TRPM4 in cardiac electrical activity

Romain Guinamard1*, Patrice Bouvagnet2, Thomas Hof1, Hui Liu3, Christophe Simard1, and Laurent Salle1

1 Groupe Signalisation, Electrophysiologie et Imagerie des Lésions d’Ischémie–Reperfusion Myocardique, EA4650, Université de Caen Basse–Normandie, Sciences D, Esplanade de la Paix, CS 14032, 14032 Caen Cedex 5, France; 2 EA 4173, Université Lyon 1 and Hôpital du Nord-Ouest, Lyon, France; and 3 Department of Anatomy, Hainan Medical College, Haikou, Hainan 571101, China

Abstract

TRPM4 forms a non-selective cation channel activated by internal Ca2+. Its functional expression was demonstrated in cardiomyocytes of several mammalian species including humans, but the channel is also present in many other tissues. The recent characterization of the TRPM4 inhibitor 9-phenanthrol, and the availability of transgenic mice have helped to clarify the role of TRPM4 in cardiac electrical activity, including diastolic depolarization from the sino-atrial node cells in mouse, rat, and rabbit, as well as action potential duration in mouse cardiomyocytes. In rat and mouse, pharmacological inhibition of TRPM4 prevents cardiac ischemia–reperfusion injuries and decreases the occurrence of arrhythmias. Several studies have identified TRPM4 mutations in patients with inherited cardiac diseases including conduction blocks and Brugada syndrome. This review identifies TRPM4 as a significant actor in cardiac electrophysiology.

Keywords

TRPM4 • Calcium-activated non-selective cation channel • Cardioprotection • Brugada • Arrhythmias

1. Introduction

A Ca2+-activated non-selective monovalent cationic current (NSCCa), with no permeability for Ca2+ and a single-channel conductance close to 25 pS, was described in the early 2000s in cardiomyocytes from adult mice, rats, and humans.1–3 Identifying this additional cardiac NSCCa4–6 sparked new investigations, which were further boosted by the concomitant cloning of the transient receptor potential melastatin 4 (TRPM4) gene in these species.7–9 This gene encodes an ion channel protein (TRPM4) whose current properties resemble the aforementioned NSCCa current and is thus considered as its molecular counterpart.

Three recent developments further highlighted the multiple roles of TRPM4 current in the heart: first, the identification of the TRPM4 inhibitor 9-phenanthrol, which helped unmask the effects of TRPM4 current modulation on small mammals heart functions10; secondly, the generation of Trpm4 null mice to study the impact of gene disruption11,12; and thirdly, the discovery by positional cloning that TRPM4 mutations are associated with cardiac conduction block and Brugada syndrome in humans.13–15 These contemporary studies provide a portrait of the role(s) of TRPM4 both in cardiac physiology and in pathophysiology, as reviewed previously.16–18 In addition to influencing cardiac development and remodelling19,20 or regulating cardiac contractility,21 TRPM4 participates in cardiac electrical activity. This review will sharply focus on this last contribution by collecting existing data.

Note that in addition to its role(s) in cardiac activity, TRPM4 is implicated in other physiological functions such as the immune response, insulin secretion, breathing activity, smooth muscle contraction, arterial constriction, and cell death.16,22–24

2. TRPM4 expression in the heart, biophysics, and regulatory properties

2.1 TRPM4 expression profile in the heart

TRPM4 mRNA is present in a large variety of tissues from human, mouse, and rat, the heart being among the major TRPM4 expressing tissues.7,8,25,26 Several studies evaluated TRPM4 mRNA or protein expression in specific parts of the heart, as collected in Table 1. TRPM4 mRNA and/or protein is expressed in nodal and conductive tissue as shown in the mouse sino-atrial node (SAN) and bovine bundle branch.14,30 It is also detected in the atrium, including in humans,27 and in the ventricle.29 Interestingly, several studies reported a significant weaker expression in ventricles than in atria in rat, mouse, and bovine.28 At the functional level, TRPM4 current has been recorded in rodent as well as in human cardiomyocytes.2,3,19,28,31,33 In addition to cardiomyocytes, Trpm4 mRNA has also been detected in rat ventricular fibroblasts and arterial smooth muscle cells and human atrial fibroblasts.23,34,35 As discussed subsequently, cardiac TRPM4 expression profile can be modified by human TRPM4 gene mutations13–15,36 or cardiac diseases such as hypertrophy in rat.28

