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The transient receptor potential Ankyrin 1 signaling pathway in hypoxic preconditioning

Francesco Moccia¹ and Michele Samaja²

Hypoxic preconditioning has the potential to represent a valuable intervention to trigger and amplify the body's endogenous protection against stress. In contrast with classical ischemic preconditioning, hypoxic preconditioning is a non-invasive procedure that can be applied in multiple ways, such as by breathing gas mixtures with variable oxygen concentrations or by varying the body's oxygen consumption via physical exercise. As far as the cardiovascular system is concerned, such interventions target multiple sub-systems, triggering various systemic responses and causing elevated cellular and molecular changes. Such changes interact collectively to lead to a cardioprotection outcome. Among the potentially involved cellular and molecular changes, here we focus on the pathway that originates from the modulation of Ca^{2+} entry into the cardiac myocytes via the transient receptor potential Ankyrin 1 (TRPA1) channel. Being hypoxia-sensitive because it is strictly associated with hypoxia-inducible factors, this pathway may represent an ideal link between hypoxic preconditioning and the insurgence of cardioprotection. Here, we briefly review the mechanisms of action of TRPA1 in the cardiovascular system that could be recruited by TRPA1-mediated extracellular Ca^{2+} entry during hypoxic preconditioning. The availability of dietary and synthetic TRPA1 agonists that may potentially activate TRPA1 for cardioprotective purposes may open new frontiers to design novel therapeutic approaches for preconditioning medicine.

Keywords: hypoxic preconditioning, TRPA1, calcium entry, cardioprotection, hypoxia-inducible factor, cardiovascular system

Highlights

Hypoxic preconditioning is an emergent procedure that can be applied actively (e.g., physical exercise) or passively (e.g., breathing gas mixtures with variable oxygen levels) to raise cardioprotection. A multitude of subsystems, systemic responses, as well as cellular and molecular changes mediate the translation of hypoxic preconditioning into protective signals. Among these, the modulation of calcium entry via the interaction between the factors induced by hypoxia and the hypoxia-sensitive transient receptor potential ankyrin 1 (TRPA1) channel may be a key driver of hypoxic preconditioning in the cardiovascular system. The availability of dietary and synthetic TRPA1 agonists may open a new frontier for designing therapeutic approaches to preconditioning medicine.

Introduction

Preconditioning is broadly defined as the situation that occurs when a tissue, an organ, or even the entire body is exposed repeatedly to sublethal stresses or stimuli to gain resilience against potentially lethal stresses or stimuli (Holcombe and Weavers, 2022). The root of this phenomenon is hormesis, a complex event first examined in toxicologic studies: while a high-dose contact with a harmful agent is accompanied by toxic effects, a low-dose contact with the same agent may call for beneficial effects instead (Mattson, 2008). This biphasic dose

response to environmental agents occurs in several instances. For example, myocardial ischemic preconditioning (IschPrec), first discovered in 1986 (Reimer et al., 1986), is a representative example of preconditioning medicine. In myocardial IschPrec, previous exposures to brief periods of vascular occlusion make the heart resistant to the subsequent effects of major ischemic events. As no known pharmacological or procedural interventions are yet recognized to be as efficient, IschPrec is still the most potent maneuver to improve cardioprotection in experimental models. However, translating IschPrec into clinical

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Table 1. The subsystems that represent targets of the hypoxic preconditioning process in the cardiopulmonary system, along with the systemic, cellular, and molecular responses that underlie the hypoxic preconditioning process.

Sub-systems	Systemic responses	Cellular and molecular changes
Hemodynamic	Heart rate	Oxygen sensing
	Cardiac output	Posttranslational modifications of proteins (e.g., HIFs, PHDs)
	Blood pressure	NO handling
Pulmonary circulation	Ventilatory response	Apoptosis
	Hypocapnia and alkalosis	Autophagy
Skeletal muscle	Pulmonary vasoconstriction	Metabolic shifts
	Peripheral vasodilation	Antioxidant defense
Peripheral circulation	Aerobic capacity	Ion-channel permeability
	Exercise tolerance	Cell stress response
Oxygen transport in blood	Erythropoiesis	Mitochondria fission/fusion
	Plasma volume	Changes in hemoglobin function
	Myocardial remodeling	Mitogen-activated protein kinases
		Stem cell recruitment

practice has proved disappointing, despite the identification of the main mechanisms that confer protection, mainly endothelial nitric oxide (eNOS)-protein kinase G (PKG), reperfusion injury salvage kinase (RISK), survivor activating factor enhancement (SAFE), ATP-sensitive potassium (K^+) channels, and calcium (Ca^{2+}) overload (Comita et al., 2021). One reason for this poor clinical translation is perhaps the complex nature of IschPrec, as it involves not only the contracting cardiomyocytes but also the endothelium, the microcirculation, the immune system, cardiac remodeling, the coagulation cascade, and, more recently, the onset of inflammation (Algoet et al., 2023). Another reason is the invasive nature of classical IschPrec. To overcome the latter concern, non-invasive alternatives such as remote conditioning, post-conditioning, delayed IschPrec, perhaps physical exercise, and hypoxic preconditioning (HypPrec) have been occasionally proposed.

Here, we briefly review the mechanisms induced by HypPrec, which involves a novel key pathway that can be activated by hypoxia and targets Ca^{2+} -sensitive cardioprotective mechanisms, the transient receptor potential (TRP) ankyrin 1 (TRPA1) pathway. To this purpose, we conducted a comprehensive literature search across PubMed, Google Scholar, and Web of Science using the following keywords: TRPA1, hypoxia, hypoxic preconditioning, cardioprotection, heart, cardiovascular disorders, cardiomyocytes, endothelial cells, and cardiac fibroblasts. The present review is not intended as a systematic review that follows the PRISMA guidelines, but it nevertheless suggests that TRPA1 signaling could be activated during HypPrec and may stand out as a potential target to design novel therapeutic approaches based on preconditioning medicine.

Hypoxic preconditioning

HypPrec today is emerging as a powerful approach to raise protection in virtually all body organs, including the cardiovascular system. HypPrec can be administered in multiple ways by exposing experimental animals and human subjects to high- or low-frequency intermittent hypoxia, continuous hypoxia for sustained periods as during sojourns at high altitude, or even by alternating hypoxia and hyperoxia (Burtscher et al., 2023). Remarkably, all these interventions are noninvasive and may also be administered to passive subjects, such as unconscious or paralyzed patients.

These interventions trigger several systemic responses in virtually every body compartment to accomplish their effects.

Such responses, in turn, trigger cellular and molecular responses that collectively stimulate the processes leading to HypPrec (Burtscher et al., 2023). Table 1 reports some of the major targets for HypPrec related to the insurgence of protection in the cardiopulmonary system.

HypPrec represents the culmination of a complex interaction across several sub-systems, each of which independently triggers multiple systemic responses that affect many overlapping signaling pathways. Most, but not all, of the signaling pathways affected by HypPrec may originate from the intracellular accumulation of hypoxia-inducible factors (HIF) that act as cell and body oxygen (O_2) sensors. Remarkably, this orchestrator of the body's response to hypoxia responds to both a lack of oxygen (O_2) and excess O_2 (Mancardi et al., 2022). Thus, it is difficult to believe that HIF is the only mechanism underlying the complex response to O_2 variations. Clearly, many molecular pathways interact to yield such an extensive outcome, HypPrec, which occurs in virtually all systems in the body in response to major stress.

TRPA1 and its effect on the protection of the cardiovascular system

TRPA1: biophysics and gating

TRPA1 is a unique member of the TRP Ankyrin (TRPA) subfamily of non-selective cation channels in mammals (Talavera et al., 2020). The eponymous feature of TRPA1 is the presence of sixteen ankyrin repeats domain (ARD) within the NH_2 terminus of the protein channel, which regulates TRPA1 interaction with protein partners and TRPA1 activation by a variety of stimuli (Talavera et al., 2020; Thakore et al., 2020). TRPA1 is widely expressed in sensory neurons and non-neuronal cells (Negri et al., 2019; Talavera et al., 2020; Thakore et al., 2020; Liu et al., 2021; Oguri et al., 2021; Berra-Romani et al., 2023; McGarr et al., 2023), and modulates cellular activity by mediating an inward Na^+ and Ca^{2+} current that depolarizes the membrane, thereby activating voltage-gated channels in excitable cells. In addition, the ensuing increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) can recruit a plethora of Ca^{2+} -dependent signaling pathways that confer TRPA1 the ability to modulate diverse functions in different cell types (Talavera et al., 2020; Thakore et al., 2020).

The fractional Ca^{2+} current carried by TRPA1 is relatively high (~17%) as compared to other TRP channels and can even be increased to ~23% by agonist-dependent modulation of

Table 2. The protective effects of TRPA1 activation on the cardiovascular system.

