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Review

Physiologic and pathophysiologic consequences of altered sialylation and glycosylation on ion channel function

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ABSTRACT

Voltage-gated ion channels are transmembrane proteins that regulate electrical excitability in cells and are essential components of the electrically active tissues of nerves, muscle and the heart. Potassium channels are one of the largest subfamilies of voltage sensitive channels and are among the most-studied of the voltage-gated ion channels. Voltage-gated channels can be glycosylated and changes in the glycosylation pattern can affect ion channel function, leading to neurological and neuromuscular disorders and congenital disorders of glycosylation (CDG). Alterations in glycosylation can also be acquired and appear to play a role in development and aging. Recent studies have focused on the impact of glycosylation and sialylation on ion channels, particularly for voltage-gated potassium and sodium channels. The terminal step of sialylation often affects channel activation and inactivation kinetics. The presence of sialic acids on *O* or *N*-glycans can alter the gating mechanism and cause conformational changes in the voltage-sensing domains due to sialic acid's negative charges. This manuscript will provide an overview of sialic acids, potassium and sodium channel function, and the impact of sialylation on channel activation and deactivation.

Contents

1. Introduction

Ion channels are integral membrane proteins residing in the lipid bilayer of the plasma membrane. The hydrophobic characteristics of the membrane do not permit ion transmission through the channel. For this reason, ion channels are vital for all organisms since many physiological processes such as cell signaling, fertilization, muscle contraction, immune responses, and electrical signaling through synapses are controlled by channel gating mechanisms [1]. Ion channels can be voltage-gated, ligand-gated, or activated by other mechanisms. Voltage-gated ion

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channels have an essential role in the transmission of neuronal impulses, regulation of synaptic currents, and nerve and muscle action potentials [2,3]. Neuronal excitability in the central nervous system is mainly controlled by voltage-gated potassium channels [4]. Furthermore, neurological and neuromuscular defects in potassium channels can lead to a number of pathological conditions called channelopathies [5]. Interestingly, many of these channelopathies, such as episodic ataxia and myokymia, are common diseases also faced by the patients suffering from one of several classes of congenital disorders of glycosylation (CDG) [6–9].

CDG are a large and growing family of genetic disorders with 45 known diseases caused by glycosylation deficiencies.[8] Defects in the *N*-glycosylation or *O*-glycosylation pathways or the generation of glycan precursors cause CDG. Glycosylation is one of the most important post-translational modifications that takes place in the endoplasmic reticulum (ER) and Golgi apparatus, and plays a vital role in protein folding and the functioning of transmembrane proteins [10]. Voltage-gated potassium and other channels are targets of glycosylation and some of their glycan moieties can be capped with sialic acid [6]. Indeed, sialic acid is the only negatively charged sugar group found in mammals which is extensively discussed in the following section. Recent studies have shown that sialylation can have an important impact on the voltage-gated potassium channels [11]. While there are a number of reviews on ion channel function [12], this review focuses specifically on the effect of sialylation on neurological, neuromuscular and cardiac channelopathies involving potassium and sodium channels. Furthermore glycosylation and sialylation effects on CDGs and their association with ion channels as well as sialylation effect on development and aging are extensively discussed.

2. Sialylation

Sialic acids are nine carbon monosaccharides that are added at the terminal ends of sugar conjugated structures such as *N*-glycans, *O*-glycans, and glycolipids. The carbon-1 has a carboxylate group, which is ionized at physiological pH to give a negative charge. The side group of the fifth carbon determines the type of the sialic acid. If the 5-carbon has an N-acetyl group, it is known as N-acetylneuraminic acid (Neu5Ac). If the N-acetyl group is hydroxylated, Neu5Ac becomes N-glycolylneuraminic acid (Neu5Gc), as shown below. If the N-acetyl group is de-acetylated to an amino group, it is known as neuraminic acid (Neu) [13]. 2-keto-3-deoxynonic acid (KDN), another type of sialic acid, has a hydroxyl group at the 5-carbon. Neu5Ac, Neu5Gc, Neu, and KDN are core sialic acids. The carbon at the second position can form glycosidic linkages with other sugar chains. Various substitutions can be made to the hydroxyl groups at the 4-, 7-, 8-, and 9-carbon positions to form other sialic acids and over forty sialic acids are now known [14].

In mammals, the primary sialic acid generated is Neu5Ac, which is produced in a multi-step pathway. The metabolic pathway begins with ManNAc-6-P being synthesized from UDP-GlcNAc by the bifunctional enzyme UDP-GlcNAc 2-epimerase/ManNAc-kinase [15]. The enzyme Neu5Ac-9-P synthetase combines ManNAc-6-P with phosphoenolpyruvate (PEP) to form Neu5Ac-9-P. Then, Neu5Ac-9-P phosphatase dephosphorylates Neu5Ac-9-P to Neu5Ac which is transported into the nucleus. Neu5Ac is converted to CMP-Neu5Ac by CMP-Neu5Ac synthetase and is transported to the Golgi by a CMP-Neu5Ac transporter (CST) [16]. In the Golgi apparatus, Neu5Ac is transferred to glycoconjugates via a sialyltransferase using CMP-Neu5Ac [17]. However, other forms of sialic acid may also be transferred to glycoconjugates. For example, Neu5Gc can be made from Neu5Ac by CMP-Neu5Ac hydroxylase [14]. Neu5Gc is not found in humans because the human homologue for CMP-Neu5Ac hydroxylase lacks the active N-terminal domain present in other mammals [18]. KDN can be synthesized from two pathways. One pathway requires the deacetylation and deamination of Neu5Ac. The other pathway is similar to that of Neu5Ac with mannose as the starting substrate and KDN-9-phosphatase condensing KDN-6-P from PEP [19].

