Channels 9:3, 139–144; May/June 2015; Published with license by Taylor & Francis Group, LLC

Gating pore currents, a new pathological mechanism underlying cardiac arrhythmias associated with dilated cardiomyopathy

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Voltage-gated ion channels (VGIC) are transmembrane proteins responsible for the generation of electrical signals in excitable cells. VGIC were first described in 1952 by Hodgkin and Huxley,[1] and have since been associated with various physiological functions such as propagating nerve impulses, locomotion, and cardiac excitability. VGIC include channels specialized in the selective passage of K^+, Ca^{2+} Na^+, or H^+. They are composed of 2 main structures: the pore domain (PD) and the voltage sensor domain (VSD). The PD ensures the physiological flow of ions and is typically composed of 8 transmembrane segments (TM). The VSD detects voltage variations and is composed of 4 TM (S1-S4). Given their crucial physiological role, VGIC dysfunctions are associated with diverse pathologies known as ion channelopathies. These dysfunctions usually affect the membrane expression of ion channels or voltage-dependent conformational changes of the pore. However, an increasing number of ion channelopathies, including periodic paralysis, dilated cardiomyopathy (DCM) associated with cardiac arrhythmias, and peripheral nerve hyperexcitability (PNH), have been linked to the appearance of a new pathological mechanism involving the creation of an alternative permeation pathway through the normally non-conductive VSD of VGIC. This permeation pathway is called the gating pore or omega pore.

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Submitted: 03/16/2015

Accepted: 03/16/2015

http://dx.doi.org/10.1080/19336950.2015.1031937

Molecular Basis of Gating Pores

The VSD is a specialized, highly conserved structure that modulates the activity of the PD, notably by detecting voltage variations through the movement of the positively charged S4 segments, which are surrounded by the relatively fixed S1-S3 segments. The S1-S3 segments are "pillars" that catalyze the movement of the S4 TM segment. These segments contain a highly conserved structure known as the gating charge transfer center (GCTC) as can be seen in the conservation map (**Fig. 1**).[2] The GCTC is composed of 2 negatively charged residues (aspartate or glutamate) on the S2-S3 segments and an aromatic residue (phenylalanine or tyrosine) on S2. During activation, the arginines or lysines in the S4 segment sequentially interact with the GCTC to form a hydrophobic septum that isolates the water crevices on the 2 sides of the membrane (**Fig. 2**).[3-6]

Starace and Bezanilla showed that specific mutations of positively charged residues in the S4 segment of the VSD of the Shaker channel can lead to the appearance of a dedicated permeation pathway through the VSD.[7-9] This permeation pathway was later called the gating pore or omega pore.[10,11] These pores are created by mutations that disrupt interactions between the S4 segment and the GCTC and that create a water crevice spanning the membrane, opening a continuous aqueous pathway (**Fig. 3**). The biophysical characteristics of gating pores depend largely on the structure and movement of the VSD. These pores activate very rapidly (within 1 ms) and do not possess a dedicated inactivation mechanism. Their opening and closing are thus related to the kinetics and voltage dependence of the S4

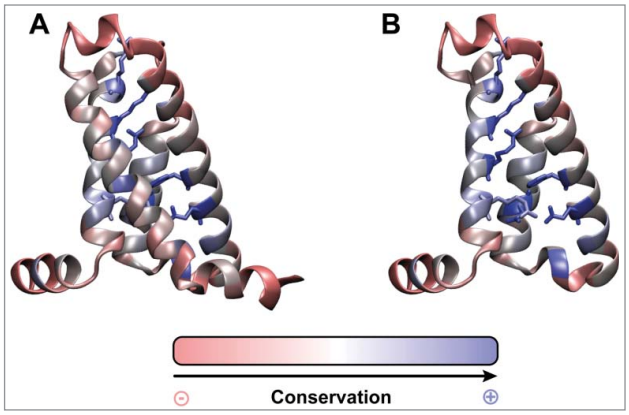

Figure 1. Residue conservation of the VSD motifs mapped on the NavAb crystal structure. Residue conservation was mapped by calculating the Shannon entropy at every position for the 6652 VSD sequences alignment reported by Palovcak et al. 2014.[41] The residues which were not present in the sequence alignment but were depicted in the crystal structure were assigned the maximal entropy value calculated for the whole alignment. Those residues are located before S1 and in the S4-S5 linker.

