v$ channel expression and neuronal excitability [108].

In summary, the biophysical analysis of the alteration of neuronal activity involved in polyQ diseases, and NDDs in general, is a challenging field directed to the
finding of new therapeutic options which require an increasing need of new models and multidisciplinary approaches to study and tackle these intractable diseases.

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