Trends in Cell & Molecular Biology
Vol. 7, 2012

Review

Diverse functions of neuronal calcium sensor-1 (NCS-1) in excitable cells

Tomoe Y. Nakamura* and Shigeo Wakabayashi

Department of Molecular Physiology, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan

ABSTRACT

Calcium (Ca^{2+}) is a versatile and important intracellular messenger, regulating a variety of cellular processes, including neurotransmission, muscle contraction, and signal transduction. A large family of EF-hand Ca^{2+}-binding proteins that includes the well-known calmodulin mediates the various Ca^{2+} functions. Neuronal calcium sensor-1 (NCS-1/frequenin) is another Ca^{2+}-binding protein that is mainly expressed in excitable cells. NCS-1 has been reported to interact with phosphatidylinositol 4-kinaseIII-β and several ion channels, thereby playing crucial roles in regulating neuronal functions, including synaptic transmission, learning and memory, and cell survival. Although NCS-1 is also highly expressed in young hearts, little was known about its cardiac functions until recently. By characterizing *Ncs1*-knockout mice, NCS-1 has recently been identified as a novel regulator of immature heart contraction and cardiac hypertrophy. Here, we will describe what is currently known about biochemical characteristics, multiple targets, and functional significance of NCS-1 in excitable cells, especially those found in brain and heart cells.

KEYWORDS: neuronal calcium sensor-1, NCS-1, frequenin, ion channels, phosphatidylinositol 4-kinase, inositol 1,4,5-trisphosphate receptor, exocytosis, synaptic plasticity, neuronal growth, neuronal survival, excitation-contraction coupling, immature heart, cardiac hypertrophy

*Corresponding author
tomoen@ri.ncvc.go.jp

ABBREVIATIONS

NCS-1 : neuronal calcium sensor-1
NCS : neuronal calcium sensor
VILIPs : visinin-like proteins
GCAPs : guanylate cyclase-activating proteins
KChIPs : potassium channel interacting proteins
PI-4-Kβ : phosphatidylinositol 4-kinase III-β
ER : endoplasmic reticulum
$InsP_3R$: inositol 1,4,5-trisphosphate receptor
IL1RAPL1 : interleukin-1 receptor accessory protein like-1
GDNF : glial cell line-derived neurotrophic factor
DRIPs : dopamine receptor-interacting proteins
SR : sarcoplasmic reticulum
CaMKII : Ca^{2+}/calmodulin-dependent protein kinase II
PI-3-K : phosphatidylinositol 3-kinase
NAIP : neuronal apoptosis inhibitory protein

INTRODUCTION

Calcium (Ca^{2+}) is a second messenger regulating a variety of cellular processes, such as signal transduction, regulation of gene and protein expression, and cell death. Therefore, it is important for the maintenance of cellular homeostasis in a variety of ways. In neurons, Ca^{2+} has a crucial role in neurotransmitter release, as well as short-term and long-term synaptic plasticity, which may

underlie a certain form of learning and memory. In the myocardium, Ca^{2+} is a key modulator not only for muscle contraction but also for gene transcription that eventually leads to cardiac remodeling. The various actions of Ca^{2+} are mediated by a large family of EF-hand Ca^{2+}-binding proteins that may act either as Ca^{2+}-sensors or Ca^{2+}-buffers. Upon binding to Ca^{2+}, Ca^{2+}-sensor proteins undergo large conformational changes and transduce distinct Ca^{2+} signals into various cellular function changes through target protein regulation, whereas Ca^{2+}-buffer proteins bind Ca^{2+} but do not undergo conformational change [1]. Calmodulin is the best studied prototypical example of a Ca^{2+}-sensor [2]; it is ubiquitously expressed and is involved in many aspects of Ca^{2+} signaling through controlling its target proteins, including protein kinases, phosphatases, and ion channels. However, many other EF-hand Ca^{2+}-binding proteins have been identified.

One subfamily of EF-hand Ca^{2+}-binding proteins, the neuronal calcium sensor (NCS) proteins (25%-35% sequence identity with calmodulin [3]), is predominantly expressed in neurons, heart cells, and retinal photoreceptors, suggesting that these proteins have specialized roles within each of these cell types. The NCS family includes neuronal calcium sensor-1 (NCS-1), visinin-like proteins (VILIPs), recoverin, guanylate cyclase-activating proteins (GCAPs), and potassium channel interacting proteins (KChIPs) (Table 1). Some members of this family, specifically recoverin and GCAPs, are only expressed in photoreceptor cells and are implicated in the control of visual transduction pathways through regulating rhodopsin kinase and guanylate-cyclase activity, respectively. VILIPs, another subfamily comprising VILIP-1, VILIP-2, VILIP-3/neurocalcin δ, and hippocalcin, are reported to have specialized functions in membrane trafficking of some receptors and ion channels, thereby affecting neuronal signaling and differentiation in defined subsets of neurons [4, 5]. They also play neuroprotective roles [6]. KChIPs (KChIP 1-4) are the most divergent subfamily and are only known to exist in mammalian species. They were discovered as specific regulators of a subclass of K^+ channels (the A-type K^+ channels) that control membrane excitability in the brain and heart. In addition, KChIP3, which is almost identical to the Ca^{2+}-dependent transcriptional repressor DREAM, regulates neuronal gene expression [7]; its involvement in Alzheimer's diseases [8, 9] has also been reported.

Neuronal calcium sensor-1 (NCS-1/mammalian homolog of frequenin) appears to be an ancestor of the larger NCS family, because it is expressed in and has a high degree of sequence conservation among species as diverse as yeast [10] and humans [3]. In *Drosophila melanogaster*, recent evidence indicates that frequenin is encoded by 2 genes, freq1 and freq2, which are 95% identical in amino acid sequence [11, 12]. In terms of function, NCS-1 regulates a variety of target proteins (Table 2): phosphatidylinositol 4-kinase III-β (PI-4-Kβ) [13], some voltage-gated ion channels [14-17], and the D2 dopamine receptor [18]. NCS-1 also plays an important role in neurotransmitter release [19], both short- and long-term synaptic plasticity [20, 21], learning and memory [22], neuronal survival [23], and nerve-terminal growth. NCS-1 is not only expressed in neuronal tissue but also in the heart [24-26], and recent evidence suggests that NCS-1 plays a crucial role in regulating immature heart function and hypertrophy [27].