segment.[4-6] There are 2 main sub-types of gating pores: H^+-specific pores and cation-specific pores. H^+-specific gating pores are created by the substitution of positively charged S4 residues by histidines. The permeation of H^+ is thought to occur via a Grotthus hopping mechanism involving a proton wire.[8] Cation selective gating pores are usually narrow pores with weak binding sites that determine their selectivity. These pores are more permeable to large cations such as Cs^+, K^+, Na^+, in descending order.[4-6]

Involvement of gating pores in the development of dilated cardiomyopathy associated with arrhythmias

Voltage-gated sodium channels ($Na_v1.5$) in the heart initiate action potentials (AP) that regulate contraction. These channels are composed of 24 TM organized in 4 homologous domains (DI-DIV)), each containing 6 TM (S1-S6). The S1-S4 segments of each domain form the VSD while the S5-S6 form the PD. $Na_v1.5$ dysfunctions are usually associated with various rhythm disorders such as Brugada syndrome, long QT type 3 syndrome, sick sinus syndrome, atrial fibrillation, and progressive cardiac conduction defect.[12] While these electrical disturbances may cause severe clinical phenotypes, potentially leading to sudden death, the morphology of the heart usually appears normal. Several mutations located in the VSD of $Na_v1.5$ channels have recently been reported to cause an atypical phenotype that associates cardiac arrhythmias with dilated cardiomyopathy (**Fig. 4**).[4,13,14] Carriers, who are difficult to clearly classify into a distinct syndrome, usually present with cardiac dilatation and a variety of arrhythmias. Intriguingly, while the phenotypes are similar, the characterization of several mutations has revealed that they cause different biophysical defects.[6,13,15-18] The R225W mutation appears to cause a loss of channel function, with a marked reduction in current density, while the R222Q, R225P, and R814W mutants cause a gain of channel function, with an increased window current, notably through shifts in steady state activation and inactivation.[6,13,15-19] On the other hand, the R219H mutation does not modify the biophysical properties of the mutant channels.[13] These major discrepancies led us to hypothesize that a potentially unifying pathological mechanism may exist to explain the development of this atypical pathological phenotype.

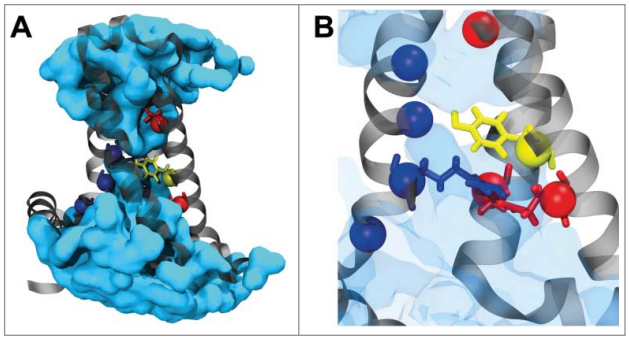

Figure 2. 3D structure of the WT $Na_v1.5$ VSD DI. 3D structure of the WT $Na_v1.5$ VSD DI in the partially activated β state. (**A**) VSD structure illustrating the water crevices, the protein and highly conserved amino acids of the S4 segment, and the GCTC. The water accessible volume is shown in cyan, and the protein helices are depicted in gray. Positively charged S4 arginines are shown in blue while the negatively charged and aromatic amino acids of the GCTC are shown in red and yellow, respectively. (**B**) Higher magnification of the hydrophobic septum formed by interactions between the S4 segment and the GCTC. This hydrophobic septum isolates the water crevices on the intracellular and extracellular sides of the membrane, resulting in a non-conductive structure characteristic of WT channels.