Cellular type	Agonist	Effects	Mechanism	Reference
Cerebral endothelial cells	4-HNE (natural) AITC (dietary)	Vasodilation Increase in cerebral blood flow	IK _{Ca} /SK _{Ca} channels	(Earley, 2011; Sullivan et al., 2015; Thakore et al., 2021; Berra-Romani et al., 2023)
Cutaneous microvascular endothelial cells	AITC (dietary) Cinnamaldehyde (dietary)	Vasodilation Increase in local blood flow	eNOS	(Pozsgai et al., 2010; McGarr et al., 2023)
Mesenteric endothelial cells	AITC (dietary) Cinnamaldehyde (dietary) Formaldehyde (both endogenous and dietary)	Vasodilation Increase in local blood flow Reduction in mean arterial blood pressure	eNOS Inwardly-rectifying K ⁺ channels	(Pozsgai et al., 2010; Jin et al., 2019)
Heart	ASP7663 (synthetic) Optovin (synthetic) IcyHot (synthetic) Cinnamaldehyde (dietary)	Protection against ischemia/reperfusion injury, reduced infarct size Protection against monocrotaline-induced pulmonary artery hypertension, reduced fibrotic remodeling, and increased cardiac contractility	Not defined Reduced CGRP release	(Lu et al., 2016; Wu et al., 2021) (Li et al., 2020b)
Cardiomyocytes	AITC (dietary) AITC (dietary)	Positive inotropic and lusitropic effects Reduced cell death	CaMKII PI3K/Akt/eNOS	(Andrei et al., 2017) (Andrei et al., 2019)
Cardiac fibroblast	Cinnamaldehyde (dietary)	Inhibited TGF- β -induced transdifferentiation	Reduced CGRP release	(Li et al., 2020b)
Vascular macrophages	AITC (dietary) Cinnamaldehyde (dietary)	Retarded atherosclerotic progression	Increased cholesterol efflux and reduced M1 polarization	(Zhao et al., 2016; Wang et al., 2020)

the ion-conduction pathway (Gees et al., 2010; Moccia and Montagna, 2023). In this view, TRPA1 is a remarkable example of a polymodal TRP channel being able to integrate chemical, physical, and thermal stimuli (Talavera et al., 2020; Thakore et al., 2020; Moccia and Montagna, 2023). TRPA1 is also a temperature-sensitive channel that can be activated by both noxious heat (>43°C) and noxious cold (<12°C) (Moparthy et al., 2016; Vandewauw et al., 2018; Moparthy et al., 2022), although its role in the cold sensation by thermal A δ fibers is still controversial (Zhang et al., 2022). In addition, TRPA1 is sensitive to many electrophilic and non-electrophilic agonists that covalently or non-covalently, respectively, modulate TRPA1 activation (Talavera et al., 2020; Thakore et al., 2020). Electrophilic activators (such as allicin, allyl isothiocyanate or AITC, and cinnamaldehyde) interact with the thiol (-SH) group of cysteine (Cys)621, Cys641, and Cys665 that are located within the NH₂ terminus between the last ARD and the first transmembrane α -helix (Michlig et al., 2016; Talavera et al., 2020; Alvarado et al., 2021; Moccia and Montagna, 2023). Sulfhydryl-reacting compounds that activate TRPA1 include many irritant compounds, such as acrolein, diesel exhaust, and anesthetics (Talavera et al., 2020; Alvarado et al., 2021; Moccia and Montagna, 2023). Covalent modifications of specific Cys residues also underlie TRPA1 sensitivity to reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are regarded as endogenous agonists of TRPA1 (Talavera et al., 2020; Alvarado et al., 2021; Moccia and Montagna, 2023). For

instance, TRPA1 can be directly gated by hydrogen peroxide (H₂O₂) (Bessac et al., 2008; Faris et al., 2023), hydrogen sulfide (H₂S) (Kimura, 2024), nitric oxide (NO) (Miyamoto et al., 2009), and peroxynitrite (ONOO⁻) (Andersson et al., 2015). A growing body of evidence suggests that the lipid peroxidation product 4-hydroxynonenal (4-HNE) is the most widespread endogenous agonist of TRPA1 in mammalian cells (Andersson et al., 2008; Talavera et al., 2020; Alvarado et al., 2021; Moccia and Montagna, 2023). Moreover, TRPA1-mediated inward currents can also be induced by two similar peroxidation products, 4-hydroxyhexenal and 4-oxo-nonenal (Andersson et al., 2008). Finally, TRPA1 activation is also sensitive to intracellular acidification (Riva et al., 2018), depletion of phosphatidylinositol-4,5-bisphosphate (Kim et al., 2008), and changes in intra- and extra-cellular [Ca²⁺] (Jordt et al., 2004; Wang et al., 2008). Therefore, TRPA1 is a highly versatile channel that integrates multiple cues from the surrounding environment, thereby setting the most appropriate cellular response in motion.

TRPA1 in cardiovascular protection

The pharmacological modulation of TRP channels, including by TRP vanilloid 1 (TRPV1), TRP melastatin 8 (TRPM8), and TRPA1, is coming of age as a novel strategy that protects against a growing number of cardiovascular disorders, including ischemia/reperfusion injury, heart failure, atherosclerosis, hypertension, and critical limb ischemia (Moran, 2018; Moccia et al., 2019; Wang et al., 2019; Negri et al., 2020; Szabados et

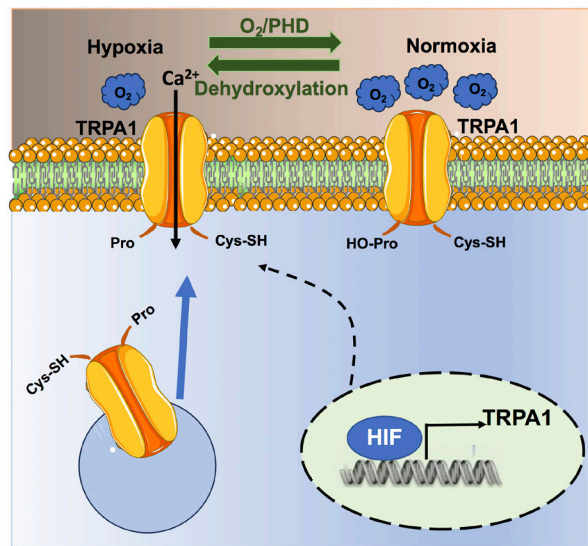


Figure 1. TRPA1-mediated Ca^{2+} entry recruits Ca^{2+} -dependent vasorelaxant pathways in proximity to the vascular wall. Extracellular Ca^{2+} entry through TRPA1 can stimulate eNOS activity, resulting in robust NO release. In addition, TRPA1-mediated Ca^{2+} signals can activate the small- and intermediate-conductance Ca^{2+} -dependent K^+ channels, SK_{Ca} and IK_{Ca} , which causes endothelium-dependent hyperpolarization (EDH). EDH is then electrotonically propagated to adjacent vascular smooth muscle cells (VSMCs) through myo-endothelial gap junctions, thereby causing VSMC hyperpolarization and relaxation. An alternative mechanism by which TRPA1 activation can induce vasorelaxation is by stimulating the release of NO and CGRP (as well as substance P) from the sensory fibers in close contact with resistance vessels.

al., 2020; Moccia et al., 2022a; Moccia et al., 2022b; Bao et al., 2023; Jesus et al., 2023). Understanding which of the multiple cellular components of the cardiovascular system are endowed with TRPA1 is an essential step to designing an effective multitarget strategy based on HypPrec (Davidson et al., 2019). TRPA1 is widely expressed in the cardiovascular system (Wang et al., 2019), especially in vascular endothelial cells (Sullivan et al., 2015; Thakore et al., 2021; Berra-Romani et al., 2023; McGarr et al., 2023), cardiac myocytes (Andrei et al., 2016; Andrei et al., 2019; Conklin et al., 2019), fibroblasts (Oguri et al., 2021), and intracardiac nociceptive fibers (Hoebart et al., 2021; Wang et al., 2022). Although a recent investigation has raised doubt against TRPA1 (and TRPV1) expression in cardiac myocytes (Hoebart et al., 2021), this discrepancy could be due to the different sensitivities of the anti-TRPA1 antibodies (Bao et al., 2023), some of which fail to detect TRPA1 protein either in Western blot or immunocytochemistry assays (Virk et al., 2019), or inappropriate signal-to-noise ratio (Bao et al., 2023). Conversely, TRPA1 is expressed at the mRNA level (Bugert and Kluter, 2006) but not as a protein in platelets (Albarra et al., 2013), while its expression in vascular smooth muscle cells (VSMCs) has never been reported.

