

Calcium dysregulation in heart diseases: targeting calcium channels to achieve a correct calcium homeostasis

Giampaolo Morciano ^{1,2,*}, Alessandro Rimessi ¹, Simone Patergnani ¹, Veronica A.M. Vitto ¹,
Alberto Danese ¹, Asrat Kahsay ¹, Laura Palumbo ¹, Massimo Bonora ¹, Mariusz R. Wieckowski ³,
Carlotta Giorgi ¹, Paolo Pinton ^{1,2,*}

¹ Laboratory for Technologies of Advanced Therapies (LTTA), Section of Experimental Medicine, Department of Medical Science, University of Ferrara, 44121 Ferrara, Italy;

² Maria Cecilia Hospital, GVM Care & Research, 48033 Cotignola (RA), Italy;

³ Laboratory of Mitochondrial Biology and Metabolism. Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093 Warsaw, Poland.

* Corresponding authors

Email: paolo.pinton@unife.it / tel: 0532455802

Email: giampaolo.morciano@unife.it / tel: 0532455804

Via Fossato di Mortara 70, 44121 Ferrara, Italy

28 **Abstract**

29 Intracellular calcium signaling is a universal language source shared by the most part of biological
30 entities inside cells that, all together, give rise to physiological and functional anatomical units, the
31 organ. Although preferentially recognized as signaling between cell life and death processes, in the
32 heart it assumes additional relevance considered the importance of calcium cycling coupled to ATP
33 consumption in excitation-contraction coupling. The concerted action of a plethora of exchangers,
34 channels and pumps inward and outward calcium fluxes where needed, to convert energy and electric
35 impulses in muscle contraction. All this without realizing it, thousands of times, every day. An
36 improper function of those proteins (i.e., variation in expression, mutations onset, dysregulated
37 channeling, differential protein-protein interactions) being part of this signaling network triggers a
38 short circuit with severe acute and chronic pathological consequences reported as arrhythmias,
39 cardiac remodeling, heart failure, reperfusion injury and cardiomyopathies. By acting with chemical,
40 peptide-based and pharmacological modulators of these players, a correction of calcium homeostasis
41 can be achieved accompanied by an amelioration of clinical symptoms.

42 This review will focus on all those defects in calcium homeostasis which occur in the most common
43 cardiac diseases, including myocardial infarction, arrhythmia, hypertrophy, heart failure and
44 cardiomyopathies. This part will be introduced by the state of the art on the proteins involved in
45 calcium homeostasis in cardiomyocytes and followed by the therapeutic treatments that to date, are
46 able to target them and to revert the pathological phenotype.

47

48

49

50 **Keywords**

51 Calcium channels, mitochondria, sarcoplasmic reticulum, heart disease, therapy

52

53

1. Intracellular calcium handling and calcium cycling

1.1 Calcium from Plasma Membrane

Plasma membrane (PM, or sarcolemma in the cardiac muscle cells) is a selectively permeable structure which finely regulate the inner content of the cell by the concerted action of numerous systems of passive and active transport. More, the sarcolemma connects the basement membrane to other muscle cells and actively contribute to excitation and conduction of neuronal impulses. At PM, among all membrane-spanning proteins, there are a wide diversity of calcium (Ca^{2+}) channel types that diffuse Ca^{2+} ions down its electromechanical gradient from the extracellular space to the cytoplasm; these channels are mostly classified on the basis of their activation mechanism.

Voltage-gated Ca^{2+} channels (VGCCs) are transmembrane proteins activated by membrane depolarization; their activation allows Ca^{2+} influx in the cell, they are essential for the induction of physiological processes in the heart [1]. This is a peculiarity of excitable cells; cardiomyocytes, mainly express the L-type Ca^{2+} channels (LTCCs) $\text{Ca}_v1.2$ with major roles in excitation-contraction coupling (ECC) [2]. Conversely, in non-excitable cells the main pathway for Ca^{2+} influx is performed by store-operated calcium channels (SOCCs) [3] that generate the store-operated calcium entry (SOCE), activated in response to endoplasmic reticulum (ER)- Ca^{2+} stores depletion and following stimulation of PM receptors that couple to phosphatidyl inositol-bisphosphate (PIP_2) hydrolysis and inositol-triphosphate (IP_3) generation [4]. The knowledge about presence and function of SOCCs and molecular partners in cardiomyocytes has undergone a remarkable evolution: in principle, they were excluded from this cell type; later, they have been reported to be expressed only in the embryonal cardiomyocyte with a progressive downregulation until shutdown in adult and differentiated cells. Nevertheless, latest discoveries highlight not only the presence of SOCE mechanism in cardiomyocytes but also its involvement in disease [5].