2.2 TRPM4 biophysical properties

The biophysical and regulatory properties of TRPM4 were described in the early of 2000s7,25,37 and have been reviewed extensively.16,38 The Trpm4 gene is located on human chromosome 19 and encodes a
1214-amino-acid protein,\textsuperscript{7} whose tertiary structure features six transmembrane domains. The functional channel is a homotetramer. TRPM4 has calmodulin-binding sites, Walker B motifs, ATP, and phosphatidyl inositol 4,5-bisphosphate (PIP\(_2\)) binding sites, a glycosylation site, and putative phosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC).\textsuperscript{16,38–40}

TRPM4-transfected HEK-293 cells develop an ionic current with a linear unitary current–voltage relationship and a conductance between 20 and 25 pS,\textsuperscript{7,34} similar to endogenous TRPM4 currents recorded in cardiomyocytes (Figure 1). The channel is equally permeable to Na\(^+\) and K\(^+\), but not permeable to Ca\(^{2+}\) (permeability sequence: Na\(^+\) ∼ K\(^+\) > Cs\(^+\) > Li\(^+\) > Ca\(^{2+}\)).\textsuperscript{7,41} Channel open probability increases with membrane depolarization, resulting in an outwardly rectifying current at the whole-cell level.\textsuperscript{7,25}

### 2.3 Regulatory properties of the TRPM4 current

The intracellular effector PKC as well as PIP\(_2\) and oxygen species such as H\(_2\)O\(_2\) increase TRPM4 activity.\textsuperscript{14–16} Conversely, TRPM4 activity is inhibited by internal adenosine nucleotides such as ATP and ADP.\textsuperscript{37}

Ca\(^{2+}\) activates the TRPM4 channel with an EC\(_{50}\) up to 370 \(\mu\)mol/L when measured in the inside-out configuration, but from 20 to 0.5 \(\mu\)mol/L in the whole-cell configuration after overexpression in cell lines.\textsuperscript{7,42} This discrepancy unmask that TRPM4 sensitivity to Ca\(^{2+}\) is modulated by regulators such as calmodulin and PIP\(_2\).\textsuperscript{42–43}

Inside-out recordings of TRPM4 current from human atrial cardiomyocytes revealed an EC\(_{50}\) at 21 \(\mu\)mol/L with channel openings recorded even at 0.1 \(\mu\)mol/L (Figure 1).\textsuperscript{2} This EC\(_{50}\) is probably lower in intact cells, similar to heterologous TRPM4 in HEK cells\textsuperscript{25,37} and thus TRPM4 might be able to activate at physiological [Ca\(^{2+}\)]. Accordingly, whole-cell recordings in mouse ventricular cardiomyocytes exhibit a Ca\(^{2+}\)-transient-activated current attributed to TRPM4 that was absent from TRPM4 knock-out mice.\textsuperscript{21} In addition, unitary TRPM4 currents are detectable in cell-attached patches from dedifferentiated rat ventricular cardiomyocytes, indicating that the current can activate in an intact cell with physiological [Ca\(^{2+}\)].\textsuperscript{45}

### 3. Tools to unmask TRPM4 contributions to cardiac electrical activity

#### 3.1 Pharmacological inhibitors

Flufenamic acid inhibits TRPM4 (IC\(_{50}\) = 3 \(\mu\)mol/L),\textsuperscript{36} but this promiscuous drug also affects a variety of other channels.\textsuperscript{7} In addition, TRPM4 is inhibited by glibenclamide (10 \(\mu\)mol/L),\textsuperscript{30} spermine (IC\(_{50}\) = 61 \(\mu\)mol/L),\textsuperscript{37} and quinine (IC\(_{50}\) from 113 to 450 \(\mu\)mol/L),\textsuperscript{48} but again, these drugs also inhibit other channels.\textsuperscript{38}

More recently, 9-phenanthrol was shown to inhibit TRPM4 (IC\(_{50}\) = 17 \(\mu\)mol/L).\textsuperscript{10} It can be applied to the inside or the outside of the membrane and most probably acts directly on the channel as it is effective in cell-free patches.\textsuperscript{10} 9-Phenanthrol has been tested on other channels, including various transient receptor potential (i.e. TRP) channels (including TRPC3, TRPC6, TRPM5, and TRPM7), and so far has shown a specificity for TRPM4 within this family.\textsuperscript{10,49–51} Nevertheless, high concentrations of 9-phenanthrol (0.1 \(\mu\)mol/L) inhibit the delayed outward rectifier K\(^+\) current and the voltage-gated Ca\(^{2+}\) current in mouse cardiomyocytes, whereas lower concentrations do not.\textsuperscript{32} Furthermore, a recent study showed that 9-phenanthrol inhibits the Cl\(^−\) channel TMEM16A over a concentration range that was previously associated with TRPM4-specific effects.\textsuperscript{52} In addition to modulating these ion channels, 9-phenanthrol also inhibits bovine heart cyclic-AMP-dependent PKA catalytic subunit, although these data were collected only using biochemical assays in vitro and were never confirmed in cellular models.\textsuperscript{53} As described subsequently, comparing the effect of 9-phenanthrol in wild-type (WT) vs. knock-out animals has been a valuable complement to confirm that TRPM4 is the primary target of 9-phenanthrol.