TRPA1-mediated Ca^{2+} entry recruits vasodilating pathways in vascular endothelial cells

The functional expression of endothelial TRPA1 channels has been reported in several vascular regions (Table 2), including brain (Sullivan et al., 2015; Thakore et al., 2021; Berra-Romani et al., 2023), cutaneous (Pozsgai et al., 2010; McGarr et al., 2023), and mesenteric districts (Pozsgai et al., 2010; Jin et al., 2019), where TRPA1 activation leads to vasodilation and increased local blood flow (Gao et al., 2020; Thakore et al., 2020; Alvarado et al., 2021; Moccia et al., 2022a). TRPA1-mediated Ca^{2+} entry can recruit at least two distinct endothelium-dependent vasorelaxant pathways (Figure 1), i.e., eNOS and intermediate- and small-conductance Ca^{2+} -activated K^+ channels (IK_{Ca} and SK_{Ca} , respectively) (Earley et al., 2009; Gao et al., 2020; Thakore et al., 2020; Alvarado et al., 2021; Negri et al., 2021; Moccia et al., 2022a). The eNOS and $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ channels can act in synergy to induce a local NO-dependent increase in blood flow at the capillary level,

which then spreads to upstream arterioles through $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ -mediated endothelium-dependent hyperpolarization (EDH) to steal blood from inactive tissue areas (Pozsgai et al., 2010; Earley, 2011; Sullivan et al., 2015; Guerra et al., 2018; Thakore et al., 2021; Moccia et al., 2022a; McGarr et al., 2023). It has been reported that cinnamaldehyde exerts endothelium-independent vasorelaxation in ex vivo aortic rings (Yanaga et al., 2006). However, high doses of cinnamaldehydes can inhibit the voltage-gated L-type Ca^{2+} channels that are expressed in VSMCs (Alvarez-Collazo et al., 2014), which might explain the endothelium- and TRPA1-independent vasorelaxing effect of this compound (Talavera et al., 2020).

Vasodilation can also be induced by the pharmacological activation of TRPA1 expressed on nociceptive neurons of dorsal root ganglion (Gao et al., 2020), which causes peripheral vasodilation through the release of the vasorelaxant neuropeptides, calcitonin gene-related peptide (CGRP) and substance P, and of the gasotransmitter NO (Kunkler et al., 2011; Pozsgai et al., 2012; Aubdool et al., 2016; Hajna et al., 2016). Endothelial TRPA1 channels may also stimulate angiogenesis in the corneal stroma and prostate cancer (Negri et al., 2019) while negatively regulating neovessel formation in coronary circulation (Li et al., 2020a). The dual role of TRPA1 in angiogenesis could be explained by its selective coupling to distinct Ca^{2+} -dependent pathways in different vascular beds, which is a typical feature of many Ca^{2+} -permeable pathways in the vascular endothelium (Moccia et al., 2023a).

TRPA1-mediated Ca^{2+} entry activates cardioprotective pathways in cardiomyocytes

Several studies have shown that TRPA1 activation in the heart is cardioprotective against ischemia/reperfusion injury (Lu et al., 2016; Andrei et al., 2019; Alizadehasl et al., 2024) and prevents myocardial fibrosis (Li et al., 2020b; Ma and Wang, 2022). An early study showed that the pharmacological stimulation of TRPA1 prior to ischemia significantly reduced the infarct size in a rat model of acute myocardial infarction (Lu et al., 2016). Similarly, topical application of the analgesic cream, IcyHot, prior to myocardial ischemia also reduced the infarct size in a TRPA1-dependent manner (Wu et al., 2021). IcyHot was found to release methyl salicylate into the bloodstream

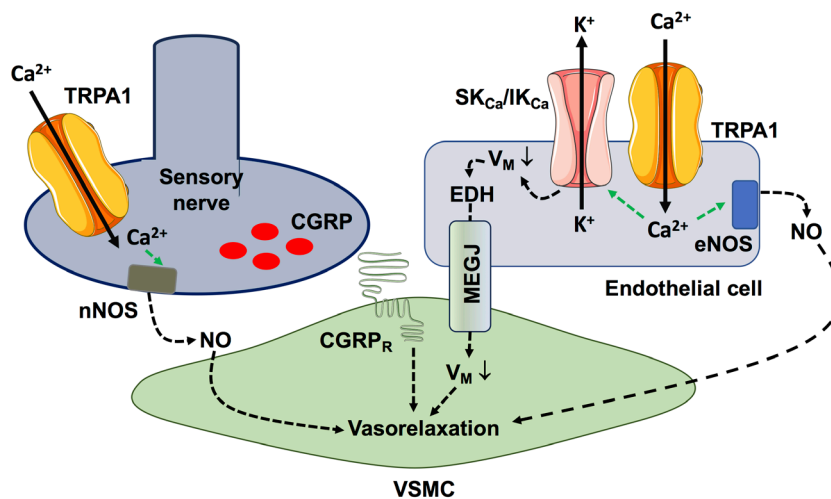


Figure 2. TRPA1-mediated Ca^{2+} entry stimulates Ca^{2+} -dependent cardioprotective pathways in cardiac myocytes. TRPA1-mediated Ca^{2+} entry stimulates CaMKII, which in turn phosphorylates sarcoendoplasmic reticulum Ca^{2+} -ATPase 2a (SERCA) and type 2 ryanodine receptors (RyR2) to, respectively, sequester cytosolic Ca^{2+} more avidly (lusitropic effect) and release sarcoplasmic reticulum (SR) Ca^{2+} more efficiently (inotropic effect). In addition, TRPA1-mediated Ca^{2+} signals stimulate NO release by inducing Akt-dependent eNOS phosphorylation and, possibly, via direct eNOS activation.

during reperfusion, thereby triggering remote cardioprotection by activating TRPA1-dependent cardioprotective pathways (Wu et al., 2021). In vitro studies revealed that TRPA1-mediated Ca^{2+} entry can both enhance cardiomyocyte contraction and prevent cardiomyocyte cell death (Figure 2) (Alizadehasl et al., 2024). In accord, TRPA1 can induce a positive inotropic and lusitropic effect by engaging Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) (Andrei et al., 2017), which has long been known to stimulate the Sarcoplasmic Reticulum Ca^{2+} cycling (Figure 3) (Maione et al., 2020; Reyes Gaido et al., 2023). In addition, TRPA1 stimulation can prevent ischemia-induced cardiomyocyte cell death by recruiting the protein kinase B (Akt)/eNOS signaling pathway (Andrei et al., 2019). It should, however, be pointed out that TRPA1 is also sensitive to ROS, such as H_2O_2 , and aldehydes (Conklin et al., 2019) that can be produced upon reperfusion (Wang et al., 2022). Therefore, the pharmacological activation of TRPA1 prior to inducing ischemia recruits Ca^{2+} -dependent cardioprotective pathways that overcome the detrimental effects of Ca^{2+} overload at the time of reperfusion (Santulli et al., 2015; Rocca et al., 2023). A major hurdle of these studies consists in the assessment of cardiomyocyte cell death by the lactate dehydrogenase assay (Andrei et al., 2019; Alizadehasl et al., 2024), which reflects membrane damage without discriminating the multiple modes of cell death (Kumar et al., 2018). The mechanisms responsible for cardiac injury during reperfusion do not include only apoptosis and necrosis but also necroptosis, ferroptosis, and pyroptosis (Davidson et al., 2020; Xiang et al., 2024). Future work should explore if TRPA1 activation protects against specific modes of cell death or if it results in global cardioprotective effects. Preliminary evidence suggests that TRPA1-mediated Ca^{2+} entry reduces the expression of the Ca^{2+} -binding inflammatory protein, S100A8, thereby reducing pyroptosis in rat primary cardiomyocytes (Wang et al., 2023a). Interestingly, Lu et al. (2016) demonstrated that the selective, photosensitive TRPA1 agonist, optovin (Kokel et al., 2013), exerts a strong cardioprotective effect against ischemia/reperfusion injury. Furthermore, optovin has been shown to stimulate cardiomyocyte pacing in both zebrafish hearts in vivo and human stem cell-derived cardiomyocytes in vitro (Lam et al., 2017). Optovin primarily targets the reactive cysteine residues

that confer TRPA1 sensitivity to oxidative stress (Trevisani et al., 2007; Kokel et al., 2013; Moccia and Montagna, 2023). However, optovin-induced cysteine modifications are reversible (Kokel et al., 2013), while those induced by ROS and aldehydes are more resistant to endogenous antioxidant systems (Lu et al., 2016). The cardioprotective role of TRPA1 is further supported by the increased cardiovascular risk in patients treated with classical pain relievers that can block TRPA1 activation, such as cyclooxygenase-2 inhibitors or some nonsteroidal anti-inflammatory drugs, during the perioperative period (Lu et al., 2016).

