Biochimica et Biophysica Acta 1859 (2017) 662–668

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Glycosylation of voltage-gated calcium channels in health and disease

Joanna Lazniewska, Norbert Weiss *

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 9 November 2016
Received in revised form 10 January 2017
Accepted 16 January 2017
Available online 19 January 2017

Keywords:
Calcium channels
Voltage-gated calcium channels
N-glycosylation
Ancillary subunit
Trafficking
Stability
Plasma membrane
Diabetes
Neuropathic pain

ABSTRACT

Voltage-gated calcium channels (VGCCs) are transmembrane proteins that translate electrical activities into intracellular calcium elevations and downstream signaling pathways. They serve essential physiological functions including communication between nerve cells, muscle contraction, cardiac activity, and release of hormones and neurotransmitters. Asparagine-linked glycosylation has emerged as an essential post-translational modification to control the number of channels embedded in the plasma membrane but also their functional gating properties. This review provides a comprehensive overview about the current state of knowledge on the role of glycosylation in the expression and functioning of VGCCs, and discusses how variations in the glycosylation of the channel proteins can contribute to pathological conditions.

Contents

1. Introduction

Calcium ion (Ca^{2+}) is an essential signaling molecule responsible for a plethora of cellular functions. In order to make use of the Ca^{2+} signal, cells have developed complex yet fundamentally essential machinery so-called the Ca^{2+} signaling toolkit [1]. Indeed, cell "well-being" is only possible with a strict timely and localized control of intracellular Ca^{2+} concentrations. In excitable cells, voltage-gated Ca^{2+} channels (VGCCs) have emerged as key players responsible for translating electrical membrane depolarizations into intracellular Ca^{2+} elevations. VGCCs are large proteins (around 250 kDa) embedded in the plasma membrane of most excitable cells. They are comprised of a pore-forming subunit ($Ca_v\alpha_1$) that consists of four domains each containing six transmembrane segments, connected with intracellular and

* Corresponding author at: Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam. 2, 166 10 Prague, Czech Republic.
E-mail address: weiss@uochb.cas.cz (N. Weiss).

http://dx.doi.org/10.1016/j.bbamem.2017.01.018
0005-2736/

extracellular linkers, and responsible for the Ca^{2+} permeability and selectivity [2,3]. In addition to the main pore-forming subunit, high voltage-activated Ca^{2+} channels (HVA, $Ca_v1.x$ and $Ca_v2.x$) associate with a number of ancillary subunits namely $Ca_v\beta$, $Ca_v\alpha_2\delta$, and in some circumstances $Ca_v\gamma$, which are essential for cell surface expression and gating of the channel [4]. In contrast, these ancillary subunits have little, if any, influence on low-voltage-activated channels (LVA, $Ca_v3.x$) [5–7].

The regulation of VGCCs occurs *via* a myriad of signaling pathways that are essential to fine-tune their expression and activity [8–11]. Post-translational modifications are recognized to play important regulatory roles on VGCCs. For instance, phosphorylation [12–15], ubiquitination [16–20] as well as S-palmitoylation [21,22] were reported to significantly impact the functioning of VGCCs. In recent years, asparagine (N)-linked glycosylation has emerged as an essential modulator of ion channels, providing an additional level of control over the expression and functioning of the channel proteins [23]. In this review, we provide a comprehensive overview of the role of N-glycosylation in the maturation, trafficking, and functional expression of VGCCs, and discuss the potential implications in disease states.

2. N-glycosylation in protein biogenesis and sorting

N-glycosylation, as opposed to glycation, is an enzymatic process that relies on the activity of various enzymes acting all the way long the glycosylation pathway [24]. It consists in the orchestral building of an oligosaccharide tree (glycan) to an asparagine (N) residue in a consensus motif N-X-S/T (X being any amino acid) within the target protein. However, the presence of this consensus locus does not always lead to glycosylation that can be influenced by a number of factors including the location of this sequence within the target protein and the nature of the X residue [25,26]. N-glycosylation is initiated in the endoplasmic reticulum (ER), where the oligosaccharide precursor is transferred from the dolichol donor onto the asparagine residue of the target protein (for review see [27]). Trimming of the terminal glucose residues within the immature N-glycan allows the nascent polypeptide chain to interact with a number of ER resident chaperon proteins including calnexin and calreticulin. This interaction is essential for the proper folding of newly synthesized proteins and represents a checkpoint for quality control that determines the fate of the protein [28,29]. While repeated addition/trimming of new glucose moieties allows the protein to reenter the calnexin/calreticulin cycle, misfolded proteins are in contrast directed to the ER associated degradation (ERAD) pathway. Correctly folded proteins that enter the secretory pathway undergo additional maturation of the glycan tree in the Golgi apparatus including branching and additional chemical modifications such as addition of sialic acid moieties, and resulting in bisecting, hybrid, and complex glycan structures [30,31] (Fig. 1). The composition of the oligosaccharide structure represents an important readout that determines the delivery of the protein to its final destination [32,33]. We note that all of these aspects have been extensively reviewed in the recent years and readers interested in additional details on the role of N-glycosylation in the biogenesis and trafficking of proteins are redirected to some of the recent reviews [34,35].

3. N-glycosylation of the $Ca_v\alpha_1$ pore-forming subunit

Based on amino-acid sequence analysis, canonical N-glycosylation sites are virtually present in all of the ten mammalian VGCC isoforms (Fig. 2). In this section, we summarize our current knowledge of the glycosylation of the $Ca_v\alpha_1$ pore-forming subunit of VGCCs.

