SUMOylation and calcium signalling: potential roles in the brain and beyond

Leticia Coelho-Silva1, Gary J. Stephens2 and Helena Cimarosti1

1Department of Pharmacology, Federal University of Santa Catarina, Florianopolis, Brazil; 2School of Pharmacy, University of Reading, Reading, U.K.

Correspondence: Helena Cimarosti (helena.cimarosti@ufsc.br)

SUMOylation pathways

Post-translational modifications of proteins can affect their function, localization and degradation depending on the stimulus applied, to control cellular response [16,17]. SUMOylation is a reversible lysine-targeted post-translational modification, whereby covalently conjugated SUMO regulates proteins in numerous pathways [18,19]. Currently, there are five proposed SUMO isoforms, with SUMO-1, 2...
and 3 being the best-characterized paralogs. SUMO-1 shares approximately 50% of its amino acid sequence with both SUMO-2 and SUMO-3, which are typically known as SUMO-2/3 since they differ by only three N-terminal amino acids and antibodies are usually unable to distinguish between them [20,21]. Despite the similarities, there are functional differences between SUMO-1 and SUMO-2/3. For instance, under basal conditions, unconjugated SUMO-1 is scarce, but free SUMO-2/3 is widely expressed in mammalian cells [22]. Although the exact role for SUMO-4 remains uncertain, it has been associated with the pathophysiological mechanisms underlying diabetes [23,24]. Finally, the existence of a fifth SUMO isoform, SUMO-5, that regulates promyelocytic leukaemia nuclear bodies, has recently been suggested [25]. The same enzymes conjugate all SUMO isoforms [19].

The first step in the SUMOylation process requires the maturation of SUMO by SUMO-specific isopeptidases/proteases; next, SUMO is activated in an ATP-dependent step by E1 complex, which in humans consists of an heterodimer formed by SUMO-activating enzyme subunits 1 and 2 (SAE1 and SAE2 respectively). Subsequently, SUMO is transferred from the E1 activating enzyme to the E2 conjugating enzyme, also known as Ubc9, which is able to conjugate SUMO to target proteins both in E3 ligase-dependent and -independent manners. Most target proteins carry the same consensus motif that is directly recognized by Ubc9: the –K–x–D/E sequence, with representing a large hydrophobic residue (commonly isoleucine, leucine or valine), K is the modified lysine, x is any residue and D/E are acidic residues [22,26]. Nevertheless, non-covalent interactions between SUMO and target proteins can occur through SUMO interacting motifs (SIMs) [17,27]. These SIMs consist of a short stretch of branched hydrophobic residues, typically comprising isoleucine (I) or valine (V) residues organized as (V/I)–x–(V/I)–(V/I) or (V/I)–(V/I)–x–(V/I), flanked NH₂– or COOH– terminally by serine residues and/or acidic residues [28]. Alternatively, SUMO E3 ligases can directly bind to target proteins [17]. The SUMOylation process is highly reversible by the same enzymes responsible for SUMO maturation and also SUMO deconjugation from substrate proteins [29].

Recently, three distinct families of SUMO-specific isopeptidases and proteases have been identified in mammals: the ubiquitin-like protease/sentrin-specific protease (Ulp/SNOP), the deSUMOYlating isopeptidase (Desi) and ubiquitin-specific peptidase-like protein 1 (USPL1) [30,31]. The SENPs are the best characterized and, so far, six SENP isoforms have been identified in humans: SENP1, 2, 3, 5, 6 and 7 [17]. SENP1 is highly expressed in the nucleus, in the nuclear pore and as discrete nuclear ‘dots’ [32], but can also be found in all neuronal processes and at synapses at lower levels [33-35]. During the maturation phase, SENP1 cleaves pro-SUMO preferentially to generate SUMO-1 and SUMO-2/3 [36,37], while it deconjugates both SUMO isoforms [37,38]. SENP2 is similar to SENP1 with respect to its localization and characteristics regarding the maturation step, but differs from SENP1 regarding its highly selectivity for SUMO-2/3 deconjugation [37-40]. SENP3 is found in the nucleus, but also in the mitochondria and participates in neuronal signalling [41]. The role of SENP3 in cleaving pro-SUMO has not been elucidated as yet, but it is suggested that SENP3 is somehow selective for removing SUMO-2/3 from target proteins [37,38]. As for SENP3, SENP5 has a nuclear localization [37,42] and is important for SUMO-2/3 maturation and deconjugation [37,38,43]. Finally, SENP6 and SENP7 are located throughout the nucleoplasm [17,44] and, although neither participates in the maturation step, they are both important for removal of SUMO-2/3 [17,44,45]. Regarding the Desi family, two isoforms have been identified so far: Desi-1 and Desi-2. Whereas Desi-1 is found both in the cytoplasm and the nucleus, where it promotes deconjugation of all SUMO isoforms, Desi-2 is exclusively cytoplasmatic and its properties remain undefined [30,31]. Lastly, USPL1 preferably promotes SUMO-2/3 deconjugation and is located in Cajal bodies [30,31].

Roles of SUMOylation in neurological diseases
Disruption of basal SUMOylation has been implicated in multiple neurological disorders, including neurodegenerative diseases, such as Alzheimer and Parkinson’s diseases (AD and PD respectively), spinocerebellar ataxias (SCAs), cerebral ischaemia and epilepsy [46]. More specifically, amyloid precursor protein (APP) and tau, which are key proteins in AD, have been identified as SUMO targets in HeLa and HEK293 cells [47-49]. APP undergoes proteolytic cleavage by α- or β-secretases, and both are followed by further γ-secretase processing [50]. While α-secretases cleave APP to peptides that are proposed to participate in neuroprotection and neuroplasticity, characterizing the non-amyloidogenic pathway [51], cleavage by β-secretases leads to the amyloidogenic pathway, generating toxic amyloid β (Aβ) that accumulates and forms amyloid plaques [52]. A reduction in Aβ aggregates was found in HeLa cells when APP was SUMOylated by either SUMO-1 or SUMO-2 at lysines 587 and 595, which are located adjacentally to the β-secretase site [48]. Moreover, poly-SUMOylation of APP by SUMO-3 has been reported to regulate APP cleavage and decrease Aβ production in HEK293 cells [53]. Conversely, SUMO-3, as well as SUMO-1, was found to increase γ-secretase levels [54], thus increasing Aβ production in a transgenic mice model for AD [55]. It is important to note
that SUMO-3 effects on Aβ deposition might not be dependent on the ability of SUMO-3 to conjugate to target proteins [54]. Another AD hallmark is the hyperphosphorylation of tau [56] that decreases its affinity for microtubules, resulting in tau accumulation and formation of neurofibrillary tangles [57]. Tau can undergo SUMOylation at lysine 340 in HEK293 cells, which triggered its phosphorylation and inhibited its degradation by the ubiquitin–proteasome pathway, thus increasing tau aggregation [47].

As for mouse models of AD [55], increased levels of SUMO-1 were found in the plasma of patients with dementia [58]. Conversely, SUMO-1 conjugates were not altered in the post-mortem hippocampus of AD patients, whereas SUMO-2/3 high molecular weight conjugates were decreased [59]. These observations are in agreement with previous reports that found increased SUMO-1 and decreased SUMO-2 conjugation levels in the cortex and hippocampus respectively, of Tg2576 mice [60,61]. However, a recent study demonstrated absence of gross changes in global SUMOylation levels in the post-mortem cortex of AD patients [62].

α-Synuclein, parkin and DJ-1 are examples of SUMO targets relevant to PD [17,63,64]. Cytosolic inclusions known as Lewy bodies, comprised mostly by aggregated α-synuclein, contribute to the synaptic dysfunction and consequent dopaminergic neuronal death predominantly in the substantia nigra, a well-described characteristic of PD [65-68]. Promisingly, SUMO-1 conjugation to α-synuclein reduced its aggregation and toxicity in a transgenic mice model for PD [69]. Interestingly, in an early communication, lysosomal SUMO-1 labelling was identified in human olfactory mucosa-neurospheres obtained from biopsies of patients with idiopathic PD [70]. A similar finding was observed in post-mortem tissue from patients with multiple system atrophy and progressive supranuclear palsy, diseases in which α-synuclein and tau seem to be involved [70,71]. In both familial and sporadic PD, parkin, which is an ubiquitin ligase, can be found together with α-synuclein in Lewy bodies, where SUMO-1 was shown to non-covalently and selectively interact with parkin, increasing its auto-ubiquitination and transport to the nucleus [72]. Moreover, SUMOylation of DJ-1, a transcriptional regulator mutated in 1–2% of early-onset PD cases, maintained its cytoprotective function in response to oxidative stress [73,74], whereas incomplete SUMOylation of DJ-1 led to its proteasomal degradation [75]. In a similar way to SUMOylated α-synuclein, increased SUMO conjugation to ataxin-7 decreased its aggregation and cytotoxicity in SCAs [76].

