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NALCN: A Regulator of Pacemaker Activity

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Abstract Pacemaker cells play a fundamental role in generating or regulating many essential biological rhythms. Spontaneous pacemaker activity is dependent on the function of an array of ion channels expressed in these cells. Recent characterization of a Na^+ leak channel (NALCN) has linked to its role in conducting the background Na^+ current that depolarizes resting membrane properties of pacemaker neurons. NALCN, along with Unc79 and Unc80, forms a protein complex that is involved in regulating intrinsic membrane and synaptic activities. In this review, we will discuss the current understanding of NALCN channel physiology and its role in regulating cell excitability and pacemaker activity.

Keywords NALCN · Sodium leak current · Pacemaker cells · Rhythm activity · Resting membrane potential

Introduction

Pacemaker activity of excitable cells is essential for many biological functions, including heartbeat [1], locomotion [2], and respiration [3, 4]. The primary pacemaker cells are characterized by their ability of generating spontaneous action potential spikes. This ability is largely attributed to the complex composition of ion channels found in different pacemaker systems [1, 5]; however, one common prerequisite for all pacemaker cell activities is the baseline or resting membrane potential.

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The resting membrane potential largely relies on passive potassium conductance through potassium leak channels [6, 7]. However, the resting membrane potential of most pacemaker cells is in the range of −45 to −60 mV, which is far more depolarized than the potassium equilibrium potential of near −95 mV [1, 5, 8], suggesting permeability to other ions having more depolarized equilibrium potentials. Background Na^+ current has been described in numerous pacemaker cells as a major contributor toward regulation of resting membrane potential and action potential spike activity [4, 8]. A number of ion channels contributes to the background Na^+ current, including hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels [9, 10] and persistent sodium channels [11, 12].

Recent studies showed that the resting membrane potential can also be regulated by a TTX-insensitive non-selective cation channel (Na^+ leak channel, non-selective (NALCN)) [13] or NALCN-like channels [14]. Knockdown of NALCN-like channels in *Lymnaea stagnalis* suppresses rhythmic activity of the pacemaker neurons [14]. *Drosophila* melanogaster mutants of NALCN-like protein (na gene) affects circadian rhythms [15] and locomotion [16]. The *Caenorhabditis elegans* orthologues, the NCA-1 and NCA-2 are widely expressed in motor neurons [17, 18]. The *NALCN* gene has been found in various tissues including brain [13, 19, 20], pancreas [19, 20], and heart [19, 20] in mammals. Targeted deletion of this channel in mice resulted in a fatal postnatal phenotype characterized by an abnormal respiratory rhythm [13]. Na^+ leak conductance of the NALCN in non-pacemaker cells, however, requires regulatory mechanisms [20–22]. The potential role of NALCN in regulating insulin release [21, 22] and regulating neuronal excitability through extracellular Ca^{2+} sensitivity [23] has been previously reviewed. The focus of this review is to

discuss current understanding of the genetic, molecular, biophysical, and pharmacological properties of the NALCN channel and integrate these understanding into a possible physiological model for pacemaker activity.

Protein Structures and Homology

Gene and Protein Structures

Human NALCN (formerly *VGCNL1*) is located on chromosome 13, at location 13q32.3. It is encoded by 44 exons spanning approximately 6.9 kb of genomic sequence. Processed NALCN mRNA of 5.2 kb encodes a large membrane channel protein of 1,738 amino acids. In rodents, mouse, and rat *NALCN* genes are located on chromosome 14 at 14E5;14 and chromosome 15 at 15q25, respectively. Genomic transcript of mouse and rat NALCN are 7.1 and 6.7 kb, respectively. Processed mouse and rat NALCN mRNAs are 5.2 and 4.3 kb, respectively. NALCN proteins in all three mammalian systems are approximately 1,700 residues in length.

