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Pathophysiological Roles of TRPV4 Channel in the Heart

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Abstract: Transient Receptor Potential Vanilloid 4 (TRPV4) channel is a non-selective cation channel, mostly permeable to calcium (Ca^{2+}), which participates to intracellular Ca^{2+} handling in cardiac cells. It is widely expressed through the body and is activated by a large spectrum of physicochemical stimuli, conferring it a role in a variety of sensorial and physiological functions. Within the cardiovascular system, TRPV4 expression was reported in cardiomyocytes, endothelial cells (ECs) and smooth muscle cells (SMCs) where it modulates mitochondrial activity, Ca^{2+} homeostasis, cardiomyocytes electrical activity and contractility, cardiac embryonic development, fibroblast proliferation but also vascular permeability, dilatation and constriction. On the other hand, TRPV4 channels participate in several cardiac pathological processes such as development of cardiac fibrosis, hypertrophy, ischemia-reperfusion injuries, heart failure, myocardial infarction, and arrhythmia. In this manuscript, we provide an overview of TRPV4 channel implications in cardiac physiology and discuss the potential of TRPV4 channel as therapeutic target against cardiovascular diseases.

Keywords: TRPV4 channels; TRPV4-KO mice; heart; myocytes; fibroblasts; action potential; calcium homeostasis

1. Introduction

The mammalian TRP channels are widely expressed in the heart and can be considered as real ''cellular switches'' able to respond to numerous physical and chemical stimuli related to sensory physiology [1], [2], [3]. In mammals, TRP family is composed of 28 members and classified according to amino acid sequence homology into six families: TRPA (ankyrin; TRPA1), TRPC (canonical; TRPC1–TRPC7), TRPM (melastatin; TRPM1–TRPM8), TRPML (mucolipin; TRPML1–TRPML3), TRPP (polycystin; TRPP1–TRPP3) and TRPV (vanilloid; TRPV1–TRPV6) [3], [4].   With some exceptions, most of the TRP channels are able to conduct Ca^{2+} which plays an important role in stimulus-response reactions of cardiac cells. Interestingly, altered expression, localization and regulation of TRP channels have already been related to cardiovascular disorders due to Ca^{2+} handling dysregulation [4]. Among the TRPV channels family [5], TRPV4 channel emerged as a key modulator in cardiac cell structure and activity [6], [7]. Recent identification of pharmacological modulators and construction of TRPV4 knockout (KO) mice unmasked several physiopathological roles of this channel [5], [8], [9]. However, the mechanisms that regulate cardiac TRPV4 channels still remain not well apprehended. In this context, this review will discuss the latest findings on the pathophysiological role of TRPV4 channel in the cardiovascular system and why understanding of TRPV4 regulation may lead to novel therapeutical strategies related to cardiac diseases.

2. Gene, structure, function and electrical properties

TRPV4 channel have received a particular attention according to its large expression in the cardiovascular system [5], [8], [10], [11]. TRPV4 protein is encoded by the *trpv4* gene, present on the long (q) arm of chromosome 12 at position 24.1 in human [4]. The corresponding locus is found between the bases 109,783,087 & 109,783,406 of the genome and is composed of 15 exons. Alternative exon-splicing leads to 5 channel isoforms (TRPV4-A to E). Only TRPV4-A and TRPV4-D isoforms are localized at the plasma membrane and display identical biophysical properties [12], while the other splice variants (N-terminal deletions) remain in the endoplasmic reticulum (ER) [4] and do not form functional ion channels [12]. In the following sections, we will refer to TRPV4 as the TRPV4-A and TRPV4-D isoforms without distinction. TRPV4 has tetrameric structure, each subunit being composed of six transmembrane segments (S1 to S6), a pattern shared with other TRP channels and voltage-gated ion channel subunits (VGIC). The S1 to S4 segments constitute the peripheral structure while the central S5-S6 loops border the pore of the channel [13], [14], [15], [16]. The N and C-terminal extremities are intracellular and contain a variety of functional domains [17]. A recent crystallographic study combined to X-ray approach insights more details about the structure of *Xenopus tropicalis* TRPV4 with a resolution of 3.8 Å [16].

The N-terminal region represents more than half of the protein and plays a critical role in channel assembly, trafficking and regulation [16], [18], [19], [20], [21], [22]. It harbours six Ankyrin repeated domains (ARD1–6) that participate in channel oligomerization, protein-protein interaction and trafficking [4]. Note that the absence of these specific domains blocks the TRPV4 trafficking at the ER level [12]. This region also presents a Ca^{2+}-calmodulin kinase type II (CaMKII) regulation site conferring channel sensitivity to intracellular Ca^{2+}. In mammalian cells, a proline-rich sequence in TRPV4 N-terminal can interact with the cytoskeleton protein PACSIN 3 (protein kinase C and casein kinase substrate in neurons 3) thereby regulating channel trafficking, sensitivity to membrane stretch [23] and preventing/reducing TRPV4 activation by heat [24]. In this context, PACSIN3 can be considered as a TRPV4 channel regulatory protein that regulates both the TRPV4 subcellular localization and its function. Obviously, further studies on cardiomyocytes are needed to better understand the mechanism and the role of this interaction. In addition, the TRPV4 N-terminal region is able to interact with OS-9, an ER-resident lectin, in order to prevent channel trafficking to the plasma membrane [25] suggesting that OS-9 is an important auxiliary protein for TRPV4 maturation.

TRPV4 C-terminal extremity contains different domains: i) "TRPbox" (carrying the consensus sequence WKFQR) [26], ii) a CaM binding domain which modulates tetramerization, gate opening [27] and Ca^{2+} influx [28], iii) a PDZ domain which interacts with numerous cell auxiliary proteins [4], [13]. Furthermore, the microtubule-associated protein 7 (MAP7) interaction with the C-terminal extremity of TRPV4 increases both expression and functional activity at the plasma membrane in Chinese hamster ovary cells [29]. Because MAP7 is directly related to the cytoskeletal filaments, this interaction may underline the TRPV4 mechanotransduction mechanism [29] nevertheless this hypothesis requires further attention in myocytes.

