TOPICS IN REVIEW

Uncoupling cytosolic calcium from membrane voltage by transient receptor potential melastatin 4 channel (TRPM4) modulation: A novel strategy to treat ventricular arrhythmias

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The current antiarrhythmic paradigm is mainly centered around modulating membrane voltage. However, abnormal cytosolic calcium (Ca^{2+}) signaling, which plays an important role in driving membrane voltage, has not been targeted for therapeutic purposes in arrhythmogenesis. There is clear evidence for bidirectional coupling between membrane voltage and intracellular Ca^{2+}. Cytosolic Ca^{2+} regulates membrane voltage through Ca^{2+}-sensitive membrane currents. As a component of Ca^{2+}-sensitive currents, Ca^{2+}-activated nonspecific cationic current through the TRPM4 (transient receptor potential melastatin 4) channel plays a significant role in Ca^{2+}-driven changes in membrane electrophysiology. In myopathic and ischemic ventricles, upregulation and/or enhanced activity of this current is associated with the generation of afterdepolarization (both early and delayed), reduction of repolarization reserve, and increased propensity to ventricular arrhythmias. In this review, we describe a novel concept for the management of ventricular arrhythmias in the remodeled ventricle based on mechanistic concepts from experimental studies, by uncoupling the Ca^{2+}-induced changes in membrane voltage by inhibition of this TRPM4-mediated current.

KEYWORDS Ventricular arrhythmia; Myopathic ventricle; TRPM4; 9-Phenanthrol

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Introduction

Ventricular arrhythmia contributes to significant morbidity and mortality in patients with cardiovascular disease. Conventional antiarrhythmic approaches attempt to terminate the arrhythmias by alteration of membrane electrophysiology through modulation of ion-channel homeostasis. However, the pre-existing structural and electrophysiological remodeling of myopathic or ischemic myocardium predisposes to the proarrhythmic effects of ion-channel modulation.[1,2] Hemodynamic deterioration due to the negative inotropic effect of some agents is another challenge with current antiarrhythmic therapy.[3] Dysregulation of cytosolic calcium (Ca^{2+}) dynamics has emerged as one of the key pathophysiological factors in the generation of ventricular arrhythmia.[4,5] Cytosolic Ca^{2+} overload and dispersion of Ca^{2+} transient amplitude (or Ca^{2+} transient amplitude alternans) are known to generate arrhythmia trigger and substrate, respectively.[6–8] Considering the challenges with current conventional antiarrhythmic agents, development of novel antiarrhythmic strategies remains an important focus of arrhythmia research.[9]

Transient receptor potential (TRP) channels are nonselective cation channels and are activated by a host of physical and chemical stimuli.[10] TRP channels are widely expressed in different organs, including the heart, and cardiac TRP channels play an essential role in cardiac growth and development, excitation-contraction, fibrosis, and remodeling.[10] The transient receptor potential melastatin 4 (TRPM4) channel is principally linked to cardiac electrophysiology.[10,11] Although the expression and functional role of TRPM4 channels in normal ventricular cardiomyocytes is debated, the

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KEY FINDINGS

- Transient receptor potential melastatin 4 (TRPM4) channels are calcium (Ca^{2+})-regulated membrane ion channels that principally carry inward current during membrane cardiac repolarization.
- In structurally normal ventricles, TRPM4 channels are mainly expressed in Purkinje fibers. However, higher expression and current density are reported in myopathic ventricular cardiomyocytes.
- Increased activity of the TRPM4 channel plays an important role in the pathogenesis of Ca^{2+} overload–induced ventricular arrhythmias.
- Inhibition of TRPM4 is shown to inhibit ventricular arrhythmias in conditions like coronary ischemia, ischemic heart failure, and catecholaminergic polymorphic ventricular tachycardia without significantly altering the intracellular Ca^{2+} homeostasis.
- TRPM4 inhibition is also associated with improvement of the inotropic state of myopathic ventricle.

channels are known to be upregulated in diseased ventricles.[11–15] TRPM4 channels are involved in the regulation of membrane voltage in response to intracellular Ca^{2+}.[11,12] The role of TRPM4 current in cardiac electrophysiology and arrhythmogenesis has been extensively discussed.[11,16,17] The current review aims to evaluate the mechanistic concept of whether mitigation of intracellular Ca^{2+} overload–induced membrane voltage changes by TRPM4 inhibition may have a potential role in the modification of the trigger and substrate in ventricular arrhythmia. Given the paucity of clinical data, the objective is to review the experimental data that support targeting TRPM4 modulation and its potential mechanistic impact on substrate and triggers of ventricular arrhythmia.