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**Table 1** Cardiac expression of TRPM4 mRNA, protein, and current

<table>
<thead>
<tr>
<th>Species</th>
<th>Heart</th>
<th>SAN</th>
<th>Atrium</th>
<th>Ventricle</th>
<th>Conductive tissue</th>
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<td>mRNA (higher)</td>
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<td>Current</td>
<td>Protein</td>
<td>Guinamard et al.\textsuperscript{28} Guinamard et al.\textsuperscript{28} Piao et al.\textsuperscript{29} Zhainazarov\textsuperscript{3}</td>
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<td>mA</td>
<td>mRNA (lower)</td>
<td>Current</td>
<td>Current</td>
<td>Murakami et al.\textsuperscript{8} Nilius et al.\textsuperscript{25} Jang et al.\textsuperscript{26} Demion et al.\textsuperscript{19} Demion et al.\textsuperscript{16} Demion et al.\textsuperscript{16} Mathar et al.\textsuperscript{21} Simard et al.\textsuperscript{32} Mathar et al.\textsuperscript{21} Liu et al.\textsuperscript{14}</td>
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<td>mRNA, protein, current</td>
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</table>

Reported currents were recorded from freshly isolated cardiomyocytes. Higher and lower indicate the level of mRNA or protein expression compared with other parts of the heart.
knock-out second strain develops cardiac hypertrophy and exhibits ECG perturbations that include conduction blocks, sinus pauses, and enhanced ectopic atrial activity. This discrepancy between the phenotypes of the two strains is not yet understood. However, experiments conducted on multicellular preparations or isolated cardiomyocytes from these mice have given valuable data regarding the role of TRPM4 in cardiac electrical activity, as described in the following sections.

Although pharmacological approaches reveal the direct effects of ion channels on electrical activity, transgenic (knock-out) animals can reveal more integrated functions of ion channels in situ, but their phenotypes can be more difficult to interpret due to developmental modifications or compensatory effects. From the standpoint of cardiac electrophysiology, major currents (voltage-gated Ca$^{2+}$, K$^{+}$, and Na$^{+}$ currents) as well as Ca$^{2+}$ transients are not affected in Trpm4 knock-out mice, which indicate that the global cardiac channel pattern is not strongly modified so the model remains useful to unmask TRPM4 contributions to electrical activity.

Note that the newly designed cardiac-specific Trpm4 knock-out mice may give valuable results in the future by excluding extracardiac impact of TRPM4 disruption. It is already known that this mouse does not show ECG variations in basal conditions, compared with WT.

### 3.3 Contribution of human genetics

Exploration of TRPM4 function in electrical cardiac activity benefited from mutational screening of patients with cardiac disturbances. Cardiac dysfunction in patients with TRPM4 mutations does not necessarily indicate, by itself, that TRPM4 is directly implicated in cardiac electrical activity because developmental or regulatory modifications can occur, such as those in transgenic animals. However, as described subsequently, it was a valuable complement to animal models and in vitro experiments.

## 4. TRPM4 in the control of membrane potential in cardiac cells

### 4.1 Modulation of diastolic depolarization and beating rate in the SAN

Sinus rhythm, at the onset of cardiac activity, arises from spontaneous action potential (AP) generation in the sino-atrial pacemaker cells. These cells express a specific pattern of ion channels and transporters that produce a spontaneous diastolic depolarization (DD), which causes membrane potential trajectory to pass the activation threshold that produce a spontaneous diastolic depolarization (DD). The DD is mediated by the hyperpolarization-activated cyclic nucleotide-gated ion channel (HCN)$^{44,55}$ and an inward Ca$^{2+}$-activated current evoked by local Ca$^{2+}$-releases from the sarcoplasmic reticulum.$^{56}$ In mouse SAN, the main component of the Ca$^{2+}$-activated current is due to the Na$^{+}$/Ca$^{2+}$ exchanger, but an additional Ca$^{2+}$-activated current can be recorded in the presence of Li$^{+}$. This last current conducts monovalent cations (Na$^{+}$ and K$^{+}$), as observed in rabbit SAN cells.$^{58}$ Because functional TRPM4 single-channel currents were reported in mouse isolated SAN cells$^{30}$ and two different mutations in the TRPM4 gene have been reported in patients with bradycardia,$^{36}$ TRPM4 was considered as a candidate to support this current.