Despite high sequence similarity and similar primary structures, NCS protein genes have distinctive expression patterns and thus perform distinct functions specific for the particular cell types they are expressed in [28]. Their functions also depend on the interacting proteins they associate with. In this review, we have summarized the current knowledge of NCS-1 in terms of its biochemical and biophysical properties, targets, and various functions in excitable tissues-especially those that have been reported recently; we also include a discussion on the conflicting data present in the current literature. We also describe in detail the physiological and pathological roles of NCS-1 in cardiac tissues. For more detail about other subjects regarding NCS-1 that are not covered in this review, readers can refer to these excellent reviews [3, 29-31].

Structure and localization of NCS-1

NCS-1 is a small (22kDa) Ca^{2+}-binding protein containing 4 EF-hand motifs; of these, 3 (EF2-4)

Table 1. Members of the NCS protein family and their tissue distribution.

Name of subfamily and its members	Main tissue distribution
NCS-1 (neuronal Ca^{2+} sensor-1/mammalian homolog of frequenin)	Entire brain Neuroendocrine cells Heart
VILIPs (visinin-like proteins) VILIP1, VILIP2, VILIP3 /neurocalcin, hippocalcin	Entire brain Heart, Lung, Pancreatic island etc…
Recoverin	Photoreceptor cells in the retina
GCAPs (guanylate cyclase-activating proteins) GCAP1, GCAP2, GCAP3	
KChIPs (potassium channel-interacting proteins) KChIP1, KChIP2, KChIP3/DREAM, KChIP4	Brain Heart

Table 2. Target proteins and suggested cellular functions of NCS-1 in excitable cells.

Target proteins	Effects	Suggested cellular functions	References
PI-4-Kβ	Activation	• Stimulation of exocytosis in neuroendocrine cells. • Increase in plasma membrane phosphoinositides • Facilitation of synaptic transmission in neurons • Enhancement of glucose-induced insulin secretion in pancreatic β cells • Enhancement of inflammatory reactions in mast cells	[13, 45-47] [13, 44] [41, 48] [51] [52, 53]
Voltage-gated Kv4 K^+ channel	Activation (increases the surface expression, and decreases the rate of inactivation)	• Increase in A-type currents in neurons, cardiomyocytes and zebra fish • Regulation of the duration, timing, and frequency of action potential	[14, 25, 60]
Voltage-gated Ca^{2+} channels	Inhibition	• Reduction of neurite elongation in PC12 cells • Inhibition of autocrine pathway in adrenal chromaffin cells	[62] [17]
	Activation	• Enhancement of neurotransmitter release • Activity-dependent synaptic facilitation • Enhancement of nerve-terminal growth	[16, 63] [15] [63]
D2 dopamine receptor	Activation (inhibits desensitization)	• Promotion of exploration, synaptic plasticity, rapid acquisition of spacial memory • Up-regulated in schizophrenia and bipolar disorder	[21] [65]
$InsP_3R$	Activation	• Enhancement of Ca^{2+} -signaling, related to bipolar disorder • Reduction of neurite outgrowth • Related to taxol-induced Ca^{2+}-oscillation and neuropathy • Involved in metabotropic glutamate receptor-mediated long-term depression • Promotes immature heart function • Regulation of cardiac hypertrophy	[71] [72] [73-75] [76] [27] [27]
unknown		• Neuronal survival	[23]

Figure 1. Schematic structure of NCS-1.

bind to Ca^{2+} within physiological Ca^{2+} concentration ranges [3] (Figure 1). Bacterially expressed NCS-1 induces Ca^{2+}-mediated large conformational changes [32]. High-affinity Ca^{2+} binding *in vitro* is observed in this model, which reaches half-maximal binding below 500 nM free Ca^{2+} and is co-operative with a Hill coefficient value of approximately 2 [33]. Calmodulin, on the other hand, demonstrated several-fold lower affinity for Ca^{2+} binding. Thus, NCS-1 is able to sense small, incremental changes in intracellular Ca^{2+} levels within a range that would only activate a small proportion of calmodulin.

Structurally, NCS-1 is N-terminally myristoylated, which is critical for membrane association [32]. Based on structural and biochemical studies performed with recoverin, one model for the Ca^{2+}-induced conformational change proposes that exposure of the myristoyl group induces a "Ca^{2+}-myristoyl switch" [34]. This model suggests that Ca^{2+} binding to cytosolic recoverin leads to a large conformational change, followed by extrusion of the myristoyl group previously buried within a hydrophobic pocket, and finally resulting in the translocation and myristoyl-dependent association of Ca^{2+}-bound recoverin with the plasma membrane. It has long been thought that NCS-1 may not possess a functional myristoyl switch [32, 35], because NCS-1 appears to be localized to membranes even at low Ca^{2+} levels [32], and NMR structural studies on NCS-1 (from budding yeast) suggested that the myristoyl group of the myristoylated NCS-1 remains exposed to the solvent, regardless of Ca^{2+} level [36]. However, the NMR structures of Ca^{2+}-free myristoylated NCS-1 homolog from fission yeast (Ncs1) was recently compared with an Ca^{2+}-bound Ncs1 protein that was complexed to a PI-4-Kβ fragment (one of the NCS-1 target proteins, see below) [37]. This analysis indicates that NCS-1 also possesses a Ca^{2+}-myristoyl switch mechanism, similar to recoverin [37]. In Ca^{2+}-free Ncs1, the N-terminal arm positions the fatty-acyl chain inside a cavity near the C terminus. In Ca^{2+}-bound Ncs1, the myristoyl group is extruded (Ca^{2+}-myristoyl switch), exposing a prominent patch of hydrophobic residues that specifically contact the target protein. These authors speculated that some results from previously studied subcellular localization and cellular roles of NCS family members might be misleading because they may have been compromised by the use of tagged derivatives that may have caused significant structural perturbations during experimentation [37]. Although both recoverin and NCS-1 have the Ca^{2+}-myristoyl switch mechanism, the myristoylated NCS-1 structure (both Ca^{2+}-bound and Ca^{2+}-unbound forms) is quite different from those of recoverin [37]. The structural difference in this myristoylated domain between the related proteins is in contrast to the unmyristoylated structures, which are all similar among NCS proteins. These observations suggest that myristoylation remodels NCS proteins in a way that is unique for each individual NCS protein. They further suggest that the Ca^{2+}-dependent unmasking of different residues unique to each NCS protein may explain how each family member can recognize distinct target proteins, despite a high degree of sequence similarity.