Coupling between cytosolic Ca^{2+} and membrane electrophysiology

Cytosolic Ca^{2+} plays a significant role in regulating membrane voltage and in the generation of membrane action potential. A bidirectional coupling exists between membrane voltage and intracellular Ca^{2+} dynamics, and the relationship may be concordant or discordant depending on the underlying condition.[7,18] Membrane voltage-driven Ca^{2+} alternans principally results from reduced activation of voltage-dependent Ca^{2+} channels (VDCCs) following a short diastolic interval during action potential duration (APD) restitution, whereas intracellular Ca^{2+} cycling can regulate membrane voltage through feedback control of Ca^{2+}-modulated currents during repolarization.[7,19–21] Hence, abnormal intracellular Ca^{2+} cycling plays an important role in ventricular arrhythmogenesis by triggering ectopic beats (also known as early afterdepolarization [EAD], and delayed afterdepolarization [DAD]) as well as facilitating re-entry due to membrane APD alternans and functional block.[7,20,21] Cytosolic Ca^{2+} overload activates inward currents (sodium-calcium exchanger [NCX] and Ca^{2+}-activated nonselective cation channels [CAN]), leading to the generation of afterdepolarizations (EAD and DAD) and prolongation of APD, whereas Ca^{2+} inactivation of VDCCs tends to abbreviate APD.[19] Inhibition of VDCCs and NCX is found to be associated with inhibition of afterdepolarization, shortening of APD, and mitigation of APD alternans translating into reduction of ventricular arrhythmia.[21,22] However, Ca^{2+} signaling is also important for various physiological processes, such as cardiac contraction, cell death, and apoptosis. Hence, modulations of VDCCs and NCX are known to exert adverse influences on Ca^{2+} homeostasis like negative inotropic effects with VDCC inhibition and cytosolic Ca^{2+} overload with NCX inhibition.[23] Apart from NCX, the Ca^{2+}-activated nonselective cation channel current (I_{CAN}) is found to be a crucial component of Ca^{2+}-sensitive inward membrane current,[19,24] and manipulation of I_{CAN} may modulate the Ca^{2+}-induced changes in membrane voltage without influencing the Ca^{2+} homeostasis. The most commonly described cardiac CAN channel conducts a 20- to 40-pS current and permeable to monovalent cations including sodium (Na^+) and potassium (K^+) but nonpermeable to Ca^{2+}.[25] I_{CAN} is linked to both Ca^{2+}-induced afterdepolarization[26] and cyclic alteration of APD.[27] Recent evidence suggests that the TRPM4 channel is the principal molecular candidate for the common cardiac CAN channel.[28]

Structure of TRPM4

TRP channels consist of a group of cationic channels expressed in a wide variety of cells and share structural homology.[10] TRP channels are divided into 6 subfamilies, that is, TRPA (ankyrin; TRPA1), TRPC (canonic; TRPC1–TRPC7), TRPM (melastatin; TRPM1–TRPM8), TRPML (mucolipin; TRPML1–TRPML3), TRPP (polycystin; TRPP1–TRPP3), and TRPV (vanilloid; TRPV1–TRPV6), and they are involved in a spectrum of cellular signaling processes in mammalian organs.[10] Although several TRP channels are reported to be involved in cardiac development, hypertrophy, remodeling, and heart failure,[29] TRPM4 has emerged as a key player in cardiac electrophysiology.[11] The TRPM4 channel differs from other TRP channels by the absence of ankyrin in the N-terminus and impermeability to the Ca^{2+} ion.[28,30] The human TRPM4 gene is located on chromosome 19. TRPM4b, the longest variant with 1214 amino acids, is considered the canonical isoform, while other isoforms are TRPM4a and TRPM4c.[25,30] Like voltage-gated potassium (K^+) channels and hyperpolarization-activated cyclic nucleotide-gated channels, the TRPM4 channel consists of 4 subunits (Figure 1). The pore of this tetrameric channel is surrounded by 4 α subunits, and each α subunit contains 6 transmembrane-spanning segments (TM1–TM6) with N- and C-terminal regions located within cytoplasm.[31,32] The central pore is constituted by TM5 and TM6 along with the P-loop segment. An aspartate-rich