Molecular components of SOCE are the mammalian transient receptor potential canonical (TRPC) family of Ca^{2+} permeable channels (TRPC1–TRPC7), Stromal Interaction Molecule (STIM1) and ORAI Calcium Release-Activated Calcium Modulator 1 (ORAI1). The lowering of ER- Ca^{2+} concentrations stimulate STIM1 oligomerization and the interaction with ORAI1 and/or TRPC. Remarkably, TRPC and ORAI1 generate different patterns of Ca^{2+} motifs that are decoded for the regulation of precise cellular functions [6]. Several types of TRP exist; in this review we take into consideration the canonical family, considered its main contribution in cardiac diseases discussed below. TRPC3 is the most abundant form in the heart [7]; it was found at the sarcolemma of pacemaker cells and it triggers CaMKII activation by channeling Ca^{2+} ; also, it provides for the phosphorylation of several proteins including PLB and RyR2 and thus increasing sarcoplasmic reticulum (SR) Ca^{2+} release [8]. There exist also evidence about TRCP3 involvement in sinoatrial node pacemaker activity trough SOCE and its kinase activity [8].

Postulated to be part of the SOCE pathway [9], Acid-sensing ion channel (ASIC) allows the passage of cations through the PM; ASICs generally sense changes in extracellular acidity ($\text{pH}<7$) and in cardiomyocytes they generate intracellular Ca^{2+} transients [10]. Since their expression is significant high in in neonatal cells and very low in adult cardiomyocytes, a role in development is supposed [11]. Widely expressed in neurons, they were found also in cardiomyocytes with pivotal roles in development and cardioprotection [11,12].

P2X receptors (P2X7Rs) are PM trimeric assemblies, defined as ATP-gated channels inasmuch they bind ATP for ionic permeation through the PM. There are seven P2XR subunits with very different trafficking properties and, consequently, different membrane subcellular distributions (PM, ER, endosomes and lysosomes) [13]. Their activation allow for Na^+ and Ca^{2+} influx into the SR and cytosol, contributing to stimulate sarcomere contraction [14]. ATP-induced Ca^{2+} mobilization can also induce pathological states [15].

ATP is therefore one of the most important elements in controlling intracellular Ca^{2+} and this is also reflected in the extrusion of this important second messenger from the intracellular to the extracellular environment.

The $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) is a PM ion transporter that works in two directions. Normally, the exchanger transports one Ca^{2+} ion in the extracellular environment and three Na^+ ions into the cell and this is known as the Ca^{2+} exit, or "forward" mode. However, under certain conditions, the exchanger can reverse and transport Ca^{2+} ions into the cell putting into action what is called the " Ca^{2+} entry mode" [16].

PM Ca^{2+} ATPase (PMCA) belongs to the superfamily of P-type ATPases and have a peculiar feature that make them unique as they are activated by calmodulin (CaM), which binds at a C-terminal extension that functions as a pump autoinhibitory domain. PMCA undergo conformational changes during the reaction cycle for which energy is provided by ATP forming a high energy acyl phosphate to permit the pumps to transfer Ca^{2+} across the membrane against the Ca^{2+} ion gradient [17]. In mammals there exist 4 different isoforms (PMCA1-4) where the most represented in the heart are PMCA 1 and PMCA4 [18]. Overall, consequently to indirect findings which attributed to NCX and SR the greater ability to remove "useless" cytosolic Ca^{2+} , it is believed that PMCA contributes only minimally to Ca^{2+} cycling in the heart. However, increasingly evidence due to the discovery of mutations in the genes encoding PMCA found among a wide variety of ethnics groups, show its their differential expression or activity produces severe defects in cardiac function [18,19].