Gating pore currents have been proposed as a common unifying mechanism given the location of the mutations associated with this particular phenotype. Most of the reported mutations are located in the VSD of $Na_v1.5$ channels.[4,14,20] The R219H mutation consists of the substitution of the first arginine on the S4 segment of the first $Na_v1.5$ VSD by a histidine. This mutation creates a H^+-selective gating pore that is activated by hyperpolarization.[13] On the other hand, the R222Q and R225W mutations (second and third S4 arginines of the first $Na_v1.5$ VSD) both create a cation-selective gating pore that forms upon depolarization.[6] To date, gating pore currents have been observed and characterized for 3 unrelated mutations located on the first VSD of $Na_v1.5$ (R219H, R222Q, and R225W).[6,13] These observations strengthen the hypothesis that gating

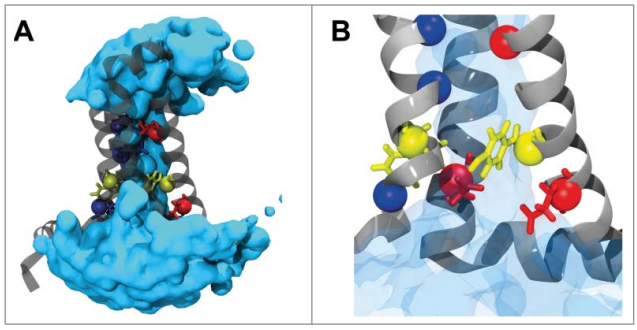

Figure 3. 3D structure of the R225W $Na_v1.5$ VSD DI. 3D structure of the R225W $Na_v1.5$ VSD DI in the partially activated β state. (**A**) VSD structure illustrating the continuous water crevice, the protein and highly conserved amino acids of the S4 segment, and the GCTC. The water accessible volume is shown in cyan, and the protein helices are depicted in gray. Positively charged S4 arginines are shown in blue, and the arginine substituted by a tryptophan is shown in yellow. The negatively charged and aromatic amino acids of the GCTC are shown in red and yellow, respectively. (**B**) Higher magnification of the disrupted interactions between the S4 segment and the GCTC. The tryptophan (yellow) in the S4 segment slightly twists the bottom of the S4 segment and does not interact with the GCTC. The disruption of interactions between the S4 segment and the GCTC creates a continuous water crevice through the membrane, resulting in a permeation pathway or gating pore.

pores may be the common unifying mechanism underlying the development of the atypical phenotype associating dilatation of cardiac chambers and cardiac arrhythmias.

R219H, R222Q, and R225W gating pore currents may disrupt ionic homeostasis through, for example, the Na^+/H^+ (R219H) and Na^+/Ca^{2+} exchangers.[4,14] The disruption in ionic homeostasis, especially the dysregulation of Ca^{2+} homeostasis, may affect the entire contractile apparatus, ionic conductance at the plasma membrane, and gap junction coupling, resulting in the atypical clinical phenotype associating DCM with several types of cardiac arrhythmias.[4,14]

Gating pores can be created at either hyperpolarized or depolarized potentials

Gating pores are created by the disruption of interactions between the GCTC and the S4 segment. Depending on the location of the mutation in the S4 segment, gating pores can be open at either hyperpolarized or depolarized potentials. Mutations on the outermost part of the S4 segment disrupt interactions between the S4 and the GCTC when the S4 is in its resting state (downward position), opening the permeation pathway at hyperpolarized potentials, while mutations on the innermost part of the S4 segment disrupt interactions when the S4 segment is in its activated conformation (upward position), creating a continuous water crevice through the membrane at depolarized potentials.[4-6,8]

This difference appears to be crucial in pathophysiological conditions. In the case of cation-selective gating pores, which do not possess a specialized selectivity filter, the permeation of ions mainly depends on their driving forces. As such, the opening of a gating pore at hyperpolarized potentials should cause a Na^+ leak while the opening of a gating pore at depolarized potential should cause a K^+ leak.