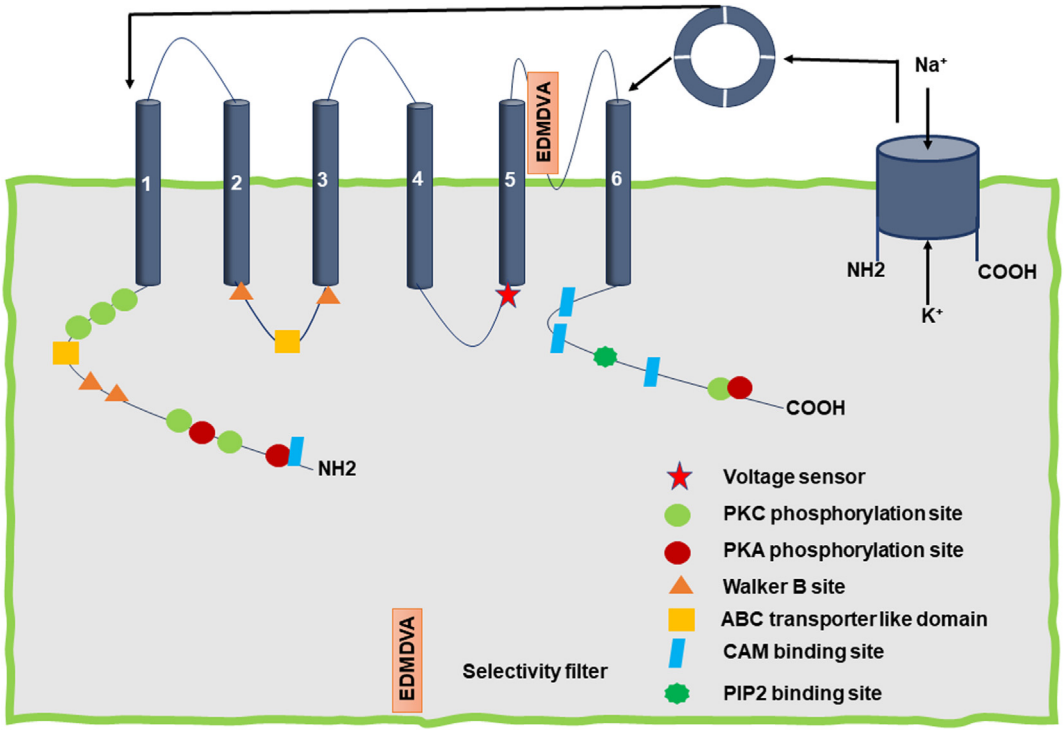

Figure 1 Structure of the TRPM4 (transient receptor potential melastatin 4) channel. The pore of the channel is surrounded by 4 α subunits. The numbers 1 to 6 indicate membrane-spanning segments. The pore is situated between the fifth and sixth segments. CAM = calmodulin; EDMDVA = glutamate-aspartate-methionine-aspartate-valine-alanine residues; PIP2 = phosphatidylinositol 4,5-bisphosphate; PKA = protein kinase A; PKC = protein kinase C; PLC = phospholipase C; SR = sarcoplasmic reticulum.

EDMDVA (glutamate-aspartate-methionine-aspartate-valine-alanine) domain in this region forms the selectivity filter of the channel.[30] The N- and C-terminal regions consist of 2 ABC transporter motifs, multiple protein kinase C (PKC) phosphorylation sites, 4 Walker B sites, 5 calmodulin-binding sites, and an arginine-lysine–rich PIP2 binding site.[25,30,32] These regulatory domains modulate the Ca^{2+} sensitivity of the channels (Figure 2).

Function and regulation cardiac TRPM4

TRPM4, a Ca^{2+}-activated, and voltage-dependent channel with a single-channel conductance of 25 pS, selectively conducts Na^+ and K^+ ions but is impermeable to Ca^{2+}.[33,34] A rise of intracellular Ca^{2+} causes transient activation of the channel. The current is principally active in a range of intracellular Ca^{2+} from 0.4 μM to 9.8 μM, and the value is only achieved during peak systole in physiological conditions.[34] The brief activation is followed by deactivation due to rapid Ca^{2+} desensitization.[35] Even in the presence of high intracellular Ca^{2+}, the current is modulated by membrane voltage with activation in positive membrane potential and fast deactivation in negative membrane potentials.[36] However, higher intracellular Ca^{2+} and Ca^{2+} sensitizers shift the activation curve toward more negative potentials.[30] As this channel is equally permeable to Na^+ and K^+ ions, the current through the active channel is dependent on membrane voltage, at positive membrane voltage it carries outward K^+ current, while inward depolarizing Na^+ current is activated at negative membrane voltage.[28,37] The Ca^{2+} sensitivity of the channel is increased by calmodulin binding, diacylglycerol (DAG), and PKC-mediated phosphorylation (Figure 2).[25,35,38] Calcium–calmodulin–dependent protein kinase II–mediated activation of TRPM4 is also reported in the stressed heart.[39] The role of adenosine triphosphate (ATP) in TRPM4 modulation is complex with inhibition by increased cytosolic ATP, while activation by extracellular ATP through stimulation of purinergic receptors (Figure 2).[25,35,36] During cellular stress, posttranslational modification by SUMOylation is known to potentiate the channel activity.[32,40]