Despite several reports from our group and others showing that SUMOylation can protect cells from metabolic stress caused by low levels of oxygen and glucose in different models of cerebral ischaemia and hypoxic conditions [77-81], disease-modified SUMO targets remain largely unknown. However, one such target is the mitochondrial GTPase dynamin-related protein 1 (Drp1), which regulates mitochondrial fission [41,82]. Under stress conditions, Drp1-mediated mitochondrial fission can release cytochrome c and induce caspase cleavage followed by cell apoptosis [83]. In an in vitro model of ischaemia, oxygen and glucose deprivation led to SENP3 degradation and consequent increase in SUMO-2/3 conjugation to Drp1, thus preventing mitochondrial fission and cytochrome c release, as well as promoting cell survival [41]. Another ischaemia-modified SUMO target is the isofrom 3 of the sodium (Na+)/Ca2+ exchanger (NCX), which controls ionic homoeostasis during cerebral ischaemia [84]. NCX3 f-loop lysine 590 is required for SUMOylation, and the absence of this residue increased NCX3 degradation, exacerbating ischaemic damage induced by permanent and transient middle cerebral artery occlusion (MCAO) [85]. Following preconditioning and transient MCAO, SUMO-1 basal expression led to increased NCX3 levels, whereas SUMO silencing decreased NCX3 levels, suggesting that NCX3 SUMOylation participates in the protective role that SUMO-1 plays during ischaemic preconditioning [85].

Evidence shows that SUMOylation may be involved in mechanisms implicated in the development and maintenance of epilepsy, since it was demonstrated that neuronal K+ channels could be SUMOylated, thus modulating neuronal excitability [3,6-10]. Moreover, SUMOylation of excitatory receptor subunits can modulate receptor trafficking and interfere with synaptic transmission [86-90]. For example, SUMOylation of the GluK2 subunit of kainate receptors led to receptor internalization, which could be neuroprotective against excitotoxicity [33]. More recently, the major cause of premature death in epilepsy, known as sudden unexplained death in epilepsy, has been linked with the hyper-SUMOylation of the Kv7 K+ channel, which functionally reduces the depolarizing M-current conducted by this channel [13].

Ca2+ channels

Unique amongst other ions, Ca2+ can modulate both membrane potential and function as an important signalling entity. Several cellular processes, ranging from neurotransmitter/hormone release [91] and muscle contraction [92] to gene transcription [93,94], require an increase in the intracellular Ca2+ levels, which under basal conditions are maintained approximately 100 nM [95]. This temporary increase occurs by either release from intracellular Ca2+
stores or influx into the cell by agonist-operated channels, G-protein coupled receptors, store-operated channels and, predominantly, through VGCCs located at the plasma membrane [96].

VGCCs were initially classified based on their voltage-dependent activation (high or low voltage-activated channels) [97,98] and subsequently subdivided by pharmacological and biophysical function (high voltage-activated and low voltage-activated) [99] and then by CaVα1 subunits [100]. CaVα1 structure allows selectivity for Ca2+ over monovalent ions and contains a sensor motif that detects membrane depolarization leading to channel opening [96]. Based on their CaVα1 subunits, three families of VGCCs have been defined: Cav1 – present mainly in skeletal muscle, heart, neurons and endocrine cells, Cav2 – found mainly at presynaptic terminals in the CNS, but also in peripheral synapses, and Cav3 – localized mainly in the sinoatrial node, adrenal glomerulosa cells, neurons and sperm acrosome [100,101]. Cav1 subunits form L-type Ca2+ current; Cav2.1 forms P/Q-type, Cav2.2 N-type and Cav2.3 form R-type current, whereas Cav3 subunits form T-type current. In addition to the three CaVα1 family subunits (Cav1, Cav2 and Cav3), there are auxiliary β, α2, δ and also γ subunits that comprise the channel complex and have various functions including transporting channels from the endoplasmic reticulum to the plasma membrane, maintaining channel stability and contributing to physiological and pharmacological properties [100].

Roles of Ca2+ channels in neurological disorders

Pathological changes in Ca2+ homoeostasis and deregulation of Ca2+ channels are implicated in a range of neurological disorders, including epilepsy, cerebral ischaemia, pain, neurodegenerative, and psychiatric diseases [102-104]. Ca2+ levels control neuronal hyperexcitability and mutations in VGCCs have been identified in familial CNS diseases (so-called ‘channelopathies’). For example, Cav2.1 and Cav3.2 channelopathies have been widely associated with forms of absence epilepsy and episodic ataxia [105]. Furthermore, acquired epilepsy and cerebral ischaemia can occur due to insults resulting from increased Ca2+ influx [105,106]. Moreover, exocytosis of synaptic vesicles mediated by VGCCs, whereby membrane depolarization triggered by action potentials causes transmitter release, may be targeted in pain pathways, in particular at central terminals of sensory nociceptive afferents. For example, both Cav2.2 and Cav3.2 channels are crucial for control of neurotransmitter release at the dorsal horn [107,108]. Cav2.2 is targeted therapeutically by ziconotide [109,110], a drug used to treat cancer-derived pain, and other drugs targeting Cav2.2 are in development [96]. Cav3.2 also acts to regulate afferent fibre excitability [111] and there is good evidence that these channels are up-regulated under chronic pain conditions [112-115].

Neurodegenerative diseases and psychiatric disorders have been related to Ca2+ handling often with respect to mitochondrial function, since rises in Ca2+ levels lead to mitochondrial stress and generation of reactive oxygen species [96]. In AD, deregulation of Ca2+ homoeostasis contributes to Aβ production and accumulated Aβ interferes with Ca2+ influx. Under physiological conditions, Ca2+ entry is reported to contribute to APP cleavage by α-secretase, while improper intracellular Ca2+ mobilization can affect APP processing and lead to increased Aβ levels, neuroinflammation and metabolic stress [115,116]. Aβ is proposed to modulate Ca2+ influx in various ways including: by direct effects of oligomeric Aβ on the Cavα1 subunit [117,118], inducing membrane-associated oxidative stress or contributing to excitotoxicity [116,119]. Moreover, mutations in Cav1.2 and Cavβ2 have been linked to both bipolar disorder and schizophrenia, while mutations in Cav1.3 have also been linked to bipolar disorder [96]. In addition, Cav1.3 contributed to neuronal loss in PD as a consequence of inherent voltage-dependent activation of the subunit, rather than their selectivity for Ca2+ [120]. Moreover, α-synuclein aggregation can modulate the influx of Ca2+, and, in turn, increases in Ca2+ concentration can promote α-synuclein aggregation [121,122].

SUMOylation and Ca2+ signalling in neurotransmission

SUMOylation of proteins involved in Ca2+ signalling affects the maintenance of neurotransmission from synapse formation (Figure 1A) to neurotransmitter release (Figure 1B) and synaptic plasticity. Mutations in the CACNA1A gene, which encodes the Cav2.1 subunit, are found in SCA type 6 (SCA6) and lead to impaired VGCC function [123].

In an early communication, SUMO-1 overexpression was reported to decrease wild-type Cav2.1 current density in HEK293 cells, whereas it had no effects on SCA6 Cav2.1 mutants [124]. Interestingly, either SUMO-1 overexpression or SENP1 silencing enhanced cAMP-dependent exocytosis and glucagon secretion from both mouse and human pancreatic α-cells via effects on Cav1 channels [14].