TRPA1-mediated Ca^{2+} entry prevents myocardial fibrosis in cardiac fibroblasts

An additional mechanism by which TRPA1 activation can induce cardioprotection is by interfering with cardiac fibrosis. Cardiac fibroblasts are critical determinants of maladaptive ventricular remodeling after an ischemic event (Ma et al., 2017). Intracellular Ca^{2+} signaling is also involved in cellular differentiation by engaging several Ca^{2+} -dependent transcription factors that regulate cellular fate (Moccia et al., 2015; Maione et al., 2022; Moccia et al., 2023c). Cardiac fibroblasts undergo programmed conversion into myofibroblasts after cardiac injury, thereby releasing a greater amount of extracellular matrix components and favoring cardiac fibrosis (Ma et al., 2017). A recent study showed that the genetic knockout of TRPA1 accelerated aging-induced myocardial fibrosis, ventricular dilation, and cardiac dysfunction, which further hints at TRPA1 as a suitable molecular target to promote cardioprotection (Ma and Wang, 2022). Consistent with this, Li et al. (2020b) showed that the pharmacological stimulation of TRPA1 can also ameliorate myocardial fibrosis by stimulating CGRP release from cardiac fibroblasts both in vitro and in a mouse model of cardiac fibrosis (Li et al., 2020b). CGRP can then autocrinally act by preventing transforming growth factor- β (TGF- β)-induced cardiac fibroblast trans-differentiation via nuclear factor kappa-light-chain-enhancer of activated B cell activation. This mechanism could potentially be enhanced by TRPA1-regulated CGRP release from intracardiac nociceptive fibers (Hoebart et al., 2021; Wang et al., 2022), which also supports cardiomyocyte survival during ischemia/reperfusion injury (Hoebart et al., 2023). Other studies confirmed that TRPA1

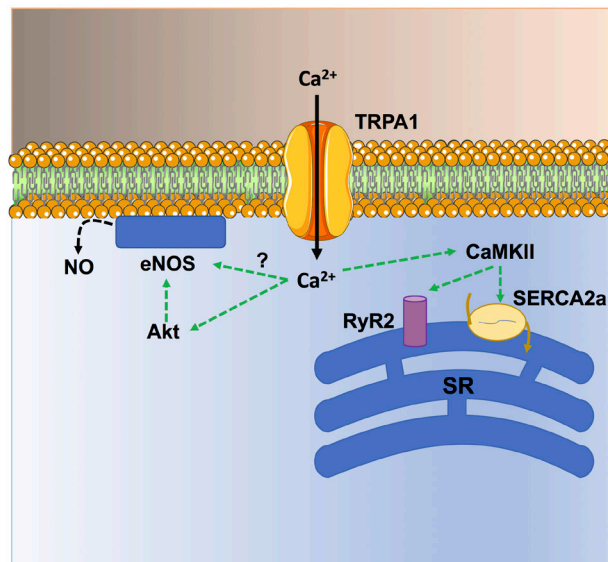


Figure 3. Hypoxia regulates TRPA1 signalling. Under normoxic conditions, PHDs hydroxylate Pro354 on the NH₂-terminal ARD domain of the TRPA1 protein, thereby inhibiting the channel and preventing basal Ca²⁺ entry. The drop in local %O₂ under hypoxia inhibits PHD activity, which results in Pro354 dehydroxylation and TRPA1 activation. In addition, hypoxia can lead to the insertion of non-hydroxylated TRPA1 proteins on the plasma membrane, which also results in the increase of basal TRPA1-mediated Ca²⁺ entry. Finally, hypoxia can increase the expression of TRPA1 protein as the TRPA1 gene contains a hypoxia-sensitive element that is bound by HIF-1.

deficiency can accelerate cardiac fibrosis in other cardiovascular disorders, such as dilated cardiomyopathy (Wang et al., 2023a). It should, however, be noted that other studies provided contrasting results showing that TRPA1 activation causes TGF- β -induced cardiac fibroblast trans-differentiation both in vitro and in vivo (Wang et al., 2018; Li et al., 2019; Naert et al., 2021; Wang et al., 2023b). It has been suggested that the dual role of TRPA1 in cardiac fibrosis could depend either on the signaling pathway that triggers scar formation or on the stage of myocardial fibrosis (Gao et al., 2020). In addition, the role of TRPA1 signaling in immune cells should be considered (Naert et al., 2021). TRPA1 could drive macrophages towards the M2 phenotype, favoring the infiltration of M2 macrophages in the heart, thereby aggravating pressure-overload induced cardiac hypertrophy and fibrosis (Wang et al., 2018). Therefore, the extent of macrophage polarization and macrophage recruitment into the cardiac interstitium could also determine whether TRPA1 activation primarily protects or exacerbates cardiac fibrosis.

TRPA1-mediated Ca²⁺ entry in macrophages inhibits atherosclerosis

TRPA1 activation in vascular macrophages, which are the major immune cell population in atherosclerotic lesions (Xu et al., 2019), counteracts M1 polarization, slowing atherosclerosis progression (Gao et al., 2020). TRPA1 expression was found to be increased in macrophage foam cells from the aorta of apolipoprotein E-deficient mice (Zhao et al., 2016). TRPA1 stimulation with AITC retarded atherosclerosis progression and reduced the inflammatory response in atherosclerotic mice. Conversely, either the pharmacological blockade of TRPA1 activity with HC-030031 or the genetic ablation of the TRPA1 protein worsened atherosclerotic lesions, hyperlipidemia, and systemic inflammation (Zhao et al., 2016). A subsequent study confirmed these findings, showing that cinnamaldehyde could also decrease the atherosclerosis plaques through TRPA1 activation. It was further shown that TRPA1-mediated Ca²⁺ entry reduced macrophage transition toward the inflammatory M1 phenotype while it favored M2 polarization (Wang et al., 2020; Wang et al., 2023a), thereby contributing to activating anti-inflammatory and tissue repair responses (Perez and Rius-Perez, 2022). Therefore, these findings strongly suggest that

TRPA1 activation represents a promising strategy for anti-atherosclerotic treatments (Gao et al., 2020).

TRPA1 in HypPrec

An intimate relationship between hypoxia and TRPA1 has recently been established (Figure 3) (Takahashi et al., 2011). Curiously, TRPA1 is not only sensitive to hypoxia but also to hyperoxia (Takahashi et al., 2011), which is also a crucial feature of HIF signaling (see above).

Association of hypoxia with TRPA1

TRPA1 expression is regulated by HIF-1 α (Figure 3) (Hatano et al., 2012). In accord, the TRPA1 gene contains a specific hypoxia response element-like motif that can be bound by HIF-1 α , which can increase TRPA1 expression (Hatano et al., 2012). Moreover, both HIF-1 α and TRPA1 proteins can be regulated by the hypoxia-sensing prolyl hydroxylases (PHDs) (Mori et al., 2017; Talavera et al., 2020). Under normoxic conditions (~20% O₂), PHD tonically inhibit TRPA1 activity via hydroxylation of Pro394, which is located on the NH₂-terminal ARD domain of the channel protein (Takahashi et al., 2011). A reduction in PO₂, therefore, relieves TRPA1 from PHD-dependent inhibition and stimulates TRPA1-mediated Ca²⁺ entry under hypoxia (Takahashi et al., 2011). The consensus sequence for PHD-dependent hydroxylation flanking the Pro394 residue in TRPA1 protein is the same as that present in the prolyl hydroxylation motif of HIF-1 and HIF-2 (Takahashi et al., 2011; Mori et al., 2017). In addition, hypoxia can stimulate the insertion of non-hydroxylated TRPA1 channel proteins on the plasma membrane, thereby further enhancing TRPA1-mediated Ca²⁺ entry (Takahashi et al., 2011). Currently, TRPA1 is regarded as a molecular sensor of local %O₂ changes (Mori et al., 2017; Talavera et al., 2020). Therefore, hypoxia can potentially stimulate TRPA1 activation in the cardiovascular system and could play a role in HypPrec. In the next paragraph, we discuss the evidence that appropriate TRPA1 activation by hypoxia could recruit cardioprotective Ca²⁺-dependent pathways.

The role of hypoxia-induced TRPA1 activation in the cardiovascular system

Cerebrovascular endothelial cells are enriched with TRPA1, which is sensitive to ROS produced during neuronal activity

and triggers an increase in local cerebral blood flow to match oxygen and nutrient perfusion with the increased neuronal demand (Alvarado et al., 2021; Thakore et al., 2021). Pires and Earley (2018) demonstrated that hypoxia stimulated TRPA1-mediated Ca^{2+} entry in the endothelial cells lining of mouse cerebral arteries, as also reviewed in (Alvarado et al., 2021). TRPA1-mediated Ca^{2+} signals mediated hypoxia-induced vasodilation of cerebral arteries by recruiting $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ channels and promoting EDH. Consistent with these findings, cerebral damage after brain ischemia was significantly enhanced in TRPA1 knockout mice. Furthermore, the pharmacological activation of TRPA1 after the induction of brain ischemia reduced the infarct size and proved to be neuroprotective (Pires and Earley, 2018). Although future studies could compare the neuroprotective effect of TRPA1 activation prior to brain ischemia, these pieces of evidence strongly suggest that endothelial TRPA1 activity can reduce cerebral injury associated with brain stroke.

Hypoxia could also lead to TRPA1 activation in cardiomyocytes. Liu et al. (2021) showed that the pharmacological and genetic blockade of PHD2 activity resulted in TRPA1-mediated Ca^{2+} entry in cardiomyocytes, followed by sarcoplasmic reticulum Ca^{2+} release via Ca^{2+} -induced Ca^{2+} release through ryanodine receptors. TRPA1-mediated Ca^{2+} signals activated both CaMKII and adenosine monophosphate (AMP)-dependent protein kinase (AMPK) (Liu et al., 2021). CaMKII can enhance cardiac contractility, as discussed above, whereas AMPK can rescue energy metabolism and promote cell survival during hypoxia (Naryzhnaya et al., 2020; Popov et al., 2023). Therefore, these findings strongly suggest that TRPA1 activation during hypoxia can exert a cardioprotective effect on the heart.