Although a small amount of NCS-1 expression is found in almost all tissues, high levels are detected in all neuronal tissues, neuroendocrine cells, and in the cardiac cells of the heart [3, 24, 27]. Depending on the particular cell type, subcellular localization analysis revealed that a large proportion of NCS-1 proteins are localized to the membranous regions, such as the Golgi complex [38], the Ca^{2+}-storage organelle endoplasmic reticulum (ER), and the plasma membrane [27, 39, 40], in addition to the cytosol. A small amount of NCS-1 is also detected in synaptic and secretory vesicles [40].

Target proteins and function of NCS-1 in neuronal tissues

NCS-1 was originally identified in *Drosophila* during a screen for neuronal hyper-excitability in V7 mutants [41] that naturally overexpressed NCS-1 protein. Indeed, NCS-1 overexpression in *Drosophila* [19] and in *Xenopus* spinal neurons

[42] facilitates neurotransmission. NCS-1 is also present in adrenal chromaffin and PC12 neuroendocrine cells, and its overexpression has been shown to increase Ca^{2+}-regulated exocytosis of growth hormones [43]. However, the mechanism(s) by which NCS-1/frequenin regulates such vesicular release was unclear. A wide array of binding partners has been identified for NCS-1/frequenin; some of them suggest possible mechanisms for NCS-1 function on neurotransmission, exocytosis, and modulation of membrane excitability. These include interactions with PI-4-Kβ, a voltage-gated K^+ channel, several voltage-gated Ca^{2+} channels (P/Q and N-type), D2 dopamine receptor, inositol 1,4,5-trisphosphate receptor ($InsP_3R$), TRPC1/5 channels, and interleukin-1 receptor accessory protein like-1 (IL1RAPL1).

We have summarized various target proteins and functions of NCS-1 in excitable cells in Table 2.

a) Phosphatidylinositol 4-kinase

Phosphatidylinositol 4-kinase III-β (PI-4K-β) catalyzes the synthesis of phosphatidylinositol 4-phosphate, which is a late limiting step to generate phosphatidylinositol 4,5-bisphosphate, an important lipid regulator of several cellular functions including intracellular vesicle trafficking, exocytosis, and ion channel regulation. A yeast homolog of NCS-1 was initially reported to interact with and activate yeast PI-4-K, both of which are indispensable for yeast survival [10]. This interaction is also detected in neuroendocrine PC12 cells, where it is implicated to enhance basal and stimulated exocytosis in these cells [13, 44-46]. Furthermore, NCS-1 overexpression also increased membrane phosphoinositide levels [13, 45]. Since NCS-1 enhanced the phospholipase C-coupled receptor agonist (UTP)-evoked exocytosis, but not the depolarization-evoked Ca^{2+} responses, phospholipase C-linked receptor-mediated Ca^{2+} signals rather than the exocytotic machinery itself was suggested to enhance NCS-1-mediated exocytosis [13]. Receptor stimulation-induced intracellular Ca^{2+} rise was also reported to enhance the recruitment of NCS-1 and PI-4-Kβ from the intracellular compartment toward the plasma membrane [46], further enhancing NCS-1-mediated exocytosis.

This NCS-1 and PI-4-Kβ association was also detected in neurons [40, 47] and was shown to facilitate neuronal exocytosis. However, this contradicted an earlier study reporting that no direct interaction was detected between these two proteins in neurons [48]. This contradiction may be explained by the existence of newly discovered PI-4-Kβ regulators, calneuron-1 and calneuron-2. While calneurons interact with PI-4-Kβ at low Ca^{2+} levels to inhibit PI-4-Kβ enzyme activity, NCS-1 binds to PI-4-Kβ at high Ca^{2+} levels to activate it [49]. These results indicate that calneurons and NCS-1 compete for PI-4-Kβ interaction depending upon intracellular Ca^{2+} levels; therefore, interactions between NCS-1 and PI-4-Kβ might be difficult to detect at low intracellular Ca^{2+} levels. Activation of PI-4-Kβ by NCS-1 was also demonstrated in other cell types: in pancreatic beta cells, it regulates glucose-induced insulin secretion [50]; in mast cells, it controls regulated exocytosis and inflammatory reactions [51, 52].

As described above, the NMR structure of Ca^{2+}-bound yeast NCS-1 (Ncs1) complexed to an N-terminal yeast PI-4-K (Pik1) fragment was recently determined. From this binding evidence, a possible mechanism of PI-4-Kβ activation by NCS-1 was proposed [53]: in the presence of Ca^{2+}, Ncs1 binding induces a U-shaped helix-loop-helix structure in Pik1 (amino acids 121-174) that may permit a functional interaction between the N-terminal lipid kinase unique (LKU) motif and the C-terminal catalytic domain of Pik1 that are both necessary for its lipid kinase activity. The structure of the Ncs1-Pik1 complex reveals that the N-terminal myristoyl group of Ncs1 is exposed and is thus able to recruit the Ncs1-Pik1 complex to membranes. Thus, Ncs1 may activate Pik1 by facilitating membrane targeting via the exposed *N*-myristoyl group and by imposing a structural transition that promotes association of the LKU motif with the kinase domain.

b) Voltage gated K^+ channel

Experiments with the NCS-1-overexpressing V7 *Drosophila* mutant suggested a role for NCS-1 in neurotransmission, possibly by affecting ion channel activity [19, 54]. Indeed, we previously demonstrated that NCS-1 is a Ca^{2+}-sensitive regulatory component important in regulating the

native K^+ current [14]. In the brain and heart, rapidly inactivating (A-type) voltage-gated K^+ currents operate at sub-threshold membrane potentials to control the excitability of neurons and cardiac myocytes. Although the pore-forming alpha-subunits of these channels are thought to be Kv4 channels [55, 56, 57], their kinetic properties differ significantly from native A-type currents; therefore, Kv4 channels are thought to have regulatory beta-subunits that may alter channel kinetics. As one such a regulatory subunit, KChIPs (an NCS protein subfamily) were initially reported to specifically interact with and modulate Kv4.2 and Kv4.3 currents [58].

