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Review

TRPC channels: Dysregulation and Ca^{2+} Mishandling In Ischemic Heart Disease

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Abstract: Transient receptor potential canonical (TRPC) channels are ubiquitously expressed in excitable and non-excitable cardiac cells where they sense and respond to a wide variety of physical and chemical stimuli. As other TRP, TRPC may form homo or heterotetrameric ion channel and they can associate with other membrane receptors and ion channels to regulate intracellular calcium concentration. Dysfunctions of TRPC channels are involved in many types of cardiovascular diseases. Significant increase of the expression of different TRPC isoforms has been observed in different animal model of heart infarcts and *in vitro* experimental model of ischemia and reperfusion. TRPC-mediated increase of the intracellular Ca^{2+} concentration seems required for the activation of signaling pathway that plays minor roles in the healthy heart, but they are more relevant for cardiac responses to ischemia, such as the activation of different factors of transcription and cardiac hypertrophy, fibrosis and angiogenesis. In this review, we will highlight the current knowledge regarding TRPC implication in different cellular processes related to ischemia and reperfusion and to heart infarction.

Keywords: TRPC channel; Ca^{2+} entry; Cardiac infarction; Cardiac repair

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Abbreviations:

Diacylglycerol (DAG); Dominant-negative (dn); Endothelial cells (ECs); Hypoxia-reoxygenation (H/R); Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$); Ischemia and reperfusion (I/R); Heart failure (HF); Knockout (KO); Myocardial infarction (MI); Neonatal rat ventricular myocytes (NRVM); 1-Oleoyl-2-Acetyl-sn-Glycerol (OAG); Sarco-Endoplasmic Reticulum Ca^{2+} ATPase (SERCA); Sarcoplasmic Na^+/Ca^{2+} exchanger (NCX); Transient Receptor Potential (TRP); Thoracic Aortic Constriction (TAC); Vascular endothelial growth factor (VEGF); Wild-type (WT).

1. Introduction

The heart rate of a healthy adult ranges between 60 and 100 beats/min, which is mainly achieved by adequate function of the cardiac contraction/relaxation cycle. Adequate ventricular contraction is strongly dependent on effective excitation-contraction (EC) coupling in cardiac cells [1]. Electrical stimuli travel across conducting cardiac tissues to the cardiomyocytes, inducing a cell-membrane depolarization which activates ion channel trafficking and finally causes the cell contractile machinery [2]. EC coupling and cell contraction are critically dependent on Ca^{2+} influx and Ca^{2+} channel trafficking. The initial cell-membrane depolarization stimulates sarcolemma L-type Ca^{2+} channels, prompting a small influx of Ca^{2+} from the extracellular medium. Ca^{2+} entry triggers a large release of Ca^{2+} from the sarcoplasmic reticulum via ryanodine receptors (RyR), resulting in an increase of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The rise in $[Ca^{2+}]_i$ boosts Ca^{2+} binding to troponin C which activates the contractile machinery. After contraction, $[Ca^{2+}]_i$ must decrease to allow cell relaxation, which is achieved mainly via two mechanisms: Ca^{2+} re-uptake by the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump and Ca^{2+} efflux by the sarcoplasmic Na^+/Ca^{2+} exchanger (NCX) [2,3]. Dysregulation of any of these Ca^{2+} handling processes is commonly associated with cardiac dysfunction.

Recently, other players have emerged as key partners in the regulation of cardiac Ca^{2+} handling. Among these partners are the transient receptor potential (TRP) channels that are classified in a superfamily, including 28 mammalian TRP proteins divided according their genetic and functional homology into six families: TRPP (polycystin), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPML (mucolipin) and TRPC (canonical). TRP channels are composed of six transmembrane domains (TM1–TM6), with a preserved sequence called "TRP domain" adjacent to C-terminus of TM6 and a cation-permeable pore region formed by a loop between TM5 and TM6 [4]. TRP channels are located in the plasma membrane and their activation allows the entry of Ca^{2+} and/or Na^+, with higher permeability for Ca^{2+}. Although most TRP channels lack a voltage sensor, they can be activated by physical or biochemical changes, regulating Ca^{2+} dynamics by directly conducting Ca^{2+} or prompting Ca^{2+} entry secondary to membrane depolarization and modulation of voltage-gated Ca^{2+} channels [5]. The activation of different isoform of TRP has been associated with cell-membrane depolarization, for example in smooth muscle cells (for review [6–8]) and in cardiac cells [9–11]. There are substantial evidences that TRP channels have important roles in mediating cardiac pathological processes, including cardiac hypertrophy and fibrosis [4,12–14],which all lead to deleterious cardiac remodeling and subsequent heart failure (HF). This review focusses on the role of TRPC channels and provides an overview of the most relevant and recent findings related to this channels and ischemia related disease in the heart.