TRPM4 in ventricular myocardium

The heart is one of the major TRPM4-expressing tissues, and TRPM4 gene expression, protein, and current activity have been detected in animal and human cardiomyocytes.[11,28] Although TRPM4 is highly expressed in Purkinje fibers in structurally normal hearts, the functional importance of the channel is still debated in normal ventricular myocytes. Most studies have demonstrated scanty presence.[12,40–42] However, a study by Mathar and colleagues[15] showed shortening of APD in ventricular cardiomyocytes from a TRPM4-deleted (*Trpm4*$^{-/-}$) murine model. More importantly, other studies in genetically modified murine models as well as following pharmacological inhibition of TRPM4 could not demonstrate any change in APD.[42,43] Unlike other studies that used isolated ventricular cardiomyocytes, Mathar and colleagues[15] used papillary muscle strips as representative of ventricular cardiomyocytes whereas papillary muscle tissue is an admixture of ventricular cardiomyocytes and

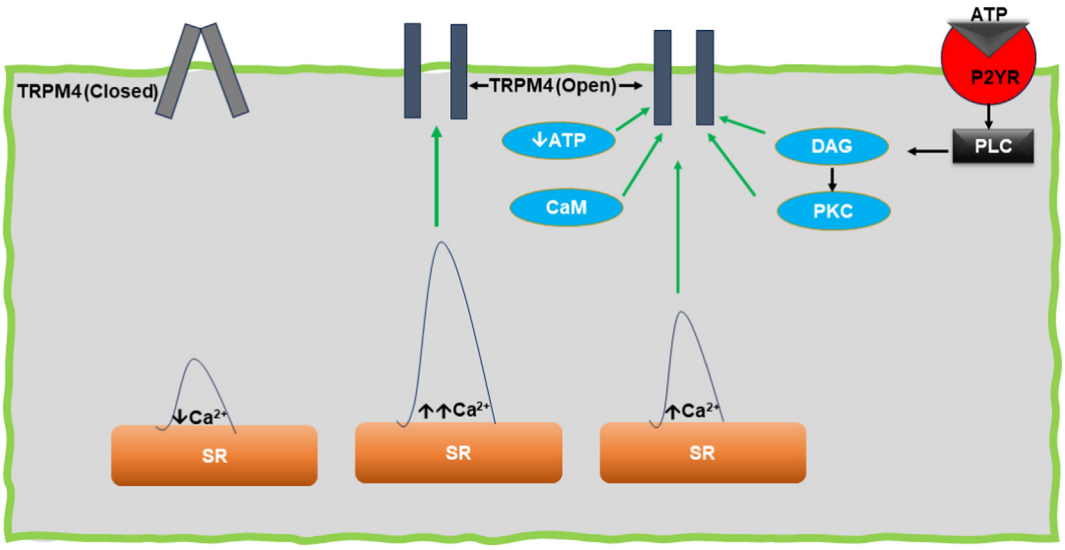

Figure 2 Regulation of the transient receptor potential melastatin 4 (TRPM4) channel. The channel is not activated below a critical cytosolic calcium (Ca^{2+}). Higher intracellular Ca^{2+} activates the channel. The Ca^{2+} sensitivity is modulated by intracellular and extracellular adenosine triphosphate (ATP) (reduced intracellular ATP and high extracellular ATP increase the Ca^{2+} sensitivity), calmodulin (CaM), protein kinase C (PKC)–mediated phosphorylation, and diacylglycerol (DAG). In the presence of Ca^{2+} sensitizer, the channel is activated by relatively low intracellular Ca^{2+}. P2YR = Purinergic receptor; PLC = phospholipase C; SR = sarcoplasmic reticulum.

extensive Purkinje fiber network.[44] However, upregulation of the TRPM4 channel in the ventricular myocardium is reported with chronic myopathic remodeling. Higher expression and TRPM4 current activity have been documented in the ventricular tissue of spontaneously hypertensive rats compared with normotensive Wistar-Kyoto rats.[41] Altered cellular DAG and PKC content leading to enhanced Ca^{2+} sensitivity of the channel is also reported with hypertrophic remodeling.[38] As observed in animal models, increased human TRPM4 expression has also been reported in ventricular tissue in hearts explanted from end-stage heart failure patients.[13] Unlike the chronic remodeling process, upregulation of the TRPM4 channel is not reported in acute myocardial ischemia in myocardial tissue.[45] Instead, increased activity of existing TRPM4 channels in Purkinje fibers contributes to increased ventricular arrhythmia vulnerability in ischemia-reperfusion injury.[46]