Increased SUMO-1 conjugation to presynaptic target proteins was shown to regulate Ca2+ influx and neurotransmitter release in synaptosomes [125]. Depending on the applied stimulus, SUMOylation of presynaptic proteins could either increase or decrease neurotransmitter release. For example, loading synaptosomes with SUMO-1 and SENP1 peptides decreased and increased Ca2+ influx and KCl-evoked glutamate release respectively. Conversely, kainate-induced Ca2+ influx and neurotransmitter release were increased in synaptosomes loaded with SUMO-1 and...
Figure 1. Potential roles played by SUMO on Ca^{2+} signalling in neurotransmission

(A) Decreased calcium signalling leads to phosphorylation and SUMOylation of MEF2A, thus promoting synapse formation. As a result of VGCC activation, MEF2 is dephosphorylated and switches SUMOylation to acetylation inhibiting synaptic processes. (B) SUMOylated RIM1α facilitates the clustering of Ca_{v2.1} Ca^{2+} channels and enhances Ca^{2+} influx necessary for vesicular release. When SUMO is conjugated to CRMP2, it inhibits Ca^{2+} entry through Ca_{v2.2} channels, and increases surface expression of NaV1.7 channels. SUMOylation of syntaxin-1A, synaptotagmin-1 and synapsin Ia can regulate neurotransmission by participating in docking/priming of synaptic vesicles; CRMP2, collapsin response mediator protein 2; MEF2, myocyte enhancer factor 2.

decreased in synaptosomes loaded with SENP1 [125]. These results suggest that SUMO may be conjugated to distinct presynaptic proteins and act in an activity-dependent and stimulus-specific manner to modulate presynaptic release.

Crucial proteins in neurotransmitter release, CRMP2 and Rab3a-interacting molecule (RIM) have been identified as members of the Ca_{v2} proteome [126]. SUMOylation of VGCC interacting proteins has been reported to play an important role in neurotransmission within pain pathways. CRMP2 interacts with Ca_{v2.2} subunits in sensory neurons or nociceptors to modulate neurotransmitter release [127]. SUMO-1–3 modified CRMP2 at lysine 374 in cultured catecholamine A differentiated cells [128]. Overexpression of SUMO, Ubc9 and CRMP2 in adult dorsal root ganglion neurons decreased, whereas overexpression of non-SUMOylatable CRMP2 increased, KCl depolarization-induced Ca^{2+} entry. In addition, CRMP2 SUMOylation increased surface expression of NaV1.7 channels [129]. Mutations in NaV1.7 channels, which are highly expressed in peripheral sensory neurons, where they are responsible for regulating neuronal excitability, are directly related with pain disorders [130].

RIM1α interacts directly or indirectly with most presynaptic active zone proteins and participates in the docking and priming of synaptic vesicles [131] by modulating Ca^{2+} influx through regulation of VGCCs clustering [132,133]. SUMO-1 conjugation to RIM1α at lysine 502 was shown to be crucial for normal presynaptic exocytosis in neurons [133]. Knockdown of endogenous RIM1α, and its replacement with a non-SUMOylatable mutant, led to impairment of Ca^{2+}-induced depolarization and consequent removal of the fast component of vesicle exocytosis. SUMOylated RIM1α facilitated the clustering of Ca_{v2.1} channels and enhanced Ca^{2+} influx necessary for vesicular release, whereas de-SUMOylated RIM1α participated in the docking/priming of synaptic vesicles and structural maintenance of the active zone [133].

Presynaptic soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNARE) proteins, such as syntaxin 1, are fundamental for neurotransmitter release [134] and might also participate in vesicle endocytosis [135,136]. Syntaxin 1A can be modified by SUMO-1 at any of three lysine residues (K252, K253 or K256) near the C-terminal transmembrane domain [137]. Preventing syntaxin 1A SUMOylation reduced its interaction with other SNARE proteins and disrupted the balance of synaptic vesicle endo/exocytosis, resulting in increased endocytosis. Another key
protein that is SUMOylated is synapsin Ia: preventing SUMO-1 conjugation to synapsin Ia at lysine 687 caused impaired exocytosis due to a reduction in the number of releasable synaptic vesicles [138]. Proteomic analysis from a neuron-specific SUMO-1 overexpressing transgenic mouse model led to the identification of a number of previously unrecognized SUMO-1 targets in vivo, including the Ca²⁺ sensor synaptotagmin-1 [139]. Increased SUMO-1 conjugation to synaptotagmin-1 resulted in impaired performed paired pulse facilitation (PPF), which involves the facilitation of neurotransmitter release caused by residual Ca²⁺ from a previous stimulus.

Homologs of the SUMOylation machinery were identified in Drosophila, and an interaction with Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) that modulates synaptic plasticity by regulating glutamatergic synapses [140] was demonstrated by yeast two-hybrid screening [141]. Drosophila SUMO-1 (DmSUMO-1) modification has potential to change the subcellular localization of CaMKII, but the functional consequences for this interaction remain to be confirmed.

Dendritic claws in cerebellar granular neurons, in which mossy fibre terminals and Golgi neurons form synapses [142], are regulated by the myocyte enhancer factor 2A (MEF2A). MEF2A transcription factor activity is regulated by several post-translational protein modifications, including phosphorylation [143-145], ubiquitination [146] and SUMOylation [147]. Lack of Ca²⁺ signalling led to phosphorylation of MEF2A at serine 408, which in turn led to SUMO-1 conjugation at lysine 403 and inactivation of MEF2A, promoting dendritic claw differentiation, synapse formation and maturation. Activity-dependent Ca²⁺ signalling via CaV1 VGCCs induced calcineurin-mediated dephosphorylation of MEF2A at serine 408, promoting a switch from SUMOylation to acetylation at lysine 403, which in turn activated MEF2A and inhibited dendritic claw differentiation and synapse formation [147].

As previously described, deregulation of Ca²⁺ homoeostasis contributes to aggregation of proteins such as Aβ and α-synuclein, known as aggregation-prone proteins, which can interfere with neurotransmission. Also, production and accumulation of these proteins interfere with Ca²⁺ influx [148]. Two lysines of APP can be modified by SUMO in vivo leading to decreased levels of Aβ aggregates [48]. SUMOylation of α-synuclein seems to inhibit α-synuclein aggregation and toxicity both in vitro and in vivo [149]. This inhibition depends on the SUMO isoform (SUMO-1 conjugation is better than SUMO-3) and on the SUMOylated lysine (K102 is better than K96) [150]. Interestingly, raised concentrations of monomeric α-synuclein in the extracellular medium promoted dopamine release in the striatum via CaV2.2 channels in vivo and in vitro, modifying plasma membrane structure and altering raft partitioning of this channel, suggesting the early reorganization of synaptic terminals as the mechanism to sensitizing dopaminergic neurons [151]. Paradoxically, SUMOylation of α-synuclein promoted its aggregation in COS-7 cells and had an intriguing protective effect [152].

Roles of SUMOylation outside the brain and effects of SUMO on other channels

Other than the brain, SUMOylation is well characterized in the heart. Both Ubc9 inhibition and SUMO-2 knockout caused early embryonic lethality in mice [2,153], whereas SUMO-1 knockout led to specific cardiac septal defects [154]. Activating the SUMOylation pathway can also evoke cardiac abnormalities, such as cardiac specific SUMO-2 overexpression that induced premature death and severe cardiomyopathy [155]. Conversely, SUMO-1 overexpression improved heart failure [154-156], suggesting that tightly regulated SUMOylation levels are essential for normal cardiac development [154,157].

SUMOylation also influences cardiac metabolism, controlling crucial proteins for the maintenance of cardiac energy homoeostasis and mitochondrial biogenesis, such as peroxisome proliferator-activated receptor (PPAR) and its associated co-regulators [158]. Similarly, under metabolic stress conditions, increased cellular SUMOylation (mainly by SUMO-2/3) can protect the brain during ischaemia or hibernation torpor [158-160]. Both in animal models and human patients, a fine balance between SUMO conjugation/deconjugation is critical for cardiac stress adaptation [155,156,161,162].