2.1. Polysialylation

Polysialic acids (PSA) are linear homopolymers of sialic acids joined by α-2,8 glycosidic linkages [20] and are found in human tissue, fish eggs, *Drosophila* embryos, and certain bacteria [21]. In some mammals, especially humans, the predominant form of PSA is a homopolymer of Neu5Ac. The main carrier of polysialic acid in humans is the neuronal cell adhesion molecule (NCAM) [22]. NCAMs are cell surface proteins containing five Immunoglobin (Ig)-like domains and two fibronectin type III repeats [23]. Polysialyltransferase-1 (ST8Sia IV) and sialyltransferase X (STX or ST8Sia II) are responsible for adding the sialic acid to glycoproteins such as NCAM [21]. PSA play an important role during development and neuronal regeneration. High levels of PSA are found on NCAMs in vertebrate embryonic tissues such as the brain, heart, kidney, and muscle [21]. PSA decrease the binding efficiency of NCAM to itself (hemophilic binding) and to cell surface receptors (heterophilic binding) while polysialylated NCAMs promote cell migration [24], neurite outgrowth, axonal growth, and neuronal sprouting. As vertebrates approach adult stages, PSA levels decrease until PSA remains only in brain tissue [23]. Thus, high levels of PSA are associated with neuronal plasticity [13]. Besides NCAM, voltage sensitive sodium channels are one of the rare proteins known to be polysialylated [25]. It has been observed that the presence of polysialylation provides stabilization of the voltage dependent sodium channel gating [26] which demonstrates the importance of both polysialylation and sialylation on the neuronal system and ion channels.

3. Potassium channels

Potassium channels are membrane proteins composed of four pore-forming α-subunits and auxiliary β-subunits, found in both neuronal and non-neuronal cells. These channels regulate neuronal excitability, membrane potential, repolarization of action potentials, and membrane resting state by selectively permitting the passage of K^+ ions through a membrane [27]. Potassium channels can be classified into four groups: (1) voltage-gated K^+ channels (K_v) (2), Ca^{2+}-activated K^+ channels (K_{Ca}) (3), two pore K^+ channels (K_{2P}) (4), and inward-rectifying K^+ channels (K_{IR}). The membrane topology of these channels differs from each other. Both K_v and K_{Ca} channels are formed with six or seven transmembrane domains (Fig. 1), whereas K_{IR} and K_{2P} channels are formed with two and four transmembrane domains, respectively [27,28] (See Fig. 2).

Shown in Fig. 3 is the topology of a K_v channel subunit. Each of the four subunits contains six membrane domains and these subunits encircle a pore, which permits the flow of potassium ions. The loop between the last two domains, S5 and S6, is the selectivity filter for the K^+ ions and the first four domains, S1, S2, S3 and S4 are the voltage sensors. A key component of the voltage sensing domains is the S4 domain, due to its positive charge [28].

The activity of the voltage-gated potassium channel is essential for many biological events, including muscle contraction, neuronal signaling, and neurotransmitter and hormone release [4]. In excitable tissues, action potentials are generated by an influx of ions, typically Na^+ and Ca^{2+}, controlled by specific voltage-gated channels and directed intracellularly along existing ionic gradients maintained at considerable metabolic expense. The gating mechanism of the voltage channels starting an action potential

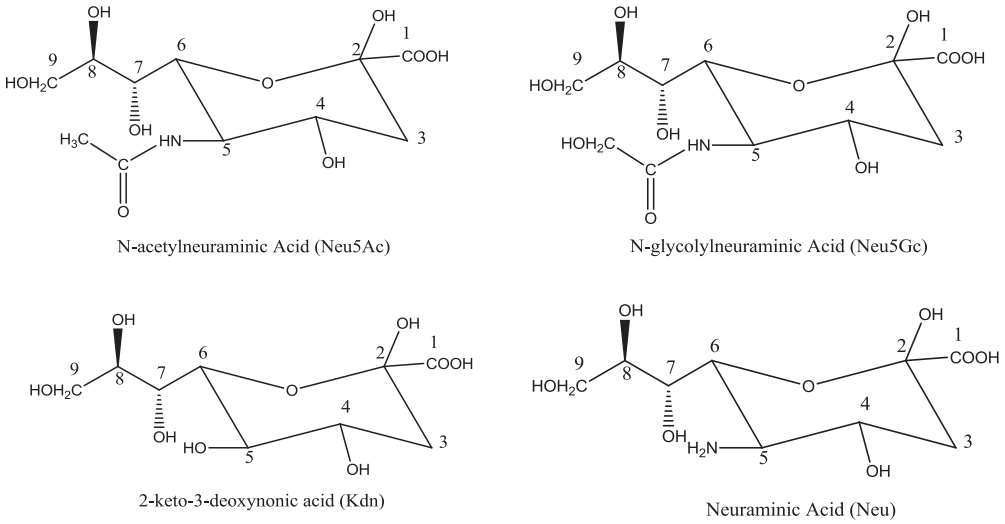

Fig. 1. Types of sialic acids.

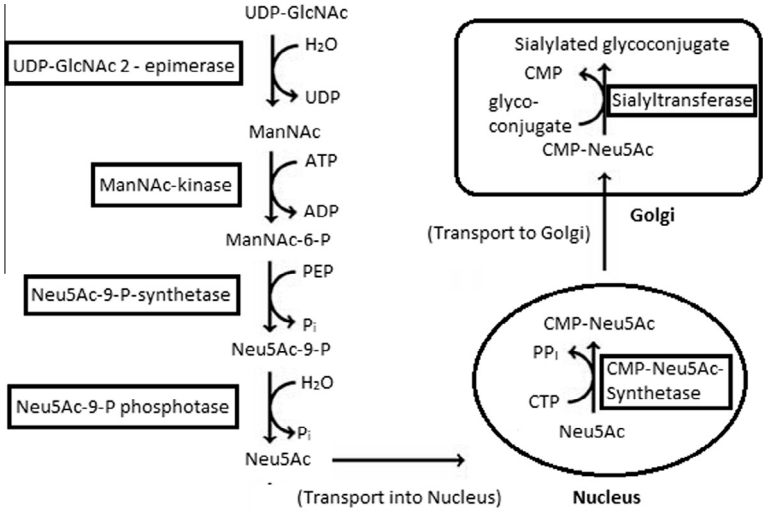

Fig. 2. Sialylation pathway in mammals.