TRPV4 principally assemble as homotetramers [13], [30] to form a functional channel but can also form heterotetramers with few other members of the TRP channels family : TRPC1 [31], [32] and TRPP2 [30], [32]. It can also interact with the α-subunit of few ion channels and aquaporins [33], [34], [35], [36], [37], [38]. This heteromerization was shown to modify TRPV4 channel biophysical properties in heterologous expression systems [32], [39], [40]. TRPV4 channel shows a lower voltage sensitivity compared to other TRP channels but still harbors an outward rectification current when expressed in heterologous expression system, constituting the signature of most TRP channels. To date, this weak voltage dependence of TRPV4 is not yet clearly elucidated. Nevertheless, the low density of positive charges in the S4 voltage sensor domain [41] combined with a better understanding of its crystal structure [16], allows to get a plausible mechanism of its gating. Indeed, TRPV4 adopts a domain-swapped arrangement between the S1-S4 domain and the pore domain S5-S6, similarly to TRPV1, TRPV2 and numerous VGICs whereas the connector between these last two domains adopts an ordered loop structure rather than an α-helix segment present within TRPV1 and TRPV2. This last structural aspect is important since VGICs connector acts as a mechanical lever to

couple the pore opening and the voltage sensor activation [42], [43] and its absence can change the gating behavior [44] [45]. Taken together, the interface behavior between the S1–S4 and pore domains in TRPV4 is unique among TRP channels and not closely related to the VGICs.

The TRPV4 single channel conductances are ~ 30-60 pS and ~ 80-100 pS for inward and outward currents respectively [46]. Like most of TRP channels, TRPV4 is mainly permeable to Ca^{2+} over other ions and its permeability sequence was established to be as follows: $Ca^{2+} \gg Mg^{2+} > K^+ > Na^+$ (P_{Ca}/P_{Na} ~ 10 and P_{Mg}/P_{Na} ~ 2-3) [31], [47], [48] . Surprisingly, the macroscopic TRPV4 current in cardiomyocytes has not been unmasked, yet. Recent structural characterization performed by Yuan group has reported a larger selectivity filter (10.6-12.6 Å) within the upper gate of TRPV4 compared to other TRPV channels (TRPV1, TRPV2 & TRPV6 ranging from 4.2 to 7.4 Å) (Figure 1- [16]).
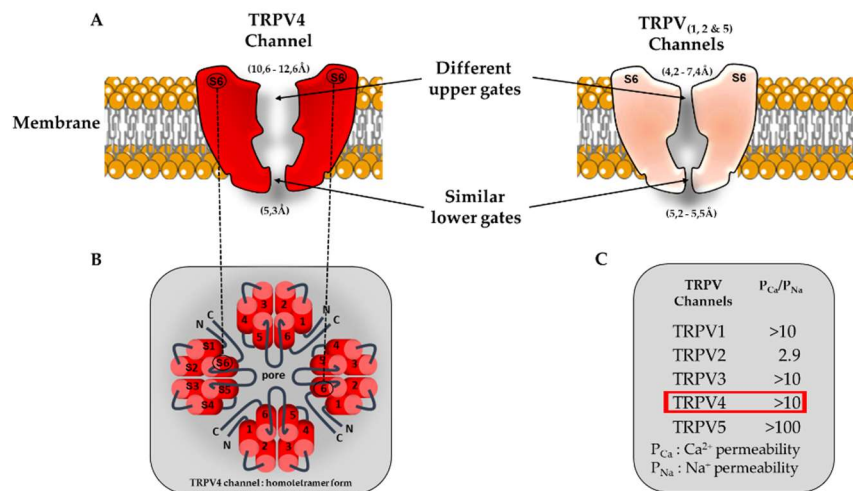
Figure 1. Schematic view of TRPV channel structures and associated ion permeation pathway. **(A)**. Comparison of TRPV pore structures. For clarity, only two opposing subunits are shown (segment S6). Interestingly the TRPV4 upper gate is larger compared to the other members of TRPV family (TRPV1, TRPV2 and TRPV5). **(B)** Homotetrameric structure of TRPV4. **(C)** Functional characteristics of TRPV channels (permeability ratio P_{Ca}/P_{Na}).

Note that the presence of this specificity does not significantly increases the degree of Ca^{2+} permeability within this ion channel family but should potentially be considered as an important structural feature when TRPV4 has to deal with mechanical forces associated with increased membrane tension.

3. Available tools to investigate TRPV4 roles

Intracellular Ca^{2+} concentration plays a pivotal role in living organisms because it is involved in several physiological regulations but also in response to various pathological states [4], [49]. In the myocardium, cellular Ca^{2+} homeostasis maintains normal heart function which requires various specialized proteins such as ion channels and exchangers [50]. The central role Ca^{2+} in cardiac excitation-contraction is well established where Ca^{2+} is at the interface of electrical membrane activity (Action potential (AP)) and cell contraction [51]. Any disturbance in intracellular Ca^{2+} homeostasis may thus lead to contractile and/or electrical defects in the myocardium. In this context, TRPV4 function related to Ca^{2+} permeability is evaluated with the greatest attention [52], [53]. Consequently, synthetic specific antagonists of TRPV4 have been designed and evaluated to treat human diseases including cardiac pathologies [49], [53], [54].

3.1. Pharmacological modulators

To date, several synthetic molecules that modulate TRPV4 channels were developed to aim at treating diseases such as osteoarthritis, respiratory diseases, cancers, gastrointestinal disorders, and pain [53]. Recently, some of these compounds entered safety trials to treat heart failure

4

[53]. Progress in the development of these agents led to a last generation of more potent and specific modulators that have been used to unmask TRPV4 channels pathophysiological roles in heart.