Cardiac TRPM4 modulation

Evaluation of mutations of the TRPM4 gene in electrical disorders of the heart, experiments on Trpm4 knockout (*Trpm4*$^{-/-}$) animals,[15,37,47] and pharmacological inhibition of TRPM4 in animal models[42,46,48,49] have allowed a better understanding of the role of the channel in cardiac electrophysiology. *Trpm4*$^{-/-}$ murine models have been used to explore the role of this channel in and the effects of its modulation on membrane electrophysiology.[15,37] Flufenamic acid and 9-phenanthrol are most commonly used as pharmacological inhibitors of the TRPM4 channel in experimental settings.[42,46,48,49] Although 9-phenanthrol can inhibit a host of other channels including voltage-gated Ca^{2+} and K^+ channels, it is considered a specific TRPM4 channel inhibitor at low concentrations (i.e., $<10^{-4}$ mol/L).[14] Meclofenamate, another nonsteroidal anti-inflammatory agent like flufenamic acid, is also shown to act as a selective TRPM4 inhibitor in a dose range of 10 to 30 μM.[50] Other components of inward currents, including NCX, Na^+ current, and VDCCs, are not found to be affected by the above-mentioned dose range of meclofenamate. A few other clinically available agents, such as glibenclamide, quinine, and clotrimazole, inhibit the TRPM4 current, but again, these drugs also inhibit other channels.[11,12,32]

TRPM4 activation and ventricular arrhythmia trigger

TRPM4 activation plays an important role in the genesis of arrhythmia triggers. Spontaneous diastolic depolarization, generated because of diastolic Ca^{2+} overload, acts as a trigger for arrhythmia. The role of I_{CAN}, a functional equivalent of TRPM4 current in cardiac tissue, was established in the genesis of oscillatory current rabbit ventricular myocytes.[26] In the setting of high intracellular Ca^{2+}, activation of transient inward current (I_{ti}) leads to the generation of spontaneous diastolic depolarization.[51–53] TRPM4-mediated I_{CAN} plays an important component in Ca^{2+}-induced I_{ti} (Figure 3).[25,51] Although TRPM4 channels are not permeable to Ca^{2+} and do not have any direct modulatory role on sarcoplasmic Ca^{2+} handling, persistent activation of the channel is associated with diastolic Ca^{2+} overload.[43,54] The indirect effects of TRPM4 activation on VDCCs and NCX may explain the diastolic rise of intracellular Ca^{2+} (Figure 3). TRPM4 activation–induced prolongation of the plateau phase of repolarization causes delayed inactivation of VDCCs, and slow Ca^{2+} influx continues even after cessation of systole and induces persistent release of Ca^{2+} from sarcoplasmic reticulum. Hence, inhibition of TRPM4 current is associated with reduction of Ca^{2+} transient duration, a marker of diastolic Ca^{2+} clearance.[15] Prolonged activation