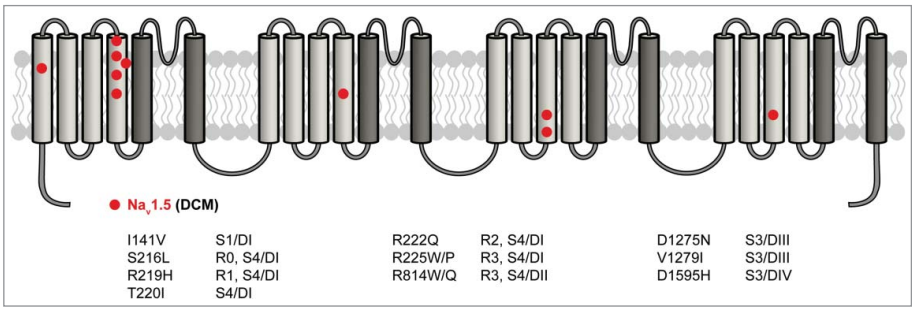

Figure 4. $Na_v1.5$ mutations associated with the development of DCM. 2D structure of $Na_v1.5$ illustrating the 4 homologous domains of the channel. The VSD formed by the S1-S4 segments of each domain are represented by light segments. The dark segments assemble to form the α pore of the channel. The red circles indicate the locations of the $Na_v1.5$ VSD mutations associated with the development of an atypical phenotype associating DCM with cardiac arrhythmias.

Depolarization-activated gating pores

It is interesting to note that an unusual mechanism has been described for gating pores that open at depolarized voltages. Mutations on the S4 segment should enhance the naturally occurring freezing of the S4 segment.[5,6,21-23] After long depolarization periods, the S4 segment of VSD should be naturally immobilized, resulting in a delay in the deactivation of the S4 segment.[24] While the molecular determinants of this process remain unclear, several hypotheses have been proposed, including the impact of α pore inactivation and the arrival of the S4 segment in a relaxed state.[25-28] However, it is interesting to note that a relaxed state has also been observed for the *Ciona intestinalis* voltage-sensitive phosphatase (Ci-VSP)[29] protein, which lacks a PD. This strengthens the hypothesis that the relaxed state of the S4 segment is an intrinsic property of VSD. Nevertheless, the immobilization process observed with mutant VSD may involve another mechanism causing the neutralization of charged S4 residues. This would lead to differences in the focused electric field, which would modify the voltage dependence of the S4 segment.

Due to the enhanced immobilization process observed for mutated S4 segments, depolarization-activated gating pores appear to have another characteristic. In addition to being permeable at depolarized voltages, depolarization-activated gating pores are also temporarily permeable at hyperpolarized potentials due to the immobilization process.[5,6,21-23,30] This results in a cation leak that is mainly enabled by the passage of Na^+ ions and that can last several tens of milliseconds. It

is also interesting to note that very little is known about the kinetics of the deactivation of mutant S4 segments.[30]

Hypokalemic periodic paralysis (HypoPP) is usually associated with the appearance of a hyperpolarization-activated gating pore, while normokalemic periodic paralysis (NormoPP) is usually associated with a depolarization-activated gating pore.[21,23,31-34] Nevertheless, the association between a hyperpolarization-activated or depolarization-activated gating pore and a specific clinical phenotype may not be as simple as associating hyperpolarization with HypoPP and depolarization with NormoPP. For example, the recently characterized R1128H/C mutation on $Na_v1.4$ creates a depolarization-activated gating pore while the clinical phenotype is HypoPP.[22] In the same vein, several mutations have been associated with the development of DCM associated with cardiac arrhythmias. However, while the R219H mutation on $Na_v1.5$ causes a H^+-specific hyperpolarization-activated gating pore, the R222Q and R225W mutations are cation-selective and are depolarization activated.[6,13] The pathological mechanism underlying depolarization-activated gating pores has been hypothetically associated with a Na^+ leak at hyperpolarized potentials due to the temporary immobilization of the S4 segment.[6,21,22] It is important to note that the exact downstream consequences of a leak mainly enabled by K^+ during the AP remain unknown. To date, only one gain of function mutation on an ATP-activated K^+ channel has been identified and associated with the development of a DCM phenotype.[35]More studies are thus clearly warranted to understand the pathological impact of such a K^+ leak.