Similar to KChIPs, NCS-1 has been shown to modulate Kv4 currents [14]; this was the first demonstration that NCS-1 could modulate cloned ion channels. NCS-1 increases Kv4.2 current amplitudes, partly by enhancing surface expression of Kv4.2 proteins. In addition, NCS-1 slows the inactivation rate of Kv4.2 currents in a Ca^{2+}-dependent manner. The interaction between NCS-1 and Kv4 channels is fairly specific, as more closely related NCS proteins, such as VILIP and neurocalcin, had no such effect on Kv4.2 proteins. Furthermore, NCS-1 also does not modulate other voltage-gated rapidly inactivating currents Kv1.4 or Kv3.4. Indeed, NCS-1 and Kv4.2 channel proteins are co-immunoprecipitated and co-localized in adult mouse brain, demonstrating that NCS-1 is one of the regulators of A-type K^+ currents in neuronal tissues. Furthermore, such interactions were also present in pyloric neurons in the lobster [59] and in zebra fish [60], where no KChIPs exist. The functional importance of this interaction in *in vivo* tissues should be further investigated, possibly using the Ncs1-knockout mice that are now available [27].

c) Voltage-gated Ca^{2+} channels

Numerous reports demonstrate that NCS-1 regulates voltage-gated Ca^{2+} channel function. However, the reported effects of NCS-1 on Ca^{2+} channels were diverse, with some studies showing positive regulation and others showing negative regulation. NCS-1 inhibits Ca^{2+} channels in the following examples: in neuroendocrine cells such as adrenal chromaffin cells, NCS-1 inhibits P/Q-type Ca^{2+} channels [17, 61]; in PC12 cells, NCS-1 overexpression results in an N-type Ca^{2+} channel loss-of-function leading to the reduction of neurite elongation [62]. In contrast, NCS-1 can activate Ca^{2+} channels, according to the following studies. In neurons, NCS-1 was reported to mediate the glial cell line-derived neurotrophic factor (GDNF)-induced enhancement of neurotransmitter release through the activation of an N-type Ca^{2+} channel [16]. NCS-1 also contributes to activity-dependent synaptic facilitation by P/Q-type Ca^{2+} channel activation in the nerve terminal [15]. In addition, a recent report using an NCS-1 null mutant in *Drosophila* suggests that NCS-1 promotes Ca^{2+} entry through a functional interaction with the alpha-subunit of voltage-gated Ca^{2+}-channel (but not through interaction with PI-4-K); this interaction enhances neurotransmission and nerve-terminal growth [63]. Overall, the mechanisms for the diverse effects of NCS-1 on several voltage-gated Ca^{2+} channels are presently unknown. However, we can speculate that NCS-1 negatively regulates Ca^{2+} channels in neuroendocrine cells while positively regulating the same channels in neurons. The modulatory effects of NCS-1 are reported to be specific for the beta-subunits of Ca^{2+} channels [64]. In addition, other NCS-1 interacting proteins, such as IL1RAPL1, are reported to regulate the N-type Ca^{2+} channel by cooperating with NCS-1 [62]. Thus, the type of Ca^{2+} channel beta-subunits or other known and/or unknown factors differentially expressed between neuroendocrine cells and neurons may explain the diverse effect of NCS-1 on voltage-gated Ca^{2+} channels.

d) D2 dopamine receptor

Abnormal activity of the dopamine system has been implicated in several psychiatric and neurological illnesses. Studies suggest that dopamine transmission is regulated via dopamine receptor-interacting proteins (DRIPs), which includes NCS-1, calcyon, and DARPP-32. NCS-1 has been found to interact with D2 dopamine receptor, reducing D2 receptor phosphorylation and attenuating agonist-induced receptor internalization [18]. Thus NCS-1 preserves the D2 receptor signaling. Subsequently, NCS-1 was reported to be upregulated in the prefrontal cortex of schizophrenic and bipolar patients [65, 66]. Since the levels of other DRIP members were also

altered in schizophrenic patients (calcyon was increased [66] whereas DARPP-32 was decreased [67]), DRIP signaling is suggested to be involved in psychiatric illness. Indeed, modest over-expression of NCS-1 in the adult murine dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory. All these effects are reversed by a cell-permeant peptide (DNIP) designed to interfere with NCS-1 binding to the D2 receptor. These results demonstrate that NCS-1 interaction with D2 receptor plays a role in the synaptic plasticity of the dentate gyrus [21]. However, several reports demonstrated that typical or atypical anti-psychotics had no effect on the NCS-1 or DARPP-32 expression levels, either in the rat brain [67] or in PC12 cells overexpressing NCS-1 [68]. These results suggest that the altered levels of DRIPs observed in the prefrontal cortex of schizophrenic patients is not related to anti-psychotic treatment, but may instead be related to psychopathology. Recent biochemical analysis using NMR or fluorescence anisotropy further support the specific interaction between NCS-1 and D2 dopamine receptor; a short 16-residue C-terminus of D2 receptor binds to NCS-1 [69], and the first 60 residues of NCS-1 binds to the D2 receptor [70] (although the data measuring binding stoichiometry between these two proteins is conflicting). Knowledge of these interaction domains may present a targeting opportunity in the future to screen for drugs that can specifically block the interaction between NCS-1 and the D2 receptor.

e) Inositol 1,4,5-trisphosphate receptor ($InsP_3R$)

Regulation and dysregulation of intracellular Ca^{2+} signaling via $InsP_3R$ has been linked to many cellular processes and pathological conditions. NCS-1 immunoprecipitated and co-localized with $InsP_3R1$ in the rat brain, as well as enhanced the Ca^{2+}-signaling in intact cells. Indeed, addition of NCS-1 to purified $InsP_3R1$ in the lipid bilayer increased $InsP_3R$ channel activity in both a Ca^{2+}- and $InsP_3$-dependent manner [71]. These results indicate that NCS-1 directly promotes the activity of $InsP_3R$ channels and enhances $InsP_3$-dependent Ca^{2+}-signaling. Since lithium inhibited the enhancing effect of NCS-1 on $InsP_3R1$ function, and we know that lithium is widely used for treatment of bipolar disorders, the $InsP_3R1$/NCS-1 interaction has been suggested to be an essential pathomechanism component of bipolar disorder [71]. Previous studies demonstrated that NCS-1 was distributed throughout the growth cone region located at the neurites in cultured chick neurons; it also co-localized with $InsP_3R1$ in these cells. Both pharmacological inhibition of $InsP_3R$ and loss of NCS-1 function in the growth cone inhibited neurite outgrowth, suggesting a functional link between the NCS-1 and $InsP_3R$ interaction and its involvement in neurite outgrowth [72]. The importance of the NCS-1/$InsP_3R$ interaction in neuropathy was also suggested in a report investigating the mechanism behind the side effects experienced with paclitaxel (taxol) treatment, a microtubule-stabilizing chemotherapeutic agent used to treat solid cancers [73-75]. Taxol interacts with NCS-1; acute application of taxol increases NCS-1 binding to $InsP_3R$ and induces Ca^{2+}-oscillation in a neuronal cell line, which is independent of taxol's microtubule-stabilizing effect [73]. In contrast, prolonged exposure to taxol activates calpain, which leads to the NCS-1 degradation, particularly at the N-terminal pseudo EF hand domain; in turn, this calpain-induced degradation attenuates $InsP_3$-mediated Ca^{2+} signaling [74, 75]. These results provide a previously undescribed approach to understanding and treating taxol-induced peripheral neuropathy. Furthermore, the important role of NCS-1 and $InsP_3$ in metabotropic glutamate receptor-mediated long-term depression (LTD) was also reported [76].