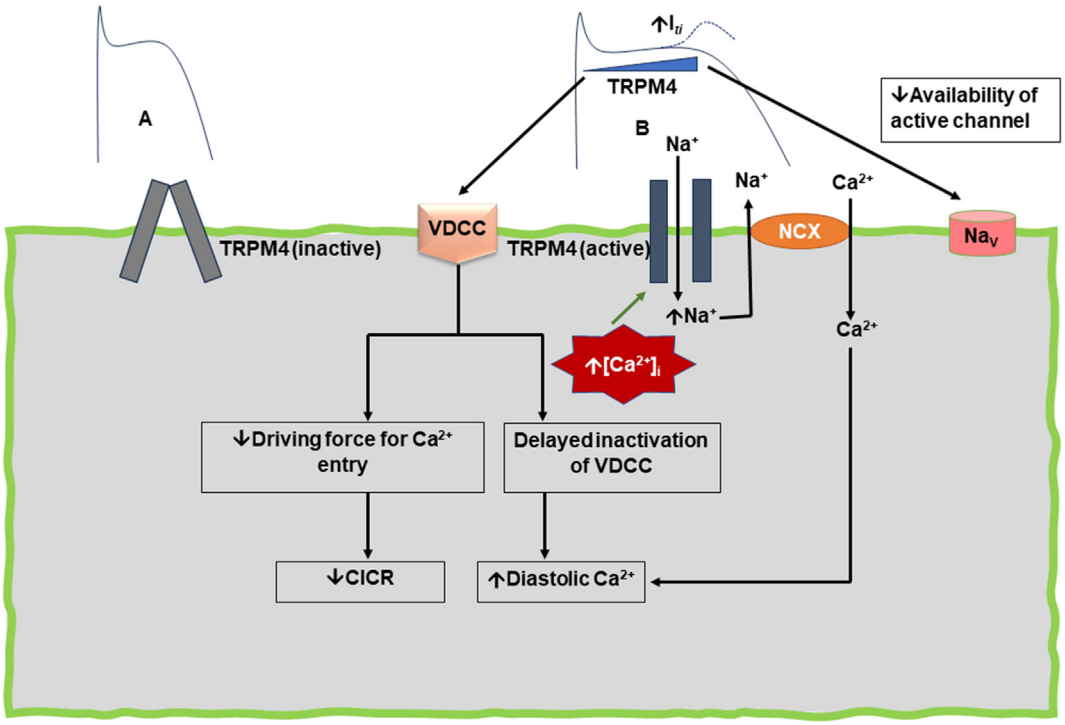

Figure 3 Transient receptor potential melastatin 4 (TRPM4) activation and membrane electrophysiology. Closure of the TRPM4 channel is associated with shortening of action potential duration (APD) (action potential A). Activation of the channel: phase 2 and early phase 3 allow sodium (Na^+) ion entry. This transient inward current activation is associated with APD prolongation (action potential B) and generation of afterdepolarization (dotted curve). A relatively positive membrane voltage by TRPM4 activation reduces the driving force for calcium (Ca^{2+}) entry through voltage-dependent calcium channels (VDCCs), and the inactivation of VDCCs is delayed due to the depolarized state of membrane voltage, leading to decreased Ca^{2+} transient amplitude with concurrent Ca^{2+} overload during diastole. Diastolic Ca^{2+} overload may also result from Na^+ overload and sodium-calcium exchange (NCX) activation. The APD prolongation also delays the conversion of inactive voltage-gated sodium channels (Na_V) to the resting stage and reduces the availability of active Na_V for subsequent depolarization. CICR = calcium-induced calcium release; I_{ti} = transient inward current.

of TRPM4 current during phase 2 of ventricular repolarization allows Na^+ entry into the cell, leading to intracellular Na^+ overload. The Na^+ overload activates the NCX in reverse mode with the subsequent intrusion of Ca^+ into the cell, leading to Ca^{2+} overload.[55]

Spontaneous generation of afterdepolarizations from the Purkinje fibers plays a crucial role in ventricular arrhythmia during coronary ischemia.[56] Due to the abundance of the channel in normal Purkinje fibers, TRPM4 has become an important candidate in the genesis of ischemic ventricular arrhythmias. Intracellular Ca^{2+} overload from hypoxic stress along with an abundance of Ca^{2+} sensitizers such as ATP depletion, and SUMOylation lead to increased activation of this channel with subsequent generation of EAD.[24,32,40] In a murine model of ventricular hypoxia–reoxygenation, TRPM4 inhibition by 9-phenanthrol was associated with abolition of EAD in a dose-dependent manner.[49] TRPM4 inhibition is also reported to eliminate EADs in anemone toxin (ATX-II)–treated ventricular cardiomyocytes.[57] Upregulation of TRPM4 channels may contribute to EAD generation in hypertrophic ventricular myocardium.[41]

TRPM4 activation and ventricular arrhythmia substrate

TRPM4-mediated I_{ti} tends to prolong APD, especially phase 2 and early phase 3 (Figure 3).[37] Prolongation of QT interval, an electrocardiographic surrogate of reduced repolarization reserve, is reported to be associated with upregulation of the TRPM4 channel in ventricular tissue in spontaneously hypertensive rats.[41] Importantly, reduced repolarization reserve in the myopathic ventricle plays an important role in the pathogenesis of ventricular arrhythmia.[58] Therefore, improving repolarization reserve by TRPM4 modulation may modify the ventricular arrhythmia substrate. The abbreviation of APD is reported with TRPM4 inhibition in Purkinje fibers from normal rabbit ventricles as well as from ATX-II–treated ventricular myocytes.[42,57] Similarly, APD in papillary muscle in *Trpm4*$^{-/-}$ mice was short compared with wild-type mice.[15] Although the effect of TRPM4 inhibition on APD alternans is currently not explored, a short APD despite high Ca^{2+} transient amplitude in *Trpm4*$^{-/-}$ mice suggests uncoupling of APD from the influence of intracellular Ca^{2+} following inhibition of TRPM4 current.[15] Hence, a reduction of APD alternans despite continuation of Ca^{2+} alternans is plausible in the setting of TRPM4 inhibition. Interestingly, the elimination of electrical alternans despite the presence of mechanical alternans has been reported in the setting of inhibition of other Ca^{2+}-modulated membrane currents.[59]