The predicated structure of mammalian NALCN is similar to α_1 subunits of voltage-gated Na^+ and Ca^{2+} channels [13, 19]. It has four homologues repeats (domains I–IV) with six transmembrane segments (S1–S6) [13, 19] (Fig. 1a, b). Four pore forming loops (P-loops) spanning from S5–S6 make up the ion selectivity filter. Unlike voltage-gated Na^+ and Ca^{2+} channels with pore selectivity filters motif of DEKA and EEEE, respectively, NALCN has a hybrid putative selectivity filter with an EEKE motif [13]. In addition, in comparison to the voltage-gated Na^+ and Ca^{2+} channels, the fourth transmembrane segment that typically functions as a voltage sensor (S4) lack many positively charged amino acids. Mutation analysis of the NALCN orthologue, NCA in *C. elegans*, identified two gain-of-function alleles located in S6 segment of domain II and the cytosolic interconnecting loop of domain I and II [18]. NCA interacts with UNC-7, an invertebrate gap junction protein [24]. The cytosolic loop linking domains I and II of NALCN interacts with M_3 muscarinic receptor (M3R) when co-expressed in human embryonic kidney-293 (HEK293) cells [20] (Fig. 1a). Further mutation analysis of the distal C-terminus showed an interaction domain necessary for modulation of NALCN current by extracellular Ca^{2+}, possibly through association with its cytosolic subunit, Unc80 [23, 25] (Fig. 1a).

Homology

Currently, NALCN and NALCN-like genes have been identified in more than 20 different species. Within most vertebrates, NALCN is highly conserved with over 96 % identities with its orthologues. Human NALCN channel also shows 48 % homology with both *C. elegans* orthologues (NCA-1 and NCA-2), 57 % homology with *Drosophila melanogaster* orthologue (Dma1U) and 55 % homology with *L. stagnalis* orthologue (U-type) (Table 1). Although NALCN protein sequences vary between phyla, the pore forming and S4 domains of NALCN and NALCN-like proteins are both highly conserved [14], suggesting functionally conserved roles of NALCN as an ion channel.

Biophysical and Pharmacological Properties

Biophysical Properties

Much of our current knowledge about the biophysical properties of NALCN channel is through overexpression studies in HEK cell line, which do not endogenously express NALCN channels. NALCN is a non-selective cationic channel with the permeability profile of: $Na^+ > K^+ > Cs^+ > Ca^{2+}$ [13]. Current–voltage relation of NALCN whole-cell current indicates a reversal potential at approximately 0 mV. These properties were shown to be contributed largely by the EEKE pore selectivity motif [13]. Unlike other voltage-gated Na^+ and Ca^{2+} channels, activation and inactivation of NALCN does not dependent on voltage. The reduced number of positive amino acids on the S4 region of NALCN was hypothesized to contribute to these properties [13]. Given the persistent activation of NALCN at rest, it has been shown that NALCN and NALCN-like channels could potentially be a major contributor to the neuronal background Na^+ current [13, 14]. However, the open probability of NALCN channels contributing toward background Na^+ current remains unclear.

Regulation of NALCN activation/inactivation and localization requires auxiliary subunits. Currently, the most studied subunits of NALCN are Unc80 and Unc79. Both Unc80 and Unc79 are two highly conserved proteins and their interaction with NALCN orthologues was initially demonstrated in *C. elegans* [17, 18] and *D. melanogaster* [10] and then confirmed in mammals [25, 26]. NALCN interacts directly with Unc80 [25, 26] and indirectly with Unc79 [25]. Unc79 affects membrane expression of Unc80 [25]. Although Na^+ leak current was recorded from the recombinant NALCN pore forming subunit when expressed alone in HEK293 cells [13], Unc80 and Unc79 auxiliary subunits are critical for the membrane localization NALCN channels [17, 18]. Recent studies showed that membrane expression of the functional NALCN channels requires NALCN-Unc80-Unc79 complex [25, 27]. NALCN also binds to the M3R in a pancreatic beta-cell line, Min6 [20]. The leak conductance of the NALCN in Min6 requires M3R and Src activation. Co-expression of M3R with NALCN was sufficient to reconstitute the NALCN current in HEK293 cells and in *Xenopus* oocytes [20].