2. TRPC channels in the cardiovascular system

TRPC channels are classified into seven members (TRPC1-7) that are distributed based on biochemical and functional similarities into TRPC1/4/5, TRPC3/6/7 and TRPC2, which is a pseudogene in humans [5]. The expression of TRPC isoforms in the heart has been examined in different stages of animal development, animal model and areas of the heart. They are expressed at very low levels in normal adult cardiac myocytes but their expression and activity might increase in pathological processes [15]. Also, TRPC isoforms are ubiquitously expressed in almost all cell types in heart. However, they likely display different patterns' expression in cardiac myocytes isolated

from sinoatrial node, atrial or ventricular heart [14,16–19]. In human cardiac tissues and/or neonatal rat cardiomyocytes mRNA of TRPC5 [20,21] and TRPC6 [22] were detected. In animal model, the expression of TRPC1/3-7 have been confirmed in adult rat and mouse ventricle and atrial cardiac myocytes either at mRNA or protein levels [13,23,24]. Others reports showed that TRPC1/3-6 are expressed in rat ventricular myocytes of fetal and neonatal ventricular myocytes [19,25]. In sinoatrial node cells, TRPC1, 2, 3, 4, 6 and 7 mRNA expression levels are detected using RT-qPCR, whereas TRPC5 expression is not observed. Furthermore, experiments using immunohistochemistry confirmed protein expression of TRPC1, 3, 4 and 6, but not TRPC7 in mouse sinoatrial node and in isolated pacemaker cells [26]. In the case of cardiac fibroblasts, all TRPC isoforms have been described. In particular, the mRNAs of TRPC1, 3, 4 and 6 are detected in mouse cardiac fibroblast [27]. Meanwhile, isolated rat ventricular fibroblasts have significant mRNA expression of TRPC2, 3 and 5 [28]. Experiments using immunocytochemistry and western blot also revealed the expression of TRPC1, 3, 4 and 6 proteins in rat and human cardiac fibroblasts [29–31].

Functional TRPC channel is composed by four proteins so it can be formed as homo or heterotetramers [32]. However, the concept of TRPC multimerization has been barely addressed in cardiac myocytes. A previous study from Molkentin's group suggested multimerization of TRPC3 and his homotypic TRPC6 in adult mouse cardiac myocytes since they demonstrate, using immunoprecipitation approach, that TRPC3 can associate with TRPC4 protein [5]. More recently, TRPC6 was suggested to form heteromeric complex with TRPC3 and NADPH oxidase 2 (NOX2) protein in diabetic mouse heart. Nonetheless, this study used HEK293 cells to confirm the interaction between TRPC3 and TRPC6 by immunoprecipitation [33].

It should be noted that other studies indicated that TRPC channels can form macromolecule complex with NCX [34,35], Na+/K+ pump [36] and SERCA pump [37]. Thereby, they might create a microenvironment facilitating the fine-tuning of Ca^{2+} homeostasis [5,38,39] and excitation-contraction coupling [40]. In fact, recent evidences confirmed that TRPC3 mediates Ca^{2+} and Na^{+} entry in proximity of NCX, elevating Ca^{2+} levels and cardiac contractility [35]. Certainly, more precise investigations about TRPC heteromerization will be welcome to reveal whether this concept is similar to that observed in other cells such as smooth muscle cell [41] platelets [42]; hippocampus [43]; or in rat brain [44].