Mutations targeting non-positively charged residues on the VSD

As previously mentioned, gating pores are created by the disruption of interactions between the S4 segment and the GCTC.[5] Most studies on the biophysical characterization of gating pore currents have investigated gating pores caused by the substitution of S4 positively charged residues in the S4 segment. However, interactions may also be disrupted by mutations on the S1-S3 segments, which make up a more rigid structure. If such mutations do create a continuous water crevice through the membrane, a gating pore should open. To our knowledge, only one study on mutations in the S1 and the S2 segments has raised the possibility of gating pores being created due to mutations outside the S4 segment.[36] A recent poster communication also dealt with HypoPP-related mutations located outside the S4 segment.[37]

Interestingly, several mutations in patients harboring HypoPP or DCM associated with arrhythmias are located in the VSD but do not target the positively charged residues or even the S4 segment (**Fig. 4**).[4,13] Recording and characterizing gating pores caused by such mutations is challenging given that their voltage dependence and selectivity are unknown and may in fact differ from those of previously characterized gating pores.[5]

Gating pore currents may be involved in many pathologies

The VSD is a highly conserved voltage sensing structure. Approximately 140 human VGIC are known, and they all share a common VSD structure that confers voltage sensitivity. Gating pore currents have been observed for mutations associated with the development of 4 unrelated pathologies: HypoPP, NormoPP, PNH, and DCM associated with cardiac arrhythmias.[4] However, based on their locations in the VSD, at least 61 other mutations cause a gating pore current that may be involved in the development of 10 different pathologies. These mutations are located on the VSD of the Na_v (1.1, 1.2, 1.4, 1.5, 1.9), Ca_v (1.1, 2.1), and K_v (3.1, 3.3, 7.2) channels. Muscular pathologies associated with gating pore currents (heart and skeletal muscle diseases) present atypical morphological abnormalities, indicating that such leak currents might impact the entire ionic homeostasis of cells.[6,38,39] Only a few mutations have been reported on the VSD of K_v channels. This could be related to their tetrameric nature, implying that one mutation may cause a gating pore 4 times larger than the gating pores in Na_v and Ca_v channels. Such non-physiological leak currents could potentially be lethal. However, caution is warranted if one assumes that this pathological mechanism is only based on the location of a mutation. While VSD are highly conserved structures in VGIC, they all possess unique properties that confer unique tridimensional structures and biophysical characteristics. For example, a single arginine mutation is not sufficient to open a gating pore in the VSD of DIV of $Na_v1.4$.[40] This characteristic is closely related to the structure and function of DIV. In fact, the 4th domain has been associated with the inactivation of the α pore of the channel. DIV possesses slower kinetics of activation to ensure that inactivation immediately follows activation. This is likely due to a larger hydrophobic septum in the VSD. Thus, a single mutation is insufficient to enable the junction of water crevices and create a continuous water pathway through the membrane. This example clearly highlights the fact that caution is warranted when associating the creation of a gating pore with the location of a mutation.

Conclusion

Gating pore currents are a novel pathophysiological mechanism that may cause DCM associated with cardiac arrhythmias. However, for similar phenotypes, only mutations on the first VSD of $Na_v1.5$ have been characterized. As such, other biophysical characterizations are required to strengthen the genotype/phenotype correlation. There are a number of hurdles that remain to be overcome to fully characterize and understand gating pores. No mutations targeting neutral S4 or S1-S3 residues have been characterized to date. The kinetics of the immobilization of mutated S4 segments are poorly understood, and the exact pathological consequences of the appearance of a gating pore are also largely unknown and mainly hypothetical.

Gating pores can be powerful tools for studying the detailed structures of VSD. They may also be valuable for characterizing the structural differences between VSD in the same family of channels and in specific channels. For example, it would be possible to investigate the differences between the DIV of Na_v and Ca_v channels

using gating pores given that gating pores caused by a single mutation have been reported for the 4th VSD of Ca_v while similar mutations are not sufficient to create gating pores in the 4th VSD of Na_v. Lastly, gating pore currents are a novel pathological mechanism that should be taken into consideration in the case of mutations located in the VSD of VGIC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This study was supported by grants from the Heart and Stroke Foundation of Quebec (HSFQ), the Canadian Institutes of Health Research (CIHR, MOP-111072 and MOP-130373),

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