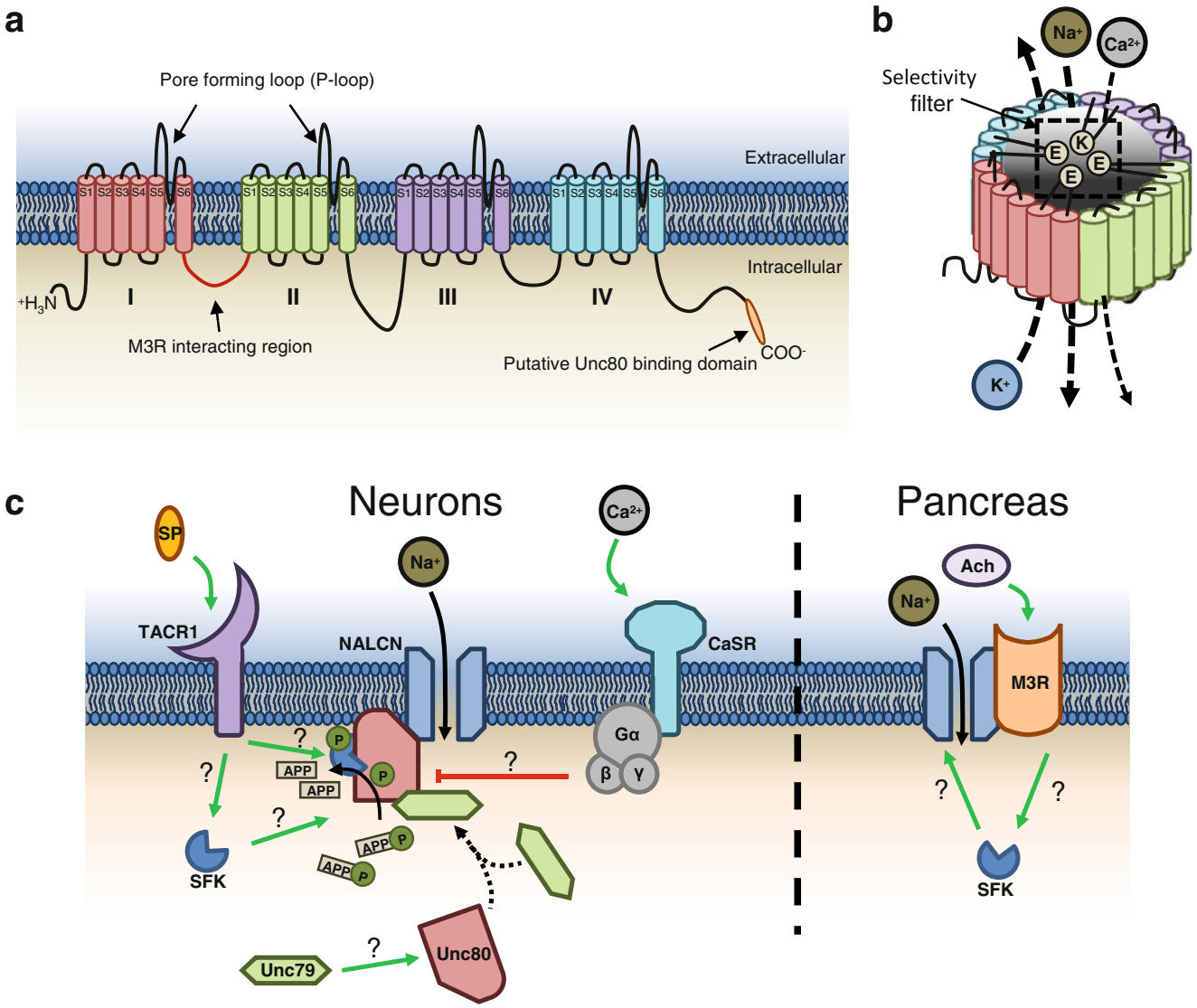

Fig. 1 Schematic diagram of functional NALCN complex in neurons and pancreatic beta-cells. **a** Schematic diagram of hypothesized NALCN transmembrane structure. It has four homologous repeats (domains I–IV) with six transmembrane segments (S1–S6). Four pore forming loops (P-loops) spanning from S5–S6 make up the ion selectivity filter. Mutation analysis identified a putative Unc80 binding domain located in the distal C-termius of NALCN. M3R interacts with NALCN via the cytosolic loop between domain I and II. **b** NALCN has a putative selectivity filter with an EEKE motif, which contributes to the non-selective cation channel properties. NALCN permeability profile is as follows: $Na^+ \approx K^+ > Ca^{2+}$. **c** NALCN indirectly interacts with Unc79 via Unc80. Unc80 protein expression requires Unc79. Unc80 function as a scaffolding protein for SFK-coupled signaling from receptors. In neurons, one of the identified receptor in this pathway is activation of TACR1 by SP. Both Unc80 and SFK are capable of tyrosine phosphorylation. In addition, Unc80 also function as an intermediate protein for G-protein dependent inhibition of NALCN channel through activation of CaSR with extracellular Ca^{2+}. Similar to neurons, NALCN channel can be activated in pancreatic beta-cells by Ach binding to M3R in a SFK-dependent pathway. Co-expression of M3R and NALCN in HEK-293 cells and *Xenopus* oocytes indicated physical coupling of receptor–channel complex, which were important in establishing ACh activation of NALCN current. *NALCN* Na^+ leak non-selective, *TACR1* tachykinin receptor 1, *SP* substance P, *SFK* Src-family kinase, *APP* adenosine diphosphate, *P* phosphate, *CaSR*, calcium-sensing receptor, *Ach* acetylcholine, *M3R* M3 muscarinic receptor