1.2 Contacts with Sarcoplasmic Reticulum: a functional role

Sarcolemma invaginates to face some highly specific region of the SR (called junctional SR, jSR) with transversal tubules (TT). Here, the most percentage of $\text{Ca}_v1.2$ are located on TT and are very close to Ryanodine Receptors (RyRs). In jSR is located a large amount

of Ca^{2+} which is bound to calsequestrin (CSQ), a protein able to store most of Ca^{2+} needed for rapid and frequent contractions [20].

In turn, SR perform contacts also with mitochondria. While playing different biological roles and being two distinct organelles, mitochondria and the SR are not completely independent structures; specifically, the dynamic and tight association between both organelles give rise to a microdomain called mitochondria-associated ER/SR membranes (MAMs) [21]. MAMs play a crucial role in many signaling pathways, providing an excellent platform for the SR-mitochondria crosstalk and, in this way, allowing the rapid exchange of biological molecules to maintain cellular health. It has been described, especially in recent years, as the correct signaling between mitochondria and SR guaranteed by the passage of the second messenger Ca^{2+} , is a biological event of fundamental importance for the maintenance of normal physiological functions; the perturbation of this delicate balance leads to pathologies [22].

Focusing on SR-mitochondria contact sites, the SR locally canalize Ca^{2+} signals through the RyRs to Voltage Dependent Anion-selective Channel (VDAC) on the mitochondrial side after both electrical and chemical cell stimulation. SR is the major intracellular Ca^{2+} storage organelle; on its lumen Ca^{2+} is accumulated via active Ca^{2+} transport mediated by ER Ca^{2+} ATPases (SERCA) followed by intraluminal Ca^{2+} buffering by calnexin, calreticulin and CSQ [20]. The cardiac specific isoform of SERCA in the heart is SERCA2a. RyRs are the most important Ca^{2+} outflow channels on the SR surface, mediating Ca^{2+} release to the cytoplasm following the functional interaction of agonists on the PM receptors and intracellular second messenger IP3. In mammals exists three different isoforms of IP3R (IP3R1, -2 and -3) that are ubiquitously expressed [23] though in cardiomyocytes they are 50-fold less present [24] than the RyRs (RyR1 primarily expressed in skeletal muscles, -2 in cardiac muscle and -3 mostly in hippocampal neurons) [25]. A huge number of proteins modulate Ca^{2+} efflux from the ER/SR, at MAMs, through

interactions with IP3Rs. An emblematic example is represented by the sigma-1 receptor (S1R), a protein defined as a Ca^{2+} -sensitive chaperone that exerts a protective function in cells in various ways, including the modulation of MAMs Ca^{2+} signaling [26]. In 2020, a systematic review and metanalysis on S1R function in cardiac pathologies, ascribed to its cardioprotective roles by triggering the Akt-eNOS pathway and the modulation of intracellular Ca^{2+} signaling [26].

The ER Ca^{2+} content preserves the ability to regulate the channel opening. While being IP3 and Ca^{2+} essential for IP3R channel activation, other physiological ligands are not essential but can finely modulate channel Ca^{2+} sensitivity; for example, the IP3R modulation by ATP is biphasic. ATP micromolar concentrations exerts a stimulatory effect of IP3Rs while inhibits channel opening in the millimolar range [27]. Thanks to SR proximity to mitochondria, that juxtapose at a distance that can range from ~10 to ~25 nm [28], Ca^{2+} is taken up into mitochondria through VDACs. Established proteins involved in maintaining the structure of MAMs which guarantee a correct tethering between the two organelles are the Vesicle-associated membrane protein-associated protein B/C (VAPB)- Protein tyrosine phosphatase interacting protein 51 (PTPIP51) complex [29] and Mitofusins (Mfn1-Mfn2) complex.