In freely beating isolated right atrial preparations from mouse, rat, and rabbit, which retain the SAN, 9-phenanthrol reversibly reduces

### 3.2 Trpm4 knock-out mice

Two groups had developed Trpm4 knock-out mice from the 129/Svl and C57bl/6j strains.$^{11,12}$ The two groups first reported altered immune response, and mice were later used to evaluate cardiovascular roles of TRPM4.$^{19,31}$ Surprisingly, the cardiac phenotypes of these two Trpm4 knock-out mice differ one from the other. The first one does not develop a specific cardiac phenotype under basal conditions and its electrocardiogram (ECG) remains normal. However, the increase in contractility in response to β-adrenergic stimulation is enhanced in this first Trpm4 knock-out strain.$^{21}$ In contrast, the

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**Figure 1** Biophysical properties of TRPM4. (A) TRPM4 current/voltage relationship in inside-out membrane patches from human atrial cardiomyocytes with 140 mmol/L NaCl in the pipette and 140 mmol/L (black squares) or 14 mmol/L (open circles) NaCl in the bath. Vm corresponds to the membrane potential. Equilibrium potentials are indicated for Na$^{+}$ and Cl$^{-}$. (B) Cardiac TRPM4 open probability ($P_o$) as a function of [Ca$^{2+}$]$_i$. Upper inset shows inside-out recordings with various [Ca$^{2+}$]$_i$ (Vm = +40 mV), c and o correspond to the close and open states. (C) Cardiac TRPM4 open probability ($P_o$) as a function of voltage (see Guinamard et al.$^{2}$ for details).
the beating rate with an IC₅₀ matching TRPM4 inhibition. It has no effect when the experiments are conducted in Trpm4 knock-out mice isolated atrium (Figure 2).⁵⁹ which indicates that 9-phenanthrol acts via Trpm4 inhibition and not by modulating other ion channel targets in this preparation. Moreover, in rabbit SAN cells, 9-phenanthrol reduces the slope of the DD.⁵⁹ Thus TRPM4 participates in sinus rhythm generation by influencing the DD (Figure 2). Because the experiments were performed on isolated atria, it excludes any participation of external regulatory processes such as modifications of neuro-hormonal regulations by 9-phenanthrol. Because isolated right atria have a lower beating rate than the heart rate of conscious animals (300 vs. 500 b.p.m.), 9-phenanthrol may have elucidated a phenomenon that is absent in vivo. Unfortunately, no data are available yet regarding in vivo sinus rhythm regulation by pharmacological modulation of TRPM4. However, a recent study using telemetric ECG recordings in conscious and unrestrained mice showed a higher incidence of sinus pauses in Trpm4 knock-out mice than in littermate controls, which is consistent with a contribution of TRPM4 in sinus activity in vivo.¹⁹

The precise level of [Ca²⁺], reached during the local release in SAN cells is not well known. A computer simulation study indicates that [Ca²⁺], variations of the order of 1 µmol/L would be sufficient to activate TRPM4 and thus augment the DD slope in SAN cells.⁶⁰ Interestingly, the effect of 9-phenanthrol is inversely proportional to initial beating rate: the lower the initial beating rate, the stronger its reduction by 9-phenanthrol.⁵⁹ These data suggest that TRPM4 would activate only during bradycardia. In addition, these in vitro results may explain the lack of disparity in the heart rates measured in vivo in WT vs. Trpm4 knock-out mice,¹⁹,²¹ as well as in cardiac-specific Trpm4 knock-out mice.²⁰ Indeed, these mice are not spontaneously bradycardic and thus do not express TRPM4 activity. In vivo, isoprenaline infusion increases the heart rate slightly more in Trpm4 knock-out mouse than in WT controls.²¹ However, that effect is not reproduced in isolated right atria from those animals, suggesting that the difference in vivo does not occur through a direct regulation of cardiac TRPM4 activity by β-adrenergic stimulation.²¹

The stronger effects of 9-phenanthrol at low beating rate further suggest that the channel may have a stronger influence in humans than in mice because the resting heart rate is around 60 b.p.m. instead of 500 b.p.m. Bradycardia was reported in three patients with Y790H TRPM4 mutations.³⁶ It is tempting to speculate that bradycardia may be due to a decrease in TRPM4 expression in these patients. Unfortunately, biophysical and expression properties of this mutant were not evaluated. Moreover, the meta-analysis of data from patients with TRPM4 mutations¹³–¹⁵,³⁶ does not provide any persuasive results regarding a relation between TRPM4 expression level and heart rate. The lack of clarity may be due to the small cohort size of patients with such mutations.