Pharmacological Properties

Currently, there is no specific blocker for NALCN channel. NALCN shares many structural similarities with voltage-gated Na^+ and Ca^{2+} channels; hence, sensitivities to these blockers were tested. NALCN is insensitive to (1) TTX, a Na_v channel blocker, in overexpressed HEK293 cells [13], Min6 cells [20], and neurons [13, 14]; (2) Cs^+ ions, a nonspecific I_h blocker, in rodent neurons [13]; and (3) various Ca_v channel blockers, such as, nifedipine, dialtizem, D-600, and mibefradil [13]. Interestingly, the current conductance via recombinant [13] or native [13, 14, 20] NALCN channels

Table 1 Homologous of NALCN between species

Identity to Homo sapiens	Genes	Alternate names	Accession no.		Phylum	Species	Reference
			mRNA	Protein			
100 %	*NALCN*	VGCNL1	XM_001149573	XP_001149573	Chordata	*Pan troglodytes*	
99 %	*NALCN*	VGCNL1	NM_177393	NP_796367	Chordata	*Mus musculus*	[13, 19]
99 %	*NALCN*	VGCNL1	XM_542654	XP_542654	Chordata	*Canis lupus familiaris*	
99 %	*NALCN*		XM_001492809	XP_001492859	Chordata	*Equus caballus*	
99 %	*NALCN*		XM_003270152	XP_003270200	Chordata	*Nomascus leucogenys*	
99 %	*NALCN*		XM_002713013	XP_002713059	Chordata	*Oryctolagus cuniculus*	
98 %	*NALCN*		XM_001513615	XP_001513665	Chordata	*Ornithorhynchus anatinus*	
98 %	*NALCN*	VGCNL1	XM_416967	XP_416967	Chordata	*Gallus gallus*	
98 %	*NALCN*		XM_001366005	XP_001366042	Chordata	*Monodelphis domestica*	
98 %	*NALCN*		XM_002914851	XP_002914897	Chordata	*Ailuropoda melanoleuca*	
96 %	*NALCN*	VGCNL1	XM_002691980	XP_002692026	Chordata	*Bos taurus*	
96 %	*NALCN*	VGCNL1	NM_153630	NP_705894	Chordata	*Rattus norvegicus*	[13, 19]
96 %	*NALCN*		XM_003218712	XP_003218760	Chordata	*Anolis carolinensis*	
92 %	*NALCN*		XM_003451547	XP_003451595	Chordata	*Oreochromis niloticus*	
90 %	*NALCN*		NM_001017549	NP_001017549	Chordata	*Danio rerio*	
79 %	*NALCN*		XM_003482928	XP_003482976	Chordata	*Sus scrofa*	
57 %	*na*	alpha1U, Dmα1U	NM_001103511	NP_001096981	Arthropoda	*D. melanogaster*	[13, 16]
55 %	*U*-type		AF484086	AAO84496	Mollusca	*Lymnaea stagnalis*	[14]
48 %	*nca-1*		NM_171352	NP_741413	Nematoda	*Caenorhabditis elegans*	[10, 13, 17, 18]
48 %	*nca-2*		NM_065653	NP_498054	Nematoda	*Caenorhabditis elegans*	[10, 13, 17, 18]

can be partially blocked by other channel blockers such as Gd^{3+} (IC_{50}, 1.4 μM), Cd^{2+} (IC_{50}, 0.15 mM), Co^{2+} (IC_{50}, 0.26 mM), and verapamil (IC_{50}, 0.38 mM). The sensitivity of NALCN to Gd^{3+} block is higher than other channels such as transient receptor potential (TRP) [28], voltage-gated calcium channels [29–31], stretch-activated channel [32] and other ion channels [33, 34]. The absence of a specific NALCN blocker is a major barrier for most conventional pharmacological studies. Therefore, developing specific pharmacological tools for NALCN channels becomes essential in future physiology studies.