f) TRPC5

NCS-1 also interacts with the C-terminal part of the transient receptor potential channel TRPC5. NCS-1 has positive impacts on TRPC5, thus also positively impacting Ca^{2+} influx [77]. Although this interaction stimulates Ca^{2+} influx, it inhibits neurite outgrowth, which is consistent with previous reports for both TRPC5 [78] and NCS-1 [79].

NCS-1 as a survival factor in neurons

NCS-1 also has an important role in neuronal survival, especially under stressed conditions [23]. Numerous stressors, including physical or chemical injury and genetic abnormalities, lead to

neuronal degeneration. This may underlie many human neurodegenerative disorders, such as Alzheimer's and Parkinson's disease. Several intrinsic and extrinsic factors, including neurotrophic factors, can activate the antiapoptotic process to rescue neurons from death under these conditions. However, the signaling pathway leading to cell survival is not yet completely understood.

While NCS-1 overexpression rendered cultured neurons more tolerant to cell death induced by several kinds of stressors, such as oxidative stress and trophic factor withdrawal, the dominant-negative EF-hand mutant (E120Q) accelerated the cell death, suggesting that endogenous NCS-1 is important for the survival of cultured neurons. These results further suggest that Ca^{2+} binding is required for NCS-1-mediated cell survival. In addition, NCS-1 proteins increased upon neurotrophic factor GDNF treatment and mediated GDNF survival signals in an Akt-dependent manner. Furthermore, NCS-1 is significantly upregulated in the affected neurons of adult rats in response to axotomy-induced injury *in vivo*, and adenoviral overexpression of E120Q resulted in a significant loss of surviving neurons, suggesting that NCS-1 is involved in an antiapoptotic mechanism in adult motor neurons. Based on these results, NCS-1 has been proposed to be a novel survival-promoting factor, upregulated in injured neurons, which mediates the GDNF survival signal via the phosphatidylinositol-3-kinase (PI-3-K)-Akt pathway [23].

Although the precise mechanism underlying the NCS-1-mediated neuronal survival is presently unknown, possible target proteins are suggested. Since NCS-1 activates PI-4-Kβ, which could increase the phospholipid level of phosphatidylinositol 4-phosphate, leading to production of the PI-3-K substrates phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-triphosphate. These substrates can then activate the PI-3-K/Akt pathway for survival. Another possible mechanism could involve NCS-1's ability to activate voltage-gated K^+ channels [14]. In this scenario, NCS-1-induced increase of outward K^+ current would prevent neurons from reaching their firing threshold and thereby prevent the Ca^{2+} overload that normally leads to cell death. It is also interesting to note that another closely related Ca^{2+}-sensor, hippocalcin, protects neurons against Ca^{2+}-induced cell death [6] by interacting with neuronal apoptosis inhibitory protein (NAIP) and preventing apoptosis partly through inhibiting caspase activation. Since NAIP is upregulated by GDNF and is thought to be essential for GDNF-mediated neuroprotective effects in injured neurons *in vivo* [80], a similar mechanism might exist between the analogous NCS-1- and hippocalcin-mediated neuronal survival mechanisms.

NCS-1 roles in heart

Although NCS-1 is expressed at high levels in neurons and neuroendocrine cells (which explains the origin of the name "neuronal calcium sensor-1"), we and others detected that NCS-1 is also expressed in the heart. It is expressed at especially high levels in immature-stage cardiomyocytes [24, 27]. As demonstrated in neurons [14], NCS-1 interacts with the voltage-gated K^+ channel Kv4, one of molecular components of the transient outward K^+ currents (I_{to}) in adult mouse ventricular cells [25]. Co-expression studies in HEK293 cells revealed that NCS-1 increases both membrane expressions of Kv4 channel proteins and functional Kv4-encoded K^+ current densities. It also decreases the inactivation rate of Kv4 K^+ currents. Based on these results, NCS-1 is suggested to be another regulator of I_{to} in the heart, in addition to KChIPs are. The relationship between NCS-1 and KChIPs for modulation of Kv4 channels *in vivo* should be investigated.

In addition, NCS-1 is reported to be contribute to taxol-induced cardiac arrhythmia [26], based on the following evidence. Taxol is used for cancer chemotherapy but is known to have serious side effects, including cardiac arrhythmia. As described above, taxol is also a binding partner of NCS-1 [73]. Treatment of cardiomyocytes with taxol increases the spontaneous calcium oscillation frequency in an $InsP_3R$-dependent manner. Since NCS-1interacts with cardiac IP_3Rs, it is suggested that taxol-induced calcium oscillation occurs by a mechanism involving NCS-1-mediated activation of $InsP_3R$ [26].

Despite the above findings, little evidence was available to support the physiological and pathological roles of NCS-1 in cardiac functions