SUMOylation is not only essential for cardiac development, predominantly by regulating transcription factors, but also implicated in the onset of cardiac diseases [163-165]. Several K⁺ channels found in the heart can be modulated by SUMO, such as Kv2.1 [11,12], a channel that helps set the cell resting potential [166]; Kv1.5 [10], which controls excitability of atrial cells [167]; and K2P1 [3,6-9], which helps set resting membrane potential. SUMOylation also regulates the cardiac non-selective cationic channel TRPM4, which is localized predominantly in human atrial myocardin, and can act as a Ca²⁺ regulator [15,168]. Progressive familial heart block type I, an autosomal dominant disease, has been linked to a mutation in the TRPM4 amino-terminal region that leads to increased TRPM4 SUMOylation and prevention of its ubiquitination and consequent proteosomal degradation [15]. Other proteins crucial for
Table 1 Potential functional consequences of SUMOylation in Ca\(^{2+}\) signalling

| Target (direct or indirect) | SUMO isoform | Modified lysine | Mechanism or Ca\(^{2+}\) channel type | Proposed SUMOylation effect | Reference |
|----------------------------|--------------|-----------------|-------------------------------------|---------------------------|-----------|
| CaV2.1 subunit (indirect)  | SUMO-1       | Unknown         | Inhibition of P/Q-type Ca\(^{2+}\) channels | Role in SCA6 pathogenesis [124] |
| CAMKII (indirect)          | SUMO-1       | Unknown         |                                      | Differentiation of Drosophila’s nervous system [141] |
| CRMP2 (direct)             | SUMO-1, SUMO-2/3 | K374           | Inhibition of N-type Ca\(^{2+}\) channels | Reduces Ca\(^{2+}\) influx in sensory neurons [128] |
| MEF2 (direct)              | SUMO-1       | K403            | –                                   | Promotes dendritic claw differentiation [145,147] |
| NCX3 (direct)              | SUMO-1       | K590            | –                                   | Inhibits NCX3 degradation [85] |
| NFAT (indirect)            | SUMO-2       | Unknown         |                                      | Activates pro-hypertrophic genes [173] |
| RIM1\(\alpha\) (direct)    | SUMO-1       | K502            | Increase in P/Q-type Ca\(^{2+}\) channel activity | Promotes synaptic vesicles release [133] |
| SERCA2a (direct)           | SUMO-1       | K480 and K585   | –                                   | Increases Ca\(^{2+}\) reuptake to sarcoplasmic reticulum [156,177] |
| Synapsin Ia (direct)       | SUMO-1       | K687            | –                                   | Sets up releasable synaptic vesicles [138] |
| Synaptotagmin-1 (indirect) | SUMO-1       | Unknown         | –                                   | Impairs neurotransmitter release [139] |
| Syntaxin 1A (direct)       | SUMO-1       | K252, K253 or K256 | –                                   | Increases vesicular endocytosis [137] |

Abbreviations: CAMKII, Ca\(^{2+}\)/calmodulin-dependent protein kinase II; CRMP2, collapsin response mediator protein 2; MEF2, myocyte enhancer factor 2; NCX3, isoform 3 of the Na\(^+\)/Ca\(^{2+}\) exchanger; NFAT, N-terminal serine residues of the nuclear factor of activated T-cells; RIM1\(\alpha\), Rab3a-interacting molecule 1\(\alpha\); SERCA2a, isoform 2a of sarcoendoplasmic reticulum Ca\(^{2+}\) ATPase.

the maintenance of cardiomyocyte physiology, such as lamin A that plays a structural and functional role in the nucleus, are also reported to be SUMOylated [169,170]. Familial cardiomyopathy has been linked with mutations in the human laminin A gene, which were in turn associated with decreases in laminin A SUMOylation and accelerated cell death [169].

Disrupting Ca\(^{2+}\) dynamics by interfering with other proteins or transcriptional factors that maintain Ca\(^{2+}\) homeostasis, such as some of TRP protein Ca\(^{2+}\) entry channels or N-terminal serine residues of the nuclear factor of activated T cells (NFAT), can contribute to the onset of cardiac dysfunctions [171]. Increased intracellular Ca\(^{2+}\) levels activate calcineurin, a Ca\(^{2+}\)/calmodulin dependent serine–threonine protein phosphatase that dephosphorylates NFATs, leading to nuclear translocation of NFATs and activation of pro-hypertrophic genes [172]. SUMO-2 can activate calcineurin-NFAT signalling in cardiomyocytes leading to a hypertrophic phenotype, both in vitro and in vivo [173]. Unexpectedly, a conjugation-deficient SUMO-2 mutant (SUMO-2\(\Delta G\)) was equally capable to activate the pathway and promote hypertrophic effects, suggesting a SUMOylation-independent mechanism.

Proteins such as sarcoendoplasmic reticulum calcium ATPase (SERCA) in the sarcoplasmic reticulum and NCX in the cardiomyocyte membrane help to restore Ca\(^{2+}\) concentrations at baseline following contraction [174]. The reduced expression or activity of SERCA2a is a hallmark of heart failure [175]. A proteomic screen has identified SERCA2a as a target for SUMO-1 (but not SUMO-2/3) at lysines 480 and 585 [156]. SUMO-1 and SERCA2a protein levels were decreased in animal models of heart failure, as well as in human cardiomyocytes isolated from failing ventricles. SUMO-1 overexpression restored SERCA2a levels, whereas either SUMO-1 or SERCA2a overexpression improved Ca\(^{2+}\) handling, improving cardiac function. However, increased global SUMOylation in SERCA2a knockdown cardiomyocytes did not prevent contractile dysfunction, further confirming that SUMOylated SERCA2a is essential for cardiac function [156]. The small molecule N106 (N-(4-methoxybenzo[d]thiazol-2-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine) was identified using an \(\alpha\)-screen assay that detects SUMO-1 conjugation to nuclear RanGAP1 (the first and one of the most stable SUMO targets identified so far [176]). N106 promoted SERCA2a SUMOylation, resulting in enhanced contractility both in cultured cardiomyocytes and in vivo, significantly improving ventricular function in mice with heart failure [177]. N106 was proposed to directly activate the SUMO-activating enzyme [177].
Concluding remarks

Both alterations in Ca\(^{2+}\) homeostasis and protein SUMOylation may lead to severe neurological, and also, cardiac pathologies. For example, SUMOylation of proteins involved in Ca\(^{2+}\) signalling can modulate synapse formation and alter neurotransmitter release. Furthermore, SUMOylation of proteins can modulate Ca\(^{2+}\) reuptake in cardiomyocytes and thus affect contractility. As described above and summarized in Table 1, it is clear that a wide range of proteins involved in these key physiological processes are subject to, potentially temporal, post-translational modification by different SUMO isoforms. Thus, at the presynapse, proteins involved in Ca\(^{2+}\) homeostasis, including VGCCs and their proteome, are emerging as SUMO targets; equally, synaptic proteins involved in excytosis and endocytosis are known to be SUMOylated. Postsynaptic receptor SUMOylation can also impact synaptic function. There is clear potential to exploit this knowledge to improve synaptic function in neurodegenerative and hyperexcitability disorders and to improve cardiac function. Thus, understanding how SUMOylation affects Ca\(^{2+}\) signalling in physiological and pathophysiological conditions is key to novel therapeutic strategies to prevent and/or cure important human diseases.

Acknowledgements
Royal Society Newton Advanced and CNPq Fellowships together with IBRO and ISN/CAEN Return Home Awards to H.C. supported this work. L.C.S. is recipient of a CAPES MSc Studentship.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

\(\text{A}_\beta\), amyloid \(\beta\); AD, Alzheimer’s disease; APP, amyloid precursor protein; CNS, central nervous system; CRMP2, collapsin response mediator protein 2; Desi, deSUMOylating isopeptidase; DJ-1, PD (autosomal recessive, early onset) 7; DmSUMO-1, Drosophila SUMO-1; Drp1, dynamin-related protein 1; MEF2A, myocyte enhancer factor 2A; NCX, sodium/calcium exchanger; NFAT, N-terminal serine residues of the nuclear factor of activated T cells; PD, Parkinson’s disease; PPAR, peroxisome proliferator-activated receptor; PPF, paired pulse facilitation; RIM, Rab3a-interacting molecule; SCA, spinocerebellar ataxia; SERCA, sarcoendoplasmic reticulum calcium ATPase; SiM, SUMO interacting motif; SNARE, soluble N-ethylmaleimide sensitive factor attachment protein receptors; SUMO, small ubiquitin-like modifier; TRP, transient receptor potential; TRPM4, transient receptor potential cation channel subfamily M member 4; Ubc9, ubiquitin-like conjugating enzyme 9; USPL1, ubiquitin-specific peptidase-like protein 1; VGCC, voltage-gated calcium channel.