NALCN Channel Regulation

NALCN conductance is modulated by various membrane receptors (Fig. 1c). In neurons, substance P and neurotensin has been shown to activate NALCN current [35]. Binding of substance P with tachykinin receptor 1 (TACR1) activates a G-protein independent pathway and recruits Src family of tyrosin kinases that interact with Unc80 to activate NALCN currents [35]. In pancreatic beta-cells, NALCN current can also be modulated by acetylcholine (Ach) through activation of M3R, and the ACh-sensitive NALCN current is also Src-dependent [20] (Fig. 1c). In addition to Src regulation, lowing extracellular Ca^{2+} to 0.1 mM in the mouse hippocampal neurons [25] and to 0.5 mM in *Lymnaea* pacemaker neurons [14] activates the NALCN current. In hippocampal neurons, binding of Ca^{2+} to a Ca^{2+} sensing G-protein-coupled receptor (CaSR) inactivates NALCN currents [25]. As part of the NALCN protein complex, Unc80 and Unc79 are involved in endogenous extracellular Ca^{2+} sensitivity. Unc80 interacts directly with NALCN channel and is essential for Ca^{2+} sensitivity, whereas Unc79 is indirectly involved [25]. Co-expression of NALCN, Unc80 and CaSR in HEK293 cells was sufficient to reconstitute extracellular Ca^{2+} sensitivity of NALCN current [25]. The conserved regulatory mechanism represents a functional importance in regulating basal neuronal activity and excitability through NALCN. In addition, it would be of great interest to investigate how NALCN is involved in many pathophysiological conditions involving large fluctuation of extracellular Ca^{2+} levels [23, 25].

Expression and Distribution

In humans, the mRNA of NALCN is widely expressed in the brain, heart, and other glandular tissues [13, 19, 20]. Lee and colleagues first cloned the NALCN channel and quantified the mRNA expression profile through northern blot analysis of human brain regions as: amygdale = corpus callosum > caudate nucleus > hippocampus > substantia nigra = subthalamic

nucleus > thalamus [19]. Further study by Swayne and colleagues also confirmed high expression by using dot blot analysis of mRNA from various brain regions as well as the spinal cord [20]. In addition, moderate NALCN mRNA expression was also identified in the heart, aorta, lymph node, pancreas, adrenal gland, and thyroid gland [20]. A similar high NALCN mRNA expression profile was also identified in the brain tissues of both rat through northern blot analysis [19] and mouse through real-time PCR [20] and in situ hybridization [13]. Within the pancreas, NALCN mRNA was almost exclusively expressed within endocrine tissues of the islets rather than the exocrine tissues [20]. Currently, the cellular localization pattern of mammalian NALCN protein has not been described in detail. However, distribution of NCA along with Unc-80 and Unc-79 in *C. elegans* shows high localization throughout the non-synaptic regions of the axon [18].

Physiological Functions

Rhythm Activity

Physiological roles of NALCN were mostly derived from studies using genetic modulation. Mutation in *C. elegans* of NCA-1 and NCA-2 resulted in impaired synaptic transmission producing abnormalities in locomotion [17, 18]. In *D. melanogaster*, one mutation of Dmα1U allele showed a narrow abdomen phenotype [15, 16] and two mutations of Dmα1U alleles altered sensitivity to halothane [36, 37]. All three mutations of Dmα1U exhibit dysfunction in light sensitivity and locomotion [16]. Dmα1U are also highly expressed in the pacemaker cells responsible for the diurnal rhythm network [15]. Channel and pore selectivity motif mutation analyses indicate that Dmα1U function as an ion channel that is involved in regulating circadian rhythm [15]. In mice, targeted deletion of the NALCN exon resulted in a knockout strain with a lethal postnatal phenotype characterized by abnormal rhythmic respiratory activity. C4 nerve root recording, which indirectly measures respiratory network output showed a substantial reduction in spontaneous burst activities [13]. Knockdown of NALCN-like U-type channel in *L. stagnalis* reduced respiratory activity [14]. Detailed analysis of the isolated respiratory pacemaker interneuron identified U-type channel as an essential component to pacemaker generation, which directly affects respiratory rhythm output [14]. These studies demonstrated that NALCN regulates the intrinsic membrane properties that are essential for a network activity.

Resting Membrane Potential and Pacemaker Activity

The rhythmic activity of pacemaker cells is dependent on the composition and properties of a large array of ion channels (Fig. 2). These includes the hyperpolarizing activated currents (I_h) [38], leak K^+ current ($I_{K\text{-}LEAK}$) [39, 40], small conductance Ca^{2+}-activated potassium currents (I_{SK}) [41–43], persistent sodium currents (I_{NaP}) [12, 44, 45], and subthreshold Ca^{2+} currents ($I_{Ca\text{-}T}$) [46, 47]. Similarly in the heart, pacemaker activity requires multiple ion channels, including the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels [48–51], voltage-dependent calcium channels [52–55], voltage-dependent sodium channels [56, 57], voltage-dependent potassium channels [58, 59], and chloride channels [60].