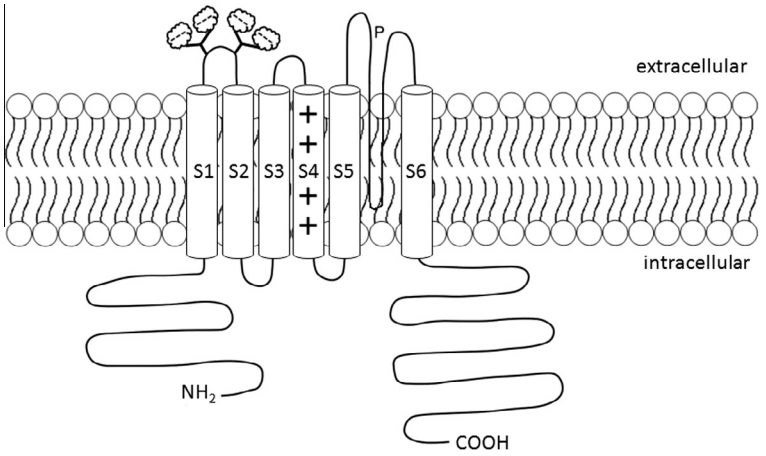

Fig. 3. Topology of a kV channel subunit. The sialylation sites specific to 2 N-glycosylation sites between S1 and S2 linker are shown. Other glycosylation sites can also appear on the S3–S4 linker (not shown here).

initiates inactivation of the channel preventing continued membrane depolarization. However, the membrane potential is returned to its resting level by the delayed opening of the voltage-gated potassium channels, which permit the efflux of intracellular potassium down its concentration gradient. The interplay of the voltage-gated channels that produce an action potential and return the membrane to its rest potential is depicted in Fig. 4, which was generated from the now classic model of Hodgkin and Huxley [29]. Multiple types of potassium channels, each with a unique kinetic profile, are frequently involved in the process of repolarization [30].

3.1. Glycosylation and sialylation effect on ion channels and nervous system channelopathies

The variety of potassium channels and differential expression levels in excitable cells result in a diverse collection of physiological channel functions in the central nervous system. Voltage sensitive potassium channel dysfunctions are called potassium channelopathies. Examples are episodic ataxia and hereditary deafness syndromes which are caused by acute and long-standing dysfunction of potassium channels, respectively [5,31]. In recent years, molecular biology techniques have enabled a better understanding of the effect of ion channel mutations on the neuronal system of organisms, both at the single channel and behavioral/phenotypic level.

Patients with episodic ataxia 1 suffer from intermittent incoordination often caused by fatigue and stress. Myokymia, which is the rippling of skeletal muscles due to hyperexcitability of motor neurons, also happens between and during episodic ataxia 1 attacks [32]. Localization, functional, and clinical studies have shown that certain mutations in the $K_v1.1$ (KCNA1) channel are associated with episodic ataxia 1 [31,33,34]. These mutations slow the repolarization rate, leading to membrane excitability. Furthermore, a decrease in the channel current increases the neuronal excitability both in the cerebellum and the peripheral nervous system, which causes ataxia and myokemia, respectively [31].

In addition, benign familial neonatal convulsion (BFNC) is epilepsy caused by channel mutations which usually begins in the first week of life. Mutations in the $K_v7.2$ and $K_v7.3$ (KCNQ2 and KCNQ3) channels can result in reduced currents which slightly depolarize the neurons and rhythmically cause multiple action potential firing. This delayed membrane hyperpolarization causes BFNC in the neonatal period [5,34].

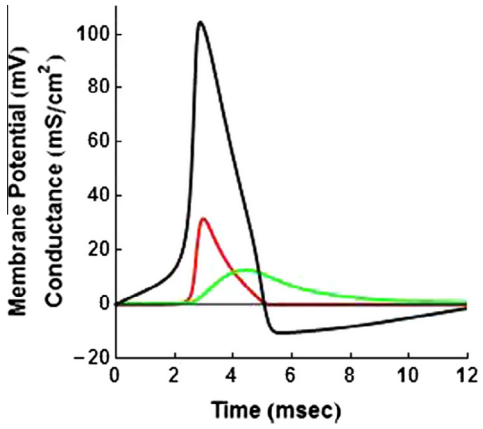

Fig. 4. An action potential generated by the model of Hodgkin and Huxley in response to application of a steady depolarizing current. In response to this depolarizing current, the voltage-gated Na+ channel permits conductance of Na+ through the channel along its concentration gradient. Once the voltage-gated Na+ channel begins to inactivate, the voltage-gated K+ channel increases its conductance, permitting efflux of K+ to return the membrane potential to its rest level (0 volts). Membrane potential is given in black, Na conductance is in red and K conductance is in green. There is a horizontal line at the resting membrane potential of 0 volts. Ionic currents are the product of the membrane potential (shown) minus the reversal potential (positive for Na and negative for K, given by the corresponding Nernst potentials) times the conductance (shown).

Mutations that affect channel action can be due to changes in amino acid sequences or post-translational modifications. The secretory pathway, where post-translational modifications take place, plays a very important role in the folding and density of the ion channel proteins that are transported from the endoplasmic reticulum to the Golgi apparatus and cell membrane. Post translational changes, including glycosylation and especially sialylation, occur on many potassium channels and can affect the function expression levels and activity of membrane proteins including ion channels.

Knowledge about the effect of glycosylation on ion channel function can help to elucidate if neurological diseases are caused by changes in ion channel glycosylation. However, until now, there has been a limited number of studies on the role of glycosylation and sialylation on ion channel functioning. This review will examine some of those studies relating glycosylation to potassium channel function as well as sodium channels.