The role of TRPA1 signaling in HypPrec: current perspectives

TRPA1 is emerging as a critical molecular tool to translate short hypoxic bursts into a cardioprotective signal. Future work is needed to solve the controversy regarding the ability of TRPA1 to modulate cardiac fibrosis and to assess whether TRPA1 activation in coronary endothelial cells may induce vasodilation and, possibly, post-ischemic neo-angiogenesis. Nevertheless, available evidence highlights TRPA1-based mechanisms as key drivers of the HypPrec process. In agreement with these observations, other members of the TRP superfamily have been shown to protect the cardiovascular system from ischemic insults (Randhawa and Jaggi, 2015). For instance, the ability of the TRPV1 pathway to induce cerebral IschPrec (Thushara Vijayakumar et al., 2016) as well as HypPrec in rat hearts (Lu et al., 2014) has been elucidated. Furthermore, TRPV1 could also activate cardioprotective pathways during ischemic post-conditioning and remote ischemic post-conditioning (Randhawa and Jaggi, 2017). Vascular HypPrec also relies on TRPV4-dependent Ca^{2+} influx and proper intercellular gap junctions' communication (Rath et al., 2012). TRP-based mechanisms are also involved in sevoflurane-mediated preconditioning, which promotes mesenchymal stem cells to relieve myocardial ischemia/reperfusion injury via TRP canonical 6 (TRPC6)-induced angiogenesis via HIF-1 α , C-X-C chemokine receptor type 4, and vascular endothelial growth factor (Yang et al., 2021). It is worth pointing out that several dietary and synthetic TRPA1 agonists are now available that could pharmacologically exploit the activation of TRPA1 for cardioprotective purposes (Moccia et al., 2019; Talavera et al., 2020; Thakore et al., 2020; Alvarado et al., 2021). TRPA1 signaling is likely to play a critical role during HypPrec. Confirming TRPA1 contribution could be instrumental in designing novel therapeutic approaches for preconditioning medicine. The cardioprotective effects of TRPA1 activation

by the photosensitive agonist, optovin, shed novel light on the pharmacological manipulation of TRPA1 (Kokel et al., 2013; Lam et al., 2017). Optical stimulation of the target proteins offers an unprecedented spatio-temporal resolution to rescue the injured functions of a specific organ target (Di Maria et al., 2018; Moccia et al., 2022b; Moccia et al., 2023b; Vurro et al., 2023). Being itself photosensitive, optovin does not require the genetic manipulation of host cells, as required by optogenetics and chemogenetics (Di Maria et al., 2018). Unfortunately, optovin is sensitive to relatively short wavelengths (~400 nm) that do not effectively penetrate tissue and are, therefore, not easily exploitable for therapeutic purposes. Nevertheless, a number of photosensitive nanomaterials, which are highly conformable, biocompatible, and suitable for in vivo applications, have recently been developed (Zucchetti et al., 2017; Di Maria et al., 2018; Antognazza et al., 2019; Aziz and Antognazza, 2020). These nanomaterials, including regioregular Poly (3-hexyl-thiophene) (P3HT) (Lodola et al., 2019b; Moccia et al., 2022b; Negri et al., 2022; Criado-Gonzalez et al., 2023), poly[2,1,3-benzothiadiazole-4,7-diyl[4,4-bis(2-ethylhexyl)-4H-cyclopenta[2,1-b:3,4-b']dithiophene-2,6-diyl]] (PCPDTBT) (Ronchi et al., 2024), poly[(2,6-(4,8-bis(5-(2-ethylhexyl)thiophen-2-yl)-benzo[1,2-b:4,5-b']dithiophene))-alt-(5,5-(1',3'-di-2-thienyl-5',7'-bis(2-ethylhexyl)benzo[1',2'-c:4',5'-c']dithiophene-4,8-dione)] (PBDB-T), and poly[[4,8-bis[(2-ethylhexyl)oxy]benzo[1,2-b:4,5-b']dithiophene-2,6-diyl][3-fluoro-2-[(2-ethylhexyl)carbonyl]thieno[3,4-b]thiophenediyl]] (PTB7) (Tullii et al., 2023), are sensitive to longer wavelengths and generate ROS in response to light. It has been demonstrated that optical stimulation of photosensitive conjugated polymers, in the form of either thin films or nanoparticles, can stimulate angiogenesis and cardiac contraction through a mechanism that involves both ROS and TRPV1-mediated Ca^{2+} entry (Lodola et al., 2019a; Negri et al., 2020; Moccia et al., 2022b). However, this same signaling pathway has also been proposed to activate TRPA1 (Negri et al., 2020; Moccia et al., 2022b). Therefore, future studies that assess whether the photostimulation of conjugated polymers also results in cardioprotective effects upon TRPA1 activation would provide therapeutically relevant results.

Conclusions

TRPA1 signaling is likely to play a critical role during HypPrec. Confirming TRPA1 contribution could be instrumental in designing novel therapeutic approaches for preconditioning medicine. A critical step is to determine how TRPA1-mediated Ca^{2+} entry protects cardiomyocytes from cell death. As outlined above, multiple mechanisms can contribute to cardiac injury or reperfusion, and although all of them ultimately lead to cell death, some are more prone to induce an inflammatory response than others (e.g., pyroptosis vs. necrosis and apoptosis) (Davidson et al., 2019; Davidson et al., 2020). The current evidence shows that TRPA1-mediated Ca^{2+} entry may inhibit pyroptosis (Wang et al., 2023a). Moreover, in some cancer cell types, TRPA1 is coupled with an antioxidant system that mitigates the oxidative stress and the mitochondrial Ca^{2+} overload associated with apoptosis (Moccia and Montagna, 2023). Therefore, the cardiac TRPA1 could engage multifaceted signaling pathways that protect cardiomyocytes from various regulated cell death processes. The therapeutic translation of TRPA1 for HypPrec strategies also requires the elucidation of its functional role in coronary VSMCs (if present) and endothelial cells (Davidson et al., 2019), as well as in pericytes (if present) (Frangogiannis, 2024), which regulate coronary flow and permeability. Being expressed in multiple cellular compartments of the cardiovascular system, TRPA1 could provide an ideal molecular target to activate the multitarget strategy that has recently been proposed as the most suitable

approach to reduce myocardial injury (Davidson et al., 2019).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