3. TRPC channels mediated Ca^{2+} influx in cardiac myocytes

The activation of TRPC channels has not been completely clarified yet. It is known that TRPC1, 4 and 5 are IP3-sensitives [45–47]; whereas TRPC3, 6 and 7 are activated by diacylglycerol (DAG), the cleavage product of phosphatidylinositol 4,5-bisphosphate (PIP2) [18,48,49]. TRPC4 and 5 channels become also sensitive to DAG when the interaction between PKC and Na^{+}/H^{+} exchanger regulatory factor (NHERF) are inhibited [50].

There are considerable indications that in cardiac myocytes isolated from the atrium, the ventricle or from neonatal rat ventricular myocytes (NRVM), TRPC channels participate both in store operated Ca^{2+} entry (SOCE) and receptor operated Ca^{2+} entry (ROCE) pathways, and their activation and/or upregulation is essential for cardiac Ca^{2+} signaling, particularly under pathological situations [34,51,52]. Independent studies have shown that DAG, which works as an important mediator of G-protein coupled receptor (GPCR)-stimulated Ca^{2+} signaling pathway, activates TRPC3 and 6. For instance, Onohara et *al.* [11] demonstrated that stimulation of NRVM with angiotensin-II and 1-oleoyl-2-acetyl-sn-glycerol (OAG), membrane permeable DAG analogue, activates TRPC3 and 6

channels, causing membrane depolarization. They further demonstrated that siRNA of TRPC3 and 6 reduces significantly responses to angiotensin-II. OAG also activates a cation current in mouse cardiac myocyte that is significantly reduced by cell dialysis with anti-TRPC3 [53]. Moreover, the activation of A1 adenosine receptor in atrial and ventricular myocytes activates TRPC3, through DAG, since Ca^{2+} influx is inhibited by Pyr3, considered specific inhibitor of TRPC3 [24].

It should be noted that other studies focused on the role of TRPC channels in SOCE activation in cardiac myocytes. For instance, a recent study by Wen et *al.* [54] demonstrated the presence of SOCE in normal adult mouse ventricular myocyte and the participation of TRPC1, 3 and 6 since antibodies against these TRPC channels reduce store depletion mediated-Ca^{2+} entry. Previously, an Wu *et al.* [5] characterized the participation of TRPC3, 4 and 6 in the exacerbated SOCE observed in mouse cardiac myocyte from hypertrophic hearts. They demonstrated significant reduction of SOCE mediated by specific inhibition of SERCA with cyclopiazonic acid in cardiac-specific transgenic mice expressing dominant-negative (dn) of TRPC3 (dn-TRPC3), dn-TRPC6, or dn-TRPC4. The participation of TRPC3 and 4 in SOCE has been also characterized in adult rat ventricular myocyte induced by specific activation of EPAC (Exchange Protein directly Activated by cAMP) with 8-pCPT [12]. This study revealed significant upregulation of TRPC3 and 4 which correlates with SOCE increase in 8-CPT-treated cardiac myocyte. In addition, thapsigargin-induced SOCE is inhibited by Pyr3, TRPC3 inhibitor [12]. Another study suggested a role of TRPC1, 4 and 5 in SOCE caused by aldosterone stimulation of NRVM. Indeed, thapsigargin-induced SOCE is inhibited in aldosterone-treated NRVM transfected with dn-TRPC1, dn-TRPC4 and with siRNA against TRPC5, whereas dn-TRPC3 did not alter SOCE [55]. Moreover, TRPC1 and 4 overexpression correlates with CRAC-like current recorded in isolated hypertrophied right ventricular myocytes treated with monocrotaline [56]. Recently, we proposed that at least TRPC5 may be critical in SOCE since its downregulation inhibits thapsigargin-induced potentiated SOCE in NRVM under ischemia and reperfusion. We further demonstrated that TRPC5 colocalizes with Orai1, the pore forming sub-unit of store operated Ca^{2+} channel (SOCC) [13].

The role of TRPC channels in SOCE is still controversial despite the increasing number of studies in cardiac myocytes. Most of these reports use agents that selectively deplete sarcoplasmic reticulum Ca^{2+} stores (e.g. cyclopiazonic acid, thapsigargin) to activate SOCC and avoid contribution of ROCE pathways. The combination of using different TRPC inhibitors together with functional pore inhibitory antibodies for TRPC proteins and RNA silencing, suggest that TRPC channels account for the prominent SOCE in cardiac myocytes specially under pathological conditions.