The role of TRPM4 inhibition in action potential maximum upstroke velocity and ventricular conduction is debated. Genetic knockout of TRPM4 or its pharmacological

inhibition in Purkinje fibers and ventricular cardiomyocytes from normal hearts failed to modulate the maximum upstroke velocity in normoxic conditions.[15,42,50] However, slow conduction and propagation failure are predicted in the His/Purkinje system following TRPM4 overexpression, most probably due to the inadequate recovery of Na^+ channels due to APD prolongation.[33,60] The heterogeneity of conduction in the His/Purkinje system is reported to be associated with the remodeling in heart failure and is responsible for re-entrant tachycardias, such as bundle branch re-entry, interfascicular, and intrafascicular ventricular tachycardias.[61] Keeping in mind that upregulation of TRPM4 expression/activity is reported in myopathic ventricles,[13,34] TRPM4 inhibition may improve Purkinje fiber conduction in this setting and is supposed to suppress reentrant ventricular arrhythmias. However, this speculation requires experimental validation.

On the other hand, as discussed subsequently, loss-of-function TRPM4 mutations are also associated with abnormal cardiac conduction.[62] In a *Trpm4*$^{-/-}$ murine model, abnormal intraventricular conduction is linked to reduced activity of peak Na^+ current.[63] However, observation from the TRPM4 knockout model should be interpreted with caution because the expression and function of TRPM4 are shown to be influenced by other background genes.[64] It should also be remembered that although TRPM4 overexpression is pathological, a background TRPM4 activity may be essential for the physiological function of Na^+ current. Hence, complete ablation of the TRPM4 current may also adversely affect cardiac conduction. The structural remodeling following complete TRPM4 knockout may also contribute to abnormal ventricular conduction.[43] Interestingly, 9-phenanthrol also inhibits peak Na^+ current in the ventricle.[57] Hence, future studies with selective pharmacological TRPM4 inhibition in experimental models of ischemia and cardiomyopathies may further elucidate the effects of TRPM4 modulation on ventricular conduction in structural heart diseases.

TRPM4 modulation as a target for ventricular arrhythmia therapy

Considering the fact that TRPM4 channels are highly expressed in Purkinje fibers and that the Purkinje fibers play an important role in the genesis of ventricular arrhythmia in a variety of conditions including acute myocardial infarction, ischemic and nonischemic cardiomyopathies, and primary electrical disorders such as catecholaminergic polymorphic ventricular tachycardia, long QT syndrome, short QT syndrome, and Brugada syndrome, TRPM4 inhibition may offer potential avenues for mitigating a broad spectrum of ventricular arrhythmias.[61] Ventricular arrhythmia suppression in the ischemic ventricle is demonstrated by TRPM4 inhibition. Deletion of the TRPM4 gene was associated with the reduction of mortality in an in vivo murine model of ischemic heart failure.[47] A trend toward reduction of both early and late ventricular arrhythmia events was noted in TRPM4-deficient mice.[47] The cardioprotective effect of pharmacological TRPM4 inhibition was also associated with a significant reduction of ventricular arrhythmia in a rodent model of ischemia-reperfusion.[46] In another model of ischemia-reperfusion, treatment with 9-phenanthrol was associated with significant ventricular arrhythmia suppression.[37] The reduction of ventricular arrhythmia was associated with a reduction of EAD.[37] In a murine model of catecholaminergic polymorphic ventricular tachycardia, inhibition of Ca^{2+} overload–induced TRPM4 current by meclofenamate was translated into suppression of stress-induced ventricular arrhythmia.[50] TRPM4 inhibition is also shown to reduce drug-induced ventricular arrhythmias following treatment with isoprenaline and the Na^+-channel activator ATX-II.[57,65]

TRPM4 channels and clinical arrhythmia syndrome

Mutations in the TRPM4 gene are reported in patients with arrhythmic conditions. In corollary with the distribution of TRPM4 channels in the cardiac conducting system, the clinical manifestations of TRPM4 mutations include progressive familial heart block type I, isolated cardiac conduction disease, atrioventricular block, right bundle branch block, and Brugada syndrome.[32,62] Interestingly, both loss- and gain-of-function mutations are associated with disease phenotypes.[32,40,62] The molecular mechanisms of clinical arrhythmias are unclear. It is postulated that both depolarization by TRPM4 gain-of-function and hyperpolarization by loss-of-function may reduce the availability of Na^+ channels and thus delay ventricular conduction.[66,67]