3.1.1. TRPV4 agonists

Four generations of TRPV4 agonists (see Table 1) have been used on different tissues and cell types to determine its physiological and pathological functions in the myocardium. The 4α-phorbol 12,13-didecanoate (4α-PDD, a semisynthetic derivatives of the diterpenoid phorbol), 5,6 epoxyeicosatrienoicacid (5,6-EET, an oxidative metabolite of arachidonic acid), the RN-1747 (a benzenesulfonamide), and the GSK1016790A agonist (fully synthetic) which has been extensively used in cardiovascular investigations [8], [11], [55], [56], [57]. In addition, Atobe and al., have recently reported the new TRPV4 agonist quinazolin-4(3 H)-one derivative intended to treat osteoarthritis [58]. (see table 1)

3.1.2. TRPV4 antagonists

Over the last decade, several TRPV4 antagonists were used to highlight TRPV4's roles in the cardiovascular system. Initial studies used several non-selective TRPV4 antagonists such as ruthenium red (RuR) and gadolinium [59]. Subsequent studies have evaluated the effect of more selective antagonists including: RN-1734 which completely blocks TRPV4 channels without affecting other TRP channels [60] or the widely-used HC067047   antagonist which was used in cardiac investigations in the context of ischemia/reperfusion [56], [61]. Recently, a promising clinical antagonist GSK2193874 which possesses high TRPV4 affinity, has been shown to efficiently prevent and treat lung edema in heart failure models and congestive heart failure [62] (Table 2). Several optimized antagonists exhibiting better *in vivo* availability (RN-9893) [63], solubility (GSK3527497) [64], efficacy (novel 2',4'-dimethyl-[4,5'-bithiazol]-2-yl amino derivatives [65], [66] and GSK2798745 [53]) or with a decreased toxicity (Sulfone Pyrrolidine Sulfonamide) [54], [67] have been developed since then. Knowing that TRPV4 antagonist GSK2798745 [53] is the first inhibitor tested in humans (phase 2), more homologs are likely to appear in the near future (see below Table 2). Indeed, the recently unveiled Cryo-EM structure [16] of TRPV4 channels combined with the pharmaceutical industry efforts should accelerate knowledge related to the mechanism of action of current modulators and may help to identify the next generation of TRPV4 drugs.

Table 1. Overview of the evolution of TRPV4 agonist, the associated physiological processes and their clinical application. 4α-PDD: 4alpha-Phorbol 12,13-didecanoate; CF: Cardiofibroblasts; PKC: Protein kinase C; 5,6-EET: 5,6 epoxyeicosatrienoicacid; mPTP: Mitochondrial Permeability Transition Pore; iv: intravenous injection; TNF-α: Tumor Necrosis Factor;.

Molecules	Year of identification	EC50/IC50	Other targets	Features	Cardiovascular effects	Clinical trials / uses	References
Agonists							
4α-PDD	2003	50 μM	● Dorsal root ganglia neurons independently of TRPV4	● Negative control for phorbol esters (PKC inhibitors)	● Ca²⁺ influx in CF & myocytes ● Ca²⁺ entry in pulmonary artery smooth muscle cells and increased isometric tension in artery rings	● None	[68], [69], [70] & [71]
5,6-EET	2003	0.13 μM	● mPTP	● Metabolite of arachidonic acid by cytochrome P450	● Reduction in: vascular tone, inflammatory response, pathological cardiac remodeling (fibrosis, hypertrophy) & apoptosis ● Improvement in cardiomyocytes function ● Cardioprotection ● Promotes angiogenesis	● None because of poor solubility and short half life	[72], [73], [74] & [75]
RN-1747	2009	5.9 - 7.7 μM	● TRPM8 antagonist (IC50 = 4 μM)	● Benzenesulfonamide derivative	● None reported	● None	[60]
GSK1016790 A	2008	1-18 nM	● Unknown	● Oral administration ● iv	● Endothelial failure and circulatory collapse ● Reduction of TNF-α induced monocyte adhesion to human endothelial cells and atherosclerosis ● Cationic non-selective current activation in rat atrial fibroblasts ● Ca²⁺ influx in CF and differentiation into myofibroblasts and cardiomyocytes ● Worsening of ischemia-reperfusion injuries in isolated mice hearts and in H9c2 cell line and neonatal rat myocytes ● Decrease in systemic arterial pressure, small decrease in pulmonary arterial pressure, and small increase in cardiac output	● None	[8], [52], [56], [76], [77], [78], [79], [80], [81] & [82]

| Quinazolin-4(3H) | 2019 | 280 nM | ● Unknown | ● Orally bioactive ? None reported | | ● None in cardiovascular diseases | [58] |

Table 2. Overview of the evolution of TRPV4 antagonists, the associated physiological processes and their clinical application. mPTP: Mitochondrial Permeability Transition Pore; iv: intravenous injection; RISK: Reperfusion injury salvage kinase; DCM-hiPSC-CMs: Dilated cardiomyopathy induced pluripotent stem cell.

Molecules	Year of identification	EC50/IC50	Other targets	Features	Cardiovascular effects	Clinical trials / uses	References
Antagonists							
RN-1734	2009	2 to 6 µM	● Poor pharmacokinetics and toxicity	● Highly selective	● Prevention of Ca^{2+} entry mediated vasorelaxation of mesenteric arteries ● Abolition of stretch-activated Ca^{2+}-entry in human induced pluripotent stem cells-derived cardiomyocytes ● Inhibition of the phenylephrine-induced contraction in pulmonary artery smooth muscle cells but nonspecific off-target effects	● None	[60], [83], [84], [85], [86] & [87]