Thanks to the growing evidence that is increasingly characterizing MAMs, it is becoming more and more evident that the SR-mitochondria interfaces have a crucial role in Ca^{2+} homeostasis regulation and are fundamental for different functional outcomes, such as cell metabolism or induction of cell death [22].

Summarizing the route of Ca^{2+} waves, what happens following an action potential along the sarcolemma and the TT of each cardiomyocyte, is the Ca^{2+} flow from $\text{Ca}_v1.2$ to stimulate RyR2 at jSR (forming diads), in turn releasing the ion from the SR and, at the same time, the accumulation by SERCA2a. In this way Ca^{2+} transiently reside in the cytosol and finely regulates ECC.

1.3 Mitochondrial calcium entry

The mitochondrial Ca^{2+} uptake plays a pivotal role in fundamental cellular processes, it modulates the metabolism, the Ca^{2+} homeostasis, cell fate and autophagy [30].

Ca^{2+} transiently enters the mitochondrial matrix driven by the electronegative mitochondrial membrane potential (MMP), which favors the Ca^{2+} transit across the permeable outer mitochondrial membrane (OMM) and impermeable inner mitochondrial membrane (IMM) relying on the activity of selective channels [31]. In basal condition, the mitochondrial Ca^{2+} uptake is limited, it rapidly rises when the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]$) reaches orders of micromolar in close proximity to mitochondria. This is due to the dynamic apposition between the main intracellular Ca^{2+} stores, such as the SR, Golgi or lysosomes, and the mitochondria that permit to generate Ca^{2+} hot spots, microdomains of high $[\text{Ca}^{2+}]$ formed, for example at TT-SR and MAMs by which born the mitochondrial Ca^{2+} signals [32].

Ca^{2+} presumably across the OMM mediating an inert diffusion via VDAC. VDAC is the most abundant protein at the OMM, which also allows the exchange of ATP and ADP as well as electron transport chain (ETC) substrates pyruvate, malate, succinate, and NADH [33–35]. When open, the channel is typically anion-selective but becomes cation-selective when the channel assumes a half-open state, favoring the diffusion of Ca^{2+} and other cationic metabolites [36]. Three individual genes codify for the three mammalian isoforms of VDAC (VDAC1, -2 and -3), respectively. The three isoforms display similarities in both structure and function, but each VDAC isoform presents a tissue-specific expression pattern and plays a distinct role in mitochondrial processes, including metabolism or apoptosis. In general, VDAC1 and VDAC2 show a higher protein expression level than VDAC3 with exception in the testis [37]. Among the three isoforms, only VDAC2 is lethal in embryonic knockout model while VDAC1 and VDAC3 knockout mice present only

bioenergetic defects and/or infertility [38–40]. All three isoforms are expressed in the heart.

To date, it is still unknown whether VDAC really plays an active role in Ca^{2+} regulation or it is only an inert Ca^{2+} diffusion pore. Tom40 could be a further candidate for the Ca^{2+} -transfer across the OMM. Component of the Translocase of Outer Membrane (TOM complex), this channel shows high cation selectivity, resulting a more favorable candidate for active Ca^{2+} -transport respect to the anion-selective VDAC [41]. VDAC is distributed at the level of SR-mitochondria contact sites and it is inserted in the multi-protein complex containing the ER-IP3R1 and the adaptor protein GRP75, responsible of SR-mitochondria Ca^{2+} microdomains [42]. Experiments performed in VDACs knockout cells showed that the IP3-induced Ca^{2+} -transfer to mitochondria was impaired while the overexpression of individual isoform increased the mitochondrial Ca^{2+} uptake, without affecting the number of SR-mitochondria contact sites [43]. These data indicate that VDAC is important for Ca^{2+} transfer to mitochondria because increase the permeability of OMM to Ca^{2+} . In particular, VDAC2 and VDAC3 had a slightly higher effect on mitochondrial Ca^{2+} uptake respect to VDAC1, being the last more anionic and thus less Ca^{2+} selective among the three isoforms [44]. Mediating immunoprecipitation assay it has been validated that VDAC1, but not VDAC2 or VDAC3, is responsible of Ca^{2+} transfer to mitochondria from lysosomes, via Transient Receptor Potential Mucolipin 1 (TRPML1) channel at the mitochondria-lysosomes contact sites [45]. All these data suggest that the distinct role of each isoform depends by their differential regulation and interaction with specific protein partners. **Only VDAC1 and VDAC3 seem to have high specificity for the skeletal muscle.** Their influence the Ca^{2+} -transfer to mitochondria forming specific multiprotein complex with Ca^{2+} channels express in several organelles or binding cytosolic and/or OMM proteins, such as the cytosolic free dimeric tubulin and PD-associated protein α -synuclein or the OMM protein Translocator Protein 18 kDa (TSPO) [44,46,47].