- Albarran L, Lopez JJ, Dionisio N, Smani T, Salido GM, Rosado JA (2013) Transient receptor potential ankyrin-1 (TRPA1) modulates store-operated Ca²⁺ entry by regulation of STIM1-Orai1 association. *Biochim Biophys Acta* 1833:3025-3034.
- Algoet M, Janssens S, Himmelreich U, Gsell W, Pusovnik M, Van den Eynde J, Oosterlinck W (2023) Myocardial ischemia-reperfusion injury and the influence of inflammation. *Trends Cardiovasc Med* 33:357-366.
- Alizadehasl A, Alavi MS, Alavi MS, Roohbakhsh A (2024) TRPA1 as a promising target in ischemia/reperfusion: A comprehensive review. *Iran J Basic Med Sci* 27:270-278.
- Alvarado MG, Thakore P, Earley S (2021) Transient receptor potential channel ankyrin 1: A unique regulator of vascular function. *Cells* 10:1167.
- Alvarez-Collazo J, Alonso-Carbajo L, Lopez-Medina AI, Alpizar YA, Tajada S, Nilius B, Voets T, Lopez-Lopez JR, Talavera K, Perez-Garcia MT, Alvarez JL (2014) Cinnamaldehyde inhibits L-type calcium channels in mouse ventricular cardiomyocytes and vascular smooth muscle cells. *Pflugers Arch* 466:2089-2099.
- Andersson DA, Gentry C, Moss S, Bevan S (2008) Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci* 28:2485-2494.
- Andersson DA, Filipovic MR, Gentry C, Eberhardt M, Vastani N, Leffler A, Reeh P, Bevan S (2015) Streptozotocin stimulates the ion channel TRPA1 directly: involvement of peroxynitrite. *J Biol Chem* 290:15185-15196.
- Andrei SR, Sinharoy P, Bratz IN, Damron DS (2016) TRPA1 is functionally co-expressed with TRPV1 in cardiac muscle: Co-localization at z-discs, costameres and intercalated discs. *Channels* 10:395-409.
- Andrei SR, Ghosh M, Sinharoy P, Damron DS (2019) Stimulation of TRPA1 attenuates ischemia-induced cardiomyocyte cell death through an eNOS-mediated mechanism. *Channels* 13:192-206.
- Andrei SR, Ghosh M, Sinharoy P, Dey S, Bratz IN, Damron DS (2017) TRPA1 ion channel stimulation enhances cardiomyocyte contractile function via a CaMKII-dependent pathway. *Channels* 11:587-603.
- Antognazza MR, Abdel Aziz I, Lodola F (2019) Use of exogenous and endogenous photomediators as efficient ROS modulation tools: Results and perspectives for therapeutic purposes. *Oxid Med Cell Longev* 2019:2867516.
- Aubdool AA, Kodji X, Abdul-Kader N, Heads R, Fernandes ES, Bevan S, Brain SD (2016) TRPA1 activation leads to neurogenic vasodilatation: involvement of reactive oxygen nitrogen species in addition to CGRP and NO. *Br J Pharmacol* 173:2419-2433.
- Aziz IA, Antognazza MR (2020) Wireless nanotechnologies light up the next frontier in cell Calcium signalling. *MRS Advances* 5:3473 - 3489.
- Bao J, Gao Z, Hu Y, Ye L, Wang L (2023) Transient receptor potential vanilloid type 1: cardioprotective effects in diabetic models. *Channels* 17:2281743.
- Berra-Romani R, Brunetti V, Pellavio G, Soda T, Laforenza U, Scarpellino G, Moccia F (2023) Allyl isothiocyanate induces Ca²⁺ signals and nitric oxide release by inducing reactive oxygen species production in the human cerebrovascular endothelial cell line hCMEC/D3. *Cells* 12:1732.
- Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, Jordt SE (2008) TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J Clin Invest* 118:1899-1910.
- Bugert P, Kluter H (2006) Profiling of gene transcripts in human platelets: an update of the platelet transcriptome. *Platelets* 17:503-504.
- Burtscher J et al. (2023) Mechanisms underlying the health benefits of intermittent hypoxia conditioning. *J Physiol*.
- Comita S, Femmino S, Thairi C, Alloatti G, Boengler K, Pagliaro P, Penna C (2021) Regulation of STAT3 and its role in cardioprotection by conditioning: focus on non-genomic roles targeting mitochondrial function. *Basic Res Cardiol* 116:56.
- Conklin DJ, Guo Y, Nystoriak MA, Jagatheesan G, Obal D, Kilfoil PJ, Hoetker JD, Guo L, Bolli R, Bhatnagar A (2019) TRPA1 channel contributes to myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 316:H889-H899.
- Criado-Gonzalez M, Bondi L, Marzuoli C, Gutierrez-Fernandez E, Tullii G, Ronchi C, Gabirondo E, Sardon H, Rapino S, Malferrari M, Cramer T, Antognazza MR, Mecerreyes D (2023) Semiconducting polymer nanoporous thin films as a tool to regulate intracellular ROS balance in endothelial cells. *ACS Appl Mater Interfaces* 15:35973-35985.
- Davidson SM, Adameova A, Barile L, Cabrera-Fuentes HA, Lazou A, Pagliaro P, Stenslokken KO, Garcia-Dorado D, Action E-CC (2020) Mitochondrial and mitochondrial-independent pathways of myocardial cell death during ischaemia and reperfusion injury. *J Cell Mol Med* 24:3795-3806.
- Davidson SM, Ferdinandy P, Andreadou I, Botker HE, Heusch G, Ibanez B, Ovize M, Schulz R, Yellon DM, Hausenloy DJ, Garcia-Dorado D, Action CC (2019) Multitarget strategies to reduce myocardial ischemia/reperfusion injury: JACC Review Topic of the Week. *J Am Coll Cardiol* 73:89-99.
- Di Maria F, Lodola F, Zucchetti E, Benfenati F, Lanzani G (2018) The evolution of artificial light actuators in living systems: from planar to nanostructured interfaces. *Chem Soc Rev* 47:4757-4780.
- Earley S (2011) Endothelium-dependent cerebral artery dilation mediated by transient receptor potential and Ca²⁺-activated K⁺ channels. *J Cardiovasc Pharmacol* 57:148-153.
- Earley S, Gonzales AL, Crnich R (2009) Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca²⁺-Activated K⁺ channels. *Circ Res* 104:987-994.
- Faris P, Rumolo A, Pellavio G, Tanzi M, Vismara M, Berra-Romani R, Gerbino A, Corallo S, Pedrazzoli P, Laforenza U, Montagna D, Moccia F (2023) Transient receptor potential ankyrin 1 (TRPA1) mediates reactive oxygen species-induced Ca²⁺ entry, mitochondrial dysfunction, and caspase-3/7 activation in primary cultures of metastatic colorectal carcinoma cells. *Cell Death Discov* 9:213.
- Frangogiannis NG (2024) The fate and role of the pericytes in myocardial diseases. *Eur J Clin Invest*:e14204.
- Gao S, Kaudimba KK, Guo S, Zhang S, Liu T, Chen P, Wang R (2020) Transient receptor potential ankyrin type-1 channels as a potential target for the treatment of cardiovascular diseases. *Front Physiol* 11:836.
- Gees M, Colsohl B, Nilius B (2010) The role of transient receptor potential cation channels in Ca²⁺ signaling. *Cold Spring Harb Perspect Biol* 2:a003962.
- Guerra G, Lucariello A, Perna A, Botta L, De Luca A, Moccia F (2018) The role of endothelial Ca²⁺ signaling in

- neurovascular coupling: A view from the lumen. *Int J Mol Sci* 19:938.
- Hajna Z, Saghy E, Payrits M, Aubdool AA, Szoke E, Pozsgai G, Batai IZ, Nagy L, Filotas D, Helyes Z, Brain SD, Pinter E (2016) Capsaicin-sensitive sensory nerves mediate the cellular and microvascular effects of H₂S via TRPA1 receptor activation and neuropeptide release. *J Mol Neurosci* 60:157-170.
- Hatano N, Itoh Y, Suzuki H, Muraki Y, Hayashi H, Onozaki K, Wood IC, Beech DJ, Muraki K (2012) Hypoxia-inducible factor-1 α (HIF1 α) switches on transient receptor potential ankyrin repeat 1 (TRPA1) gene expression via a hypoxia response element-like motif to modulate cytokine release. *J Biol Chem* 287:31962-31972.
- Hoebart C, Kiss A, Pilz PM, Szabo PL, Podesser BK, Fischer MJM, Heber S (2023) TRPA1 as target in myocardial infarction. *Int J Mol Sci* 24:2516.
- Hoebart C, Rojas-Galvan NS, Ciotu CI, Aykac I, Reissig LF, Weninger WJ, Kiss A, Podesser BK, Fischer MJM, Heber S (2021) No functional TRPA1 in cardiomyocytes. *Acta Physiol* 232:e13659.
- Holcombe J, Weavers H (2022) The role of preconditioning in the development of resilience: Mechanistic insights. *Curr Opin Toxicol* 30:100338.
- Jesus RLC, Araujo FA, Alves QL, Dourado KC, Silva DF (2023) Targeting temperature-sensitive transient receptor potential channels in hypertension: far beyond the perception of hot and cold. *J Hypertens* 41:1351-1370.
- Jin L, Jagatheesan G, Guo L, Nystoriak M, Malovichko M, Lorkiewicz P, Bhatnagar A, Srivastava S, Conklin DJ (2019) Formaldehyde induces mesenteric artery relaxation via a sensitive transient receptor potential ankyrin-1 (TRPA1) and endothelium-dependent mechanism: Potential role in postprandial hyperemia. *Front Physiol* 10:277.
- Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427:260-265.
- Kim D, Cavanaugh EJ, Simkin D (2008) Inhibition of transient receptor potential A1 channel by phosphatidylinositol-4,5-bisphosphate. *Am J Physiol Cell Physiol* 295:C92-99.
- Kimura H (2024) Hydrogen sulfide (H₂S)/polysulfides (H₂S_n) signalling and TRPA1 channels modification on sulfur metabolism. *Biomolecules* 14:129.
- Kokel D, Cheung CY, Mills R, Coutinho-Budd J, Huang L, Setola V, Sprague J, Jin S, Jin YN, Huang XP, Bruni G, Woolf CJ, Roth BL, Hamblin MR, Zylka MJ, Milan DJ, Peterson RT (2013) Photochemical activation of TRPA1 channels in neurons and animals. *Nat Chem Biol* 9:257-263.
- Kumar P, Nagarajan A, Uchil PD (2018) Analysis of cell viability by the lactate dehydrogenase assay. *Cold Spring Harbor protocols* 2018.
- Kunkler PE, Ballard CJ, Oxford GS, Hurley JH (2011) TRPA1 receptors mediate environmental irritant-induced meningeal vasodilatation. *Pain* 152:38-44.
- Lam PY, Mendu SK, Mills RW, Zheng B, Padilla H, Milan DJ, Desai BN, Peterson RT (2017) A high-conductance chemo-optogenetic system based on the vertebrate channel Trpa1b. *Sci Rep* 7:11839.
- Li R, Liu R, Yan F, Zhuang X, Shi H, Gao X (2020a) Inhibition of TRPA1 promotes cardiac repair in mice after myocardial infarction. *J Cardiovasc Pharmacol* 75:240-249.
- Li S, Sun X, Wu H, Yu P, Wang X, Jiang Z, Gao E, Chen J, Li D, Qiu C, Song B, Chen K, He K, Yang D, Yang Y (2019) TRPA1 promotes cardiac myofibroblast transdifferentiation after myocardial infarction injury via the calcineurin-NFAT-DYRK1A signaling pathway. *Oxid Med Cell Longev* 2019:6408352.
- Li W, Zhang Z, Li X, Cai J, Li D, Du J, Zhang B, Xiang D, Li N, Li Y (2020b) CGRP derived from cardiac fibroblasts is an endogenous suppressor of cardiac fibrosis. *Cardiovasc Res* 116:1335-1348.
- Liu L, Liu X, Liu M, Xie D, Yan H (2021) Proline hydroxylase domain-containing enzymes regulate calcium levels in cardiomyocytes by TRPA1 ion channel. *Exp Cell Res* 407:112777.
- Lodola F, Vurro V, Crasto S, Di Pasquale E, Lanzani G (2019a) Optical pacing of human-induced pluripotent stem cell-derived cardiomyocytes mediated by a conjugated polymer interface. *Adv Healthc Mater* 8:e1900198.
- Lodola F, Rosti V, Tullii G, Desii A, Tapella L, Catarsi P, Lim D, Moccia F, Antognazza MR (2019b) Conjugated polymers optically regulate the fate of endothelial colony-forming cells. *Sci Adv* 5:eaav4620.
- Lu MJ, Chen YS, Huang HS, Ma MC (2014) Hypoxic preconditioning protects rat hearts against ischemia-reperfusion injury via the arachidonate 12-lipoxygenase/transient receptor potential vanilloid 1 pathway. *Basic Res Cardiol* 109:414.
- Lu Y, Piplani H, McAllister SL, Hurt CM, Gross ER (2016) Transient receptor potential ankyrin 1 activation within the cardiac myocyte limits ischemia-reperfusion injury in rodents. *Anesthesiology* 125:1171-1180.
- Ma S, Wang DH (2022) Knockout of Trpa1 accelerates age-related cardiac fibrosis and dysfunction. *PLoS One* 17:e0274618.
- Ma Y, Iyer RP, Jung M, Czubyrt MP, Lindsey ML (2017) Cardiac fibroblast activation post-myocardial infarction: current knowledge gaps. *Trends Pharmacol Sci* 38:448-458.
- Maione AS, Faris P, Bissonni L, Lodola F, Casella M, Catto V, Pompilio G, Moccia F, Sommariva E (2020) Imbalance of calcium-dependent mechanisms in cardiac mesenchymal stromal cells from arrhythmogenic cardiomyopathy patients. *Vascul Pharmacol* 132:106732.
- Maione AS, Faris P, Iengo L, Catto V, Bissonni L, Lodola F, Negri S, Casella M, Guarino A, Polvani G, Cerrone M, Tondo C, Pompilio G, Sommariva E, Moccia F (2022) Ca(2+) dysregulation in cardiac stromal cells sustains fibro-adipose remodeling in Arrhythmogenic Cardiomyopathy and can be modulated by flecainide. *J Transl Med* 20:522.
- Mancardi D, Ottolenghi S, Attanasio U, Tocchetti CG, Paroni R, Pagliaro P, Samaja M (2022) Janus, or the inevitable battle between too much and too little oxygen. *Antioxid Redox Signal* 37:972-989.
- Mattson MP (2008) Hormesis defined. *Ageing Res Rev* 7:1-7.
- McGarr GW, King KE, Cassan CJM, Janetos KT, Fujii N, Kenny GP (2023) Involvement of nitric oxide synthase and reactive oxygen species in TRPA1-mediated cutaneous vasodilation in young and older adults. *Microvasc Res* 145:104443.
- Michlig S, Merlini JM, Beaumont M, Ledda M, Tavenard A, Mukherjee R, Camacho S, le Coutre J (2016) Effects of TRP channel agonist ingestion on metabolism and autonomic nervous system in a randomized clinical trial of healthy subjects. *Sci Rep* 6:20795.
- Miyamoto T, Dubin AE, Petrus MJ, Patapoutian A (2009) TRPV1 and TRPA1 mediate peripheral nitric oxide-induced nociception in mice. *PLoS One* 4:e7596.
- Moccia F, Montagna D (2023) Transient receptor potential ankyrin 1 (TRPA1) channel as a sensor of oxidative stress in cancer cells. *Cells* 12:1261.