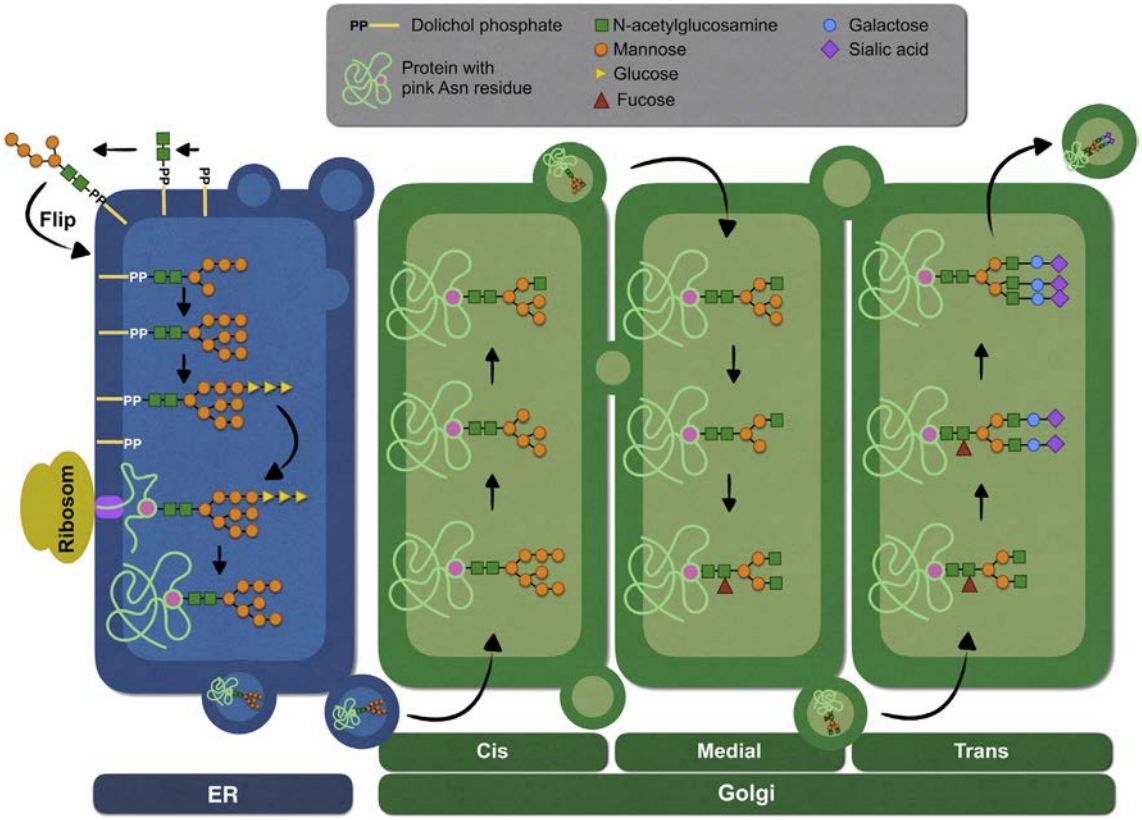

Fig. 1. Biosynthesis and processing of N-linked glycosylation. The N-glycosylation process is initiated at the membrane of the endoplasmic reticulum (ER) with the assembly of nucleotide-activated sugar precursors onto the lipid carrier dolichol by specific glycosyltransferases. The preliminary oligosaccharide is then flipped across the ER membrane and further enlarged by additional sugar species creating a branched oligosaccharide. The oligosaccharide is subsequently transferred to the asparagine residue within a consensus motif (N-X-S/T) to the nascent polypeptide chain by the oligosaccharytransferase complex (OST). The oligosaccharide is further modified in the ER and Golgi apparatus where it can be trimmed or refined, for example by addition of a terminal sialic acid moiety.

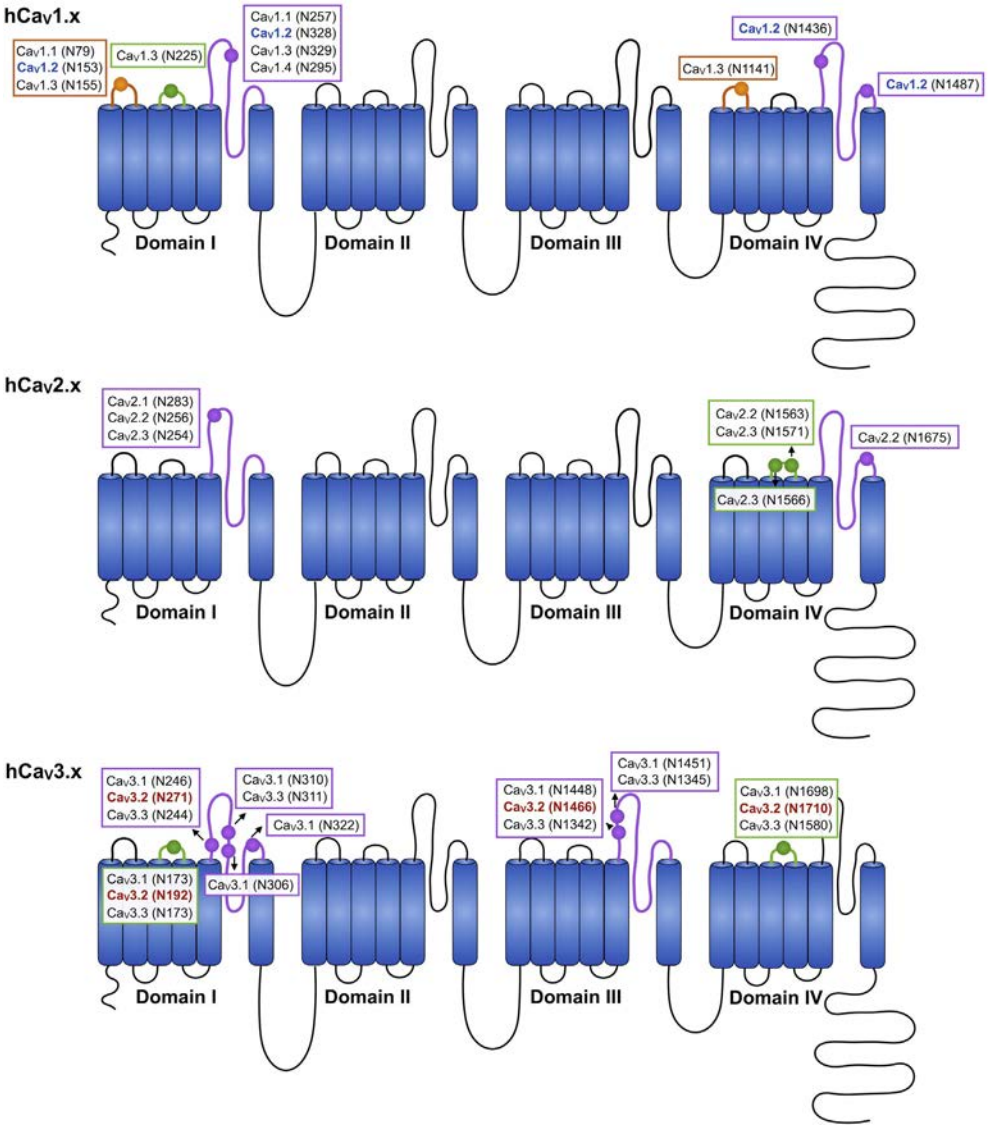

Fig. 2. N-glycosylation loci on various human voltage-gated calcium channel isoforms. The characteristic topology of the pore-forming $Ca_v\alpha_1$ subunit is shown where each hydrophobic domain (I–IV) is made up six transmembrane segments connected by a number of intracellular and extracellular linkers. N-glycosylation loci are located within domains I, III and IV of the channel. Asparagine residues indicated in red have been characterized functionally in human channel variants, whereas residues indicated in blue ($Ca_v1.2$) were characterized with the rabbit channel variant but the equivalent human loci is reported in the figure for clarity.

3.1. $Ca_v1.x$ or L-type channels

Early studies using antibody and lectin-gold labeling identified glycosylation sites in the skeletal $Ca_v1.1$ channel [36]. However, it is lately that the role of N-glycosylation in the functioning of L-type channels was assessed. Detailed information on the glycosylation of the cardiac/neuronal $Ca_v1.2$ channel was recently documented. Four potential N-glycosylation sites are present in the rabbit $Ca_v1.2$ channel and located in the domain I (N124 and N299) and domain IV (N1359 and N1410). While disruption of individual N-glycosylation sites had no significant effect on the functioning of the channel, concomitant disruption of the two glycosylation motifs located in the first domain by replacing the asparagine residues by a glutamine (double mutant N124Q/N299Q) caused a depolarizing shift of the voltage-dependence of activation of the channel expressed in *Xenopus* oocytes [37]. In addition, disruption of the two additional sites (quadruple mutant N124Q/N299Q/N1359Q/N1410Q) located in the fourth domain of $Ca_v1.2$ led to a substantial reduction of the surface expression of the glycosylation-deficient channel and a decreased current density [37] (Figs. 2 and 3).