Voltage-dependent potassium channels are heavily glycosylated in the endoplasmic reticulum and Golgi apparatus. For example, when the shaker-related K_v1 family sequences from $K_v1.1$ to $K_v1.5$ are aligned, the presence of potential *N*-glycosylation sites in each of these proteins are located in the loop between the S1 and S2 domains. Each of the glycans of these glycosylation sites could then potentially terminate with sialic acid residues, which creates negative charges on the cell surface [7].

$K_v1.1$ is one of the major voltage sensitive channels, expressed in various parts of the brain. Mutations in the $K_v1.1$ channel have been associated with episodic ataxia, as mentioned previously. Since $K_v1.1$ has glycosylation sites, $K_v1.1$ gene was expressed in both CHOPro5 (a proline auxotroph of CHO cell line) and Lec1 (a glycosylation deficient CHO) cell lines. The glycan structure of the Lec1 glycosylated cell proteins was limited to GlcNAc2-Man5, while the surface glycolipids were the same as CHOPro5 cells. In order to understand the effect of glycosylation, the glycosylation site of $K_v1.1$ gene, between the S1 and S2 loop was eliminated to generate $K_v1.1$N207Q [7]. The electrophysiological experiments showed that $K_v1.1$ with a reduced number of glycans had slower activation kinetics, and more depolarization was required [7], which can be a model of CDG Type II disease [6]. Furthermore, the G-V slope of $K_v1.1$ channel was steeper compared to the mutated channel, which indicates a reduction in the apparent charge during the mutant channel activation [7]. Moreover, the effect of glycosylation on C-type inactivation was investigated and the mutated channel in both CHOPro5 cells and Kv1.1 channel in Lec1 cells exhibited slower inactivation kinetics showing the importance of galactose and sialic acid deficiency on the voltage gated potassium channel [6,7,35].

Another study examines the effects of changes in glycosylation of the shaker channel, which is the Drosophila version of Kv1.1. Johnson et al. (2008) expressed both Shaker wild type, ShB and and shaker mutant, ShNQ, with two glycosylation sites between S1 and S2 knocked out, in the CHOPro5 and Lec2 (reduced sialylation) cell lines. The normalized conductance versus potential curve for ShB Lec2 cells had a positive voltage shift compared to the CHOPro5 cells, which indicates that increased sialylation (Pro5 cells) increases hyperpolarization in the steady state. The normalized current versus repulse potential curve, which gives information on the voltage dependence of channel inactivation, was positively shifted 5 mV in Lec 2 cells to prove that sialylation causes a hyperpolarization shift as seen in the $K_v1.1$ channel [6,7,35]. Furthermore, the presence of sialic acid increased the rate of recovery

Table 1
List of potassium channels, and glycosylation associated with them. Mutations and the disorders they cause, along with references are also listed.

Channel type	Glycosylation effect	Mutation	Symptoms	References
Kv1	Removing glycans shifts gating towards depolarization. Glycosylation of ShB is important for channel processing and stability	Both potential N-glycosylation sites were removed for N-glycosylation deficient mutant ShNQ	CDGs	[6]
Kv1.1	Gating modulated both by surface potential and by cooperative subunit mechanism	Mice: 11 bp deletion Humans: Point mutations	Mice: seizures, brain overgrowth, and epilepsy Humans: autosomal dominant disorder episodic ataxia type1	[43]
Kv1.1	Kv1.1 and the Kv1.1N207Q glycosylation deficient mutant exhibit similar single channel conductance. Loss of glycosylation affected gating mechanism	Kv1.1N207Q glycosylation mutant-glycosylation deficient	None known	[44]
Kv1.1	Galactose and sialic acid addition not required for expression on membrane. Study concluded that N-glycosylation did not modify function	Glycosylation deficiency in Lec mutants produces truncated surface glycoproteins. Lec8 due to reduced galactose levels and Lec2 because of reduced sialic acid levels	None known	[35]
Kv1.1	Deficient mutant exhibits slow activation kinetics, a positively shifted VI, slowed C-type inactivation kinetics, and a reduced extent and recovery from C-type inactivation. N-glycosylation, and terminal sialylation affected Kv1.1 gating properties	Non-glycosylated, removal of the N207 carbohydrate tree	Differences in glycosylation patterns among related channels may contribute to their functional divergence and affect physiological roles	[7]
Kv1.1, Kv1.2, Kv1.4, Kv1.5, Kv1.6, Kv2.1	Impacts channel function. Correct glycosylation of channel proteins necessary for efficient surface expression in neurons	Glycosylation deficiency	None known	[41,43]
Kv1.1, Kv1.4, Kv1.1	Kv1.4: full truncation of the C terminus inhibited trans-Golgi glycosylation. Kv1.1: trans-Golgi glycosylation conversion with truncation mutation	C-terminus truncation mutants and chimeras with their cytoplasmic C termini exchanged. Point mutations in the pore region of Kv1.4	None known	[45]
Kv1.1,1.2,1.4	Point mutations in the outer pore region of Kv1.2 changed cell surface expression of Kv1.2. S371T mutation in the deep pore region of (Kv1.4) markedly enhanced cell surface expression	Substituting amino acids in the pore region of Kv1.2 with the same position amino acid present in Kv1.1 or Kv1.4. Two sites for N-linked glycosylation were modified	None known	[46,47]
Kv1.2	Gating modulated both by surface potential and by cooperative subunit mechanism	None detected	None known	[43]
Kv1.2	N-glycan at non-native positions changed channel properties. For example, on the S1–S2 linker decreased surface protein expression but with no loss in activity. Placing a non-native N-glycan on the S3–S4 linker significantly altered function	N-glycosylation sites engineered at multiple positions on the extracellular linkers of Kv1.2 to determine effects of N-glycans on channel expression and function	None known	[48]
Kv1.2	Compared with Kv1.2, positive changes in the glycosylation state shifted the V1/2 negatively with a steeper G–V slope, while negative changes produced opposite effect	Hyperpolarized or depolarized shifts in V1/2 due to an increased or decreased level, respectively of channel sialylation	Neurological disorder.	[11]
Kv1.4, Kv1.5,	Channel activation of Kv1.5 modulated by electrostatic interactions. N-glycans produced no change on Kv1.4 gating	Mutagenesis utilized to remove N-glycosylation sites. Cell line with differing sialylation patterns tested to detect channels with no sialylation	None known	[2]
Kv2.1, Kv4.2, and Kv4.3	Kv2.1, Kv4.2 and Kv4.3 activation modulated by sialic acids	Reduction in sialylation causes depolarization shift in the G-V, specific to each channel	None known	[42]
Kv3 (Kv3.1, Kv3.2, Kv3.3, Kv3.4)	Critical for regulating the expression of K+ currents at neuronal surfaces. N-glycosylation of K+ channels influences folding, trafficking, and function	Glycosylation deficiency	Glycosylation deficiency results in neurodegenerative diseases. Deficiency of Kv3 channels could contribute to abnormal brain development and aberrant function of the nervous system in CDG group I	[39]
Kv3.1	Un-glycosylated channels showed slower activation kinetics	Kv3.1 mutants: N220Q/N229Q – un-glycosylated form of Kv3.1 made by mutating Asn residues at positions 220 and 229 to Gln residues (amino acid change)	Decrease in N-glycosylation levels may cause ER stress related neurological disorders	[38]
Kv3.1	The Kv3.1 channel showed a gradient of the speed of activation. The channel was faster when fully glycosylated and slower the less glycosylation attached	Glycosylation removal	None known	[49]
Kv4	Essential for normal electrical functioning of the heart	None detected	Incomplete glycosylation of these channels could lead to arrhythmias.	[37]