4. Role of TRPC channels in cardiac physiopathology

There is a general consensus that the overexpression and activation of TRPC channels are associated with deleterious cardiac pathology. Under physiological conditions, the function of TRPC channels in the heart seems not essential [4,57]. As reviewed recently, hearts from knockout (KO) mice of different TRPC channels do not present any significant contractile abnormalities [27]. Echocardiography analysis showed that TRPC3 KO and TRPC6 KO mice have similar resting left ventricular mass and fractional shortening as compare to their respective littermate controls [58]. Although, the induced stress-stimulated contractility, known as the Anrep effect, is diminished in isolated papillary muscles and cardiomyocytes from TRPC6 KO, but not TRPC3 KO mice [59]. In addition, TRPC1/4-double KO mice have normal basal cardiac contractility, normal systolic and

diastolic functions. In contrast, isoproterenol-induced chronotropic responses are reduced in TRPC1/4-double KO mice [60].

TRPC channels might play a role in some physiological processes. TRPC channels likely regulate cardiac pacemaking, conduction, ventricular activity and contractility during cardiogenesis, through the interaction with the $Ca_v1.2$ channel in isolated hearts obtained from 4-day-old chick embryos [18]. TRPC channels also contribute to Ca^{2+} homeostasis by directly conducting Ca^{2+} or indirectly via membrane depolarization and voltage gated Ca^{2+} channels modulation. The resulted TRPC-mediated Ca^{2+} influx is required for the activation of signaling pathways that play minor roles in the healthy heart. For instance, they are involved in the activation of transcription factors promoting cardiac hypertrophy, fibrosis and or arrythmia [13,19,27,34,61]. Here, we will discuss the role of TRPC channels in processes related to cardiac ischemic diseases.

5. Role of TRPC channels in cardiac ischemia

5.1. TRPC channels in myocardial infarction

One of the first evidences of the participation of TRPC in myocardial infarction (MI) has been proposed using bioinformatic analysis combined with experimental approaches. Zhou R, *et al.* [62] demonstrated an increase in the expression of TRPC6, which was experimentally validated in 1-month post-MI rat model, suggesting TRPC6 as a potential therapeutic target for MI. Later, other studies highlighted the induction of TRPC proteins under MI and explored the idea that Ca^{2+} influx through TRPC channels overexpressed after MI contributes to cardiac dysfunction and adverse remodeling. In fact, significant increase of TRPC1, 3, 4 and 6 mRNA levels in mice 1, 2 and 6 weeks post-MI has been observed, as compared with Sham [63]. These channels upregulation correlate with the increase of Ca^{2+} entry when myocytes isolated from MI adult mouse are stimulated with cyclopiazonic acid and OAG. Furthermore, mice expressing dn-TRPC4 has less pathological hypertrophy, better cardiac hemodynamics performances and increased survival after MI, as compared with wild-type (WT) mice [63]. Therefore, the loss of TRPC4 function likely protects against the progression of cardiac dysfunction after MI. Interestingly, Jung *et al.* [64] suggested that gain-of-function of TRPC4 due to a genetic variation (I957V) causes an increase of channel activity which has a protective effect against MI. The authors identified a single-nucleotide polymorphism (SNP) in TRPC4 that associates with MI risk in a case-control study. They further used multivariate analysis to show a protective effect of the I957V allele against MI risk, but only in diabetic patients. Therefore, the mutated TRPC4 -I957V is thought to mediate higher Ca^{2+} signals, perhaps to facilitate the generation of endothelium and nitric oxide dependent vasorelaxation. Nevertheless, the authors did not test experimentally this hypothesis. Recently, we observed significant dysregulation in the expression of several TRPC isoforms in Wistar rats' model of MI induced by transient ligation of the left coronary artery. PCR-based micro-array, qRT-PCR and western blotting demonstrate significant upregulation of TRPC1, 3, 4, 5 and 6 either in risk or in remote zones of infarcted hearts, as compared to Sham. Specific inhibition of TRPC5 in MI rats infused with urocortin-2 at the onset of reperfusion was observed, offering a role of TRPC5 in cardioprotection [13].