HC-067047	2010	17 to 133 nM	● ROS production, depolarization of mitochondrial membrane potential (Δψm) and mPTP opening during H/R	● iv administration ● Intraperitonealy injected ● Potent ● Selective?	● Cardioprotection (significantly reduced infarct size, decreased troponin T levels and improved cardiac function in murine model myocardial I/R) [57] ● Anti-apoptotic effects via the activation of RISK pathway ● Reduced TRPV4-related mechanosensitive Ca^{2+} signaling in DCM-hiPSC-CMs ● Prevent entry of divalent cation in response to myocyte-stretch & hypoosmotic stress-induced cardiomyocyte death and ischemia/reperfusion-induced cardiac damage ● Reduced significantly diabetes-induced cardiac fibrosis ● Inhibition of the PE-induced contraction in pulmonary artery smooth muscle cells	● None	[11], [56] , [62], [86], [87], [88], [89] & [90]
RN-9893	2015	320 to 660 nM	● Exhibits >15-fold selectivity for TRPV4 over TRPV1&V3 and TRPM8	● Moderate oral bioavailability ● Potent ● Selective	● Cardioprotection (blocked collagen production following stretch in human valve interstitial cells) ● Reduced cardiac fibrosis	● None	[82], [63], [89], [88], [89] & [91]
GSK2193874	2017	2 to 50 nM	● Unknown	● Orally active ● Potent ● Selective	● Abolition of pulmonary edema associated with heart failure and enhanced arterial oxygenation ● Increased tail blood flow	● None	[76], [87], [92] & [93]

GSK3527497	2019	12 nM	● Unknown	● Suitable for oral and iv administration ● Reduced bioavailability ● Poor pharmacokinetics and low solubility	● Unknown	● None	[88] & [64]
GSK2798745	2019	2 to 16 nM	● Without any clinically significant safety concerns	● Highly potent ● Selective ● Orally active	● Resolve pulmonary edema in heart failure models and attenuate lung damage induced by chemical agents	● Cardiac heart failure and respiratory diseases ● Diabetic macular edema and cough: https://clinicaltrials.gov/	[49], [53], [61], [76], [87] [88], [89] & [94]
GSK3395879	2018	1 nM	● IC50 > 10μM for TRPA1, TRPV1, TRPM2, TRPM4, TRPM8, TRPC3, TRPC4, TRPC5, TRPC6	● Orally bioactive ● Highly potent	● ● Abolition of pulmonary edema associated with heart failure	● None	[11], [91], [67] & [95]

3.2. TRPV4 knockout mice

To our knowledge, two transgenic TRPV4 knock-out mice strains were developed from two different groups. Suzuki *et al*, used the 129/SvJ strain via a cassette insertion mutagenesis of exon 5 [96] whereas Liedtke and al, used the C57bl/6J strain with a Cre-lox-mediated excision of exon 12 [97]. Both mice strains were viable and fertile [96], [97] (see Table 3).

Table 3. Comparison of 129/SvJ *trpv4*^{-/-} and C57bl/6J *trpv4*^{-/-}. Ref. : References.

Outcomes	129/SvJ *trpv4*-/-	Ref. [96]	C57bl/6J *trpv4*-/-	Ref. [97]
Generation method	129/SvJ strain via a cassette insertion mutagenesis of exon 5		C57bl/6J strain with a Cre-lox-mediated excision of exon 12	
Cardiovacular phenotypes	Impaired vasorelaxation, endothelial calcium response, systemic tonicity	[98] [99] [100] [101]	Impaired vasorelaxation	[98] [102] [103]
	Altered flow-induced vasodilatation	[104]	Loss of shear stress-induced vasodilation	[105]
	-		Cardiac electrophysiological changes	[8]
	-		Absence of VGIC remodelling (Na+, Ca2+ and K+ VGIC) in the left ventricle	[8]
Extracardiac phenotypes	Viable and fertile		Viable and fertile	[97]
	Normal appearance, growth, size, and temperature and no obvious behavioral (including drinking) abnormalities	[106]	Tendency to a lower body weight	[8]
	Reduced response to harmful stimuli caused by pressure	[96]	Reduction in water intake and serum osmolality changes	[97]
	Intact heat detection but abnormal sensory phenotype	[96]	Reduced response to noxious mechanical stimuli and impaired response to mechanical stimulation	[97]
	Altered hearing	[107]	Intact thermal sensing	[97]
	Inability to thermoregulate	[100]	Loss of the permeability response in lungs, alveolar barrier	[108]
	Deficits in renal tubular K+ secretion	[109]	Proximal tubule defect	[110]
	Increased bone mass, decreased osteoclast differentiation	[111]	Blood metabolite changes	[112]
	-		Increased bladder capacity	[88]

4. Physiological roles in cardiovascular system

4.1. TRPV4 expression profile under physiological condition

In mammals, TRPV4 channel expression is distributed in various organs and tissues including blood vessels and heart. RT-PCR, immunostainings, and functional recordings demonstrated that TRPV4 channels are expressed in cardiac cell types such as atrial and ventricular cardiomyocytes [8], [10] [11], [52], [56], [61], [113], [114], human embryonic stem cell-derived cardiomyocytes [115],

cardiofibroblasts (CFs) [52], [81], [90], [116], [117], [118], [119], endothelial cells (ECs) [84], [101], [120], [121], [122], [123] and smooth muscle cells [71], [84], [87], [122], [124], [125], [126], [127], [128], [129] (See table 4).

Taken together, TRPV4 channel is present and functional at the plasma membrane of cardiac cells where it plays important physiological roles.

Table 4. TRPV4 channel expression and demonstrated function in cardiovascular system.