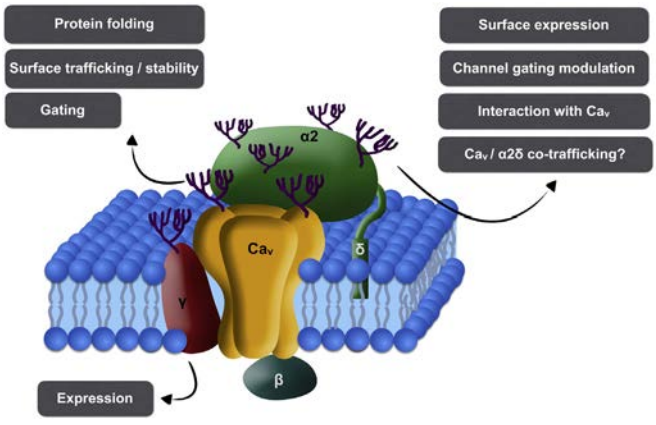

Fig. 3. Graphical summary depicting the role of N-glycosylation on the expression and functioning of the respective molecular components of the calcium channel complex.

These results support the notion that N-glycosylation of $Ca_v1.2$ serves distinct roles in the expression of the channel protein at the cell surface and also in the control of the gating properties of the channel.

3.2. $Ca_v3.x$ or T-type channels

Although it is recently that glycosylation of T-type channels was investigated, indications for the existence of major post-translational modifications of the channel proteins emerged from earlier studies. For instance, initial biochemical analysis of $Ca_v3.1$ and $Ca_v3.3$ have revealed significant differences in the apparent molecular mass of the protein between brain regions, and also during neuronal development. Indeed, a 20 KDa differential was observed between the mouse forebrain (240 kDa) and the cerebellum $Ca_v3.1$ (260 kDa) [38]. Similar observations from western blot analysis of $Ca_v3.3$ channels indicated that the mouse midbrain and diencephalon expressed a 230 kDa and 190 kDa variants, whereas other brain regions only expressed the small product [38]. In addition to brain regional differences, developmental changes were observed. For instance, a high molecular weight $Ca_v3.3$ (260 kDa) is predominantly expressed at birth and is gradually replaced by a relative lower molecular product during neuronal development [38]. Additional studies revealed that these differentials are normalized after enzymatic deglycosylation of the channel protein with PNGase F, suggesting that brain regional and developmental changes in the apparent molecular weight are essentially caused by variable degrees of glycosylation of the channel protein [39]. However, it is lately that the functional significance of N-glycosylation on the functioning of T-type channels was investigated. Recently, we and others reported a detailed characterization of the glycosylation of $Ca_v3.2$ channels [40,41]. Four potential glycosylation motifs are present within the human $Ca_v3.2$ channel and are located in the first (N192 and N271), third (N1466) and fourth membrane domain (N1710) (Fig. 2). While enzymatic deglycosylation of $Ca_v3.2$ expressed in tsA-201 cells produced an overall substantial decrease of the T-type conductance, detailed analysis of individual glycosylation loci using site directed mutagenesis revealed more subtle and specific roles of the glycan tree in the expression and functioning of the channel. For instance, disruption of glycosylation sites at asparagine residues N271 and N1710 caused an almost complete loss of the expression of $Ca_v3.2$ suggesting an essential role for these glycosylation loci in the channel biogenesis [42]. In contrast, glycosylation at asparagine residues N192 and N1466 were found essential for proper expression and stability of the channel at the plasma membrane [42,43]. In addition to control surface expression of $Ca_v3.2$, glycosylation at asparagine N192 and N1466 was found to influence the gating of the channel by modulating the Ca^{2+} permeability and/or opening probability of the channel [44]. We note that the N192 residue is located right downstream the histidine residue H191 that support metal/redox modulation of $Ca_v3.2$ channels [45–47]. This indicates that the outer IS3–IS4 loop of $Ca_v3.2$ not only supports N-glycosylation-dependent modulation of the channel but form a molecular hub for multiple regulatory pathways that may play an essential role in fine-tuning the activity of the channel.

Altogether, these data revealed that not only the nature but also the specific location within the target protein defines the functional role of the glycan tree to differentially control the expression and functioning of the channel. This aspect is further discussed in the last section of this review when considering the role of glycosylation in pathological conditions.

4. N-glycosylation of ancillary subunits

The first indication for ancillary subunit glycosylation arose from initial studies aimed at investigating the molecular composition of the dihydropyridine receptor in skeletal muscle, where the apparent mass of $Ca_v\alpha_2\delta$ and γ subunits was reduced upon glycosidase treatment [48].

4.1. $Ca_v\alpha_2\delta$ subunit

The $Ca_v\alpha_2\delta$ subunit is an integral component of the Ca^{2+} channel complex essential for proper surface trafficking and function of the channel [49,50]. $Ca_v\alpha_2\delta$ derives from a single gene and subsequently undergoes a post-translational cleavage generating α_2 and δ peptides, which remain associated with each other *via* disulphide bonds. While $Ca_v\alpha_2\delta$ is mostly extracellular, it remains anchored to the plasma membrane *via* a glycosylphosphatidylinositol (GPI) moiety attached to the C-terminal region of the δ domain [51]. $Ca_v\alpha_2\delta$ is by far the most extensively glycosylated constituent of the Ca^{2+} channel complex and the glycan accounts for about a third of the total molecular weight of the protein [52]. Initial reports suggested that N-glycosylation of $Ca_v\alpha_2\delta$ might play a role in stabilizing its interaction with the pore-forming $Ca_v\alpha_1$ subunit of the channel. This notion was further supported by the observation that $Ca_v\alpha_2\delta$-dependent potentiation of the Ca^{2+} current was abolished upon enzymatic deglycosylation of the ancillary subunit [53]. More detailed analysis where strategic glycosylation sites within $Ca_v\alpha_2\delta$-1 were disrupted by site-directed mutagenesis revealed that glycosylation at asparagine residues N136 and N184 is essential for functional expression of $Ca_v2.2$ channels [54]. Similar observations were reported for $Ca_v1.3$ channels, suggesting that modulation of the expression of the channel complex by glycosylation of $Ca_v\alpha_2\delta$-1 may be a general mechanism that exists across various channel isoforms [55]. However, it remained elusive whether this effect was mediated by an altered trafficking of the channel complex to the cell surface, or by a direct modulation of the channel activity. More recently, the importance of N-glycosylation on the trafficking of $Ca_v\alpha_2\delta$-1 was extensively analyzed. Tetreault and colleagues worked out a number of additional glycosylation sites within the $Ca_v\alpha_2\delta$-1 and demonstrated that glycosylation at asparagine N633 is essential for proper expression of the subunit at the cell surface [56,57]. In addition, and consistent with the chaperone role of $Ca_v\alpha_2\delta$-1 in the trafficking of the channel complex, $Ca_v1.2$ currents were virtually abolished when expressed in HEK-293 cells with the glycosylation-deficient $Ca_v\alpha_2\delta$-1 N633Q mutant. A similar effect was observed for several other mutants indicating that other glycosylation loci also contribute to the expression of $Ca_v\alpha_2\delta$-1 at the cell surface. In contrast, while glycosylation at asparagine N136 and N184 was found essential for functional expression of $Ca_v2.2$ channels [54], it had little influence on the surface expression of $Ca_v\alpha_2\delta$-1 [56]. Considering that these glycosylation loci are located within the VWA-N region of $Ca_v\alpha_2\delta$-1, and that the VWA domain contributes to the interaction of $Ca_v\alpha_2\delta$-1 with the $Ca_v\alpha_1$ subunit [58,59], it is a possibility that reduced functional expression of $Ca_v2.2$ channels in the presence of glycosylation-deficient $Ca_v\alpha_2\delta$-1 N136Q and N184Q mutants may have resulted from a loss of interaction with the channel, disrupting the trafficking of $Ca_v\alpha_2\delta$-1 with $Ca_v2.2$ and/or altering the functional modulation of the channel. Consistent with this notion, disruption of the glycosylation site at asparagine N348 within the VWA domain of $Ca_v\alpha_2\delta$-1 significantly altered expression of $Ca_v1.2$ channels [56]. We note that a number of glycosylation loci within $Ca_v\alpha_2\delta$-1 are located in the vicinity of the pharmacological site of action of the antiepileptic/analgesic gabapentin drug [52,60] and may have important influence on the pharmacology of the channel, although this notion remains to be explored.