### 4.2 Modulation of the AP in cardiomyocytes

TRPM4 participates in both atrial and ventricular APs in mouse. Intracellular microelectrode recordings on isolated atria revealed that 9-phenanthrol reduces AP duration without affecting the resting membrane potential or AP amplitude (Figure 3).³³ The effect was preserved in the presence of H-89, a PKA inhibitor, excluding a side effect of 9-phenanthrol on this kinase. As the 9-phenanthrol-induced AP shortening did not occur in atria from Trpm4 knock-out mice, we can be confident that 9-phenanthrol effects on other channels are not responsible for this phenomenon.³³,⁵²,⁶⁵ Moreover, a comparison of APs recorded on isolated atria from WT and Trpm4 knock-out mice revealed a 25% shortened AP induced by gene disruption.³³ This AP shortening in Trpm4 knock-out mice was confirmed by patch-clamp experiments on atrial isolated cardiomyocytes and shown to be independent of voltage-gated Ca²⁺ or K⁺ channel modulation.¹⁹

The role of TRPM4 in ventricular AP remains incompletely understood. Intracellular microelectrode recordings from papillary muscles...
in Trpm4 knock-out mice derived from the 129/SvJ strain revealed a 10% shortened AP, compared with WT. Further, using patch-clamp recordings, the authors unmasked a Ca\(^{2+}\) influx in cardiomyocytes from WT animals that disappeared in Trpm4 knock-out mice.\(^{21}\) AP shortening in these Trpm4 knock-out mice results in an enhanced Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels because of the increased driving force for Ca\(^{2+}\) influx due to earlier repolarization, while Ca\(^{2+}\) channels are still open.\(^{21}\) In contrast, intracellular microelectrode recordings in the ventricular wall of the C57bl/6j mice did not reveal any effect of 9-phenanthrol superfusion on AP parameters, even though this drug reduced AP duration in the atria.\(^{33}\) Perhaps, due to the low number of animal subjects (six WT and four knock-outs), the difference did not reach significance, according to the conventional statistical hypothesis tests. However, if these data can be replicated and the effect confirmed, then these in vivo results would be consistent with the in vitro experiment, showing that the pharmacological inhibition of TRPM4 reduced ventricular

5. TRPM4 in cardiac diseases

5.1 Hypoxia–reoxygenation arrhythmias

TRPM4 was suspected to participate in electrical perturbations following cardiac ischaemia and reperfusion episodes because of its sensitivity to Ca\(^{2+}\) and ATP.\(^{64,65}\) Myocardial ischaemia (thus hypoxia) reduces ATP levels leading to [Ca\(^{2+}\)]\(i\) accumulation, a phenomenon exacerbated at the first time of reperfusion. This [Ca\(^{2+}\)]\(i\) overload is in part responsible for triggered activity that arises before the end of AP repolarization, which has been dubbed the early afterdepolarization (EAD).\(^{66–68}\)

In a model of hypoxia–reoxygenation on isolated mouse interventricular septum, EADs were observed under reoxygenation, but both TRPM4 inhibitors 9-phenanthrol and flufenamic acid reduced their occurrence.\(^{32}\) A significant effect of 9-phenanthrol was observed at concentrations starting from 10\(^{-5}\) M, a concentration that does not modulate voltage-gated K\(^{+}\) or Ca\(^{2+}\) channels in cardiomyocytes, but it was not tested on Trpm4 knock-out mice to definitely exclude other targets. Note that the cardioprotective effect of 9-phenanthrol was also observed on hypoxia-induced arrhythmias.\(^{32}\)

Interestingly, in a mouse model of ischaemic heart failure induced by ligation of the left anterior descending (LAD) artery, there was a trend showing fewer ventricular ectopic beats in Trpm4 knock-out mouse.\(^{69}\) Perhaps, due to the low number of animal subjects (six WT and four knock-outs), the difference did not reach significance, according to the conventional statistical hypothesis tests. However, if these data can be replicated and the effect confirmed, then these in vivo results would be consistent with the in vitro experiment, showing that the pharmacological inhibition of TRPM4 reduced ventricular
hypothesis-induced arrhythmias.62 In the LAD artery model, the authors also reported a higher survival rate in the Trpm4 knock-out group a week after surgery; however, mice died from left ventricular rupture or pulmonary oedema but not from arrhythmias.69