(*continued on next page*)

Table 1 (*continued*)

Channel type	Glycosylation effect	Mutation	Symptoms	References
Kv4.2	Glycosylation does not add any significant surface area to the dimer interface	None detected	Parkinsonian symptoms can be induced by firing frequency modulation in dopaminergic neurons and alterations in firing frequencies.	[50]
Kvβ2	Kvβ2 binds to Kv1 and Kv4 α subunits	Kvβ2 null and point mutants were created	None known	[51]

from fast inactivation but this result was not seen with sodium channel opening [6].

These experiments suggest that sialylation, which produces a negative cloud on the surface of the glycan, has a greater effect on K_v1 and shaker gating than other glycan groups. In order to understand the effect of surface potential on the Shaker and $K_v1.1$ gating, mammalian CHO cells were perfused with different divalent cations, such as Ca^{2+} and Sr^{2+}.[7,36] The sensitivity of the channels to the ions was much higher for the wild type as compared to the sialic acid deficient channels and channel behavior was also observed to change with the NaCl concentration. This experiment provided more evidence that the external surface charge has a significant effect on the gating of the sialylated channels, in which the voltage domains sense different potentials in the absence and presence of sialic acid. The charge also affects the conformation of the channel such that a change in the conformation is directly transmitted to the electromechanical coupling mechanism to alter the opening and closing characteristics for the channel.

In another study, the role of sialic acid was also investigated for the Shal related-$K_v4.3$ channels. The inactivation rate of the $K_v4.3$ channel was not affected by sialidase treatment whereas the Kv1.1 channel inactivation was found to be sialic acid dependent [6,37]. This difference shows that the effect of sialic acid on the channel can be isoform-specific.

Experiments with glycosylation deficient mutants also indicated that N-linked sugars affect channel gating [6,7,38]. While the glycan-dependent mechanism of channel gating is not fully understood, sugar groups may affect the conformational stability of the channel during channel expression, activation, and deactivation. Another neuronal channel that has conserved N-glycosylation sites between S1 and S2 loop is Shaw-related K_v3 channel. Immunoblot experiments on a rat brain showed that Shaw-related K_v3 channel is heavily glycosylated and sialylated [38,39]. The influence of the sugar groups on the gating of the K_v3 channels was shown in Sf9 cells [38]. However, the sialylation effect on the K_v3 gating is still unknown due to the absence of sialylation in Sf9 cells [40]. Further investigation on the physiological effect of sialylation on K_v3 channel should be done in mammalian cells.

Computer predictions indicate that $K_v2.1$ has a consensus N-glycosylation site between the S3 and S4 loop. As a result, the N-glycosylation of this channel has been studied in rat brains and transfected mammalian cells. Tunicamycin and glycosidase treatments indicated that $K_v2.1$ is not N-glycosylated [41]. Although, $K_v2.1$, $K_v4.3$, and $K_v4.2$ are not N-glycosylated, they have shown depolarization shifts in the absence of sialylation [37,42]. This change suggests that O-linked sialylation is present and affects G-V shifts. Recently, it is shown that O-linked sialylation can have an impact on channel depolarization. However, it does not affect the steady-state inactivation and/or rate of recovery from fast inactivation [42].

Overall, sialylation of potassium channels plays an important role in channel gating. However, the effect of sialylation on the channels seems to be isoform-specific. For instance, sialylation affects the gating of $K_v1.5$ whereas sialylation does not have any impact on the gating of $K_v1.4$ channels [2]. There are many other examples of K_v channels which have different glycosylation patterns, and these are summarized in Table 1 along with the impact on disease states.

3.2. Glycosylation and sialylation effects on CDGs and their association with ion channels

Glycosylation is a critical post-translational modification since it plays a role in the proper folding, stability, and secretion of the proteins. In addition, glycosylation impacts cell adhesion, cell migration, and many other cell and protein functions [52]. However, the range of effects of glycosylation on protein function is not yet fully understood. There has been a fourfold increase in the number of identified congenital disorders of glycosylation (CDGs) since 2003. These syndromes can cause a variety of neurological disorders in the humans, such as mental retardation and epilepsy [8]. A wide variety of disorders are included under the category of CDGs, but broadly they can be known as either Type I (pre-Golgi localized defects) or Type 2 (Golgi localized defects). Defects that change the stepwise assembly of lipid-linked oligosaccharides or their transfer to proteins within the endoplasmic reticulum are considered CDG-1. Once the glycans are linked to the protein, individual sugars are removed or added to the protein-bound glycans. Defects in this pathway are considered CDG-II. The effects of glycosylation and sialylation on various types of CDGs are listed in Table 2.