Overall, N-glycosylation of $Ca_v\alpha_2\delta$-1 is undoubtedly an essential process contributing to expression and modulation of high-voltage activated Ca^{2+} channels (Fig. 3) and further studies will certainly decipher the exact underlying mechanisms taking place in this interplay.

4.2. $Ca_v\gamma$ subunit

The ancillary $Ca_v\gamma$ subunit is present in some channel complexes and associates with the $Ca_v\alpha_1$ pore-forming subunit, modulating essentially the gating of the channel. Initially described as part of the dihydropyridine complex in skeletal muscles [48], the $Ca_v\gamma$ subunit was later identified as an integral component of various neuronal and

cardiac channel complexes. It consists of four transmembrane segments, the first extracellular loop connecting the first and second segment containing at least one consensus N-glycosylation locus [61]. However, there is yet limited information on the functional role of glycosylation of the $Ca_v\gamma$ subunit. Using a similar disruptive molecular strategy as described above, it was reported that mutation of the asparagine residue N48 within the $Ca_v\gamma_2$ subunit prevents the trafficking of the protein to the cell surface, and abolishes its role as a mediator for cell-cell adhesion [62]. However, despite its reduced expression at the cell surface, disruption of asparagine N48 was not sufficient to abolish the inhibitory role of $Ca_v\gamma$ on the functioning of $Ca_v2.2$ channels, suggesting that N-glycosylation and cell surface expression of $Ca_v\gamma_2$ is not essential for the modulation of the channel [63]. In addition, the deglycosylated $Ca_v\gamma$ subunit retained its ability to biochemically interact with $Ca_v1.1$ channels, indicating that glycosylation is not required for the interaction of $Ca_v\gamma$ with the channel complex [64]. Others reported that inhibition of glycosylation with tunicamycin produced a hyperpolarizing shift of the steady-state inactivation of $Ca_v1.2$ channels only in the presence of $Ca_v\gamma_1$ [65]. However, even though tunicamycin-induced alteration of $Ca_v1.2$ gating requires the presence of $Ca_v\gamma_1$, molecular disruption of N-glycosylation loci within the $Ca_v\gamma_1$ subunit did not reproduce the effect [65]. We note that the use of tunicamycin is likely to affect all N-glycosylated proteins and it is thus important to consider the possibility that the effects observed on the functioning of the channel may have resulted from a more global alteration of the glycosylation of the proteome than from the channel subunits *per se*.

Altogether, and in contrast to what was reported for $Ca_v\alpha_1$ and $Ca_v\alpha_2\delta$ subunits, glycosylation of $Ca_v\gamma$ plays a role in the surface trafficking of the protein but has little influence on the regulation of the channel (Fig. 3).

5. Potential implications of glycosylation of VGCCs in diseased conditions

Defect in glycosylation of voltage-gated Na^+ and K^+ channels was implicated in a number of congenital disorders including long QT syndrome, cystic fibrosis, and Varitint-Waddler phenotype (for review see [66]). In contrast, and despite the broad implication of VGCCs in numerous neuronal, cardiac, and immune disorders [67–69], the role of glycosylation in the physiopathology of VGCCs has remained largely overlooked. Recently, altered glycosylation of $Ca_v3.2$ channels has emerged as a potential mechanism contributing to painful peripheral neuropathy associated with diabetes. It is well established that $Ca_v3.2$ channels are essential contributors to the initiation and maintenance of the pain signal [70–73], and contribute to synaptic transmission in the dorsal horn or the spinal cord [74] possibly by interacting with some of the proteins of the vesicular release machinery of neurotransmitters [75,76]. In addition, increased expression of $Ca_v3.2$ channels in primary afferent fibers was documented in various types of chronic and neuropathic pain [77–79] including painful diabetic neuropathy [80–87]. Although a number of signaling pathways and cellular mechanisms contribute to the expression of $Ca_v3.2$ channels [17,88,89], our group recently demonstrated that chronic elevation of extracellular glucose levels in a range consistent with diabetic hyperglycemia significantly increased surface expression of $Ca_v3.2$ both in tsA-201 cells and in primary DRG neurons in culture by promoting the trafficking of the channel to the plasma membrane [42,43]. Most importantly, increased expression of $Ca_v3.2$ channels in diabetic-like conditions was not observed when N-glycosylation at specific residues (N192 and N1466) was disrupted, suggesting that glycosylation is required for glucose-dependent potentiation of $Ca_v3.2$ channels [42,43] (Fig. 4). Although the mechanism by which glucose influence N-glycosylation is not fully understood, recent studies reported that decreased external glucose level is associated with a reduced availability of a number of glycosylation precursors and altered structure of protein N-glycan [90,91]. This regulation may be particularly important in nerve cells where the uptake of glucose relies on insulin-independent glucose transporters GLUT1 and GLUT8 and directly depends on extracellular glucose levels (for review see ([92]). In addition, increased activity of glycan processing enzymes was reported *in vitro* and *in vivo* in animal models of diabetes [93]. Consistent with the notion that altered glycosylation of $Ca_v3.2$ may contribute to the enhanced T-type current in DRG neurons during diabetes, the group of Todorovic elegantly reported that peripheral injection of neuraminidase *in vivo* in a rodent model of diabetes is sufficient to reverse T-type-dependent painful neuropathy [41]. Hence, current data strongly support the notion that N-glycosylation is a key modulator of $Ca_v3.2$ channel expression in diabetic conditions and is necessary for glucose-driven up-regulation of $Ca_v3.2$ channels. In addition, decreased expression of $Ca_v3.3$ channels was reported with loss-of-function mutations in *CACNA1I* associated with schizophrenia [94]. Considering that these mutations are located in the vicinity of canonical glycosylation loci, it was proposed that altered expression of $Ca_v3.3$ channel might have resulted from a defective glycosylation of the channel protein.