In the case of TRPC3 and 6, a previous study determined that TRPC6-KO mice had significantly higher rates of mortality due to ventricular wall rupture throughout 3–7 days post MI [65]. In contrast, TRPC3/6/7 triple KO mice subjected to transient MI (30 min of ischemia followed by 24-h reperfusion) exhibit reduced infarct size, better cardiac performance and less cardiac tissue damage

post-MI, as compared with WT animal. In addition, they have a reduced apoptosis through the inhibition of the calcineurin–NFAT signaling pathway [49]. These results suggest that TRPC3, 6 and 7 contribute significantly to worsen MI impacts on cardiac function. Further investigations will be welcome to clarify the discrepancy between these KO studies. It will be interesting to examine whether the cardioprotective effects observed in the triple KO mice affect or not the transformation of myofibroblast required during wound healing and scare formation.

5.2. TRPC channels role in ischemia and reperfusion injuries and cardioprotection

Ischemia and reperfusion (I/R) injury is the main cause of cell apoptosis and necrosis observed after a MI. Several studies have demonstrated evidences linking cytosolic Ca^{2+} increase through TRPC and apoptosis after I/R [49,66]. Studies using TRPC inhibitors examined their role in I/R injuries. For instance, Kojima *et al.* [67] showed in a Langendorff-perfused mouse heart under I/R that left ventricular functions are significantly improved by the administration of ion channels blockers (2-APB and La^{3+}) during initial 5 minutes of reperfusion, suggesting TRPC channels' role in the contractile dysfunction in reperfused ischemic myocardium. In atrial cardiac cell line, H9C2, the addition of SKF96365, another widely used inhibitor of TRPC, ameliorates injuries induced by hypoxia-reoxygenation (H/R) [49]. However, it is well known that 2-APB, La^{3+} and SKF96365 are not specific to TRPC channels and may block other cationic channels [68,69]. Therefore, these results should be supported by experiments using siRNA and/or TRPC-deficient mice. Actually, other reports used different molecular approaches to identify TRPC isoforms responsible of Ca^{2+} entry and its relationship with cardiac myocytes death under I/R. For example, Shan *et al.* [66] observed that transgenic mice overexpressing TRPC3 in myocardial cells are highly sensitive to injuries after I/R as they enhance apoptosis through increased TRPC3-mediated Ca^{2+} influx and calpain cleavage. They also demonstrated significant improvement in the viability of cardiomyocytes after SKF96365 treatment. Moreover, Meng *et al.* [70] observed *in vitro* that I/R increases TRPC6 protein expression, $[Ca^{2+}]_i$ levels and cell apoptotic rate in a time-dependent manner in H9C2 cell line. In addition, they suggested TRPC6 as possible target for cardioprotection in H9C2 cells since the administration of Danshensu, an active component of Salvia miltiorrhiza, protects against I/R injury by reducing TRPC6 expression via JNK signaling pathway [70]. Hang *et al.* [71] also demonstrated that brain-derived neurotrophic factor (BDNF) protects against MI and inhibits H/R-mediated cardiomyocyte apoptosis through TRPC3 and TRPC6 regulation.

On the other hand, the role of TRPC1 in I/R is still unclear. A recent study suggested that it is implicated in I/R injury, as the expression of mRNA and protein of TRPC1, Orai1 and STIM1 are significantly increased *in vivo* in mice subjected to myocardial I/R injury and *in vitro* in H9C2 cells after H/R [72]. Interestingly, the suppression of STIM1 by siRNA decreases the expression of TRPC1 and Orai1, leading to decreased intracellular Ca^{2+} accumulation and apoptosis produced by H/R in H9C2 cells [72]. Therefore, STIM1 likely regulates the expression of TRPC1 and Orai1 in the context of apoptosis and myocardial I/R injury. In contrast, Al-awar *et al.* [73] speculated that TRPC1 plays a cardioprotective role against I/R injury. They showed that sitagliptin, inhibitor of dipeptidyl peptidase-4 (DPP-4), decreases the infarct size in a rat model of I/R which correlates with the increase of protein levels of TRPC1, TRPV1 and calcitonin gene-related peptide in heart tissue. Nevertheless, specific experiment targeting TRPC1 has not been shown. Our recent study, through western-blot, confirms that TRPC1 and 6 are upregulated in rat model of I/R although they are not inhibited by urocortin-2 mediated cardioprotection. In contrast, urocortin-2 administration in NRVM undergoing

in vitro I/R inhibits SOCE and prevents I/R-induced protein overexpression of TRPC5 and Orai1 [13]. Taking in consideration these results further investigations are necessary to clarify the functional role of TRPC channel increase after I/R.