Potassium channels are often involved in the central nervous system function, so any glycosylation or sialylation deficiencies on the voltage sensitive protein may lead to neurological or neuromuscular disorders. Interestingly, many of the nervous or neuromuscular system diseases are associated with CDG type diseases [8,53]. For instance, conserved oligomeric Golgi complex (COG) is a CDG disease associated with the decrease of CMP-sialic acid transport and UDP-galactose transport.[9,54] In this disease, both galactosylation and sialylation of the O-glycosylated and N-glycosylated proteins are decreased. Common features of this disease include cerebrocostomandibular-like syndrome, cerebral atrophy, and dysmorphy [9,54]. Cerebral atrophy is also one of the neurological disorders observed in the patients suffering from mutations in their K_v3 channels [55]. Indeed, the large number of glycosylation disorders affecting neurological functions suggests that glycosylation and particularly sialylation of ion channels is critically important to these proteins' function within the nervous system.

3.3. Effect of sialylation on other voltage gated-channels

Although much research has focused on potassium channel sialylation, recent studies have shown that sialylation will alter other voltage gated ion channels as well, in particular sodium channels. Sodium channels consist of an alpha subunit forming the core of the channels to permit sodium ion transport across the membrane. These channels are extremely selective for sodium ions due to the pore containing negatively charged amino acid residues which attract positive ions and restrict negative ions and the β subunit modulates their activity. Sodium channels are typically classified into two types, either voltage gated or ligand gated channels. Voltage gated sodium channels are particularly prevalent in

Table 2
Effects of glycosylation and sialylation on various types of CDGs.

Disease name	Deficiency	Biochemical abnormalities	Symptoms	References
CDG-Ia (PMM2-CDG)	Conversion of mannose 6-phosphate to mannose 1-phosphate	Hypoglycosylation of proteins. Lack of mannose-1-phosphate	Developmental delay, atypical subcutaneous fat pads, facial dysmorphism, epilepsy, cerebellar ataxia/ hypoplasia/atrophy, muscular hypotonia/ atrophy, inverted nipples	[56,57]
CDG-Ic (ALG6-CDG)	ALG6- encodes $Man_9GlcNAc_2$-*PP*-Dol alpha-1,3-glucosyltransferase enzymes	Low serum plasma thromboplastin antecedent and cholesterol. Hypoglycosylation of proteins, accumulation of $Man_9GlcNAc_2$-*PP*-Dol in the ER	Proteinuria, dysmorphism, ataxia, psychomotor retardation, epilepsy, hypotonia, strabismus, cerebellar, hypoplasia	[58,59]
CDG-Id (ALG3-CDG)	Mannosyltransferase VI	Accumulation of Man5GlcNAc2-glycan	Failure to thrive, microcephaly, epilepsy, hypotonia, brain atrophy	[60,61]
CDG-Ie (DPM1-CDG)	Dolichol-phosphate-mannose synthase-1 (DPM1 gene)	Hypoglycosylation of proteins. Accumulation of $Man_5GlcNAc_2$-PP-dolichol	Facial dysmorphism, microcephaly, hypotonia, developmental delay, psychomotor retardation	[62]
CDG-If (MPDU1-CDG)	Mannose-P-dolichol utilization defect 1 (MPDU1)	Hypoglycosylation of proteins. Accumulation of Glc3Man5GlcNAc2, Man5GlcNAc2, Man9GlcNAc2, and Glc3Man9GlcNAc2	Failure to thrive, erythema, seizures, encephalopathy, motor and mental retardation, ataxia, visual impairment	[63]
CDG-Ig (ALG12-CDG)	ALG8 $Man_7GlcNAc_2$-PP-dolichyl mannosyltransferase	Accumulation of $Man_7GlcNAc_2$-PP-Dol. underglycosylation serum glycoproteins	Dimorphism, cardiomyopathy, skeletal dysplasia, motor and mental retardation, hypotonia, seizures, microcephaly, subcutaneous fat pads	[64]
CDG-Ih (ALG8-CDG)	Dolichyl-P-Glc:Glc1Man9GlcNAc2- PP-dolichyl alpha-1,3-glucosyltransferase (hALG8)	Fibroblast accumulation of Dol-PP-Glc-NAc2Man9 and Dol-PP-GlcNAc2Man9Glc1	Hypotonia, epicanthus, dysmorphy, ataxia, diarrhea, hypoplasia, mental and psychomotor retardation	[65]
CDG-Ii (ALG2-CDG)	GDP-Man:Man1GlcNAc2-PP-dolichol mannosyltransferase (hALG2)	Skin fibroblast accretion of Man1GlcNAc2-PP-dolichol and Man2GlcNAc2-PP-dolichol.	Psychomotor, mental, myelinization, and motor retardation, cleft eyelid, seizures, enlarged liver.	[66]
CDG-Ij (DPAGT1-CDG)	Dolichol phosphate N-acetyl-glucosamine-1 phosphate transferase activity	Protein hypoglycosylation. decreased N-acetyl-glucosamine-1 phosphate transferase (GPT) activity	Dimorphism, microcephaly, hypotonia, mental and psychomotor retardation, seizures, abnormal speech development	[67]
CDG-Ik (ALG1-CDG)	GDP-Man:GlcNAc2-PP-dolichol mannosyltransferase	Accumulation of GlcNAc2-PP-dolichol and GlcNAc1-PP-dolichol in skin. Reduced transferrin level.	Facial dysmorphism, epilepsy, cardiomyopathy, large liver and spleen, hypogonadism	[68]
CDG-IL (ALG9-CDG)	*ALG9* alpha-1,2 mannosyltransferase enzyme	Accretion of DolPP-GlcNAc2Man6 and DolPP-GlcNAc2Man8	Seizures, failure to thrive, hypotonia, large liver and spleen, microcephaly, mental and motor retardation	[69]
CDG-Im (DK1-CDG)	Dolichol kinase catalyzes end reaction of dolichol phosphate synthesis.	Lipid-linked oligosaccharide total quantity critically decreased. underglycosylation of serum transferrin	Dilative cardiomyopathy, death in early infancy, hair loss, hypotonia, seizures, ichthyosis	[70]
CDG-In (RFT1-CDG)	Reduced-folate transporter-1	Decreased blood coagulation factor XI, and antithrombin. Lower accumulation of Man5GlcNAc2-PP-Dol by Protein C	Failure to thrive, sensorineural deafness, hypotonia, seizures, strabismus, dysmorphism, feeding problems	[60,71]
CDG-Io (DPM3-CDG)	Dolichyl-Phosphate Mannosyltransferase polypeptide 3 (DPM3)	Decreased (Dol-P-Man) synthase	Dilated cardiomyopathy, muscle weakness, stroke like episodes	[72]
CDG-Ip (ALG11-CDG)	GDP-Man:Man3GlcNAc2-PP-dolichol-alpha-1,2-mannosyltransferase	Fibroblast accumulation of Man3GlcNAc2-PP-dolichol and Man4GlcNAc2-PP-dolichol. Decrease in glycosylation of transferrin, reduced antithrombin levels	Hypotonia, delayed development, seizures, facial dysmorphisms, feeding problems, epilepsy,	[73]
CDG-Iq (SRD5A3-CDG)	Steroid 5α-reductase type 3 (SRD5A3)	Glycosylation defects on transferrin	Mental and psychomotor retardation, facial dysmorphism, coloboma, ichthyosis, hypoplasia, cerebellar atrophy	[74]
CDG-IIf	Solute carrier family 35 (CMP-sialic acid transporter), member A1 (SLC35A1)	Lack of the sialyl-Lex antigen. Atypical megakaryocyte structure and heavy sialylation. Absence of some sialylated oligosaccharide structures	Neutropenia, macrothrombocytopenia, thrombocytopenia, hematidrosis	[75]
CDG-IIg	Conserved oligomeric Golgi complex subunit 1	Lack of sialylation and galactosylation of serum proteins. Abnormal N- and O-glycosylation	Failure to thrive, dysmorphism, feeding problems, hypotonia, growth and mental retardation, brain atrophy, microcephaly, hepatosplenomegaly, hypoplasia	[76]
CDG-IIh	Conserved oligomeric Golgi complex subunit 8	Reduced sialylation. decreased 1,3GalNAc alpha 2,3-sialyltransferase (ST3Gall) activity. Slow retrograde transport kinetics of fibroblasts. abnormal N- and O-glycosylation	Brain atrophy, mental and psychomotor retardation, encephalopathy, hypotonia, seizures, ataxia	[77,78]
CDG-IIi	Conserved oligomeric Golgi complex subunit 5	Reduced sialylation of N- and O-glycans. Slow retrograde transport kinetics of fibroblasts. reduced terminal sialylation	Ataxia, hypotonia, cerebellum and brain stem atrophy, global developmental delay, mental and motor retardation	[79]