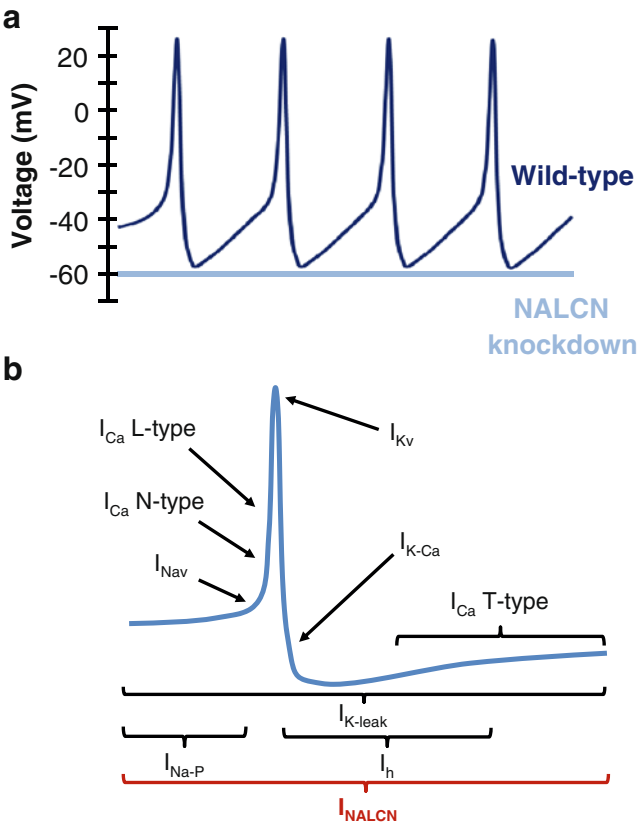

Fig. 2 Proposed model of NALCN channel function in regulating pacemaker activity. **a** NALCN conductance is essential in establishing resting membrane potential for spontaneous activity. **b** During action potential firing, voltage-dependent persistent Na^+ current ($I_{Na\text{-}P}$) depolarizes membrane potential to threshold activating voltage-gated Na^+ current (I_{Nav}). Rapid depolarization of membrane potential activates N-type and L-type voltage-gated Ca^{2+} channels (I_{Ca} N-type and I_{Ca} L-type, respectively), and voltage-gated K^+ current (I_{Kv}). After hyperpolarization is mediated by the Ca^{2+} dependent K^+ current ($I_{K\text{-}Ca}$), followed by hyperpolarizing-activated current (I_h) and T-type low voltage activated Ca^{2+} current (I_{Ca} T-type). K^+ leak current determines resting membrane potential along with Na^+ leak current through NALCN channel. In addition, Na^+ conductance through NALCN channel is a passive regulator of depolarizing phase of action potential, which potentially regulates rhythmic spiking

The vast array of ion channel composition to pacemaker cells of different tissue types suggests complex interplay of ionic conductance that contributes to pacemaker generation. For example, persistent sodium current (I_{NaP}) in combination with calcium-mediated inward cationic currents (I_{CAN}) are important for bursting activity and enhancement of rhythmogenesis in pacemaker neurons [44, 61–63]. In respiratory pacemaker neurons, specific $I_{NaP}/I_{K\text{-}leak}$ ratio is required for appropriate pacemaker generation [5, 40]. In the heart, pharmacological studies combined with genetic knockouts of HCN, Ca_v 1.3, and Ca_v 3.1, suggest a complex relationship of ionic conductance that generates cardiac pacemaker rhythm [1]. In adrenal chromaffin cells, tight coupling of Ca_v 1.3 and BK channels was found to affect pacemaker firing and action potential profile [64, 65]. Moreover, Ca_v 1.3 conductance is important in pacemaker cells found in numerous excitable tissues [55, 65, 66], which also have the propensity to contribute to the susceptibility and development of Parkinson's disease [55], cardiac arrhythmia [66], and chronic stress disorder [64, 65].