Another study evaluated the long-term effect of 9-phenanthroline when applied under pre-conditioning conditions in isolated rat heart.70 It produced a cardioprotective effect on contractile function, limited tissue damage, and proved to be prophylactic against hypoxia–reoxygenation-induced ventricular arrhythmias.70 Note that induction of ischaemia–reperfusion for 40 min in rats before lethal anaesthesia increased the expression level of Trpm7 mRNA, but not that of Trpm4 mRNA.71 Thus, regarding TRPM4, the effect of ischaemia–reperfusion most likely occurs through modulation of channel activity rather than gene expression.

Altogether, these data indicate that, even if the pathway is not clearly identified yet, TRPM4 is involved in hypoxia and hypoxia–reoxygenation injuries, including arrhythmias, and thus TRPM4 might be an interesting new target for cardioprotection.

5.2 Inherited cardiac diseases

Genetic testing for inherited cardiac arrhythmias identified a number of genes, several of those encoding ion channels.72,73 It most often leads to complex interpretations as mutations of the same ion channel can be related to several pathologies and, reciprocally, a single pathology can be attributed to the mutation of several types of ion channels. TRPM4 participates in this complex scheme.18 The TRPM4 mutations known to be related to cardiac electrical perturbations are reported in Table 2 and schematized in Figure 4.

5.2.1 Conduction blocks

A correlation between TRPM4 and human disease was first discovered in a family with a cardiac bundle branch conduction block transmitted with an autosomal dominant inheritance.13 An E7K mutation was identified in this family on the TRPM4 gene. Later, other mutations were found in unrelated families with similar autosomal dominant isolated conduction blocks.14,16 These mutations were found in a quarter of the right bundle branch blocks and a tenth of the atrioventricular blocks (AVBs).16 The impact of TRPM4 mutations on cardiac conduction is consistent with the high level of TRPM4 expression detected in bovine conductive tissue.14 In four of these mutants, TRPM4 properties were evaluated after heterologous expression in CHO or HEK-293 cells. None of the mutations affects biophysical (single-channel conductance and ion selectivity) or regulatory (sensitivity to voltage and Ca2+) properties, but an increase in the amplitude of the whole-cell current was detected for all.13,14 This was attributed to an increase in the number of channel proteins expressed at the cell membrane, due to a deregulation of small ubiquitin modifier conjugation (SUMOylation) involved in channel recycling.13,14

5.2.2 Brugada

Cohorts of patients with Brugada syndrome were screened for TRPM4 mutations. Although a first study did not observe any mutation within 27 patients,60 a second one found one mutation over 51 patients.74 A third study reported 11 different mutations of the gene in 20 of the 247 patients who did not carry any mutation on SCN5A gene,15 another major gene responsible for Brugada syndrome.75 Among these mutations, K914X results in a non-functional channel, as the protein is truncated.15 Another one, P779R, decreases whole-cell TRPM4 current compared with WT without variations of single-channel properties when expressed in HEK-293 cells. Although this may indicate that a reduction in TRPM4 functional expression could result in the Brugada syndrome, two other mutations (T873I and L1075P) showed no significant variation in TRPM4 current, compared with WT.15 More perplexing, the mutation G844D identified in a familial case with conduction block was also found in the cohort of patients with Brugada syndrome, but this particular mutation was demonstrated to lead to a TRPM4 gain of function in HEK-293 cells.14,15

Although the relation between TRPM4 mutation and inherited cardiac diseases is established, several points remain to be clarified: (i) heterologous expression in CHO or HEK-293 cells revealed that several mutations modified TRPM4 protein and current expression, but this remains to be confirmed in cardiac cells; (ii) modelling the impact of increasing or decreasing the TRPM4 current may provide one part of the mechanism, but the contribution of TRPM4 in cardiomyocytes is not totally understood and, even worse, not described in the conductive tissue; and (iii) the potential impact of TRPM4 mutations on cardiac development and global cardiovascular system, as shown in Trpm4 knock-out mice, has to be evaluated.

Note that Trpm4 knock-out mice exhibit multilevel conduction blocks, including first- and second-degree AVBs.19 Although second-degree AVB is most likely due to paroxysmal parasympathetic overdrive, first-degree AVB is not and thus is probably attributable to changes in ion channel expression. These results suggest that either increase or decrease in TRPM4 expression may lead to conduction blocks. In contrast, it can also be due to different roles of TRPM4 in human and mouse conductance.