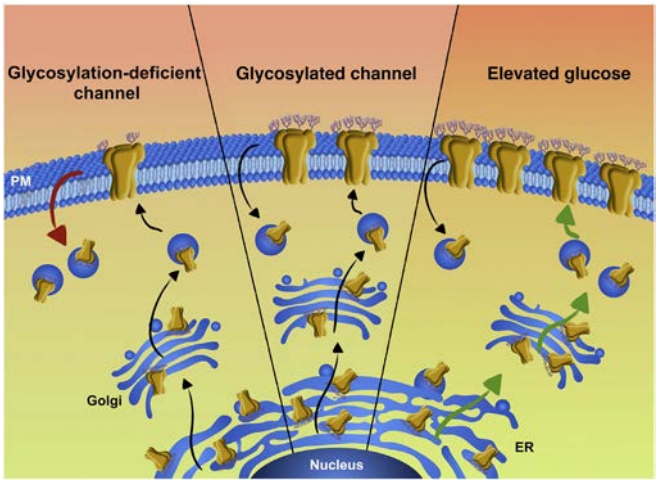

Fig. 4. Crosstalk between glucose and N-glycosylation in $Ca_v3.2$ channel expression. In normal condition, the steady-state expression of glycosylated $Ca_v3.2$ channels at the cell surface is defined by the number of channels coming to and getting removed from the plasma membrane (middle panel). Glycosylation-deficient channels do traffic to the cell surface but their stability in the plasma membrane is decreased and get internalized faster (red arrow), leading to a decreased channel density at the cell surface (left panel). In contrast, elevated extracellular glucose levels significantly accelerates the trafficking of the channel to the cell surface (green arrows) without affecting its membrane stability, causing an increased channel density in the plasma membrane, which is believed to contribute to the development of painful diabetic neuropathy (right panel). Note that glucose-dependent potentiation of $Ca_v3.2$ channel expression relies on the proper glycosylation of the channel protein.

Although the implication of N-glycosylation in the development of VGCC-associated pathologies is a new notion, emerging evidence indicate that altered glycosylation of the channel proteins may contribute to a number of congenital and chronic conditions.

6. Conclusion and perspectives

Voltage-gated Ca^{2+} channels are undeniably essential contributors to a wide range of physiological functions, including nerve cell communication, muscle contraction, regulation of cardiac function, and release of hormones and neurotransmitters. Therefore, disturbance in their functioning are responsible for a number of chronic and congenital disorders. N-glycosylation has emerged as an important post-translational modification not only controlling the expression and intrinsic functioning of VGCCs, but also mediating environment-dependent modulation of the channels. In addition, there is emerging evidence for a role of glycosylation in the pathogenesis of VGCCs. Mutations in the genes encoding for VGCCs may represent one of the primary cause of altered glycosylation of the channel proteins. In addition, glycosylation-dependent regulation of ion channels can be influenced by various factors

influencing the complex glycosylation pathway. For instance, the availability of monosaccharide [95], dolichol [96], or alteration in the activity of glycosidases [93], are all factors that may influence the glycosylation of the channel protein. In that respect, evaluation of the chemical composition of the glycan tree in normal and pathological conditions represents an important challenge in the fine understanding of the role of glycosylation in diseased states. In addition, genetic mutations in key genes of the glycosylation machinery are responsible for a growing number of congenital disorders of glycosylation (CDG) [97,98]. Although alteration in the glycosylation pathway likely represents the primary cause of these disorders, phenotypical manifestation most certainly relies on altered glycosylation of downstream target proteins possibly including ion channels. Hence, there is increased interest in identifying the molecular factors and pathways responsible for the glycosylation of VGCCs. This may uncover tremendous opportunities for rational design of new therapeutically relevant drugs targeting glycosylation of VGCCs and associated disorders, and possibly other channelopathies.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

Work in the Weiss laboratory is supported by the Czech Science Foundation (grant 15-13556S), the Czech Ministry of Education Youth and Sports (grant 7AMB15FR015), and the Institute of Organic Chemistry and Biochemistry (IOCB). J.L. is supported by an IOCB postdoctoral fellowship.