(*continued on next page*)

Table 2 (*continued*)

Disease name	Deficiency	Biochemical abnormalities	Symptoms	References
CDG-IIj	Conserved oligomeric Golgi complex subunit 4	Decreased platelet number and coagulation factors, increased serum alkaline phosphatases and transaminases. Decreased sialyation and galactosylation	Ataxia, dysmorphism, hyperreflexia, hyponia, microcephaly, psychomotor retardation, epilepsy	[80]
SIAT9-CDG	GM3 synthase enzyme (lactosylceramide a-2,3 sialyltransferase)	Increase in lactosylceramide and deficiency of GM3 gangliosides and their derivatives	Feed problems, epilepsy, brain atrophy, hypotonia, developmental retardation	[81]
CDG-IIa	N-acetyl-glucosaminyltransferase I	Low serum glycoproteins. Increased serum glutamic oxaloacetic transaminase, deficiency of proteinuria and clotting factor XII	Facial dysmorphism, hypotona, psychomotor and growth retardation, epilepsy, abnormally inactive	[82–84]
CDG-IIb	Glucosidase I (GLS1)	Glucosidase I deficient	Hypotonia, hypomotility, facial dysmorphism, enlarged liver, seizures	[85]
CDG-IIc	Leukocyte adhesion deficiency type II syndrome (LAD II)	Hypofucosylation of glycoconjugates	Hypotonia, ataxia, seizures, cerebrum and cerebellum atrophy. mental, motor, and developmental retardation	[86]
CDG-IId	β-1,4 galactosyltransferase (B4GALT1)	Deficiency of sialic acid and galactose residues on serum transferrin	Hemorrhages, hydrocephalus, hypotonia, myopathy	[87]
CDG-IIe	Component of oligomeric Golgi complex 7	Serum transferrin glycosylation abnormalities	Hepatosplenomegaly, epilepsy, dysmorphism, hypotonia	[88]

cardiac myocytes where they are responsible for signal conduction [89]. Any alterations in these can cause disturbances in electrical conduction and ventricular arrhythmias [90].

Sodium channels cause the action potential critical for signal propagation down an axon by creating localized areas that are more excitable than the rest of the cell. The sodium channels exist in three states depending on activity. Activated gates allow the passage of sodium ions into the cell until cell depolarization then the gate inactivates into the second state by the use of a plug tethered to the alpha subunit. Finally, after the membrane repolarizes, but before the gate is reactivated, the gate is deactivated, representing the third state. To finely modulate the excitability there are many different isoforms of sodium channels available for the cell's use [91].