References

1 Hay, R.T. (2005) SUMO: a history of modification. Mol. Cell. 18, 1–12
2 Nacceridine, K., Lehembr, F., Bhamik, M., Artus, J., Cohen-Tannoudji, M., Babinet, C. et al. (2005) The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev. Cell 9, 769–779
3 Plant, L.D., Dementieva, I.S., Kellewe, A., Olikara, S., Marks, J.D. and Goldstein, S.A. (2010) One SUMO is sufficient to silence the dimeric potassium channel K2P1. Proc. Natl Acad. Sci. U.S.A. 107, 10743–10748
4 Silveirinha, V., Stephens, G.J. and Cimarosti, H. (2013) Molecular targets underlying SUMO-mediated neuroprotection in brain ischemia. J. Neurochem. 127, 580–591
5 Doyle, D.A., Morais-Cabral, J., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L. et al. (1998) The structure of the potassium channel: molecular basis of \(k_1\) conduction and selectivity. Science 280, 69–77
6 Rajan, S., Plant, L.D., Rabin, M.L., Butler, M. H. and Goldstein, S.A. (2005) Sumoylation silences the plasma membrane leak K\(^{+}\) channel K2P1. Cell 121, 37–47
7 Feliciangeli, S., Bendahhou, S., Sandoz, G., Gounou, P., Reichold, M., Warth, R. et al. (2007) Does sumoylation control K2P1/TWIK1 background K\(^{+}\) channels? Cell 130, 563–569
8 Es-Salah-Lamoureux, Z., Steele, D.F. and Fedida, D. (2010) Research into the therapeutic roles of two-pore-domain potassium channels. Trends Pharmacol. Sci. 31, 587–595
9 Plant, L.D., Zuniga, L., Araki, D., Marks, J.D. and Goldstein, S.A. (2012) SUMOylation silences heterodimeric TASK potassium channels containing K2P1 subunits in cerebellar granule neurons. Sci. Signal. 5, ra84
10 Benson, M.D., Li, O.J., Kieckhafer, K., Dudek, D., Whorton, M.R., Sunahara, R.K. et al. (2007) SUMO modification regulates inactivation of the voltage-gated potassium channel K\(\beta\)1.5. Proc. Natl Acad. Sci. U.S.A. 104, 1805–1810
11 Dai, X.Q., Kolic, J., Marchi, P., Sipione, S. and Macdonald, P.E. (2009) SUMOylation regulates K\(\beta\)2.1 and modulates pancreatic beta-cell excitability. J. Cell Sci. 122, 775–779
12 Plant, L.D., Dowdell, E.J., Dementieva, I.S., Marks, J.D. and Goldstein, S.A. (2011) SUMO modification of cell surface K\(\beta\)2.1 potassium channels regulates the activity of rat hippocampal neurons. J. Gen. Physiol. 137, 441–454
13 Qi, Y., Wang, J., Bomben, V.C., Li, D.P., Chen, S.-R., Sun, H. et al. (2014) Hyper-SUMOylation of the K_27 potassium channel diminishes the M-current leading to seizures and sudden death. *Neuron* **83**, 1159–1171

14 Dai, X.Q., Spigelman, A.F., Khan, S., Braun, M., Manning-Fox, J.E. and Macdonald, P.E. (2014) SUMO1 enhances cAMP-dependent exocytosis and glucagon secretion from pancreatic β-cells. *J. Physiol.* **592**, 3715–3726

15 Kruse, M., Schulze-Bahr, E., Corfield, V., Beckmann, A., Stallmeyer, B., Kurtbay, G. et al. (2009) Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. *J. Clin. Invest.* **119**, 2737–274

16 Walsh, C.T., Garneau-Tsodikova, S. and Gatto, Jr, G.J. (2005) Protein posttranslational modifications: the chemistry of proteome diversifications. *Angew. Chem. Int. Ed. Engl.* **45**, 7342–7372

17 Henley, J.M., Craig, T.J. and Wilkinson, K.A. (2014) Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction. *Physiol. Rev.* **94**, 1249–1258

18 Hendriks, I.A., D’Souza, R.C.J., Yang, B., Verlaan-de Vries, M., Mann, M. and Vergeela, A.C.O. (2014) Uncovering Global SUMOylation Signaling Networks in a Site-Specific Manner. *Nat. Struct. Mol. Biol.* **10**, 927–936

19 Hendriks, I.A. and Vergeela, A.C.O. (2016) A comprehensive compilation of SUMO proteomics. *Nat. Rev. Mol. Cell Biol.* **17**, 581–595

20 Johnson, E.S. (2004) Protein modification by SUMO. *Annu. Rev. Biochem.* **73**, 335–382

21 Hang, J. and Dasso, M. (2002) Association of the human SUMO-1 protease SENP2 with the nuclear pore. *J. Cell Sci.* **115**, 345–348

22 Sampson, D.A., Wang, M. and Matunis, M.J. (2001) The small ubiquitin-like modifier-1 (SUMO-1) consensus sequence mediates Ubc9 binding and is essential for SUMO-1 modification. *J. Biol. Chem.* **276**, 21664–21669

23 Sozen, S., Horozoglu, C., Bireller, E.S., Karaali, Z. and Cakmakoglu, B. (2014) Association of SUMO4 M55V and -94ins/del gene variants with type-2 diabetes. *In Vivo* **28**, 919–923

24 Sinha, N., Yadav, A.K., Kumar, V., Dutta, P., Bhansali, A. and Jha, V. (2016) SUMO4 163 G-A: A variation is associated with kidney disease in Indian subjects with type 2 diabetes. *Mol. Biol. Rep.* **43**, 345–348

25 Liang, Y.C., Lee, C.C., Yao, Y.L., Lai, C.C., Schmitz, M.L. and Yang, W.M. (2016) SUMO5, a novel poly-SUMO isoform, regulates PML nuclear bodies. *Sci. Rep.* **6**, 26509

26 Rodriguez, M.S., Dargemont, C. and Hay, R.T. (2001) SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. *J. Biol. Chem.* **276**, 12654–12659

27 Jardin, C., Anselm, H.C. and Sticht, H. (2015) Binding properties of SUMO-interacting motifs (SIMs) in yeast. *J. Mol. Model.* **21**, 50

28 Flotho, A. and Melchior, F. (2013) Sumoylation: a regulatory protein modification in health and disease. *Annu. Rev. Biochem.* **82**, 357–385

29 Gareau, J.R. and Lima, C.D. (2010) The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat. Rev. Mol. Cell Biol.* **11**, 861–871

30 Nayak, A. and Müller, S. (2014) SUMO-specific proteases/isopeptidases: SENPs and beyond. *Genome Biol.* **15**, 422

31 Hickey, C.M., Wilson, N.R. and Hochstrasser, M. (2012) Function and regulation of SUMO proteases. *Nat. Rev. Mol. Cell Biol.* **13**, 755–766

32 Gong, L., Millas, S., Maul, G.G. and Yeh, E.T. (2000) Differential regulation of senescent proteins by a novel sentrin-specific protease. *J. Biol. Chem.* **275**, 3355–3359

33 Martin, S., Nishimune, A., Mellor, J.R. and Henley, J.M. (2007) SUMOylation regulates kainate-receptor-mediated synaptic transmission. *Nature* **447**, 321–325

34 Loria, C., Parisot, J., Poupan, G., Guwak, C. and Martin, S. (2012) Developmental regulation and spatiotemporal redistribution of the sumoylation machinery in the rat central nervous system. *PLoS One* **7**, e33757

35 Loria, C., Khayachi, A., Poupan, G., Guwak, C. and Martin, S. (2013) Activity-dependent regulation of the sumoylation machinery in rat hippocampal neurons. *Biol. Cell* **105**, 30–45

36 Xu, Z. and Au, S.W. (2005) Mapping residues of SUMO precursors essential in differential maturation by SUMO-specific protease, SENP1. *Biochem. J.* **386**, 325–330

37 Gong, L. and Yeh, E.T. (2006) Characterization of a family of nucleolar SUMO-specific proteases with preference for SUMO-2 or SUMO-3. *J. Biol. Chem.* **281**, 15869–15877

38 Kolli, N., Mikolajczyk, J., Drag, M., Mukhopadhyay, D., Moffatt, N., Dasso, M. et al. (2010) Distribution and paralogue specificity of mammalian deSUMOylating enzymes. *Biochem. J.* **430**, 335–344

39 Hang, J. and Dasso, M. (2002) Association of the human SUMO-1 protease SENP2 with the nuclear pore. *J. Biol. Chem.* **277**, 19961–19966

40 Reverter, D. and Lima, C.D. (2004) A basis for SUMO protease specificity provided by analysis of human Senp2 and Senp2-SUMO complex. *Structure* **12**, 1519–1531

41 Guo, C., Hildick, K.L., Luo, J., Dearden, L., Wilkinson, K.A. and Henley, J.M. (2013) SENP3-mediated deSUMOylation of dynamin-related protein 1 promotes cell death following ischaemia. *EMBO J.* **32**, 1514–1528