References

[1] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. (2003) 517–529.
[2] W.A. Catterall, Voltage-gated calcium channels, Cold Spring Harb. Perspect. Biol. (2011) a003947.
[3] B.A. Simms, G.W. Zamponi, Neuronal voltage-gated calcium channels: structure, function, and dysfunction, Neuron (2014) 24–45.
[4] J. Arikkath, K.P. Campbell, Auxiliary subunits: essential components of the voltage-gated calcium channel complex, Curr. Opin. Neurobiol. (2003) 298–307.
[5] V. Leuranguer, E. Bourinet, P. Lory, J. Nargeot, Antisense depletion of beta-subunits fails to affect T-type calcium channels properties in a neuroblastoma cell line, Neuropharmacology (1998) 701–708.
[6] S.J. Dubel, C. Altier, S. Chaumont, P. Lory, E. Bourinet, J. Nargeot, Plasma membrane expression of T-type calcium channel alpha(1) subunits is modulated by high voltage-activated auxiliary subunits, J. Biol. Chem. (2004) 29263–29269.
[7] J. Bae, E.J. Suh, C. Lee, Interaction of T-type calcium channel Ca(V)3.3 with the β-subunit, Mol. Cell (2010) 185–191.
[8] D.B. Halling, P. Aracena-Parks, S.L. Hamilton, Regulation of voltage-gated Ca^{2+} channels by calmodulin, Sci. STKE (2006) er1.
[9] N. Weiss, G.W. Zamponi, Regulation of voltage-gated calcium channels by synaptic proteins, Adv. Exp. Med. Biol. (2012) 759–775.
[10] J. Proft, N. Weiss, G protein regulation of neuronal calcium channels: back to the future, Mol. Pharmacol. (2015) 890–906.
[11] J. Huang, G.W. Zamponi, Regulation of voltage gated calcium channels by GPCRs and post-translational modification, Curr. Opin. Pharmacol. (2016) 1–8.
[12] I. Blesneac, J. Chemin, I. Bidaud, S. Huc-Brandt, F. Vandermoere, P. Lory, Phosphorylation of the Cav3.2 T-type calcium channel directly regulates its gating properties, Proc. Natl. Acad. Sci. U. S. A. (2015) 13705–13710.
[13] L. Yu, J. Xu, E. Minobe, A. Kameyama, L. Yang, R. Feng, L. Hao, M. Kameyama, Role of protein phosphatases in the run down of Guinea pig cardiac Cav1.2 Ca^{2+} channels, Am. J. Physiol. Cell Physiol. (2016) C773–C779.
[14] T. Patriarchi, H. Qian, V. Di Biase, Z.A. Malik, D. Chowdhury, J.L. Price, E.A. Hammes, O.R. Buonarati, R.E. Westenbroek, W.A. Catterall, F. Hofmann, Y.K. Xiang, G.G. Murphy, C.Y. Chen, M.F. Navedo, J.W. Hell, Phosphorylation of Cav1.2 on S1928 uncouples the L-type Ca^{2+} channel from the β2 adrenergic receptor, EMBO J. (2016) 1330–1345.
[15] Y. Liang, S.J. Tavalin, Auxiliary beta subunits differentially determine pka utilization of distinct regulatory sites on Cav1.3 L type Ca^{2+} channels, Channels (Austin) (2007) 102–112.
[16] C. Altier, A. Garcia-Caballero, B. Simms, H. You, L. Chen, J. Walcher, H.W. Tedford, T. Hermosilla, G.W. Zamponi, The Cavβ subunit prevents RFP2-mediated ubiquitination and proteasomal degradation of L-type channels, Nat. Neurosci. (2011) 173–180.
[17] A. García-Caballero, V.M. Gadotti, P. Stemkowski, N. Weiss, I.A. Souza, V. Hodgkinson, C. Bladen, L. Chen, J. Hamid, A. Pizzoccaro, M. Deage, A. François, E. Bourinet, G.W. Zamponi, The deubiquitinating enzyme USP5 modulates neuropathic and inflammatory pain by enhancing Cav3.2 channel activity, Neuron (2014) 1144–1158.
[18] D. Waithe, L. Ferron, K.M. Page, K. Chaggar, A.C. Dolphin, Beta-subunits promote the expression of Ca(V)2.2 channels by reducing their proteasomal degradation, J. Biol. Chem. (2011) 9598–9611.
[19] K.M. Page, S.W. Rothwell, A.C. Dolphin, The CaVβ subunit protects the I-II loop of the voltage-gated calcium channel CaV2.2 from proteasomal degradation but not oligoubiquitination, J. Biol. Chem. (2016) 20402–20416.
[20] R. Felix, N. Weiss, Ubiquitination and proteasome-mediated degradation of voltage-gated Ca^{2+} channels and potential pathophysiological implications, Gen. Physiol. Biophys. (2016).
[21] A.J. Chien, K.M. Carr, R.E. Shirokov, E. Rios, M.M. Hosey, Identification of palmitoylation sites within the L-type calcium channel beta2a subunit and effects on channel function, J. Biol. Chem. (1996) 26465–26468.
[22] J.H. Hurley, A.L. Cahill, K.P. Currie, A.P. Fox, The role of dynamic palmitoylation in Ca^{2+} channel inactivation, Proc. Natl. Acad. Sci. U. S. A. (2000) 9293–9298.
[23] J. Lazniewska, N. Weiss, The "sweet" side of ion channels, Rev. Physiol. Biochem. Pharmacol. (2014) 67–114.
[24] F. Schwarz, M. Aebi, Mechanisms and principles of N-linked protein glycosylation, Curr. Opin. Struct. Biol. (2011) 576–582.
[25] Y. Gavel, G. von Heijne, Sequence differences between glycosylated and non-glycosylated Asn-X-Thr/ser acceptor sites: implications for protein engineering, Protein Eng. (1990) 433–442.
[26] R.S. Rao, W. Bernd, Do N-glycoproteins have preference for specific sequons, Bioinformation (2010) 208–212.
[27] M. Aebi, N-linked protein glycosylation in the ER, Biochim. Biophys. Acta (2013) 2430–2437.
[28] J.J. Caramelo, A.J. Parodi, Getting in and out from calnexin/calreticulin cycles, J. Biol. Chem. (2008) 10221–10225.
[29] Q. Wang, J. Groenendyk, M. Michalak, Glycoprotein quality control and endoplasmic reticulum stress, Molecules (2015) 13689–13704.
[30] P. Stanley, Golgi glycosylation, Cold Spring Harb. Perspect. Biol. (2011).
[31] P. Fisher, D. Ungar, Bridging the gap between glycosylation and vesicle traffic, Front. Cell Dev. Biol. 15 (2016).
[32] M. Sano, H. Korekane, K. Ohtsubo, Y. Yamaguchi, M. Kato, Y. Shibukawa, M. Tajiri, H. Adachi, Y. Wada, M. Asahi, N. Taniguchi, N-glycans of SREC-I (scavenger receptor expressed by endothelial cells): essential role for ligand binding, trafficking and stability, Glycobiology (2012) 714–724.
[33] O. Vagin, J.A. Kraut, G. Sachs, Role of N-glycosylation in trafficking of apical membrane proteins in epithelia, Am. J. Physiol. Ren. Physiol. (2009) F459–F469.
[34] S.P. Ferris, V.K. Kodali, R.J. Kaufman, Glycoprotein folding and quality-control mechanisms in protein-folding diseases, Dis. Model. Mech. (2014) 331–341.
[35] K.W. Moremen, M. Tiemeyer, A.V. Nairn, Vertebrate protein glycosylation: diversity, synthesis and function, Nat. Rev. Mol. Cell Biol. (2012) 448–462.
[36] M.C. Wang, R.F. Collins, R.C. Ford, N.S. Berrow, A.C. Dolphin, A. Kitmitto, The three-dimensional structure of the cardiac L-type voltage-gated calcium channel: comparison with the skeletal muscle form reveals a common architectural motif, J. Biol. Chem. (2004) 7159–7168.
[37] H.J. Park, S.H. Min, Y.J. Won, J.H. Lee, Asn-linked glycosylation contributes to surface expression and voltage-dependent gating of Cav1.2 Ca^{2+} channel, J. Microbiol. Biotechnol. (2015) 1371–1379.
[38] A.M. Yunker, A.H. Sharp, S. Sundarraj, V. Ranganathan, T.D. Copeland, M.W. McEnery, Immunological characterization of T-type voltage-dependent calcium channel CaV3.1 (alpha 1G) and CaV3.3 (alpha 1I) isoforms reveal differences in their localization, expression, and neural development, Neuroscience (2003) 321–335.
[39] Y. Chen, A.H. Sharp, K. Hata, A.M. Yunker, L. Polo-Parada, L.T. Landmesser, M.W. McEnery, Site-directed antibodies to low-voltage-activated calcium channel CaV3.3 (alpha1I) subunit also target neural cell adhesion molecule-180, Neuroscience (2007) 981–996.
[40] N. Weiss, G.W. Zamponi, in: G. Stephens, S. Mochida (Eds.), Modulation of Presynaptic Calcium Channels, Springer 2013, pp. 61–78.
[41] P. Orestes, H.P. Osuru, W.E. McIntire, M.O. Jacus, R. Salajegheh, M.M. Jagodic, W. Choe, J. Lee, S.S. Lee, K.E. Rose, N. Poiro, M.R. Digruccio, K. Krishnan, D.F. Covey, J.H. Lee, P.Q. Barrett, V. Jevtovic-Todorovic, S.M. Todorovic, Reversal of neuropathic pain in diabetes by targeting glycosylation of Ca(V)3.2 T-type calcium channels, Diabetes (2013) 3828–3838.
[42] N. Weiss, S.A. Black, C. Bladen, L. Chen, G.W. Zamponi, Surface expression and function of Cav3.2 T-type calcium channels are controlled by asparagine-linked glycosylation, Pflugers Arch. (2013) 1159–1170.
[43] J. Lazniewska, Y. Rzhepetskyy, F.X. Zhang, G.W. Zamponi, N. Weiss, Cooperative roles of glucose and asparagine-linked glycosylation in T-type calcium channel expression, Pflugers Arch. (2016) 1837–1851.
[44] K. Ondacova, M. Karmazinova, J. Lazniewska, N. Weiss, L. Lacinova, Modulation of Cav3.2 T-type calcium channel permeability by asparagine-linked glycosylation, Channels (Austin) (2016) 175–184.
[45] T. Voisin, E. Bourinet, P. Lory, Genetic alteration of the metal/redox modulation of Cav3.2 T-type calcium channel reveals its role in neuronal excitability, J. Physiol. (2016) 3561–3574.
[46] H.W. Kang, J.Y. Park, S.W. Jeong, J.A. Kim, H.J. Moon, E. Perez-Reyes, J.H. Lee, A molecular determinant of nickel inhibition in Cav3.2 T-type calcium channels, J. Biol. Chem. (2006) 4823–4830.
[47] M.T. Nelson, P.M. Joksovic, P. Su, H.W. Kang, A. Van Deusen, J.P. Baumgart, L.S. David, T.P. Snutch, P.Q. Barrett, J.H. Lee, C.F. Zorumski, E. Perez-Reyes, S.M. Todorovic,