6. TRPC channels in post-ischemia cardiac repair

After MI, the heart undergoes extensive adaptative processes and myocardial remodeling, involving, angiogenesis, cardiac cell hypertrophy and accumulation of fibrous tissue in both the infarcted and non-infarcted myocardium [74–76]. Nonetheless, the role of TRPC protein in cardiac repair still remains poorly studied.

6.1. TRPC channels in post-ischemia angiogenesis

Angiogenesis rely to new blood vessels forming from pre-existing vessels and the subsequent expansion of the vascular network in the body. Post-ischemic angiogenesis is considered a protective mechanism motivated by lack of oxygen and blood supply necessary for physiological heart repair after a MI [77–79]. Angiogenesis involves the sprouting, proliferation, migration and tube formation thanks to the stimulation of endothelial cells (ECs) by growth factors such as vascular endothelial growth factor (VEGF), considered as the most potent pro-angiogenic factor specific for ECs [80]. Compelling evidences demonstrated that chronic and transient ischemia increase significantly the expression of VEGF [81–83]. Nevertheless, pre-clinical and clinical trials using solely pro-angiogenic factors, such as VEGF have not been shown to be effective in patients with stable angina or critical lower limb ischemia [84,85]. VEGF stimulates two tyrosine-kinase receptors, VEGFR-1 and VEGFR-2 [77,86] to increase $[Ca^{2+}]_i$ in ECs involving Ca^{2+} release from intracellular stores and extracellular Ca^{2+} flux through cation channels, such as TRP channels [87,88]. There is an increasing interest on the role of TRPC channels in angiogenesis, especially in studies related to cancer and diabetes [88–90]. ECs express different TRPC proteins involved in vascular function (TRPC1, 4 and 6), vascular tone remodeling (TRPC4) and oxidative stress-induced responses (TRPC3 and 4) [91,92]. It is apparent that TRPC3 and 6 are implicated in VEGF-mediated $[Ca^{2+}]_i$ increase in ECs and angiogenesis. Indeed, VEGF and OAG-induced ERK1/2 activation and tubulogenesis are significantly suppressed by TRPC3 inhibitor and siRNA in Human Umbilical Vein ECs (HUVEC) [93]. Meanwhile, the overexpression of dn-TRPC6 in human microvascular ECs inhibits the VEGF-mediated $[Ca^{2+}]_i$ increase, migration, sprouting, and proliferation, well-known hallmarks of angiogenesis [94]. In addition, TRPC4 siRNA attenuates oxLDL-induced human coronary ECs proliferation; migration and angiogenesis-tube formation [95].

Unfortunately, little is known regarding the role of TRPC channels during post-ischemic angiogenesis. In contrast, TRPC channels appear involved in hypoxia-induced angiogenesis [96,97]. For instance, the expression of TRPC4 protein is significantly upregulated in retina under hypoxic condition. TRPC4 siRNA inhibits VEGF-induced migration and tube formation of retinal microvascular ECs, which suggest a role of TRPC4 in initiating neovascularization in response to VEGF in retina under hypoxia [97]. Recently, Moccia *et al.* [98] hypothesized and debated about transfecting TRPC3 into autologous endothelial progenitor cells (EPCs) might enhance revascularization and functional recovery of ischemic hearts. However, functional experiments that test this hypothesis have not been performed. Recently, Zhu *et al.* [99] demonstrated that TRPC5 activation is necessary for ECs sprouting, angiogenesis and blood perfusion in a hind-limb ischemia model. TRPC5 downregulation prevents NFAT activation and ECs tube formation under hypoxia.

Moreover, TRPC5-KO mice have worse vascular recovery than WT mice after an ischemic injury. Finally, activation of TRPC5 by riluzole stimulates ECs sprouting and improves significantly limb's recovery from ischemia injuries [99]. Therefore, it will be interesting to confirm the beneficial role of others TRPC channels in heart's post-ischemic angiogenesis.