TRPV4	Atrium	Ventricles	Fibroblasts	Endothelial Cells	Smooth Muscle Cells
• **mRNA**	-	Mouse [61], neo rat [10], [56], rat [56]	Mouse [119] , rat [118], human [119], [82]	Mouse [101], rat [120], [122], human [121], [123]	Rat [122], [125], [126], [127]
• **Protein**	Rat [52]	Mouse [8], [11], [61], neo rat [56], [10], rat [56]	Rat [81], neo rat [90], human [82], [119]	Mouse [101], [130] , rat [120], [122], human [121], [123]	Rat [122], [71], [125], [127], [128], human [128], [129]
• **Function**	Rat [52]	Mouse [8], [11], [52], [61], [117], neo rat [10], rat [52]	Rat [52], [81], [90], human [82], [119]	Mouse [101], [130], rat [120], [122], human [121], [123]	Mouse [83], rat [122], [71], [124], [125], [128], human [128], [129]

4.2. Modulation of ventricular electrical activity

In healthy mammalian hearts, the action potential (AP) waveform initiates and modulates cardiac contractility. It occurs suddenly and transitorily when the resting membrane potential depolarizes and repolarizes according to the successive opening and closing of several ion channels. A lot of attention is paid to the ventricular repolarization phase in pre-clinical investigations, as AP prolongation is often associated with an increased risk of potentially lethal arrhythmias. Recent evidence has shown that TRP channels opening produces a depolarizing current, since the net flux of cations is inward under physiological conditions [3]. Therefore, these channels are particularly important in excitable cells, such as cardiomyocytes, where they can both trigger and modulate AP shape. To date, some TRP channels, such as TRPC3 [131], [132], TRPM4 [133], [134, 135] and TRPM7 [136] were shown to modulate both sinus node and cardiac AP. Interestingly, deletion or mutations of TRPM4 were reported to be associated with several cardiac electrical disorders (Brugada, long QT, progressive cardiac conduction disorder) [137], [138], [139], [140], [141] and hypertrophy [142]. Since other TRP channels are expressed in cardiac myocytes they may participate in cardiac electrical activity and their mutations could lead to inherited cardiac electrical disorders. Thus, it appears necessary to specifically describe the effects of each of these channels, including TRPV4, and evaluate their physiological roles in the cardiovascular system.

TRPV4 protein expression was detected in mouse [8], [56], [61] and rat [56] ventricular myocytes. It shows a membrane specific expression and location (plasma membrane and t-tubules) which is age-dependent [8], [11]. In young mice [8], TRPV4 agonist GSK1016790A induced a dose-dependent and transient increase in AP duration in $trpv4^{+/+}$ ventricular myocytes. In this study, GSK1016790A was proposed to promote the trafficking TRPV4 channel to the membrane. This phenomenon was transient, because of subsequent rapid channel endocytosis [8], [57], [143]. A similar effect of GSK1016790A was also observed on rat atrial myocytes [52]. According to its biophysical properties, TRPV4 potentiation in a physiological or pathological context would prolong AP. Conversely, the TRPV4 inhibitor GSK2193874 (100 nM) significantly shortened AP duration in $trpv4^{+/+}$ ventricular myocytes. The effect of these pharmacological modulators on the ventricular AP were not observed in $trpv4^{-/-}$ mice, highlighting the effects observed on WT cardiomyocytes were exclusively due to TRPV4 modulation. These data suggest that TRPV4 channels carry an inward current, which remains to be characterized, during the ventricular AP in basal conditions. Computational modelling was used to predict the shape of TRPV4 current during AP [8]. This modelling considered channel

permeation and open probability depending on the voltage and subsarcolemmal [Ca^{2+}]$_i$ that will be sensed by the channel. Since TRPV4 channel open probability increases with depolarization and decreases with [Ca^{2+}]$_i$ elevation, the model predicted a transient inward current, that develops rapidly after the AP upstroke and declines with the rise in [Ca^{2+}]$_i$ and membrane repolarization [8]. The consequence of *trpv4* deletion on cardiac electrical activity was also evaluated. *In vivo* electrocardiogram recordings revealed significantly shortened QT interval in *trpv4$^{-/-}$* mice compared to their wild type littermates (C57bl/6J strain). In accordance with these data, a 19% reduction in AP duration was found in ventricular myocytes from *trpv4$^{-/-}$* mice compared to *trpv4$^{+/+}$* mice. No change in the properties of the main VGICs participating in AP was observed between *trpv4$^{+/+}$* and *trpv4$^{-/-}$* myocytes, suggesting that QT and AP duration shortening in *trpv4$^{-/-}$* mice are exclusively attributable to *trpv4* deletion [8] . Finally, a recent investigation has shown that TRPV4 expression level is increased in cardiomyocytes of aged (24–27 months) mice compared with young (3–6 months) mice [11] but TRPV4 contribution to the prolonged repolarization commonly observed with aging remains to be studied.

Together, these studies suggest that TRPV4 modulates ventricular electrical activity under basal conditions and may thus be involved in cardiac arrhythmias in aging and/or under pathological conditions. [11] (see section 4 for details).