Molecular mechanisms of subtype-specific inhibition of neuronal T-type calcium channels by ascorbate, J. Neurosci. (2007) 12577–12583.
[48] M. Takahashi, M.J. Seagar, J.F. Jones, B.F. Reber, W.A. Catterall, Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle, Proc. Natl. Acad. Sci. U. S. A. (1987) 5478–5482.
[49] A.C. Dolphin, Calcium channel auxiliary α2δ and β subunits: trafficking and one step beyond, Nat. Rev. Neurosci. (2012) 542–555.
[50] S. Geisler, C.L. Schöpf, G.J. Obermair, Emerging evidence for specific neuronal functions of auxiliary calcium channel $\alpha_2\delta$ subunits, Gen. Physiol. Biophys. (2015) 105–118.
[51] A. Davies, I. Kadurin, A. Alvarez-Laviada, L. Douglas, M. Nieto-Rostro, C.S. Bauer, W.S. Pratt, A.C. Dolphin, The alpha2delta subunits of voltage-gated calcium channels form GPI-anchored proteins, a posttranslational modification essential for function, Proc. Natl. Acad. Sci. U. S. A. (2010) 1654–1659.
[52] E. Marais, N. Klugbauer, F. Hofmann, Calcium channel alpha(2)delta subunits-structure and gabapentin binding, Mol. Pharmacol. (2001) 1243–1248.
[53] C.A. Gurnett, M. De Waard, K.P. Campbell, Dual function of the voltage-dependent Ca^{2+} channel alpha 2 delta subunit in current stimulation and subunit interaction, Neuron (1996) 431–440.
[54] A. Sandoval, N. Oviedo, A. Andrade, R. Felix, Glycosylation of asparagines 136 and 184 is necessary for the alpha2delta subunit-mediated regulation of voltage-gated Ca^{2+} channels, FEBS Lett. (2004) 21–26.
[55] A. Andrade, A. Sandoval, R. González-Ramírez, D. Lipscombe, K.P. Campbell, R. Felix, The alpha(2)delta subunit augments functional expression and modifies the pharmacology of Ca(V)1.3 L-type channels, Cell Calcium (2009) 282–292.
[56] M.P. Tétreault, B. Bourdin, J. Briot, E. Segura, S. Lesage, C. Fiset, L. Parent, Identification of glycosylation sites essential for surface expression of the CaVα2δ1 subunit and modulation of the cardiac CaV1.2 channel activity, J. Biol. Chem. (2016) 4826–4843.
[57] J. Lazniewska, N. Weiss, Glycosylation of α2δ1 subunit: a sweet talk with Cav1.2 channels, Gen. Physiol. Biophys. (2016) 239–242.
[58] C.A. Gurnett, R. Felix, K.P. Campbell, Extracellular interaction of the voltage-dependent Ca^{2+} channel alpha2delta and alpha1 subunits, J. Biol. Chem. (1997) 18508–18512.
[59] J. Wu, Z. Yan, Z. Li, C. Yan, S. Lu, M. Dong, N. Yan, Structure of the voltage-gated calcium channel Cav1.1 complex, Science (2015) aad2395.
[60] M.J. Field, P.J. Cox, E. Stott, H. Melrose, J. Offord, T.Z. Su, S. Bramwell, L. Corradini, S. England, J. Winks, R.A. Kinloch, J. Hendrich, A.C. Dolphin, T. Webb, D. Williams, Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin, Proc. Natl. Acad. Sci. U. S. A. (2006) 17537–17542.
[61] M.G. Kang, K.P. Campbell, Gamma subunit of voltage-activated calcium channels, J. Biol. Chem. (2003) 21315–21318.
[62] M.G. Price, C.F. Davis, F. Deng, D.L. Burgess, The alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptor trafficking regulator "stargazin" is related to the claudin family of proteins by its ability to mediate cell-cell adhesion, J. Biol. Chem. (2005) 19711–19720.
[63] A. Sandoval, A. Andrade, A.M. Beedle, K.P. Campbell, R. Felix, Inhibition of recombinant N-type Ca(V) channels by the gamma 2 subunit involves unfolded protein response (UPR)-dependent and UPR-independent mechanisms, J. Neurosci. (2007) 3317–3327.
[64] J. Arikkath, C.C. Chen, C. Ahern, V. Allamand, J.D. Flanagan, R. Coronado, R.G. Gregg, K.P. Campbell, Gamma 1 subunit interactions within the skeletal muscle L-type voltage-gated calcium channels, J. Biol. Chem. (2003) 1212–1219.
[65] A. Angelova, A. Ulyanova, R. Shirokov, Gamma1 subunit renders Cav1. 2 channels dependent on cell cycle, Biophys. J. (2010) 694a.
[66] D. Baycin-Hizal, A. Gottschalk, E. Jacobson, S. Mai, D. Wolozny, H. Zhang, S.S. Krag, M.J. Betenbaugh, Physiologic and pathophysiologic consequences of altered sialylation and glycosylation on ion channel function, Biochem. Biophys. Res. Commun. (2014) 243–253.
[67] N. Weiss, A. Koschak, Pathologies of Calcium Channels, 2014.
[68] G.W. Zamponi, J. Striessnig, A. Koschak, A.C. Dolphin, The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential, Pharmacol. Rev. (2015) 821–870.
[69] G.W. Zamponi, Targeting voltage-gated calcium channels in neurological and psychiatric diseases, Nat. Rev. Drug Discov. (2016) 19–34.
[70] A. Dogrul, L.R. Gardell, M.H. Ossipov, F.C. Tulunay, J. Lai, F. Porreca, Reversal of experimental neuropathic pain by T-type calcium channel blockers, Pain (2003) 159–168.
[71] E. Bourinet, A. Alloui, A. Monteil, C. Barrère, B. Couette, O. Poirot, A. Pages, J. McRory, T.P. Snutch, A. Eschalier, J. Nargeot, Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception, EMBO J. (2005) 315–324.
[72] S. M'Dahoma, V.M. Gadotti, F.X. Zhang, B. Park, J.H. Nam, V. Onnis, G. Balboni, J.Y. Lee, G.W. Zamponi, Effect of the T-type channel blocker KYS-05090S in mouse models of acute and neuropathic pain, Pflugers Arch. (2015).
[73] E. Bourinet, A. Francois, S. Laffray, T-type calcium channels in neuropathic pain, Pain (2016) S15–S22.
[74] M.O. Jacus, V.N. Uebele, J.J. Renger, S.M. Todorovic, Presynaptic Cav3.2 channels regulate excitatory neurotransmission in nociceptive dorsal horn neurons, J. Neurosci. (2012) 9374–9382.