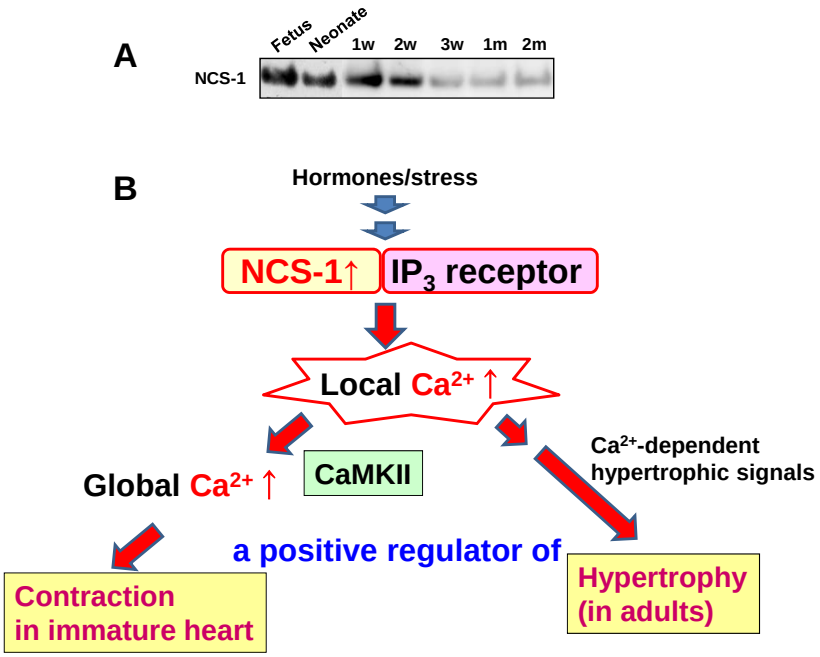

Figure 2. Role of NCS-1 in the regulation of immature heart contraction and hypertrophy.
A: Western blot showing that the expression level of NCS-1 is higher in the immature heart compared to that in adult heart. **B:** NCS-1-IP_3R interaction increases local Ca^{2+} signals, which serves as a Ca^{2+} source for the activation of CaMKII. This leads to a higher rate of SR Ca^{2+} pumping and release and induces global Ca^{2+} signaling, thus promoting contraction in immature hearts. Increase of local Ca^{2+} signals also activates some Ca^{2+}-dependent hypertrophic signals and promotes hypertrophy.

until recently, possibly because its expression is relatively low in the adult heart. Since high expression levels of NCS-1 were previously detected in immature hearts [24] and hypertrophic hearts (unpublished observation) that were comparable to the levels in neurons, NCS-1 was hypothesized to have a specific role in cardiac function, particularly at the immature stage and during progression of hypertrophy. To test these hypotheses, our group recently identified NCS-1 function in the heart by characterizing *Ncs1*-knockout (*Ncs1*$^{-/-}$) mice [27]. *Ncs1*$^{-/-}$ mice had a high mortality rate at the neonatal stage (30% of the pups died within 4 days after birth), and the surviving mice had considerably diminished ventricular function at a young age (2 weeks of age), but not in the adult (6 weeks of age or older). Intracellular Ca^{2+} levels and sarcoplasmic reticulum Ca^{2+} content were significantly lower in *Ncs1*$^{-/-}$ myocytes than in wild-type cells. In the neonatal mouse heart, the structure and function of sarcoplasmic reticulum (SR) is immature; nonetheless, it is considered a primary source of Ca^{2+} necessary for muscle contraction, suggesting the existence of factors missing at this developmental time point that promote SR-dependent excitation-contraction coupling in the postnatal stages. We found that NCS-1 interacted with $InsP_3R$ in the hearts and increased $InsP_3R$-dependent Ca^{2+} signaling. This local Ca^{2+} increment then activated Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)-dependent pathways, which phosphorylated phospholamban, a regulator of the SR Ca^{2+} pump, and resulted in a large increase in the SR Ca^{2+} content. This increased global Ca^{2+}-signals and consequently promoted contraction in young hearts (Figure 2). The importance of NCS-1, $InsP_3Rs$ and CaMKII in immature hearts was demonstrated by the observation that all these three proteins are expressed at high levels in young hearts. In addition, NCS-1 expression increased during the early stages of hypertrophy in the adult heart and promoted progression of hypertrophy, at least in part, through IP_3R activation (Figure 2). These

results demonstrate that NCS-1 is a novel regulator of immature heart contraction and of hypertrophy in adult hearts. Our results also reveal a previously unrecognized mechanism of excitation-contraction coupling in young hearts as well as another regulatory mechanism for the progression of receptor stimulation-elicited cardiac hypertrophy.

CONCLUSION

In this review, we summarized the most recent advances in the structure, function, and physiological roles of NCS-1. NCS-1 is a Ca^{2+}-dependent signal mediator that regulates a variety of target proteins, such as enzymes and ion channels, likely via a Ca^{2+}-myristoyl switch mechanism. A recent advance in the NCS-1 field demonstrates the importance of NCS-1 on immature and hypertrophic adult heart function, as NCS-1 gene disruption resulted in reduced contractile activity in immature hearts and abrogated hormone-induced cardiac remodeling. Further elucidation of the physiological roles of NCS-1 in other excitable tissues will likely be revealed using *Ncs1*-knockout mice in future studies. Given the discovery of multiple interacting proteins of NCS-1, the therapeutic potential of developing strategies to specifically block the interaction between NCS-1 and its relevant target proteins is high. We hope that this review will provide thought-provoking opportunities and compel our readers to ask more questions and extend their studies on NCS-1 and other important regulatory proteins.