42 Zunino, R., Schau, A., Rippstein, P., Andrade-Navarro, M. and McBride, H.M. (2007) The SUMO protease SENP5 is required to maintain mitochondrial morphology and function. *J. Cell Sci.* **120**, 1178–1188

43 Di-Bacco, A., Ouyang, J., Lee, H.Y., Catic, A., Ploegh, H. and Gill, G. (2006) The SUMO-specific protease SENP5 is required for cell division. *Mol. Cell Biol.* **26**, 4489–4498

44 Shen, L.N., Geoffroy, M.C., Jaffray, E.G. and Hay, R.T. (2009) Characterization of SENP7, a SUMO-2/3-specific isopeptidase. *Biochem. J.* **421**, 223–230

45 Lima, C.D. and Reverter, D. (2008) Structure of the human SENP7 catalytic domain and poly- SUMO deconjugation activities for SENP6 and SENP7. *J. Biol. Chem.* **283**, 32045–32055

46 Anderson, D.B., Zanella, C.A., Henley, J.M. and Cimarosti, H. (2017) Sumoylation: implications for neurodegenerative diseases. *Adv. Exp. Med. Biol.* **963**, 261–281
66 Dorval, V. and Fraser, P.E. (2006) Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein. J. Biol. Chem. 281, 9919–9924
67 Zhang, Y.Q. and Sarge, K.D. (2008) Sumoylation of amyloid precursor protein negatively regulates Abeta aggregate levels. Biochem. Biophys. Res. Commun. 374, 673–678
68 Geoffroy, M.C. and Hay, R.T. (2009) An additional role for SUMO in ubiquitin-mediated proteolysis. Nat. Rev. Mol. Cell Biol. 10, 564–568
69 Haass, C. and Selkoe, D.J. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid β-peptide. Nat. Rev. Mol. Cell Biol. 8, 101–112
70 Tritton, E., Vink, R., Blumbergs, P.C. and Heuvel, C.V.D. (2006) Soluble amyloid precursor protein α reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. Brain Res. 1094, 38–46
71 Harris, M.E., Wang, Y., Pedigo, Jr., N.W., Hensley, K., Butterfield, D.A. and Carney, J.M. (1996) Amyloid β peptide (25–35) inhibits Na⁺-dependent glutamate uptake in rat hippocampal astrocyte cultures. J. Neurochem. 67, 277–286
72 Li, Y., Wang, H., Wang, S., Quon, D., Liu, Y.W. and Cordell, B. (2003) Positive and negative regulation of APP amyloidogenesis by sumoylation. Proc. Natl. Acad. Sci. U.S.A. 100, 259–264
73 Dorval, V., Mazella, M.J., Mathews, P.M., Hay, R.T. and Fraser, P.E. (2007) Modulation of Abeta generation by small ubiquitin-like modifiers does not require conjugation to target proteins. Biochem. J. 404, 309–316
74 Yun, S.M., Cho, S.J., Song, J.C., Song, S.Y., Jo, S.A., Jo, C. et al. (2013) SUMO1 modulates Abeta generation via BACE1 accumulation. Neurobiol. Aging 34, 650–662
75 Weingarten, M.D., Lockwood, A.H., Hwo, S.Y. and Kirschner, M.W. (1975) A protein factor essential for microtubule assembly. Proc. Natl Acad. Sci. U.S.A. 72, 1858–1862
76 Selkoe, D. (2001) Alzheimer’s disease: genes, proteins, and therapy. Physiol. Rev. 81, 741–766
77 Cho, S.J., Yun, S.M., Lee, D.H., Jo, C., Ho-Park, M., Han, C. et al. (2015) Plasma SUMO-1 protein is elevated in Alzheimer’s disease. J. Alzheimers Dis. 56, 639–643
78 Lee, L., Dale, E., Staniszewski, A., Zhang, H., Saeed, F., Sakurai, M. et al. (2014) Regulation of synaptic plasticity and cognition by SUMO in normal physiology and Alzheimer’s disease. Sci. Rep. 4, 7110
79 McMillan, L.E., Brown, J.T., Henley, J.M. and Cimarosti, H. (2011) Profiles of SUMO and ubiquitin conjugation in an Alzheimer’s disease model. Neurosci. Lett. 502, 201–208
80 Nistico, R., Ferraina, C., Marconi, V., Blandini, F., Negri, L., Egebjerg, J. et al. (2014) Age-related changes of protein SUMOylation balance in the AbetaPP Tg2576 mouse model of Alzheimer’s disease’s front. Pharmacol. 5, 63
81 Binda, C.S., Heimann, M.J., Duda, J.K., Muller, M., Henley, J.M. and Wilkinson, K.A. (2017) Analysis of protein SUMOylation and SUMO pathway enzyme levels in Alzheimer’s disease and Down’s syndrome. Oper. Med. Phys. 3, 19–24
82 Eckermann, K. (2013) SUMO and Parkinson’s disease. Neuronmolecular Med. 15, 737–759
83 Guerra de Souza, A.C., Prediger, R.D. and Cimarosti, H. (2016) SUMO-regulated mitochondrial function in neurodegenerative diseases. J. Neurochem. 137, 673–686
84 Maroteaux, L., Campanelli, J.T. and Scheller, R.H. (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J. Neurosci. 8, 2804–2815
85 Golbe, L.I., Iorio, G., Di-Bonavita, V., Miller, D.C. and Duvoisin, R.C. (1990) A large kindred with autosomal dominant Parkinson’s disease. Ann. Neurol. 27, 276–282
86 Chandra, S., Fornai, F., Kwon, H.B., Yazdani, U., Asaso, D., Liu, X. et al. (2004) Double-knockout mice for α- and β-synucleins: effect on synaptic functions. Proc. Natl Acad. Sci. U.S.A. 101, 14966–14971
87 Chandra, S., Gallardo, G., Fernandez-Chacon, R., Schluter, O.M. and Sudhof, T.C. (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 123, 383–396
88 Oh, Y., Kim, Y.M., Mouradian, M.M. and Chung, K.C. (2011) Human polycomb protein 2 promotes alpha-synuclein aggregate formation through covalent SUMOylation. Brain Res. 1381, 78–89
89 Wong, M.J.L., Cook, A.L., Mackay-Sim, A. and Poutney, D.L. (2012) Differential SUMO-1 distribution in Parkinson’s disease patient neuropeptide-derived cells in response to proteolytic stress (Abstract). Proteostasis and Disease Symposium
90 Wong, M.B., Goodwin, J., Norazit, A., Medeniya, A.C., Richter-Landsberg, C., Meedeniya, A.C., Richter-Landsberg, C., Fernandez-Chacon, R., Schluter, O.M. and Sudhof, T.C. (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Science 299, 256–259
91 Taira, T., Saito, Y., Niki, T., Ichiguchi-Ariga, S.M., Takahashi, K. and Ariga, H. (2004) DJ-1 has a role in antioxidative stress to prevent cell death. EMBO Rep. 5, 213–218
92 Shinbo, Y., Niki, T., Taira, T., Ooe, H., Takahashi-Niki, K., Maia, C. et al. (2006) Proper SUMO-1 conjugation is essential to DJ-1 to exert its full activities. Cell Death Differ. 13, 96–108
93 Janer, A., Werner, A., Takahashi-Fujigasaki, J., Daret, A., Fujigasaki, H., Takada, K. et al. (2010) SUMOylation attenuates the aggregation propensity and cellular toxicity of the polyglutamine expanded ataxin-7. Hum. Mol. Genet. 19, 181–195
94 Lee, Y.J., Miyake, S., Waki, K., McMillan, D.C., Azuma, Y., Auh, S. et al. (2007) Protein SUMOylation is massively increased in hibernation torpor and is critical for the cytoprotection provided by ischemic preconditioning and hypothermia in SHSY5Y cells. J. Cereb. Blood Flow Metab. 27, 950–962
95 Cimarosti, H., Lindberg, C., Bomhoff, S.F., Ronn, L.C. and Henley, J.M. (2008) Increased protein SUMOylation following focal cerebral ischemia. Neuropharmacology 54, 280–289
Yang, W., Sheng, H., Horii, H.M., Warner, D.S. and Paschen, W. (2008) Cerebral ischemia/stroke and small ubiquitin-like modifier (SUMO) conjugation—a new target for therapeutic intervention? J. Neurochem. 106, 989–999