- Moccia F, Ruffinatti FA, Zuccolo E (2015) Intracellular Ca^{2+} signals to reconstruct a broken heart: Still a theoretical approach? *Curr Drug Targets* 16:793-815.
- Moccia F, Negri S, Faris P, Berra-Romani R (2019) Targeting the endothelial Ca^{2+} toolkit to rescue endothelial dysfunction in obesity associated-hypertension. *Curr Med Chem* 27:240-257.
- Moccia F, Negri S, Faris P, Angelone T (2022a) Targeting endothelial ion signalling to rescue cerebral blood flow in cerebral disorders. *Vascul Pharmacol* 145:106997.
- Moccia F, Negri S, Faris P, Ronchi C, Lodola F (2022b) Optical excitation of organic semiconductors as a highly selective strategy to induce vascular regeneration and tissue repair. *Vascul Pharmacol* 144:106998.
- Moccia F, Brunetti V, Soda T, Berra-Romani R, Scarpellino G (2023a) Cracking the endothelial calcium (Ca^{2+}) code: A matter of timing and spacing. *Int J Mol Sci* 24:16765.
- Moccia F, Fiorio Pla A, Lim D, Lodola F, Gerbino A (2023b) Intracellular Ca^{2+} signalling: unexpected new roles for the usual suspect. *Front Physiol* 14:1210085.
- Moccia F, Brunetti V, Soda T, Faris P, Scarpellino G, Berra-Romani R (2023c) Store-operated Ca^{2+} entry as a putative target of flecainide for the treatment of arrhythmogenic cardiomyopathy. *J Clin Med* 12:5295.
- Moparthy L, Sinica V, Moparthy VK, Kreir M, Vignane T, Filipovic MR, Vlachova V, Zygmunt PM (2022) The human TRPA1 intrinsic cold and heat sensitivity involves separate channel structures beyond the N-ARD domain. *Nat Comm* 13:6113.
- Moparthy L, Kichko TI, Eberhardt M, Hogestatt ED, Kjellbom P, Johanson U, Reeh PW, Leffler A, Filipovic MR, Zygmunt PM (2016) Human TRPA1 is a heat sensor displaying intrinsic U-shaped thermosensitivity. *Sci Rep* 6:28763.
- Moran MM (2018) TRP channels as potential drug targets. *Annu Rev Pharmacol Toxicol* 58:309-330.
- Mori Y, Takahashi N, Kurokawa T, Kiyonaka S (2017) TRP channels in oxygen physiology: distinctive functional properties and roles of TRPA1 in O_2 sensing. *Proc Jpn Acad Ser B Phys Biol Sci* 93:464-482.
- Naert R, Lopez-Requena A, Talavera K (2021) TRPA1 expression and pathophysiology in immune cells. *Int J Mol Sci* 22:11460.
- Naryzhnaya NV, Ma HJ, Maslov LN (2020) The involvement of protein kinases in the cardioprotective effect of chronic hypoxia. *Physiol Res* 69:933-945.
- Negri S, Faris P, Soda T, Moccia F (2021) Endothelial signaling at the core of neurovascular coupling: The emerging role of endothelial inward-rectifier K^+ ($\text{Kir}2.1$) channels and N-methyl-D-aspartate receptors in the regulation of cerebral blood flow. *Int J Biochem Cell Biol* 135:105983.
- Negri S, Faris P, Berra-Romani R, Guerra G, Moccia F (2019) Endothelial transient receptor potential channels and vascular remodeling: Extracellular Ca^{2+} entry for angiogenesis, arteriogenesis and vasculogenesis. *Front Physiol* 10:1618.
- Negri S, Faris P, Rosti V, Antognazza MR, Lodola F, Moccia F (2020) Endothelial TRPV1 as an emerging molecular target to promote therapeutic angiogenesis. *Cells* 9:1341.
- Negri S, Faris P, Tullii G, Vismara M, Pellegata AF, Lodola F, Guidetti G, Rosti V, Antognazza MR, Moccia F (2022) Conjugated polymers mediate intracellular Ca^{2+} signals in circulating endothelial colony forming cells through the reactive oxygen species-dependent activation of Transient Receptor Potential Vanilloid 1 (TRPV1). *Cell Calcium* 101:102502.
- Oguri G, Nakajima T, Kikuchi H, Obi S, Nakamura F, Komuro I (2021) Allyl isothiocyanate (AITC) activates nonselective cation currents in human cardiac fibroblasts: possible involvement of TRPA1. *Heliyon* 7:e05816.
- Perez S, Rius-Perez S (2022) Macrophage polarization and reprogramming in acute inflammation: A redox perspective. *Antioxidants* 11:1394.
- Pires PW, Earley S (2018) Neuroprotective effects of TRPA1 channels in the cerebral endothelium following ischemic stroke. *Elife* 7:e35316.
- Popov SV, Mukhomedzyanov AV, Voronkov NS, Derkachev IA, Boshchenko AA, Fu F, Sufianova GZ, Khlestkina MS, Maslov LN (2023) Regulation of autophagy of the heart in ischemia and reperfusion. *Apoptosis* 28:55-80.
- Pozsgai G, Bodkin JV, Graepel R, Bevan S, Andersson DA, Brain SD (2010) Evidence for the pathophysiological relevance of TRPA1 receptors in the cardiovascular system in vivo. *Cardiovasc Res* 87:760-768.
- Pozsgai G, Hajna Z, Bagoly T, Boros M, Kemeny A, Materazzi S, Nassini R, Helyes Z, Szolcsanyi J, Pinter E (2012) The role of transient receptor potential ankyrin 1 (TRPA1) receptor activation in hydrogen-sulphide-induced CGRP-release and vasodilation. *Eur J Pharmacol* 689:56-64.
- Randhawa PK, Jaggi AS (2015) TRPV1 and TRPV4 channels: potential therapeutic targets for ischemic conditioning-induced cardioprotection. *Eur J Pharmacol* 746:180-185.
- Randhawa PK, Jaggi AS (2017) TRPV(1) channels in cardiovascular system: A double edged sword? *Int J Cardiol* 228:103-113.
- Rath G, Saliez J, Behets G, Romero-Perez M, Leon-Gomez E, Bouzin C, Vriens J, Nilius B, Feron O, Dessy C (2012) Vascular hypoxic preconditioning relies on TRPV4-dependent calcium influx and proper intercellular gap junctions communication. *Arterioscler Thromb Vasc Biol* 32:2241-2249.
- Reimer K, Murry C, Yamasawa I, Hill M, Jennings R (1986) Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. *Am J Physiol* 251:H1306-H1315.
- Reyes Gaido OE, Nkashama LJ, Schole KL, Wang Q, Umapathi P, Mesubi OO, Konstantinidis K, Luczak ED, Anderson ME (2023) CaMKII as a therapeutic target in cardiovascular disease. *Annu Rev Pharmacol Toxicol* 63:249-272.
- Riva B, Dionisi M, Potenzieri A, Chiorazzi A, Cordero-Sanchez C, Rigolio R, Carozzi VA, Lim D, Cavaletti G, Marmioli P, Distasi C, Genazzani AA (2018) Oxaliplatin induces pH acidification in dorsal root ganglia neurons. *Sci Rep* 8:15084.
- Rocca C, Soda T, De Francesco EM, Fiorillo M, Moccia F, Viglietto G, Angelone T, Amodio N (2023) Mitochondrial dysfunction at the crossroad of cardiovascular diseases and cancer. *J Transl Med* 21:635.
- Ronchi C, Galli C, Tullii G, Marzuoli C, Mazzola M, Malferrari M, Crasto S, Rapino S, Di Pasquale E, Antognazza MR (2024) Nongenetic optical modulation of pluripotent stem cells derived cardiomyocytes function in the red spectral range. *Adv Sci* 11:e2304303.
- Santulli G, Xie W, Reiken SR, Marks AR (2015) Mitochondrial calcium overload is a key determinant in heart failure. *Proc Natl Acad Sci U S A* 112:11389-11394.
- Sullivan MN, Gonzales AL, Pires PW, Bruhl A, Leo MD, Li W, Oulidi A, Boop FA, Feng Y, Jaggar JH, Welsh DG, Earley S (2015) Localized TRPA1 channel Ca^{2+} signals stimulated by reactive oxygen species promote cerebral artery dilation. *Sci Signal* 8:ra2.
- Szabados T, Gomori K, Palvolgyi L, Gorbe A, Baczkó I, Helyes Z, Jancsó G, Ferdinandy P, Bencsik P (2020) Capsaicin-sensitive sensory nerves and the TRPV1 ion channel in cardiac physiology and pathologies. *Int J Mol Sci* 21:4472.