5.3 Cardiac hypertrophy

Electrical activity is modified by cardiac hypertrophy. In the ECG, hypotension prolongs the QT interval, modifies the cardiac axis, and may lead to cardiac arrest.76,77 At the cellular level, it prolongs the ventricular AP, which is attributable to the modified expression level of a variety of ion channels including Ca2+ channels and HCN27–29 and increases the incidence of afterdepolarizations.80 TRP channels—including TRPC1, TRPC3, and TRPC6—have also been associated with the development of cardiac hypertrophy.81

Besides TRPCs, the overexpression of a TRPM4-like current was observed in an in vitro model of cardiomyocyte dedifferentiation exhibiting cell hypertrophy1 and in the in vivo model of the spontaneously hypertensive rat (SHR) which develops cardiac hypertrophy.82 The mRNA levels similarly showed elevated Trpm4 expression in SHR myocytes compared with normotensive controls. Whether this overexpression participates in the prolongation of the QT interval and ventricular AP in SHR remains to be determined. It might also participate in the hypertrophy-induced EADs similar to what was observed in the mouse model of hypoxia–reoxygenation-induced EADs,83 but that remains to be evaluated.

TRPM4 also modulates cardiac development and hypertrophy and, by so, may impact electrical activity. Indeed, Trpm4 knock-out mice exhibit cardiac hypertrophy not due to cardiomyocyte hypertrophy but to an increase in the cardiomyocyte number.16 Cardiac hypertrophy in Trpm4 knock-out mice might also develop secondarily to chronic hypertension as these animals have elevated blood pressures due to increased catecholamine secretion.45 However, the newly designed cardiac-specific Trpm4 knock-out mice revealed an increased angiotensin II-stimulated hypertrophy, compared with WT, consistent with the idea that TRPM4 is involved in this hypertrophy, directly at the cardiac level.83 These aspects have to be evaluated in humans; however, none
of the existing reports (at present) indicates that TRPM4 mutations in humans affect cardiac development.13–15,36,74

### 6. Unanswered questions and future developments

#### 6.1 Species dependence of TRPM4 role in cardiac physiology and limitations of animal models

TRPM4 has been detected in cardiac tissue from all mammalian species examined, with relative expression between cardiac areas comparable among species (Table 1). But because cardiac properties (heart rhythm and AP parameters) show variations among species, the results obtained in small mammals must be considered cautiously for implications regarding human cardiology. However, ECG perturbations related to TRPM4 mutations already demonstrate that this channel plays a role in humans.13–15,36,74

### 6.2 TRPM4 and partner proteins SUR1, TRPC3

TRPM4 activity/expression/trafficking has to be evaluated regarding its ability to interact with partner proteins.83 Among these, co-expression of SUR1 and TRPM4 in COS-7 cells leads to the formation of SUR1–TRPM4 heteromers84 that increase TRPM4 sensitivity to ATP and glibenclamide, two known regulators of both TRPM430,37 and SUR1. It also increases the affinity of TRPM4 for Ca2+.84

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Protein location</th>
<th>ECG perturbation</th>
<th>Whole-cell current: ratio mutation/WT</th>
<th>Protein expression level at membrane</th>
<th>Effect</th>
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<td>RBBB</td>
<td>4.2</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Liu et al.14</td>
</tr>
<tr>
<td>G555R</td>
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<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Liu et al.15</td>
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<td>F773I</td>
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<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
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<td>TM2</td>
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<td>0.4</td>
<td>No change</td>
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<td>Liu et al.15</td>
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<td>Y790H</td>
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<td>AVB bradycardia</td>
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<td>n.d.</td>
<td>n.d.</td>
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<td>7.5</td>
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<td>n.d.</td>
<td>n.d.</td>
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<td>Liu et al.15</td>
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<td>C-terminus</td>
<td>BrS</td>
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<td>Increase</td>
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<td>P1204L</td>
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<td>BrS</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Liu et al.15</td>
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BrS, Brugada syndrome; RBBB, right bundle branch conduction block; PFHBI, progressive familial heart block type I.