Sarge, K.D. and Park-Sarge, O.K. (2011) SUMO and its role in human diseases. Int. Rev. Cell Mol. Biol. 288, 167–183

Cimarrosto, H., Ashikaga, E., Jaafari, N., Dearden, L., Rubin, P., Wilkinson, K.A. et al. (2012) Enhanced SUMOylation and SENP-1 protein levels following oxygen and glucose deprivation in neurons. J. Cereb. Blood Flow Metab. 32, 17–22

Wilson, T.J., Slupe, A.M. and Strack, S. (2013) Cell signaling and mitochondrial dynamics: Implications for neuronal function and neurodegenerative disease. Neurobiol. Dis. 51, 13–26

Chang, C.R. and Blackstone, C. (2010) Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. Ann. NY Acad. Sci. 1201, 34–39

Molinaro, P., Cuomo, O., Pignataro, G., Boscia, F., Sirabella, R., Pannaccione, A. et al. (2008) Targeted disruption of Na+/Ca2+ exchanger 3 (NCX3) gene leads to a worsening of ischemic brain damage. J. Neurosci. 28, 1179–1184

Cuomo, O., Pignataro, G., Sirabella, R., Molinaro, P., Anzilotti, S., Scorziello, A. et al. (2016) SUMOylation of LYS90 of NCX3 f-Loop by SUMO-1 participates in brain neuroprotection induced by ischemic preconditioning. Stroke 47, 1085–1093

Dutting, E., Schroder-Kress, N., Sticht, H. and Enz, R. (2011) SUMO E3 ligases are expressed in the retina and regulate SUMOylation of the metabotropic glutamate receptor 8b. Biochem. J. 435, 365–371

Konopacki, F.A., Jaafari, N., Rocca, D.L., Wilkinson, K.A., Chamberlain, S., Rubin, P. et al. (2011) Agonist-induced PKC phosphorylation regulates GluK2 SUMOylation and kainate receptor endocytosis. Proc. Natl. Acad. Sci. U.S.A. 108, 19772–19777

Caraci, F., Battaglia, G., Sortino, M.A., Spampinato, S., Molinaro, G., Copani, A. et al. (2012) Metabotropic glutamate receptors in neurodegeneration/neuroprotection: Still a hot topic? Neurochem. Int. 61, 559–565

Zhu, Q.J., Xu, Y., Du, C.P. and Hou, X.Y. (2012) SUMOylation of the kainate receptor subunit GluK2 contributes to the activation of the MLK3-JNK3 pathway following kainate stimulation. FEBS Lett. 586, 1259–1264

Schorova, L. and Martin, S. (2016) SUMOylation in synaptic function and dysfunction. Front. Synaptic Neurosci. 8, 1–24

Wheeler, D.B., Randall, A. and Tsien, R.W. (1994) Roles of N-type and Q-type Ca2+ channels in supporting hippocampal synaptic transmission. Science 264, 107–111

Tanabe, T., Beam, K.G., Adams, B.A., Niidome, T. and Numa, S. (1990) Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. Nature 346, 567–569

Dolmetsch, R.E., Pajvani, U., Fife, K., Spotts, J.M. and Greenberg, M.E. (2001) Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science 294, 333–339

Wheeler, D.G., Groth, R.D., Ma, H., Barret, C.F., Owen, S.F., Safa, P. et al. (2012) Ca2+1 and Ca2+2 channels engage distinct modes of Ca2+ signaling to control CREB dependent gene expression. Cell 149, 1112–1124

Clapham, D.E. (2007) Calcium signaling. Cell 131, 1047–1058

Zamponi, G.W. (2016) Targeting voltage-gated calcium channels in neurological and psychiatric diseases. Nat. Rev. 15, 19–34

Hagiwara, S., Ozawa, S. and Sand, O. (1975) Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. J. Gen. Physiol. 65, 617–644

Bean, B.P. (1989) Classes of calcium channels in vertebrate cells. Annu. Rev. Physiol. 51, 367–384

Nowyczyk, M.C., Fox, C. and Tsien, R.W. (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 316, 440–443

Zamponi, G.W., Striessnig, J., Koschak, A. and Dolphin, A.C (2015) The pathology, physiology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol. Rev. 67, 821–870

Berridge, M.J. (2014) Ion channels. Cell Signal. Biol. 1–74

Felix, R. (2006) Calcium channelopathies. Neuromolecular Med. 8, 307–318

Lory, P. and Mezghrani, A. (2010) Calcium channelopathies in inherited neurological disorders: relevance to drug screening for acquired channel disorders. Drugs 13, 467–471

Oliveira, A.M., Bading, H. and Mauceri, D. (2014) Dysfunction of neuronal calcium signaling in aging and disease. Cell Tissue Res. 2, 381–383

Steinlein, O.K. (2014) Calcium signaling and epilepsy. Cell Tissue Res. 2, 385–393

Schäfer, M.K.E., Pfeiffer, A., Jaecckel, M., Povy, A., Dolga, A.M. and Mether, A. (2014) Regulators of mitochondrial Ca2+ homeostasis in cerebral ischemia. Cell Tissue Res. 357, 395–405

Bourinet, E., Altier, C., Hildebrand, M.E., Trang, T., Salter, M.W. and Zamponi, G.W. (2014) Calcium-permeable ion channels in pain signaling. Physiol. Rev. 94, 81–140

Waxman, S.G. and Zamponi, G.W. (2014) Regulating excitability of peripheral afferents: emerging ion channel targets. Nat. Neurosci. 17, 153–163

Miljanich, P.G. (2004) Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. Curr. Med. Chem. 11, 3029–3040

Rauck, R.L., Wallace, M.S., Burton, A.W., Kapural, L. and North, J.M. (2009) Intrathecal ziconotide for neuropathic pain: a review. Pain Pract. 9, 327–337

Smith, H.S. and Deer, T.R. (2009) Safety and efficacy of intrathecal ziconotide in the management of severe chronic pain. Ther. Clin. Risk Manag. 5, 521–534

Cizkova, D., Marsala, J., Lukacova, N., Marsala, M., Jergova, S., Orendacova, J. et al. (2002) Localization of N-type Ca2+ channels in the rat spinal cord following chronic constrictive nerve injury. Exp. Brain Res. 147, 456–463

Jagodic, M.M., Pathiratnna, S., Jokovic, P.M., Lee, W., Nelson, M.T., Naik, A.K. et al. (2008) Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve. J. Neurophysiol. 99, 3151–3156

© 2017 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
114 Marget, F., Gelot, A., Alloui, A., Matronic, J., Ferrer, J.F., Barrière, C. et al. (2011) T-type calcium channels contribute to colonic hypersensitivity in a rat model of irritable bowel syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11268–11273

115 Bezprozvanny, I. and Mattson, M.P. (2008) Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.* **31**, 454–463

116 Brawek, B. and Garaschuk, O. (2014) Network-wide dysregulation of calcium homeostasis in Alzheimer's disease. *Cell Tissue Res.* **357**, 427–438

117 Mezler, M., Barghorn, S., Schoemaker, H., Gross, G. and Nimmrich, V. (2012) A β-amyloid oligomer directly modulates P/Q-type calcium currents in Xenopus oocytes. *Br. J. Pharmacol.* **165**, 1572–1583

118 Hermann, D., Mezler, M., Müller, M.K., Wicke, K., Gross, G. and Draguhn, A. et al. (2013) Synthetic Aβ oligomers (Aβ(1–42) globulomer) modulate presynaptic calcium currents: prevention of Aβ-induced synaptic deficits by calcium channel blockers. *Eur. J. Pharmacol.* **702**, 44–55

119 Rush, T. and Buisson, A. (2014) Reciprocal disruption of neuronal signaling and Aβ production mediated by extrasynaptic NMDA receptors: a downward spiral. *Cell Tissue Res.* **356**, 279–286

120 Putzier, I., Kullmann, P.H., Horn, J.P. and Levitan, E.S. (2009) CaV1.3 channel voltage dependence, not Ca2+ selectivity, drives pacemaker activity and amplifies bursts in nigral dopamine neurons. *J. Neurosci.* **29**, 15414–15419

121 Surmeier, D.J. and Schumacker, P.T. (2013) Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. *J. Biol. Chem.* **288**, 10736–10741

122 Rcom'H-cheo-Gauthier, A., Goodwin, J. and Pournejad, D.L. (2014) Interactions between calcium and alpha-synuclein in neurodegeneration. *Biomolecules* **4**, 795–811