6.2. TRPC channels in early adaptative cardiac remodeling

An early cardiac hypertrophy and fibrosis are considered compensatory events to the loss of cardiac myocytes and necessary for wound healing and scare formation after heart infarcts. However, prolonged hypertrophy could lead to the development of HF, arrhythmias and even sudden cardiac death [100,101]. Since it is known that the activation of TRPC channels mediates the Ca^{2+} influx which activate Ca^{2+} intracellular signaling pathways, such as the calcineurin/NFAT, TRPC channels are suggested as Ca^{2+} effector and transducer of hypertrophic genes in the heart. Little is known regarding TRPC channels implication in I/R-induced cardiac hypertrophy yet. In contrast, there is a general agreement regarding the role of TRPC channels in pathological cardiac hypertrophy as a consequence of aortic constriction or under chronic GPCR stimulation using endothelin-1, phenylephrine, or angiotensin-II [22,102,103]. Similarly, Makarewich *et al.* [63] revealed an up-regulation of TRPC1, 3, 4, and 6 channels in mice 6-weeks post-MI as compared to sham animals, along with the activation of fetal gene program. They also demonstrated that mice expressing dn-TRPC4 has less pathological hypertrophy, better cardiac hemodynamics performances and increased survival after MI, as compared with wild-type (WT) mice, which all suggest a critical role of TRPC4 in post-MI heart damages. Cardiac hypertrophy is also observed in rat heart tissue as early as 1 week post-I/R, which correlates with the upregulation of the expression of TRPC1, 3, 4, 5 and 6 mRNA [13] and the activation of the so-called fetal gene program, the markers of cardiac hypertrophy (unpublished data). Recently, Dragún *et al.* [104] examined the expression of TRP channels in 43 patients with end-stage HF. They discovered, among other TRP channels, significant increase of TRPC1 and 5 gene expression, meanwhile TRPC4 expression is decreased in HF patients as compared to healthy donor. Also, they detected significant correlation of the gene expression of TRPC1 and MEF2c (Myocyte Enhancer Factor 2c), considered a key transcription factor for cardiac hypertrophy [105] . Interestingly, this pilot study did not detect any significant differences in TRP expression between male and female HF patients, nor between HF based on ischemic or non-ischemic background. Another recent study observed similar increase in the expression of TRPC1 in hearts of patients with hypertrophic cardiomyopathy (HCM) or HF. This study further used human pluripotent stem cell lines of TRPC1-KO generated using CRISPR/Cas9 to confirm the role of TRPC1 in regulating cardiac myocyte hypertrophy induced by phorbol 12-myristate 13-acetate (PMA), which was associated with abnormal activation of NF-κB [106]. Altogether indicate that TRPC channels might play similar role in cardiac hypertrophy and HF independently of patients' background. Perhaps, once TRPC expression is triggered they activate several Ca^{2+} dependent factors of transcription and in cardiac hypertrophy genes leading to the same outcome: HF.

In the case of fibrosis, multiple well-known markers of fibrosis, hypertrophy and Ca^{2+} handling protein have been identified recently using genome-wide transcriptome analysis of infarcted hearts [107]. TRPC6 is consider a regulator of myofibroblast differentiation, a hallmark of fibrosis, since its silencing in human cardiac fibroblasts attenuates the TGF-β1-induced upregulation of α-SMA, a marker of myofibroblast transformation [108]. A recent study confirmed that serum level of TGF-β1 is increased 28 days after MI in mice, accelerating cardiac fibrosis [109]. On the other hand, Saliba *et*