4.3. Modulation of cardiac contractility

Muscular contractility is onset by an increase in cytoplasmic Ca^{2+}. For so, Ca^{2+} handling results from a tight balance between both the Ca^{2+} influx, intracellular store release and uptake and cell extrusion [51]. In a ventricular myocyte, Ca^{2+} influx is mainly mediated by voltage-gated calcium channels (Cav1.2) whereas the Ca^{2+} extrusion is ensured by Na/Ca exchanger (NCX1) and plasma membrane Ca^{2+} pump (PMCA) [144]. Several TRP channels are present and functional and can constitute an alternative source of Ca^{2+} entry. Furthermore, it is well established that impaired Ca^{2+} handling leads to abnormal contractility [145], [146], [142]. Therefore, a better understanding of TRP channels, involved in Ca^{2+} handling and contractility constitutes an important step to treat deleterious cardiac diseases involving cytosolic Ca^{2+} overload in myocytes [147]. Regarding TRPV4, several studies reported its implication in these processes while others did not observe obvious contribution. Indeed, its contribution to intracellular Ca^{2+} influx and myocardial contractility was examined by Li et al., using the TRPV4 agonist 4-αPDD on isolated rat papillary muscle. The authors revealed that the 4-αPDD had no effect on contractility [148]. In the same line, another study revealed in isolated perfused rat hearts, TRPV4 channels potentiation by the agonist GSK1016790A also had no effect on beating rate and contractility but induced, at the *in vivo* level, circulatory collapse which was most likely due to vascular leakage and tissue haemorrhage in the lung [80]. On the other side, our group has recently shown that the TRPV4 agonist GSK101679A perfusion induced a significant increase of the transient Ca^{2+} current influx in *trpv4$^{+/+}$* but not in *trpv4$^{-/-}$* mouse myocytes [8] confirming TRPV4 involvement in modulating ventricular intracellular Ca^{2+} concentration. Recently, Veteto et al., explored the relationship between the TRPV4 function and Ca^{2+} handling after mechanical stimulation in aged hearts [114]. To this end, the authors explored the effect of left ventricular preload elevation in working-heart perfused aged mice hearts and found that following the initial Frank-Starling response, these hearts exhibited a secondary increase in left ventricular maximal pressure which was absent when perfusing TRPV4 antagonist HC067047. Interestingly, when preload elevation was maintained for a longer period of time (20 min), maximal left ventricular pressure declined less in HC067047-treated than in untreated aged hearts. The authors then studied Ca^{2+} handling in ventricular myocytes following uniaxial stretch and found a delayed increase in intracellular Ca^{2+} in myocytes from aged mice ultimately leading to a contractured state which were both prevented by HC067047 treatment. These results suggest that TRPV4 is responsible for Ca^{2+} entry leading to a hypercontractile response secondary to myocardial stretch in the aged heart but when stretched was maintained, TRPV4-mediated Ca^{2+} influx was detrimental to cardiac contractility. Further investigations have reported that TRPV4 expression increases in mouse cardiomyocytes with advancing age [11] and after *in vivo* ischemia-reperfusion [61]. Note that TRPV4

upregulation in aged mice was associated to increased hypoosmotic stress-mediated contractility, enhanced cell death and increased ischemia-reperfusion injuries [11]. Finally, inhibition of TRPV4 by HC067047 exerts   cardioprotective effect during ischemia-reperfusion, attesting the key role of TRPV4 in Ca^{2+} handling and contractility [149]. It is important to underline that TRPV4 channel can also dock with other TRP channels and little is known about the contribution of these types of channels in intracellular Ca^{2+} dynamics and their involvement in the myocyte contractility. In this context, detailed interplay between TRPV4 channels and various α-subunits requires additional research in cardiomyocytes.

Collectively, these data suggest that TRPV4 channel represents an attractive ion channel target to prevent Ca^{2+} overload and cardiac contractility dysfunction [56], [61], [10], [150]. Indeed, TRPV4 seems to contribute to Ca^{2+} homeostasis regulation under physiological condition without impacting the contractility, whereas its implication may to be more deleterious during aging or under pathological conditions and lead to Ca^{2+} overload and altered contractility. Further investigations are undoubtedly needed to fully understand TRPV4 role in cardiac contractility in cardiovascular diseases.

4.4. Modulation of vascular tone

TRPV4 is expressed in ECs where its activation results in vasodilatation [151], [152]. TRPV4 is sensitive to shear stress [153] and is thus able to be activated by blood flow and induce Ca^{2+}-entry when expressed in endothelium. In ECs, Ca^{2+}-entry activates endothelial nitric oxide synthase (eNOS) pathway and the production of nitric oxide (NO), which can, in turn, diffuse to smooth muscle cells (SMC). In SMCs, NO activates cyclic GMP / PKG signaling inducing vasodilatation [152], leading to endothelium-derived factors release, causing SMC hyperpolarization and vasodilatation [152]. Furthermore, Ca^{2+}-entry activates in parallel intermediate (IK) and small (SK) conductance Ca^{2+}-sensitive K^+ channels in ECs, leading to hyperpolarization of ECs, and then, hyperpolarization of SMC via myoendothelial gap junctions [152]. Genetic deletion or pharmacological blockade of TRPV4 channels inhibits NO- and endothelium-derived hyperpolarizing factor (EDHF)-dependent relaxation of mouse small mesenteric arteries in response to flow [101]. Accordingly, transfection of TRPV4 channels in HEK293 cells confers them sensitivity to flow and induces shear stress-dependent Ca^{2+}-entry [101] confirming the important role of TRPV4 in sensing shear stress. TRPV4 transfected HEK293T cells are also sensitive to cell confluence, modulating TRPV4 response to hypoxia at high cell density suggesting another important feature of TRPV4 activation i.e. hypoxia[154]. Indeed, in vessels, hypoxia induces vasodilatation in systemic circulation, whereas it induces vasoconstriction in pulmonary circulation. TRPV4 activation with its agonist GSK1016790A causes endothelium-dependent relaxation of pre-contracted rat pulmonary artery rings, and this relaxation is inhibited in de-endothelized vessels. The authors of this study suggested that both NO and EDHF contribute to GSK1016790A-induced relaxation [122]. Ottolini et al. found that TRPV4 channels in mouse ECs co-localize with IK/SK channels in mesenteric arteries but not in pulmonary arteries, which explains that TRPV4 sparklets can activate IK/SK channels in mesenteric arteries but not in pulmonary arteries, where ECs TRPV4 activates preferentially eNOS [130]. Additionally, flow-induced vasorelaxation in human coronary micro vessels requires endothelial TRPV4 activation which increases mitochondrial ROS production in ECs and induces ROS-dependent vasodilation [123], [155]. TRPV4 is also expressed in SMC where it participates in vascular contraction, cell migration and proliferation[124], [127]. TRPV4 activation in pulmonary artery SMC results in a Ca^{2+} entry and Ca^{2+} release from the SR through the activation of ryanodine receptors[71] . This TRPV4-mediated Ca^{2+} elevation leading to SMC contraction [71], [87] can be triggered by serotonin [87], [128] or mechanical stimuli such as flow shear stress [129]. Dysregulation of TRPV4 expression between ECs and SMC could lead to an impaired balance between vasorelaxation and vasoconstriction and result in an altered myogenic tone [71], [156], [125].