123 Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D.W., Amos, C. et al. (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat. Genet.* **15**, 62–69

124 Davila, M.A., Chan, H. and Pinto-Renteria, E.S. (2010) SUMOylation of voltage-gated alphaA1a calcium channels. *Biophys. J.* **98**, 692a–693a

125 Refigioni, M., Nishimune, A. and Henley, J.M. (2009) Protein SUMOylation modulates calcium influx and glutamate release from presynaptic terminals. *Eur. J. Neurosci.* **29**, 1348–1356

126 Müller, C.S., Haupt, A., Bildt, W., Schindler, J., Knaus, H.G., Meissner, M. et al. (2010) Quantitative proteomics of the Ca2+ channel nano-environments in the mammalian brain. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14950–14957

127 Brittain, J.M., Piekarz, A.D., Wang, Y., Kondo, T., Cummins, T.R. and Khanna, R. (2009) An atypical role for collapsin response mediator protein 2 (CRMP-2) in neurotransmitter release via interaction with presynaptic voltage-gated calcium channels. *J. Biol. Chem.* **284**, 31375–31390

128 Ju, W., Li, Q., Wilson, S.M., Brittain, J.M., Meroueh, L. and Khanna, R. (2013) SUMOylation alters CRMP2 regulation of calcium influx in sensory neurons. *Channels (Austin)* **7**, 153–159

129 Dustrude, E.T., Wilson, S.M., Ju, W., Xiao, Y. and Khanna, R. (2013) CRMP2 protein SUMOylation modulates Nav1.7 channel trafficking. *J. Biol. Chem.* **288**, 24316–24331

130 Dib-Hajj, S. D., Yang, Y. and Waxman, S.G. (2013) The Nav1.7 sodium channel: from molecule to man. *Nat. Rev. Neurosci.* **14**, 49–62

131 Deng, L., Kaeser, P.S., Xu, W. and Sudhof, T.C. (2011) RIM proteins activate vesicle priming by reversing autoinhibitory homodimerization of Munc13. *Neuron* **69**, 317–331

132 Kaeser, P.S., Deng, L., Wang, Y., Dulubova, I., Liu, X., Rizo, J. et al. (2011) RIM proteins tether Ca2+ channels to presynaptic active zones via a direct PDZ-domain interaction. *Cell* **144**, 282–295

133 Girach, F., Craig, T.J., Rocca, D.L. and Henley, J.M. (2013) RIM1α SUMOylation is required for fast synaptic vesicle exocytosis. *Cell Rep.* **5**, 1294–1301

134 Südhof, T.C. (2013) Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. *Neuron* **80**, 675–690

135 Xu, J., Luo, F., Zhang, Z., Xue, L., Wu, X.S., Chiang, H.C. et al. (2013) SNARE proteins synaptobrevin, SNAP-25, and syntaxin are involved in rapid and slow endocytosis at synapses. *Cell Rep.* **3**, 1414–1421

136 Zhang, Z., Wang, D., Sun, T., Xu, J., Chiang, H.C., Shin, W. et al. (2013) The SNARE proteins SNAP25 and synaptobrevin are involved in endocytosis at hippocampal synapses. *J. Neurosci.* **33**, 9169–9175

137 Craig, T.J., Anderson, D., Evans, A.J., Girach, F. and Henley, J.M. (2015) SUMOylation of syntaxin1A regulates presynaptic endocytosis. *Sci. Rep.* **5**, 17669

138 Tang, L.T., Craig, T.J. and Henley, J.M. (2015) SUMOylation of synapsin Ia maintains synaptic vesicle availability and is reduced in an autism mutation. *Nat. Commun.* **6**, 7728

139 Matsuzaki, S., Lee, L., Knock, E., Srikumar, T., Sakurai, M., Hazlai, L.N. et al. (2015) SUMO-1 affects synaptic function, spine density and memory. *Sci. Rep.* **5**, 10730

140 Lismann, J., Schulman, H. and Cline, H. (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev. Neurosci.* **3**, 175–190

141 Long, X. and Griffith, L.C. (2000) Identification and characterization of a SUMO-1 conjugation system that modifies neuronal calcium/calmodulin-dependent protein kinase II in *Drosophila melanogaster*. *J. Biol. Chem.* **275**, 40765–40776

142 Flavell, S.W., Cowan, C.W., Kim, T.K., Greer, P.L., Lin, Y., Paradis, S. et al. (2006) Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. *Science* **311**, 1008–1012

143 Hietakangas, V., Ancker, J., Blomster, H.A., Fujimoto, M., Palvimo, J.J., Nakai, A. et al. (2006) PSDM, a motif for phosphorylation-dependent SUMO modification. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 45–50

144 Kang, J., Gocke, C.B. and Yu, H. (2006) Phosphorylation-facilitated SUMOylation of MEF2C negatively regulates its transcriptional activity. *BMC Biochem.* **7**, 5

145 Riquelme, C., Barthel, K.K. and Liu, X. (2006) SUMO-1 modification of MEF2A regulates its transcriptional activity. *J. Cell. Mol. Med.* **10**, 132–144
175 Meyer, M., Schillinger, W., Pieske, B., Holubarsch, C., Heilmann, C., Posival, H. et al. (1995) Alterations of sarcoplasmic reticulum proteins in failing heart. Biochem. Biophys. Res. Commun. 208, 1388–1399.

180 Wang, L., Wansleeben, C., Zhao, S., Miao, P., Paschen, W. and Yang, W. (2014) SUMO-2 is essential while SUMO3 is dispensable for mouse embryonic heart. Circ. Res. 115, 686–689.

185 Kim, E.Y., Chen, L., Ma, Y., Yu, W., Chang, J., Moskowitz, I.P. et al. (2012) Enhanced deSUMOylation in murine hearts by overexpressed SENP2 leads to congenital heart defects and cardiac dysfunction. J. Mol. Cell. Cardiol. 52, 638–649.

190 Wang, J., Feng, X.H. and Schwartz, R.J. (2004) SUMO-1 modification activated protein TPase Ran/TC4 as an essential transport factor. J. Cell. Biol. 165, 2595–2608.

195 Rougier, J.S., Albesa, M. and Abrie, I.H. (2010) Ubiquitylation and SUMOylation of cardiac ion channels. J. Cardiovasc. Pharmacol. 56, 22–28.

200 Zhang, Y.Q. and Sarge, K.D. (2008) SUMOylation regulates lamin A function and is lost in lamin A mutants associated with familial cardiomyopathies. J. Cell Biol. 182, 35–39.

205 Broers, J.L., Ramaekers, F.C., Bonne, G., Yao, R.B. and Hutchison, C.J. (2006) Nuclear lamins: lamnipathies and their role in premature ageing. Physiol. Rev. 86, 967–1008.

210 Cartwright, E.J., Mohamed, T., Oceansy, D. and Neyes, L. (2011) Calcium signaling dysfunction in heart disease. Biofactors 37, 175–181.

215 Wilkins, B.J. and Molkentin, J.D. (2004) Calcium-calciurein signaling in the regulation of cardiac hypertrophy. Biochem. Biophys. Res. Commun. 322, 1178–1181.

220 Bernt, A., Rangez, A.Y., Eden, M., Jungmann, A., Katz, S., Rohr, C. et al. (2016) SUMOylation-independent activation of Calcineurin-NFAT signaling via SUMO-2 mediates cardiomyocyte hypertrophy. Sci. Rep. 6, 35758.

225 Woodcock, E.A. and Matkovich, S.J. (2005) Cardiomyocytes structure, function and associated pathologies. Int. J. Biochem. Cell Biol. 37, 1746–1751.

230 Meyer, M., Schillinger, W., Pieske, B., Holubarsch, C., Heilmann, C., Posival, H. et al. (1995) Alterations of sarcoplasmic reticulum proteins in failing heart. Biochem. Biophys. Res. Commun. 208, 1388–1399.

235 Melchior, F., Paschal, B., Evans, J. and Gerace, L. (1993) Inhibition of nuclear protein import by nonhydrolyzable analogues of GTP and identification of the small GTPase Ran/TC4 as an essential transport factor. J. Cell Biol. 123, 1649–1659.

240 Kho, C., Lee, A., Jeong, D., Oh, J.G., Gorski, P.A., Fish, K. et al. (2015) Small-molecule activation of SERCA2a SUMOylation for the treatment of heart failure. Nat. Commun. 7, 2229.