[75] N. Weiss, S. Hameed, J.M. Fernández-Fernández, K. Fablet, M. Karmazinova, C. Poillot, J. Proft, L. Chen, I. Bidaud, A. Monteil, S. Huc-Brandt, L. Lacinova, P. Lory, G.W. Zamponi, M. De Waard, A Ca(v)3.2/syntaxin-1A signaling complex controls T-type channel activity and low-threshold exocytosis, J. Biol. Chem. (2012) 2810–2818.
[76] N. Weiss, G.W. Zamponi, Control of low-threshold exocytosis by T-type calcium channels, Biochim. Biophys. Acta (2013) 1579–1586.
[77] J. Yue, L. Liu, Z. Liu, B. Shu, Y. Zhang, Upregulation of T-type Ca^{2+} channels in primary sensory neurons in spinal nerve injury, Spine (Phila Pa 1976) (2013) 463–470.
[78] M. Watanabe, T. Ueda, Y. Shibata, N. Kumamoto, S. Shimada, S. Ugawa, Expression and regulation of Cav3.2 T-type calcium channels during inflammatory hyperalgesia in mouse dorsal root ganglion neurons, PLoS One (2015) e0127572.
[79] J. Scanzi, A. Accarie, E. Muller, B. Pereira, Y. Aissouni, M. Goutte, J. Joubert-Zakeyh, E. Picard, L. Boudieu, C. Mallet, A. Gelot, D. Ardid, F.A. Carvalho, M. Dapoigny, Colonic overexpression of the T-type calcium channel Cav 3.2 in a mouse model of visceral hypersensitivity and in irritable bowel syndrome patients, Neurogastroenterol. Motil. (2016) 1632–1640.
[80] M.M. Jagodic, S. Pathirathna, M.T. Nelson, S. Mancuso, P.M. Joksovic, E.R. Rosenberg, D.A. Bayliss, V. Jevtovic-Todorovic, S.M. Todorovic, Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons, J. Neurosci. (2007) 3305–3316.
[81] R.B. Messinger, A.K. Naik, M.M. Jagodic, M.T. Nelson, W.Y. Lee, W.J. Choe, P. Orestes, J.R. Latham, S.M. Todorovic, V. Jevtovic-Todorovic, In vivo silencing of the Ca(V)3.2 T-type calcium channels in sensory neurons alleviates hyperalgesia in rats with streptozocin-induced diabetic neuropathy, Pain (2009) 184–195.
[82] J.R. Latham, S. Pathirathna, M.M. Jagodic, W.J. Choe, M.E. Levin, M.T. Nelson, W.Y. Lee, K. Krishnan, D.F. Covey, S.M. Todorovic, V. Jevtovic-Todorovic, Selective T-type calcium channel blockade alleviates hyperalgesia in Ob/Ob mice, Diabetes (2009) 2656–2665.
[83] M.M. Jagodic, S. Pathirathna, P.M. Joksovic, W. Lee, M.T. Nelson, A.K. Naik, P. Su, V. Jevtovic-Todorovic, S.M. Todorovic, Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve, J. Neurophysiol. (2008) 3151–3156.
[84] S.M. Todorovic, V. Jevtovic-Todorovic, Neuropathic pain: role for presynaptic T-type channels in nociceptive signaling, Pflugers Arch. (2013) 921–927.
[85] A.L. Obradovic, S.M. Hwang, J. Scarpa, S.J. Hong, S.M. Todorovic, V. Jevtovic-Todorovic, CaV3.2 T-type calcium channels in peripheral sensory neurons are important for mibefradil-induced reversal of hyperalgesia and allodynia in rats with painful diabetic neuropathy, PLoS One (2014) e91467.
[86] D.E. Duzhyy, V.Y. Viatchenko-Karpinski, E.V. Khomula, N.V. Voitenko, P.V. Belan, Upregulation of T-type Ca^{2+} channels in long-term diabetes determines increased excitability of a specific type of capsaicin-insensitive DRG neurons, Mol. Pain (2015) 29.
[87] S.M. Todorovic, V. Jevtovic-Todorovic, Targeting of CaV3.2 T-type calcium channels in peripheral sensory neurons for the treatment of painful diabetic neuropathy, Pflugers Arch. (2014) 701–706.
[88] K.A. Aromolaran, K.A. Benzow, L.L. Cribbs, M.D. Koob, E.S. Piedras-Rentería, T-type current modulation by the actin-binding protein Kelch-like 1, Am. J. Physiol. Cell Physiol. (2010) C1353–C1362.
[89] Y. Rzhepetskyy, J. Lazniewska, J. Proft, M. Campiglio, B.E. Flucher, N. Weiss, A Cav3.2/Stac1 molecular complex controls T-type channel expression at the plasma membrane, Channels (Austin) (2016) 346–354.
[90] B. Liu, M. Spearman, J. Doering, E. Lattová, H. Perreault, M. Butler, The availability of glucose to CHO cells affects the intracellular lipid-linked oligosaccharide distribution, site occupancy and the N-glycosylation profile of a monoclonal antibody, J. Biotechnol. (2014) 17–27.
[91] C. Villacrés, V.S. Tayi, E. Lattová, H. Perreault, M. Butler, Low glucose depletes glycan precursors, reduces site occupancy and galactosylation of a monoclonal antibody in CHO cell culture, Biotechnol. J. (2015) 1051–1066.
[92] D.R. Tomlinson, N.J. Gardiner, Glucose neurotoxicity, Nat. Rev. Neurosci. (2008) 36–45.
[93] N. Rellier, D. Ruggiero-Lopez, M. Lecomte, M. Lagarde, N. Wiernsperger, In vitro and in vivo alterations of enzymatic glycosylation in diabetes, Life Sci. (1999) 1571–1583.
[94] A. Andrade, J. Hope, A. Allen, V. Yorgan, D. Lipscombe, J.Q. Pan, A rare schizophrenia risk variant of CACNA1I disrupts CaV3.3 channel activity, Sci. Rep. (2016) 34233.
[95] J. Zhu, J. Yan, W.B. Thornhill, N-glycosylation promotes the cell surface expression of Kv1.3 potassium channels, FEBS J. (2012) 2632–2644.
[96] H. Bar-On, G. Nesher, A. Teitelbaum, E. Ziv, Dolichol-mediated enhanced protein N-glycosylation in experimental diabetes—a possible additional deleterious effect of hyperglycemia, J. Diabetes Complicat. (1997) 236–242.
[97] H.H. Freeze, B.G. Ng, Golgi glycosylation and human inherited diseases, Cold Spring Harb. Perspect. Biol. (2011) a005371.
[98] K. Scott, T. Gadomski, T. Kozicz, E. Morava, Congenital disorders of glycosylation: new defects and still counting, J. Inherit. Metab. Dis. (2014) 609–617.