- Takahashi N, Kuwaki T, Kiyonaka S, Numata T, Kozai D, Mizuno Y, Yamamoto S, Naito S, Knevels E, Carmeliet P, Oga T, Kaneko S, Suga S, Nokami T, Yoshida J, Mori Y (2011) TRPA1 underlies a sensing mechanism for O₂. *Nat Chem Biol* 7:701-711.
- Talavera K, Startek JB, Alvarez-Collazo J, Boonen B, Alpizar YA, Sanchez A, Naert R, Nilius B (2020) Mammalian transient receptor potential TRPA1 channels: From structure to disease. *Physiol Rev* 100:725-803.
- Thakore P, Ali S, Earley S (2020) Regulation of vascular tone by transient receptor potential ankyrin 1 channels. *Curr Top Membr* 85:119-150.
- Thakore P, Alvarado MG, Ali S, Mughal A, Pires PW, Yamasaki E, Pritchard HA, Isakson BE, Tran CHT, Earley S (2021) Brain endothelial cell TRPA1 channels initiate neurovascular coupling. *eLife* 10:e63040.
- Thushara Vijayakumar N, Sangwan A, Sharma B, Majid A, Rajanikant GK (2016) Cerebral ischemic preconditioning: The road so far. *Mol Neurobiol* 53:2579-2593.
- Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andre E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, Geppetti P (2007) 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc Natl Acad Sci U S A* 104:13519-13524.
- Tullii G, Gutierrez-Fernandez E, Ronchi C, Bellacanzone C, Bondi L, Criado-Gonzalez M, Lagonegro P, Moccia F, Cramer T, Mecerreyes D, Martin J, Antognazza MR (2023) Bimodal modulation of in vitro angiogenesis with photoactive polymer nanoparticles. *Nanoscale* 15:18716-18726.
- Vandewauw I, De Clercq K, Mulier M, Held K, Pinto S, Van Ranst N, Segal A, Voet T, Vennekens R, Zimmermann K, Vriens J, Voets T (2018) A TRP channel trio mediates acute noxious heat sensing. *Nature* 555:662-666.
- Virk HS, Rekas MZ, Biddle MS, Wright AKA, Sousa J, Weston CA, Chachi L, Roach KM, Bradding P (2019) Validation of antibodies for the specific detection of human TRPA1. *Sci Rep* 9:18500.
- Vurro V, Federici B, Ronchi C, Florindi C, Sesti V, Crasto S, Maniezzi C, Galli C, Antognazza MR, Bertarelli C, Di Pasquale E, Lanzani G, Lodola F (2023) Optical modulation of excitation-contraction coupling in human-induced pluripotent stem cell-derived cardiomyocytes. *iScience* 26:106121.
- Wang M, Zhao M, Zheng Z, Pan W, Zhang J, Yin Z, Wei C, Xu Y, Wan J (2023a) TRPA1 deficiency aggravates dilated cardiomyopathy by promoting S100A8 expression to induce M1 macrophage polarization in rats. *FASEB J* 37:e22982.
- Wang M, Zhao M, Xu S, Zheng Z, Zhang J, Pan W, Yin Z, Liu J, Wei C, Wan J, Xu Y (2023b) TRPA1 deficiency attenuates cardiac fibrosis via regulating GRK5/NFAT signaling in diabetic rats. *Biochem Pharmacol* 214:115671.
- Wang Q, Chen K, Zhang F, Peng K, Wang Z, Yang D, Yang Y (2020) TRPA1 regulates macrophages phenotype plasticity and atherosclerosis progression. *Atherosclerosis* 301:44-53.
- Wang X, Kanda H, Tsujino T, Kogure Y, Zhu F, Yamamoto S, Sakaguchi T, Noguchi K, Dai Y (2022) Reactive oxygen species cause exercise-induced angina in a myocardial ischaemia-reperfusion injury model. *Int J Mol Sci* 23:2820.
- Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER (2008) The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J Biol Chem* 283:32691-32703.
- Wang Z, Xu Y, Wang M, Ye J, Liu J, Jiang H, Ye D, Wan J (2018) TRPA1 inhibition ameliorates pressure overload-induced cardiac hypertrophy and fibrosis in mice. *EBioMedicine* 36:54-62.
- Wang Z, Ye D, Ye J, Wang M, Liu J, Jiang H, Xu Y, Zhang J, Chen J, Wan J (2019) The TRPA1 channel in the cardiovascular system: Promising features and challenges. *Front Pharmacol* 10:1253.
- Wu Y, Chen AW, Goodnough CL, Lu Y, Zhang Y, Gross ER (2021) IcyHot analgesic topical cream limits cardiac injury in rodents. *Transl Res* 227:42-52.
- Xiang Q, Yi X, Zhu XH, Wei X, Jiang DS (2024) Regulated cell death in myocardial ischemia-reperfusion injury. *Trends Endocrinol Metab* 35:219-234.
- Xu H, Jiang J, Chen W, Li W, Chen Z (2019) Vascular macrophages in atherosclerosis. *J Immunol Res* 2019:4354786.
- Yanaga A, Goto H, Nakagawa T, Hikiami H, Shibahara N, Shimada Y (2006) Cinnamaldehyde induces endothelium-dependent and -independent vasorelaxant action on isolated rat aorta. *Biol Pharm Bull* 29:2415-2418.
- Yang J, Tang L, Zhang F, Yang T, Lu T, Sun K, Sun N, Ren J, Yan M (2021) Sevoflurane preconditioning promotes mesenchymal stem cells to relieve myocardial ischemia/reperfusion injury via TRPC6-induced angiogenesis. *Stem Cell Res Ther* 12:584.
- Zhang H, Wang C, Zhang K, Kamau PM, Luo A, Tian L, Lai R (2022) The role of TRPA1 channels in thermosensation. *Cell insight* 1:100059.
- Zhao JF, Shyue SK, Kou YR, Lu TM, Lee TS (2016) Transient receptor potential ankyrin 1 channel involved in atherosclerosis and macrophage-foam cell formation. *Int J Biol Sci* 12:812-823.
- Zucchetti E, Zangoli M, Bargigia I, Bossio C, Di Maria F, Barbarella G, D'Andrea C, Lanzani G, Antognazza MR (2017) Poly(3-hexylthiophene) nanoparticles for biophotonics: study of the mutual interaction with living cells. *J Mater Chem B* 5:565-574.