5. Pathological implications of TRPV4 channels

5.1. Expression remodelling under pathological condition

TRPV4 expression increases under certain pathophysiological conditions, such as aging [11] pressure overload [157], [158], ischemia-reperfusion [56], [61], increased membrane tension [114], [115], pericarditis in rats and patients with atrial fibrillation [52]. In this context, it seems to be important to investigate the TRPV4 expression profile during life but also during the development of cardiac diseases.

5.2. Arrhythmias

Atrial fibrillation is the most common cardiac arrhythmia and is related, in part, to Ca^{2+} handling defects [159]. Since TRPV4 channels are expressed in native cardiac cells   including atrial cardiomyocytes their implication in atrial fibrillation was evaluated [52]. In a model of pericarditis-related atrial fibrillation phenotype, TRPV4 channel inhibition by GSK2193874 limited abnormal electrophysiological changes, cardiac fibrosis and inflammation, resulting in a reduced vulnerability to atrial fibrillation [52]. Accordingly, TRPV4 may constitute an interesting therapeutic target to treat human atrial fibrillation. Another recent investigation pointed the importance to consider aging and his potential negative effect on Ca^{2+} handling, the resting membrane potential and the risk to develop ventricular arrhythmia after ischemia-reperfusion. In this context and because TRPV4 channel is up-regulated in cardiomyocytes of aged mice [11], the perfusion of HC067047 (TRPV4 antagonist) after ischemia-reperfusion, reduces incidence of pro-arrhythmic diastolic Ca^{2+} signaling, maintains the resting membrane potential and decreases the ventricular arrhythmia score [160]. Thereby, TRPV4 blockade may also constitute an interesting strategy to limit the occurrence of arrhythmogenic events for aged populations following ischemia-reperfusion and MI.

5.3. Cardiac Remodelling and fibrosis

There are some evidence that TRPV4 may be involved in cardiac pathological remodelling and could thus be an interesting therapeutic target in these contexts. In a recent study, TRPV4 channel expression was found to be significantly increased in the ventricles of mice with left ventricular pressure-overload induced hypertrophy and in human failing ventricles [158]. Interestingly, pressure-overload resulted in reduced cardiac hypertrophy, cardiac dysfunction, fibrosis, inflammation in TRPV4 knockout mice compared to wild-type animals. Moreover, treatment with GSK2193874 (TRPV4 antagonist) prevented the pressure-overload induced remodelling and dysfunction further confirming TRPV4 involvement. In the same study, the authors showed that the increase in CaMKII phosphorylation found in the pressure-overloaded left ventricle in wild-type mice was absent in TRPV4 knockout mice. They suggested that this TRPV4-related increase in CaMKII phosphorylation, leads to NF-κB phosphorylation and NLRP3 activation, which both contribute to the pro-inflammatory remodelling found in these pressure-overloaded hearts. Therefore, inhibiting TRPV4 appears promising to limit CaMKII phosphorylation and its multiple consequences and inflammation in cardiac hypertrophy. The role of TRPV4 in cardiac remodelling has also been studied in the context of myocardial infarction and subsequent fibrosis through channels expressed in CFs.CFs originate from mesenchymal stem cells, and represent 2/3 of the cardiac cell population [161]. They contribute to maintain the structural, biochemical, mechanical and electrical properties of the healthy myocardium and constitute the main source of extracellular matrix (ECM) protein production (collagen types I and III and metalloproteases) [162]. Fibrosis is known to be related to an expansion of the cardiac interstitial space due to an accumulation of ECM proteins. During this phenomenon, CFs proliferate, migrate and differentiate into myofibroblasts [163] under the effect of a variety of factors including transforming growth factor β1 (TGF-β1), tumor necrosis factor-α (TNF-α), Angiotensin II (Ang II), Platelet derived growth factor (PDGF), Endothelin 1 (ET-1)) and mechanical (stretch and stiffness) factors [116], [164]. Interestingly, these changes in the heart eventually lead to an increased matrix stiffness, structural damaging effect, electrical disorders [6]

and predispose it to diastolic dysfunction [165], [166]. Moreover, intracellular Ca²⁺ signaling, involved in fibroblast proliferation and differentiation, is mainly activated in CFs through TRP channels, including TRPV4 channels [7], [52], [90], [118] [167].Indeed, accumulating evidence hints that TRPV4 mechanosensor is an important factor in the progression of fibrosis and/or prevention of fibroproliferative disorders in several organs such as skin, lung [168], liver, kidney, brain, blood vessel and heart [7]. The role of TRPV4 mechanotransduction in cardiac fibrosis was demonstrated using *trpv4⁻/⁻* mice model [81]. The results suggest that the TRPV4 deletion preserves cardiac function and reduces fibrosis in *trpv4⁻/⁻* mice but not in WT mice after transverse aortic constriction and MI [167]. Interestingly, *trpv4⁻/⁻* mice displayed a marked decrease in fibrosis, as well as a decrease in the pro-fibrotic gene expression level including col1A2, α-SMA, NFAT, TGF-β1, and MRTF, a mechanosensitive transcription factor. Conversely, the WT mice subjected to MI or congestive heart failure had an up-regulation of TRPV4 channels and profibrotic genes compared to the untreated WT mice which lead, *in fine*, to increase the intracellular Ca²⁺ level and amplify the pathological fibrosis process. These findings demonstrated that TRPV4 is functional in mouse CFs and directly related to fibrosis process insofar as its genetic deletion preserves cardiac function and protects the heart against the adverse fibrosis effects [167]. In line with these results, several *in vitro* investigations of rat or human CFs have shown a rapid but sustained increase in Ca²⁺ influx in response to TRPV4 agonist perfusion (4αPDD [118] or GSK1016790A [82]) which promotes the fibroblasts differentiation into myofibroblasts. Consistently, the blockade of TRPV4 by different antagonists (AB159908 [81] or RuR [118] or GSK2193874 [52]) or TRPV4 short-interference RNA (TRPV4-siRNA) [118] inhibits TGF-β-induced fibroblast differentiation [81] or abolishes Ca²⁺ conductance [118] or decreases the Ca²⁺ influx level [52], [116]. Recently, and in agreement with the previous investigations, treatment with the pro-fibrotic cytokine, TGF-β, increases TRPV4 channel expression in human ventricular CFs [117]. Simultaneously with this change, the authors noted both an increase in a fibrosis biomarker (the Plasminogen activator inhibitor-1) and an increase in a fibroblast differentiation biomarker (the α-smooth muscle actin : α-SMA) [117]. In addition, and via the MAPK/ERK pathway, TRPV4 channel activation by its specific agonist (GSK1016790A) triggers the CFs transformation into myofibroblasts while its antagonist (RN-9893) combined with a Ca²⁺ chelating agent (BAPTA-AM) reduces it [117]. These different points are important because they show that the Ca²⁺ permeability of TRPV4 channels is an essential component of human ventricular CFs differentiation. A recent investigation has demonstrated that TRPV4 channel expression level was higher in CFs isolated from Sprague-Dawley neonatal diabetic rats and from Sprague-Dawley adult rats, cultured in glucose-rich medium. Interestingly, the TRPV4 channel agonist HC067047 perfusion abolished significantly these CF changes. It has also been reported that HC067047 decreases the collagen I synthesis and suppresses the presence of the growth factor TGF-β necessary for the differentiation of CFs into myofibroblasts. In this context, different approaches could be used directly or indirectly to manage TRPV4 channel function and imagine new therapeutic strategies to limit fibrosis within cardiac diabetic patients [90] or in various cardiac pathologies involving many different patient populations. Finally, the link between fibrosis and the increased risk for atrial [52], [169], [170], [171] or ventricular [116] arrhythmias has been reported in several cardiac diseases [166], [172]. In this context, the anti-fibrosis approach via the TRPV4 inhibition constitutes a promising therapeutic way to limit adverse outcomes and prevent arrhythmias in the heart.