TRPM4 inhibition and myocardial inotropy

Most of the currently available antiarrhythmic agents exert a negative inotropic effect on myocardium and/or vascular smooth muscles, leading to exacerbation of heart failure and hypotension, especially in the presence of structural heart disease.[3,68,69] However, the antiarrhythmic effect of TRPM4 inhibition is associated with an improvement in ventricular contractile function.[46,47] In an isolated Langendorff-perfused rodent heart, pretreatment with 9-phenanthrol was associated with the improvement of contractile function following global ischemia-reperfusion, as indicated by higher left ventricular developed pressure and maximum value of the time derivative of left ventricular pressure in the 9-phenanthrol group as compared with the control group.[46] In a murine model of ischemic heart failure, significant improvement of ventricular contractility was noted in response to β-adrenergic stimulation in *Trpm4*$^{-/-}$ as compared with wild-type mice.[47] The improved contractile response by β-adrenergic stimulation in *Trpm4*$^{-/-}$ murine ventricular myocardium was also noted in the absence of myopathic insult.[15] A fast repolarization process in presence of TRPM4 inhibition may enhance the driving force of systolic Ca^{2+} influx through VDCCs.[15] Subsequent

improvement in Ca^{2+}-induced Ca^{2+} release may contribute to the improvement of contractile function.[15]

Summary

Existing experimental studies suggest that TRPM4 current inhibition may confer an antiarrhythmic effect by inhibiting ventricular arrhythmia triggers and modification of the arrhythmia substrate. As a result of APD shortening, the TRPM4 inhibition strategy is not susceptible to QT prolongation and torsades de pointes.[15,42] It is also expected that in presence of hyperexpression/overactivity of TRPM4 channels, as in cardiomyopathy and coronary ischemia, inhibition of its current will improve conduction velocity.[33] Hence, this approach may lack the proarrhythmic effect of class I agents, especially in presence of structural heart diseases.[70] Moreover, unlike the use of conventional antiarrhythmic agents, inhibition of TRPM4 is shown to improve ventricular contractile function, which may be beneficial in acute myocardial infarction, and heart failure.[46,47] However, developing a selective pharmacological TRPM4 antagonist remains a challenge. A baseline TRPM4 activity may play important roles in multiple physiological processes, and complete disruption of the TRPM4 gene may complicate the observations by inducing structural remodeling and altering Na^{+}-channel function.[43,63] Although 9-phenanthrol is considered a selective TRPM4 inhibitor in a concentration between 10^{-5} and 10^{-4} mol/L, an independent Na^{+} current inhibition is reported at that concentration range.[14,57] It should be kept in mind that TRPM4 inhibition may enhance the vulnerability to reentrant ventricular arrhythmia in normal hearts probably due to the physiological heterogeneity of spatial distribution of the channel across the ventricle.[65] Considering the role of Ca^{2+}-induced TRPM4 current on the diastolic depolarization slope of the sinoatrial node, sinus bradycardia would also be a concern of TRPM4 inhibition.[11,71] However, in a rodent model of ischemia-reperfusion, pharmacological inhibition of TRPM4 was not associated with a significant alteration of heart rate.[46] Due to the widespread tissue distribution of TRPM4 channels, possible noncardiac toxicities are another major concern for the TRPM4 inhibition strategy. Apart from the regulation of cardiovascular function, the activity of this channel is essential in the regulation of insulin secretion, immune response, cerebrovascular tone, and spontaneous respiration.[34] So, multiple noncardiac side effects may be a limitation of TRPM4 inhibition as an antiarrhythmic strategy. However, the heart is one of the most highly TRPM4-expressing organs, and aberrant cardiac electrophysiology is the only clinical manifestation of inherited TRPM4 dysfunction. Considering these facts, noncardiac side effects may not arise while using specific TRPM4 inhibitors.

Conclusion

It is biologically plausible to correct aberrant membrane electrophysiology and mitigate ventricular arrhythmia by modulating the Ca^{2+}-regulated TRPM4 current. TRPM4 modulation is not associated with altered Ca^{2+} homeostasis or proarrhythmic QT prolongation, and it is also shown to improve myocardial contraction. The previous properties may be beneficial, especially in the presence of ventricular pathologies, such as ischemic heart disease and heart failure. Future studies with selective pharmacological inhibitors of the TRPM4 channel in models of structural heart diseases may further elucidate the role of this novel strategy.

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