The permeability of IMM to Ca^{2+} is more stringent, the Ca^{2+} -transport across this membrane is mediated by a highly Ca^{2+} selective channel, the mitochondrial calcium uptake (MCU) complex [48]. MCU complex is present in almost all mammalian tissues, it exists in a large multiprotein complex of ~500 kDa, composed by the pore forming MCU subunits (known as CCDC109A), the mitochondrial calcium uptake proteins MICU1, MICU2 and MICU3, the essential MCU regulator (EMRE) and additional partners, including the MCU dominant negative subunit, MCUB, and the MCU regulator, MCUR1 [30]. In these last years, it has been determined the structure of human MCU complex in presence or in absence of Ca^{2+} , using cryo-electron microscopy [48]. In presence of Ca^{2+} , the Ca^{2+} activated open state of the channel is compatible with a V-shaped MCU-EMRE subcomplex dimer that bridges a heterotetramer MICU1-MICU2 complex while in absence of Ca^{2+} the channel is inhibited by two MICU1-MICU2 dimers. In resting condition, a single heterodimer of MICU1-MICU2 is sufficient to gate an MCU-EMRE tetramer, where MICU1 shuts the mitochondrial Ca^{2+} uniport covering the aspartate ring on the MCU pore entrance of the MCU-EMRE subcomplex. Upon Ca^{2+} increase, the ion binds the MICU1-MICU2 heterodimer promoting a conformational change that weakens the interactions between MICU1 and MCU, leading to Ca^{2+} -activation state of the uniporter. The heterodimer MICU1-MICU2 appears to be more stable in absence of Ca^{2+} , but upon Ca^{2+} elevation, the heterodimer MICU1-MICU2 further dimerizes to form the heterotetramer mediating Ca^{2+} binding and stabilization of MICU2 [49]. The MCU complex activity is regulated by intracellular Ca^{2+} concentration, as described until now, but it may be also influenced by the levels of Ca^{2+} into the mitochondrial matrix [50]. The mitochondrial Ca^{2+} concentration ($[\text{Ca}^{2+}]_m$) influences the MCU Ca^{2+} current in a biphasic manner, where the minimum MCU Ca^{2+} current is about 400 nM, which constitutes the point of “maximal suppression” while the minimal point is at normal resting (about 100nM), and that this point changes when is altered the

composition of MCU complex, suggesting that exist a coupled of Ca^{2+} regulatory mechanism that act at level of intermembrane space and matrix, respectively. MCU complex features depend on the tissue, in fact recent findings showed that the number of Ca^{2+} activate open MCU channels is regulated by intracellular Ca^{2+} concentration in skeletal muscle but not in the heart, indicating that the MCU complex presents a functional diversity specific for the different tissues [51]. Indispensable for MCU complex Ca^{2+} transport is the contribution of the EMRE binding to MCU [52]. The interaction between EMRE and MICU1 helps to shut MCU pore in absence of Ca^{2+} , and upon Ca^{2+} elevation prevents the dissociation of MICU1 from the subcomplex [48]. To maximize the uniporter's exposure to intracellular Ca^{2+} hot spots, the uniporter can dimerize, favoring its distribution towards IMM and OMM contact sites. This spatial arrangement should facilitate the effective inter-organelle Ca^{2+} transfer [48].