Taken together, these findings have clinical significance by identifying TRPV4 as an interesting therapeutic molecular target to attenuate cardiac fibrosis but still require further attention especially during arrhythmogenesis and pathological cardiac development.

6. Conclusions and perspectives

Evidence for a crucial role of TRPV4 channel has emerged from a large range of experiments in which its inhibition preserves the physiological intracellular Ca²⁺ dynamics and protects the heart form several dysfunctions including pathological cardiac disorders   (Figure 2), HF and arrhythmia. Unfortunately, the molecular mechanism involving the transition from a healthy heart towards a cardiac pathology is poorly understood. The use of specific TRPV4 modulators combined to

transgenic animal models has given valuable information about its involvement in physiological or pathological cardiovascular remodelling. Note that the recent availability of the crystal structure of TRPV4 channel will facilitate drug development to counteract diseases including potentially pathological cardiac phenotypes. Furthermore, various genetic diseases involving abnormal TRPV4 channel function have highlighted the key role of this ion channel in a broad spectrum of cellular processes. Surprisingly, no *trpv4* mutation was found in patients with cardiac diseases so far. It would be relevant to further investigate the role of TRPV4 in the elderly population given the increase in its expression and the risk of cardiac diseases (ischemia-reperfusion lesion, MI, pressure-overload, arrhythmia) found in this population. Future work in this promising field may help to better understand the function and regulation of this channel and identify its interactions with other channels and the implication in cardiac physiological and pathological processes.
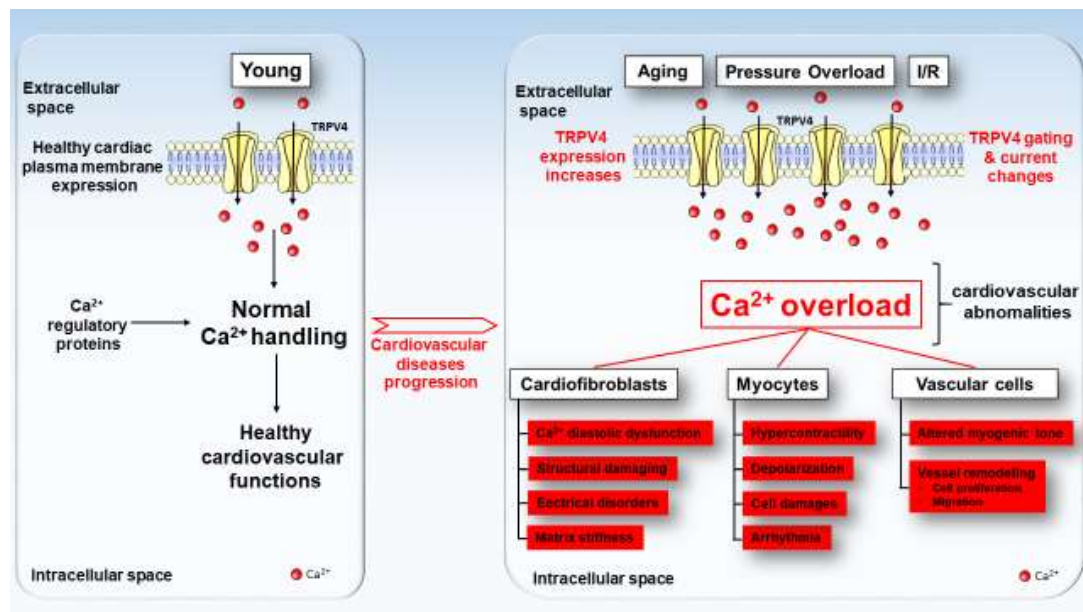
Figure 2. TRPV4 expression and function model in healthy (left) and pathologic (right) cardiac cells. Under physiological condition, TRPV4 channels (yellow channels) present a low expression at the plasma membrane of cardiac cells. With aging, pressure overload, ischemia/reperfusion and others deleterious factors, TRPV4 exhibits both an increase in expression and activity at the plasma membrane of cardiac cells which tend progression towards of cardiovascular diseases.

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