REFERENCES

1. Ikura, M. 1996, Trends Biochem. Sci., 21, 14.
2. Chin, D. and Means, A. R. 2000, Trends Cell Biol., 10, 322.
3. Burgoyne, R. D. and Weiss, J. L. 2001, Biochem. J., 353, 1.
4. Ivings, L., Pennington, S. R., Jenkins, R., Weiss, J. L. and Burgoyne, R. D. 2002, Biochem. J., 363, 599.
5. Braunewell, K. H. and Klein-Szanto, A. J. 2009, Cell Tissue Res., 335, 301.
6. Mercer, E. A., Korhonen, L., Skoglosa, Y., Olsson, P. A., Kukkonen, J. P. and Lindholm, D. 2000, EMBO J., 19, 3597.
7. Carrion, A. M., Link, W. A., Ledo, F., Mellstrom, B. and Naranjo, J. R. 1999, Nature, 398, 80.
8. Buxbaum, J. D., Choi, E. K., Luo, Y., Lilliehook, C., Crowley, A. C., Merriam, D. E. and Wasco, W. 1998, Nat. Med., 4, 1177.
9. Leissring, M. A., Yamasaki, T. R., Wasco, W., Buxbaum, J. D., Parker, I. and LaFerla, F. M. 2000, Proc. Natl. Acad. Sci. USA, 97, 8590.
10. Hendricks, K. B., Wang, B. Q., Schnieders, E. A. and Thorner, J. 1999, Nat. Cell Biol., 1, 234.
11. Romero-Pozuelo, J., Dason, J. S., Atwood, H. L. and Ferrus, A. 2007, Eur. J. Neurosci., 26, 2428.
12. Sanchez-Gracia, A., Romero-Pozuelo, J. and Ferrus, A. 2010, BMC Evol. Biol., 10, 54.
13. Koizumi, S., Rosa, P., Willars, G. B., Challiss, R. A., Taverna, E., Francolini, M., Bootman, M. D., Lipp, P., Inoue, K., Roder, J. and Jeromin, A. 2002, J. Biol. Chem., 277, 30315.
14. Nakamura, T. Y., Pountney, D. J., Ozaita, A., Nandi, S., Ueda, S., Rudy, B. and Coetzee, W. A. 2001, Proc. Natl. Acad. Sci. USA, 98, 12808.
15. Tsujimoto, T., Jeromin, A., Saitoh, N., Roder, J. C. and Takahashi, T. 2002, Science, 295, 2276.
16. Wang, C. Y., Yang, F., He, X., Chow, A., Du, J., Russell, J. T. and Lu, B. 2001, Neuron, 32, 99.
17. Weiss, J. L., Archer, D. A. and Burgoyne, R. D. 2000, J. Biol. Chem., 275, 40082.
18. Kabbani, N., Negyessy, L., Lin, R., Goldman-Rakic, P. and Levenson, R. 2002, J. Neurosci., 22, 8476.
19. Pongs, O., Lindemeier, J., Zhu, X. R., Theil, T., Engelkamp, D., Krah-Jentgens, I., Lambrecht, H. G., Koch, K. W., Schwemer, J., Rivosecchi, A., Mallart, R., Galceran, J., Canal, I., Barbas, J. A. and Ferrús, A. 1993, Neuron, 11, 15.
20. Sippy, T., Cruz-Martin, A., Jeromin, A. and Schweizer, F. E. 2003, Nat. Neurosci., 6, 1031.
21. Saab, B. J., Georgiou, J., Nath, A., Lee, F. J., Wang, M., Michalon, A., Liu, F., Mansuy, I. M. and Roder, J. C. 2009, Neuron, 63, 643.

22. Gomez, M., De Castro, E., Guarin, E., Sasakura, H., Kuhara, A., Mori, I., Bartfai, T., Bargmann, C. I. and Nef, P. 2001, Neuron, 30, 241.
23. Nakamura, T. Y., Jeromin, A., Smith, G., Kurushima, H., Koga, H., Nakabeppu, Y., Wakabayashi, S. and Nabekura, J. 2006, J. Cell Biol., 172, 1081.
24. Nakamura, T. Y., Sturm, E., Pountney, D. J., Orenzoff, B., Artman, M. and Coetzee, W. A. 2003, Pediatr. Res., 53, 554.
25. Guo, W., Malin, S. A., Johns, D. C., Jeromin, A. and Nerbonne, J. M. 2002, J. Biol. Chem., 277, 26436.
26. Zhang, K., Heidrich, F. M., DeGray, B., Boehmerle, W. and Ehrlich, B. E. 2010, J. Mol. Cell Cardiol., 49, 829.
27. Nakamura, T. Y., Jeromin, A., Mikoshiba, K. and Wakabayashi, S. 2011, Circ. Res., 109, 512.
28. Paterlini, M., Revilla, V., Grant, A. L. and Wisden, W. 2000, Neuroscience, 99, 205.
29. Hilfiker, S. 2003, Biochem. Soc. Trans., 31, 828.
30. Burgoyne, R. D. 2007, Nat. Rev. Neurosci., 8, 182.
31. Ames, J. B. and Lim, S. 2012, Biochim. Biophys. Acta, 1820, 1205.
32. McFerran, B. W., Weiss, J. L. and Burgoyne, R. D. 1999, J. Biol. Chem., 274, 30258.
33. Cox, J. A., Durussel, I., Comte, M., Nef, S., Nef, P., Lenz, S. E. and Gundelfinger, E. D. 1994, J. Biol. Chem., 269, 32807.
34. Ames, J. B., Ishima, R., Tanaka, T., Gordon, J. I., Stryer, L. and Ikura, M. 1997, Nature, 389, 198.
35. O'Callaghan, D. W. and Burgoyne, R. D. 2004, J. Biol. Chem., 279, 14347.
36. Ames, J. B., Hendricks, K. B., Strahl, T., Huttner, I. G., Hamasaki, N. and Thorner, J. 2000, Biochemistry (Mosc.), 39, 12149.
37. Lim, S., Strahl, T., Thorner, J. and Ames, J. B. 2011, J. Biol. Chem., 286, 12565.
38. Martone, M. E., Edelmann, V. M., Ellisman, M. H. and Nef, P. 1999, Cell Tissue Res., 295, 395.
39. Chen, X. L., Zhong, Z. G., Yokoyama, S., Bark, C., Meister, B., Berggren, P. O., Roder, J., Higashida, H. and Jeromin, A. 2001, J. Physiol., 532, 649.
40. Taverna, E., Francolini, M., Jeromin, A., Hilfiker, S., Roder, J. and Rosa, P. 2002, J. Cell Sci., 115, 3909.
41. Mallart, A., Angaut-Petit, D., Bourret-Poulain, C. and Ferrus, A. 1991, J. Neurogenet., 7, 75.
42. Olafsson, P., Wang, T. and Lu, B. 1995, Proc. Natl. Acad. Sci. USA, 92, 8001
43. McFerran, B. W., Graham, M. E. and Burgoyne, R. D. 1998, J. Biol. Chem., 273, 22768.
44. Scalettar, B. A., Rosa, P., Taverna, E., Francolini, M., Tsuboi, T., Terakawa, S., Koizumi, S., Roder, J. and Jeromin, A. 2002, J. Cell Sci., 115, 2399.
45. Rajebhosale, M., Greenwood, S., Vidugiriene, J., Jeromin, A. and Hilfiker, S. 2003, J. Biol. Chem., 278, 6075.
46. de Barry, J., Janoshazi, A., Dupont, J. L., Procksch, O., Chasserot-Golaz, S., Jeromin, A. and Vitale, N. 2006, J. Biol. Chem., 281, 18098.
47. Zheng, Q., Bobich, J. A., Vidugiriene, J., McFadden, S. C., Thomas, F., Roder, J. and Jeromin, A. 2005, J. Neurochem., 92, 442.
48. Bartlett, S. E., Reynolds, A. J., Weible, M., Jeromin, A., Roder, J. and Hendry, I. A. 2000, J. Neurosci. Res., 62, 216.
49. Mikhaylova, M., Reddy, P. P., Munsch, T., Landgraf, P., Suman, S. K., Smalla, K. H., Gundelfinger, E. D., Sharma, Y. and Kreutz, M. R. 2009, Proc. Natl. Acad. Sci. USA, 106, 9093.
50. Gromada, J., Bark, C., Smidt, K., Efanov, A. M., Janson, J., Mandic, S. A., Webb, D. L., Zhang, W., Meister, B., Jeromin, A. and Berggren, P. O. 2005, Proc. Natl. Acad. Sci. USA, 102, 10303.
51. Kapp-Barnea, Y., Melnikov, S., Shefler, I., Jeromin, A. and Sagi-Eisenberg, R. 2003, J. Immunol., 171, 5320.
52. Kapp-Barnea, Y., Ninio-Many, L., Hirschberg, K., Fukuda, M., Jeromin, A. and Sagi-Eisenberg, R. 2006, Mol. Biol. Cell, 17, 4130.
53. Strahl, T., Huttner, I. G., Lusin, J. D., Osawa, M., King, D., Thorner, J. and Ames, J. B. 2007, J. Biol. Chem., 282, 30949.
54. Poulain, C., Ferrus, A. and Mallart, A. 1994, Pflugers Arch., 427, 71.