The appropriate resting membrane potential is essential for pacemaker rhythmic generation in the heart pacemaker cells. Typical intrinsic regulation of resting membrane potential involves passive conductance of various currents. Numerous studies showed that linear current is important in network oscillation [40, 67, 68] and neuronal excitability [69]. The ohmic potassium leak current is the fundamental basis of establishing the resting membrane potential [70, 71]. However, the resting membrane potential of almost all pacemaker cells is more depolarized than the equilibrium potential of K^+, suggesting there are additional passive currents. One of the components that is unique is the background Na^+ current that is insensitive to TTX, Cs^+, and can be partially blocked by Gd^{3+} in pacemaker cells [8, 72, 73]. Recent findings by Lu

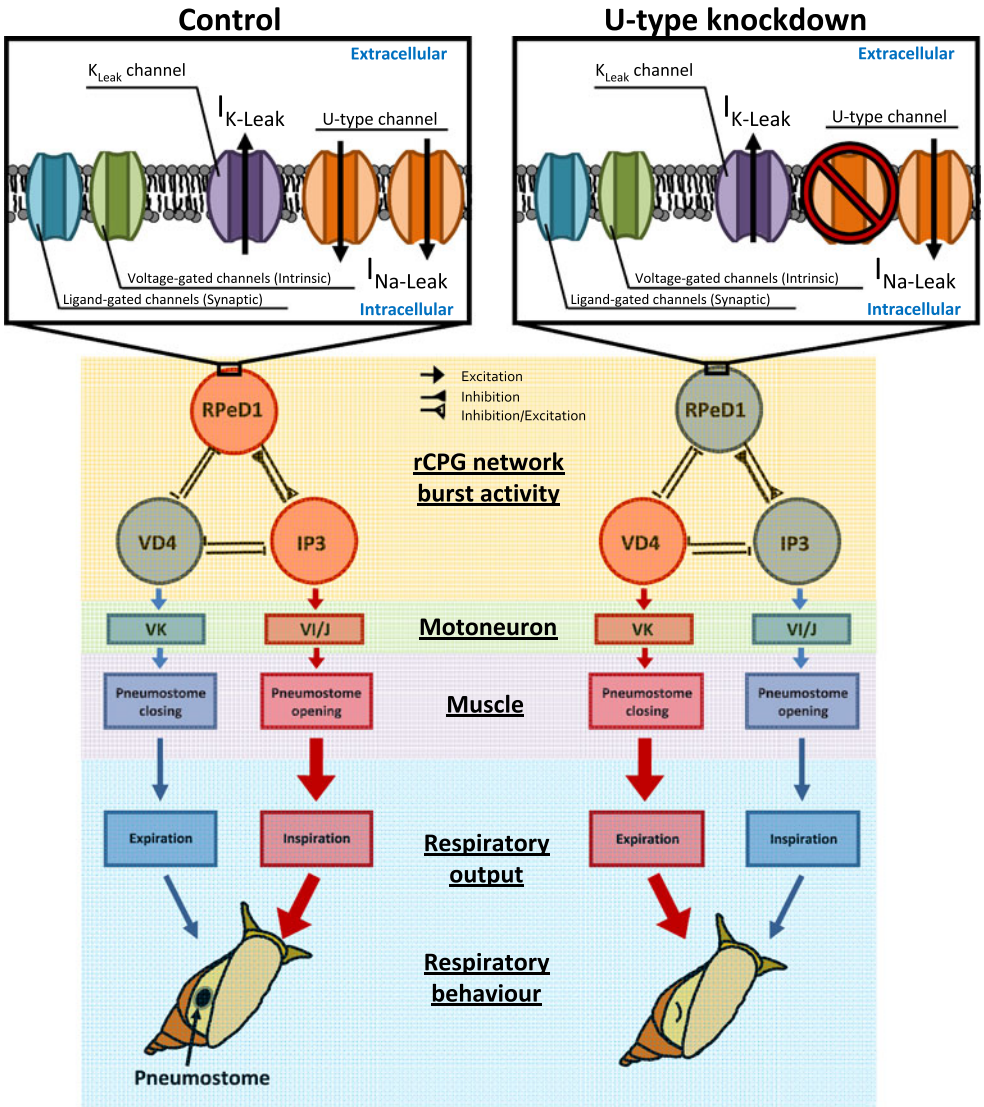

Fig. 3 Working model of U-type conductance contributing to rCPG rhythmic output and respiratory behavior. U-type channel, NALCN orthologue in *L. stagnalis*, conducts an inward leak current which combined with potassium leak channels, regulates the resting membrane potential. Blue cell body represents low activity and red cell body represents high activity. U-type knockdown hyperpolarizes basal membrane potential of right pedal dorsal 1 (RPeD1), rCPG pacemaker neuron which reduces its intrinsic activity. This results in reduction of excitation to input 3 interneuron (*IP3*) and reduction of inhibitory input to visceral dorsal 4 (*VD4*). This results in a shift in rCPG network rhythmic output toward pneumostome (respiratory orifice) closing resulting in reduced respiratory behavior in adult animal. rCPG network model adopted from [74, 75]