Until now, patients with TRPM4 mutations were not reported with any phenotype other than electrical perturbations, whereas knock-out mice develop cardiac hypertrophy due to hyperplasia15 and persistent hypertension attributed to an increased catecholamine release from the adrenal gland.31 In addition, Trpm4 knock-out mice also have altered regulations in the immune system.11,12,82 It is not clear whether patients, at least with the K914X mutation, developed such dysfunction—even if this possibility was evaluated. Anyway, the small number of patients and the heterogeneity of their symptoms are major obstacles to elucidate TRPM4 channelopathies in cardiology.
this complex protects against neuroinflammation in the context of subarachnoid haemorrhage in humans and rats,

suggesting that SUR1–TRPM4 inhibition by glibenclamide is an efficient pathway to prevent brain injuries following ischaemic stroke.

The SUR1–TRPM4 complex is still in debate as another group was not able to detect such coupling. However, it may occur in heart, as SUR1 and SUR2 are expressed. Association of these subunits with Kir proteins forms ATP-sensitive potassium channels (KATP). The expression of SUR1 in cardiac tissue in which TRPM4 is also present leaves open the possibility of their association that remains to be explored.

The physiological impact of the SUR1–TRPM4 association may counteract KATP effects. Indeed, SUR1-Kir6.2 and SUR2A-Kir6.2 as well as SUR1–TRPM4 are inhibited by internal ATP. Under conditions of metabolic stress, such as that induced by ischaemia, the reduction of internal ATP levels may naturally lead to KATP activation, which could exert a cardioprotective effect by generating outward K\(^+\) current that stabilizes the resting membrane potential and shortens the AP. In contrast, SUR1–TRPM4 might also be activated, which would produce mainly an inward Na\(^+\) current in favour of cell depolarization and AP prolongation.

TRPC3 might also be a TRPM4 partner in heart. Indeed, both proteins can interact when co-expressed in HEK-293 cells. Both channels are expressed in cardiomyocytes, and their co-expression increases during ventricular hypertrophy.

6.3 TRPM4 in the conductive tissue

Several observations strongly suggest that TRPM4 is implicated in cardiac conduction along the His and Purkinje tract: (i) immunolabelling data that revealed a high TRPM4 protein expression in bovine conductive tissue; (ii) TRPM4 mutations occur in patients with cardiac conduction blocks; (iii) conduction blocks are present in Trpm4 knock-out mice; and (iv) APs are long-lasting in conductive tissue compared with ventricular cardiomyocytes and the plateau membrane potential during the AP does not exceed 0 mV.

These data, collectively, may be of great importance for electrical cardiac perturbations as the conductive tissue is known to be a major source of arrhythmias. Unfortunately, despite these compelling indicators, no data are available yet to substantiate the direct involvement of TRPM4 in conductive tissue.

6.4 TRPM4 as a target to correct cardiac perturbations

As TRPM4 is implicated in cardiac electrical activity and its perturbations, it appears as a new target in the design of antiarrhythmic drugs. However, the widespread distribution of TRPM4 among tissues might be inconvenient for the use of such drugs because of many potential side effects. Because the heart is one of the most highly expressing tissues with regard to TRPM4 mRNA, one can argue that it will be a major target, which would potentially minimize side effects. In that sense, note that, until now, the sole human pathologies related to TRPM4 mutations are perturbations of cardiac electrical activity. It confirms that the heart would probably be one of the first targets of TRPM4 modulators. However, as TRPM4 has several roles in cardiac electrical activity, we are far from fully predicting the impact of its modulation on global cardiac electrical activity. Most probably, this will depend on several parameters such as beating rate or cause of arrhythmias (ischaemia, mutations, hypertrophy, and so on). In addition, one should keep in mind that TRPM4 may have an important role in generation and control of respiratory rhythm and breathing; thus any drug targeting cardiac TRPM4 could affect respiration in parallel.

Nevertheless, the development of new pharmacological compounds to specifically target TRPM4 would provide essential tools to further progress in the understanding of its role in heart as well as in other tissues.

7. Conclusion

Altogether, these data pointed TRPM4 as an actor in cardiac electrophysiology. Although our understanding of its roles is incomplete, particularly for inherited cardiac diseases, recent progress is valuable and predicts further developments. TRPM4 has to be included in mathematical models of cardiac AP and electrical propagation. In relation with data obtained at the cell level, it will contribute to the understanding of direct involvement of TRPM4 in controlling membrane potential of cardiac cells. TRPM4 should also be considered when developing antiarrhythmic drugs as it might be an interesting target or responsible for side effects.

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References


Figure 4  TRPM4 mutations and cardiac conduction diseases. Schematicized TRPM4 subunit with identified mutations related to cardiac conduction disease. For clarity sake, only mutations with a functional study are indicated. Numbers under mutations correspond to the ratio of mutant to WT current (asterisk, significantly different from WT).


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