55. Nakamura, T. Y., Coetzee, W. A., Vega-Saenz De Miera, E., Artman, M. and Rudy, B. 1997, Am. J. Physiol., 273, H1775.
56. Fiset, C., Clark, R. B., Shimoni, Y. and Giles, W. R. 1997, J. Physiol., 500(Pt 1), 51.
57. Serodio, P., Kentros, C. and Rudy, B. 1994, J. Neurophysiol., 72, 1516.
58. An, W. F., Bowlby, M. R., Betty, M., Cao, J., Ling, H. P., Mendoza, G., Hinson, J. W., Mattsson, K. I., Strassle, B. W., Trimmer, J. S. and Rhodes, K. J. 2000, Nature, 403, 553.
59. Zhang, Y., MacLean, J. N., An, W. F., Lanning, C. C. and Harris-Warrick, R. M. 2003, J. Neurophysiol., 89, 1902.
60. Nakamura, T. Y. and Coetzee, W. A. 2008, BMC Physiol., 8, 2.
61. Weiss, J. L. and Burgoyne, R. D. 2001, J. Biol. Chem., 276, 44804.
62. Gambino, F., Pavlowsky, A., Begle, A., Dupont, J. L., Bahi, N., Courjaret, R., Gardette, R., Hadjkacem, H., Skala, H., Poulain, B., Chelly, J., Vitale, N. and Humeau, Y. 2007, Proc. Natl. Acad. Sci. USA, 104, 9063.
63. Dason, J. S., Romero-Pozuelo, J., Marin, L., Iyengar, B. G., Klose, M. K., Ferrus, A. and Atwood, H. L. 2009, J. Cell Sci., 122, 4109.
64. Rousset, M., Cens, T., Gavarini, S., Jeromin, A. and Charnet, P. 2003, J. Biol. Chem., 278, 7019.
65. Koh, P. O., Undie, A. S., Kabbani, N., Levenson, R., Goldman-Rakic, P. S. and Lidow, M. S. 2003, Proc. Natl. Acad. Sci. USA, 100, 313.
66. Bai, J., He, F., Novikova, S. I., Undie, A. S., Dracheva, S., Haroutunian, V. and Lidow, M. S. 2004, Biol. Psychiatry, 56, 427.
67. Souza, B. R., Motta, B. S., Rosa, D. V., Torres, K. C., Castro, A. A., Comim, C. M., Sampaio, A. M., Lima, F. F., Jeromin, A., Quevedo, J. and Romano-Silva, M. A. 2008, Neurochem. Res., 33, 533.
68. Souza, B. R., Torres, K. C., Miranda, D. M., Motta, B. S., Scotti-Muzzi, E., Guimaraes, M. M., Carneiro, D. S., Rosa, D. V., Souza, R. P., Reis, H. J., Jeromin, A. and Romano-Silva, M. A. 2010, J. Negat. Results Biomed., 9, 4.
69. Lian, L. Y., Pandalaneni, S. R., Patel, P., McCue, H. V., Haynes, L. P. and Burgoyne, R. D. 2011, PLoS One, 6, e27779.
70. Woll, M. P., De Cotiis, D. A., Bewley, M. C., Tacelosky, D. M., Levenson, R. and Flanagan, J. M. 2011, Biochemistry (Mosc.), 50, 8780.
71. Schlecker, C., Boehmerle, W., Jeromin, A., DeGray, B., Varshney, A., Sharma, Y., Szigeti-Buck, K. and Ehrlich, B. E. 2006, J. Clin. Invest., 116, 1668.
72. Iketani, M., Imaizumi, C., Nakamura, F., Jeromin, A., Mikoshiba, K., Goshima, Y. and Takei, K. 2009, Neuroscience, 161, 743.
73. Boehmerle, W., Splittgerber, U., Lazarus, M. B., McKenzie, K. M., Johnston, D. G., Austin, D. J. and Ehrlich, B. E. 2006, Proc. Natl. Acad. Sci. USA, 103, 18356.
74. Boehmerle, W., Zhang, K., Sivula, M., Heidrich, F. M., Lee, Y., Jordt, S. E. and Ehrlich, B. E. 2007, Proc. Natl. Acad. Sci. USA, 104, 11103.
75. Blachford, C., Celic, A., Petri, E. T. and Ehrlich, B. E. 2009, Cell Calcium, 46, 257
76. Jo, J., Heon, S., Kim, M. J., Son, G. H., Park, Y., Henley, J. M., Weiss, J. L., Sheng, M., Collingridge, G. L. and Cho, K. 2008, Neuron, 60, 1095.
77. Hui, H., McHugh, D., Hannan, M., Zeng, F., Xu, S. Z., Khan, S. U., Levenson, R., Beech, D. J. and Weiss, J. L. 2006, J. Physiol., 572, 165.
78. Greka, A., Navarro, B., Oancea, E., Duggan, A. and Clapham, D. E. 2003, Nat. Neurosci., 6, 837.
79. Angaut-Petit, D., Toth, P., Rogero, O., Faille, L., Tejedor, F. J. and Ferrus, A. 1998, Eur. J. Neurosci., 10, 423.
80. Perrelet, D., Ferri, A., Liston, P., Muzzin, P., Korneluk, R. G. and Kato, A. C. 2002, Nat. Cell Biol., 4, 175.