and Feng demonstrated an ohmic NALCN-like conductance is necessary for spontaneous pacemaker activity [14]. First, knockdown U-type channels using a gene silencing approach led to a more hyperpolarized resting membrane potential, which was sufficient to eliminate the spontaneous activity in isolated pacemaker neurons of the *L. stagnalis* respiratory central pattern generator. Secondly, depolarizing current injection in the U-type knockdown group restored rhythmic activity to the wild-type, suggesting the U-type channel is involved in regulating intrinsic membrane properties necessary for spontaneous bursting. Thirdly, biophysical and pharmacological analysis of the U-type channel conductance showed it conducts a TTX-insensitive Na^+ leak current that can be partially blocked with Gd^{3+}. This Na^+ leak current is also sensitive to extracellular Ca^{2+}. The sequence alignment of the U-type channel shows a high level of homology with the mammalian NALCN channel. Thus, these evidences suggest that the U-type channel is a *molluscan* NALCN orthologue. Finally, the in vivo knockdown of the U-type channel resulted in reduced respiratory output (Fig. 3). This study was the first to directly test, identify, and characterize NALCN-like current as an essential component to pacemaker neuron activity.

Synaptic Regulation

Behavioural outputs of rhythmic activities require not just the intrinsic rhythmic activity of the pacemaker neurons, but also the synaptic synchronicity amongst the neuronal networks. The contribution of intrinsic and synaptic activities to rhythm generation may change throughout development. For example, the pacemaker hypothesis of respiratory network posits that the pre-Botzinger complex is mainly a respiratory rhythm generator in neonates, as the isolated brainstem and spinal cord of a neonatal rat could generate a fictive respiratory rhythm [39] with synaptic inhibition [5, 76]. The group pacemaker hypothesis suggests that periodic activity of synaptic connection between bursting respiratory neurons recruits additional burst-generating currents. In addition to its role in regulating intrinsic membrane properties, NALCN is directly involved in synaptic transmission. In *D. melanogaster*, NALCN orthologue (Dmα1U) is highly expressed at the synaptic region compared to the cell bodies [16], suggesting a functional role in mediating neurotransmitter release or postsynaptic response. Disrupted circadian rhythms of Dmα1U mutants decreased release of neuropeptide, PDF [15]. *C. elegans* orthologues of the NALCN channels (NCA-1 and NCA-2) are critical in the conduction of depolarizing signal from the soma to the axon [18]. Mutants of NCA-1 reduced Ca^{2+} transient at the synaptic terminal [18]. In addition, Unc80-dependent NCA channel activities were involved in defects in synaptic vesicle recycling in a synaptojanin mutant [17]. The highly conserved Unc80 and Unc79 proteins in *C. elegans* are essential for proper NCA-1 and NCA-2 localization [17, 18]. NALCN-like channel (U-type) knockdown in *L. stagnalis* showed a shift in respiratory network output rather than abolishment of activity (Fig. 3), suggesting NALCN contributes to both intrinsic membrane properties as well as synaptic transmission [14].

Osmoregulation

The Korstanje group recently demonstrated that NALCN is a potential player in osmoregulation [77]. Halotype association mapping was used to identify and correlate various mouse strains with differences in serum Na^+ concentration. The *NALCN* gene was identified to have the strongest correlation with Na^+ concentrations. An analysis of heterozygous NALCN strains identified hypernatremia suggesting that NALCN is possibly involved in osmoregulation. The detailed mechanism remains unclear, but Sinke and colleague proposed a model that differential NALCN expression in osmoregulatory neurons alters osmolarity signals quantified by changes in action potential frequency [78].

Conclusion

Researches within the past few years have uncovered fascinating information about NALCN channel. While the lethal nature of NALCN knockout mice indicates its vital role, the function and regulation of NALCN complex remains contentious. NALCN regulates both intrinsic membrane properties and synaptic properties, yet much is less known of NALCN on its gene regulation, protein trafficking, and intracellular signalling mechanisms. In addition, whether NALCN is involved in disease conditions remains further investigation. It is an exciting time for NALCN research as it represents a promising target for future biological rhythm study and beyond.

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