al. [110] described that polyphenol extracted from grape pomace decreases angiotensin-II induced Ca^{2+} entry through a direct regulation of TRPC3 and subsequent activation of NFATc3 in human ventricular cardiac fibroblast, which abrogates myofibroblast differentiation and fibrosis by decreasing collagen secretion. Despite the direct contribution of TRPC channels in cardiac fibrosis mediated by ischemia has been barely addressed. Different isoform of TRPC proteins are upregulated in rat showing fibrosis 1 week post-I/R, although their direct role in promoting fibrosis has been examined [13]. Interestingly, TRPC6, through calcineurin-NFAT signaling, seems required for myofibroblasts transformation after MI a critical step during which collagen deposition and scar formation happens to maintain ventricular wall structural integrity in early days post-MI. In fact, TRPC6 KO mice shows poor wound healing and less myofibroblasts, stained with α-SMA antibody, in the infarcted area [65]. Moreover and independently of studies related to ischemia and heart infarct, several reports proposed the participation of TRPC channels in the cardiac interstitial fibrosis caused by pressure overload by thoracic aortic constriction (TAC) in animal model or using vasoactive agonists, such as phenylephrine[102,111,112]. For instance, experiments performed in TRPC1/4 double KO mice revealed significant amelioration of pressure overload-induced hypertrophy and interstitial fibrosis, which is explained by a reduced activity of the TRPC1 and 4-dependent basal Ca^{2+} entry in adult ventricular myocytes [60]. At the same time, TRPC3 knockdown, using small hairpin RNA lentivirus through the tail vein of mice, efficiently suppresses the extent of atrial fibrosis induced by TAC [113].

7. Concluding remarks

In light of the revised studies, TRPC proteins stand out as key ion channels critical for cardiac cells responses under ischemic stress. A clearly defined role for specific TRPC isoform in cellular events related to ischemic heart diseases still remains elusive, perhaps reflecting the complexity of these channels, the limitations of pharmacological tools and the lack of specific inhibitors and antibodies. Nevertheless, TRPC channels have been extensively studies since they sense and respond to a plethora of endogenous and exogenous stimuli by Ca^{2+} signaling in cardiac cells. Increasing evidences indicate that TRPC channels contribute to pathophysiological consequences of heart infarction, namely cardiac hypertrophy, fibrosis and post-ischemic angiogenesis as summarized in Figure 1. The potential to influence these outcomes by specifically modulating the expression and/or function of TRPC channels requires major efforts and more investigations. Further progress in mechanistic understanding of TRPC channels will certainly help to identify new therapeutic targets for drug development to mitigate the impact of ischemia on cardiac function and to prevent cardiac transition from adaptive responses to the harmful heart failure.

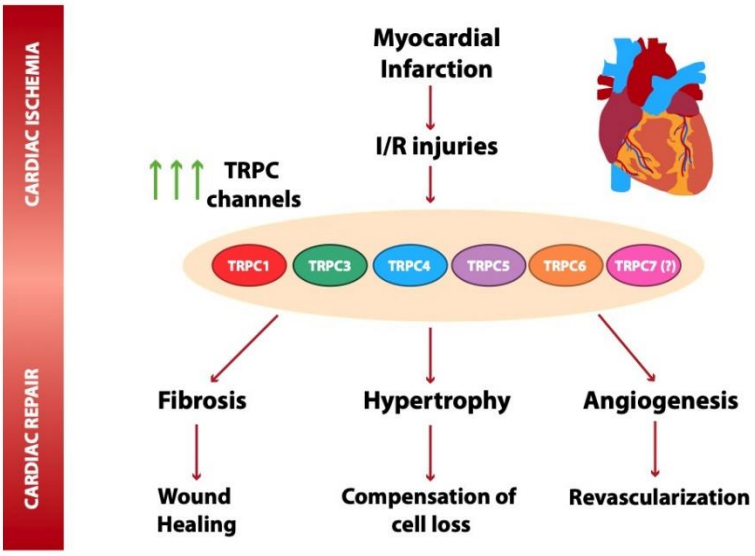

Figure 1. Scheme summarizing TRPC isoforms dysregulated under myocardial infarction (MI) and ischemia and reperfusion. TRPC1,3,4,5 and 6 are upregulated in mouse and rat animal models of MI [5,13,63]. Compelling evidence indicates TRPC channel overexpression contribute to Ca^{2+} entry, mediating the activation of Ca^{2+} sensitive signaling pathway, as calcineurin–NFAT, a critical pathway involved in apoptosis, cardiac hypertrophy and fibrosis [13,19,27,34,61]. TRPC proteins are likely involved also in cardiac repair related processes. Of note the protective role played by TRPC6 in wound healing [65]. Other studies suggested a role of TRPC, such as TRPC5, in angiogenesis and revascularization triggered post-ischemia [99].

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