Voltage gated sodium channels Na_v are complex glycoproteins and up to 30% of the mass of the channel is estimated to be glycosylated capped by sialic acids on both of the subunits [92]. Several of the α subunit isoforms such as $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.5$, $Na_v1.6$ are reported to be expressed throughout the heart. Specifically, $Na_v1.5$ is found to be one of the major channels responsible for ventricular systole and sarcolemma [93]. Changes in sialylation of voltage gated sodium channels mainly affect the excitability states of neuronal and cardiac cells, causing variations in the electrical signaling of brain and heart.[12] Studies conducted on sialic acid deficient-cell lines or transfected sialic acid deficient sodium channels have demonstrated the importance of sialylation on Na_v gating showing the shift in the depolarized direction [94–96]. Both α and β_1 linked sialylation have shown to affect the Na_v gating. In order to understand the effect of β_2 linked sialic acids, β_2 was co-expressed with $Na_v1.5$ and $Na_v1.2$ in completely sialylated cell line-Pro5CHO and sialylation deficient cell line Lec2. While β_2 sialylation has been shown to effect $Na_v1.5$, it did not show any impact on the $Na_v1.2$ gating demonstrating the complex manner of sugar and subunit dependence of sodium channels [94–96].

In order to show the pathophysiological role of sialylation on cardiac sodium channel, the beta-galactoside alpha-2,3-sialyltransferase (ST3Gal4) was knocked-out in mice. In these mice, the gating of sodium channel was shifted in the depolarizing direction and a 27% reduction in the ventricular refraction period was reported. Consequently, the deletion of ST3Gal4 increased arrhythmia probability in the ventricles of these mice. These studies clearly demonstrate the significance of sialylation in heart diseases [97]. To understand the whole glycome changes in the ventricular myocytes as well as neonatal and adult atrial, the glycogenes expression including glycosyltransferases, glycosidases and nucleotide sugars were measured by GeneChip microarrays. The glycome profiling was found to be different in atria and ventricles and also changed during the development. One of the polysialyltransferases, ST8Sia2 was found to be specific to neonatal atrium and its impact on Na_v sialylation and function was sufficient to modulate the excitability state of cardiomyocytes [92].

In another study, the effect of a loss of sialylation along the cell surface of rat hippocampal CA3 pyramidal cells was investigated. The enzyme neuraminidase (NEU), whose function is cleaving sialic acid residues from glycans, was used on cell membranes to completely remove sialic acid from the extracellular membrane. After the removal, a significant shift was observed in the Na_v activation/inactivation, and also the sensitivity of the Na_v towards extracellular calcium was decreased. On the other hand, *N*-acetyl-2,3-dehydro-2-deoxyneuraminic acid which is a neuraminidase blocker, reduced the seizure threshold. These studies demonstrate that sialylation is an important mechanism responsible for neuronal excitability and alterations in sialylation (both increases and decreases) may be used as a novel approach in seizures' therapy [98]. Indeed, the human disease epilepticus has been mimicked *in vitro* using low Mg^{2+} induction in rat hippocampal slices in which desialylation has proven to suppress the seizure-like activity [99].

Alterations in the activity of wild type sodium channels can also cause acquired channelopathies in addition to inherited ones. Evidence of this is found in peripheral nerve injury, which can cause a channelopathy in spinal sensory neurons. A set of different changes can cause hyperexcitability in which the active sodium channels can be turned-off or silent ones can be activated. These disorders can also cause dysregulations in impulse trafficking of Purkinje cells which may contribute to the multiple sclerosis (MS) [100].

3.4. Altered sialylation effect on development and aging

Although research of altered sialylation and glycosylation has focused mainly on channelopathies, there are multiple cases where alterations of sialylation and glycosylation can significantly affect development and aging. Glycoproteins are involved in nearly all molecular interactions at the cell membrane and intercellular space. Studies of rat liver during the aging helped to elucidate the relationship between glycosylation changes during

development. The arylsulfatase B, which is significantly important for glycosaminoglycan (dermatan and chondroityn-4 sulfates) turnover was extracted from six Wistar rats. The carbohydrate structures from each rat aged 18 days of gestation as well as 1 week, one month, one and a half, three and eighteen months were examined [101]. As a result, the sialylation has been found to increase whereas fucosylation decreases with the aging. An especially large increase has been observed in (α2-6) Neu5Ac and (α2-3) Neu5Ac between rats 3 and 18 months of age showing a change in sialylation during aging [101]. In order to investigate the alterations in sodium channels, the channels from 15 day rat embryo forebrains and postnatal states were compared. The sialylation level was found to be lower in the α-subunits of 15 day embryo compared to post-natal [102].

In addition, studies of rat cardiomyocytes from neonatal and adult stages showed sialylation dependent changes in voltage gated sodium channels in ventricle cells as a part of myocardium development. When the sialic acids were removed from adult atria and ventrical cells as well as neonatal atria through enzymatic digestion, significant depolarization shift was observed. Also immublot results showed heavy sialylation in neonatal atria, adult atria and adult ventricles compared to neonatal ventricles [93].

Separate studies on human plasma glycans from 1914 individuals from a normal population revealed the significance of glycosylation changes between pre-menopausal and post-menopausal ages [103]. Separate studies have also found that levels of disialylated biantennary glycans change during aging [103].

The studies reviewed in this paper show that the impact of sialylation on the potassium and other channels can be dependent on the isoform or on the type of glycan that is sialylated as O-linked sialylation and N-linked sialylation do not exhibit the same effects on the channel gating. Sialylation of ion channels in mammalian cells may increase the hyperpolarization of the steady state gating, channel activation, and inactivation kinetics. The sialic acid effect on the opening and closing of the channel is due to the electrostatic mechanisms created by its negative charge. Glycans other than sialic acid may also have a minor role in channel gating because they can stabilize the channel in different conformations. Although the most is known about glycosylation of the K^+ channels in health and disease, emerging data clearly indicates that these concepts are not exclusive to K^+ channels and that alterations in glycosylation and especially sialylation may be an essential component of normal development and aging.

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