1.4 Mitochondrial calcium efflux

The recovery of Ca^{2+} homeostasis into mitochondrial matrix is guarantee by efficient Ca^{2+} -efflux pathway, which the molecular identification is occurred only in the last decade and albeit still open to further investigations. Findings demonstrated that PM NCX isoforms are also expressed on mitochondria where contribute to Ca^{2+} and Na^+ handling [53]. In mammalian exist three NCX isoforms, named NCX1, NCX2 and NCX3, differently redistributed in the tissues. NCX1 is a ubiquitous isoform while NCX2 is mainly expressed in the brain and NCX3 is essentially found in skeletal muscles and brain [54]. Their catalyze the bidirectional and rheogenic exchange of three Na^+ and one Ca^{2+} ions across the membranes, primarily working in forward mode, where their operate the Ca^{2+} -efflux from matrix and Na^+ -influx from cytosol [55]. When the membrane potential changes and/or the mitochondrial Na^+ or Ca^{2+} concentration is altered, the NCX activity may change direction, operating in reverse mode, where the Ca^{2+} is imported to mitochondria while Na^+ is extruded.

The first evidence that documented the existence of a mitochondrial Na^+ -dependent Ca^{2+} efflux was published in the 1974, where Carafoli et al. showed that Na^+ and Li^+ , but not K^+ , Mg^{2+} and Rb^+ , were able to evoke an efficient mitochondrial Ca^{2+} release in isolated cardiac mitochondria [56,57]. In the years findings have continuously supported that PM NCX isoforms localized also within mitochondria, contributing to mitochondrial Ca^{2+} handling in different cell types [58]. The pioneering Carafoli's findings was supported by Garlid et al. that in the 1992 identified an additional mitochondrial Ca^{2+} efflux system in heart beef mitochondria, a 110 kDa $\text{Na}^+/\text{Ca}^{2+}$ antiporter, successively identified as $\text{Na}^+/\text{Ca}^{2+}/\text{Li}^+$ exchanger (NCLX) [59,60]. Encoded by Slc8b1, NCLX is expressed at the IMM and catalyzes $\text{Li}^+/\text{Ca}^{2+}$ exchange as well as $\text{Na}^+/\text{Ca}^{2+}$ exchange at similar rate, transporting Ca^{2+} outside the matrix [59,61]. The two identified mitochondrial Ca^{2+} efflux systems may cooperate in the Ca^{2+} handling, both expressed within IMM, there can form hetero or homomeric complexes. Modulating the activity or expression of one exchanger is possible to interfere with the activity of the other [62]. These exchangers exhibit similar distinctive features, including the ionic regulation of their activity, potentiated by K^+ , their activity is inhibited by Ni^{2+} , Mg^{2+} , Ba^{2+} and La^{3+} [63–65]. Also, the H^+ can significantly regulate the activity of both exchangers [66,67]. Structurally, both have two transmembrane hydrophobic regions separated by a hydrophilic loop, in which are contained the catalytic regions involved in ions transport [68,69]. Differences are emerged in lipid and kinases regulation, where lipid molecules may affect function and localization of NCX but it not clear whether the same effects are addressed to NCLX; or about Protein Kinase A (PKA) that typically stimulates NCLX activity while for NCX activity the effects are controversial [66,70,71]. A peculiar difference between NCX and NCLX is due to variations in ion-coordinating regions involved in exchange activity. NCX is allosterically regulated by Ca^{2+} mediating the binding with Calcium Binding Domain (CBD) 1 and CBD2 while in NCLX seems lack the CBD domains and the Ca^{2+} ion should

act as an indirect modulator [66]. Future findings will aim to perform the molecular characterization of these exchangers, shedding light on the real scenario of mitochondrial Ca^{2+} -efflux that involves the tandem, NCX and NCLX, in Ca^{2+} handling.

Taken together, all these systems that allow Ca^{2+} to enter and escape from the cell constitute an essential toolkit for the maintenance of Ca^{2+} homeostasis (Figure 1) and their perturbation often leads to imbalances and various pathologies.

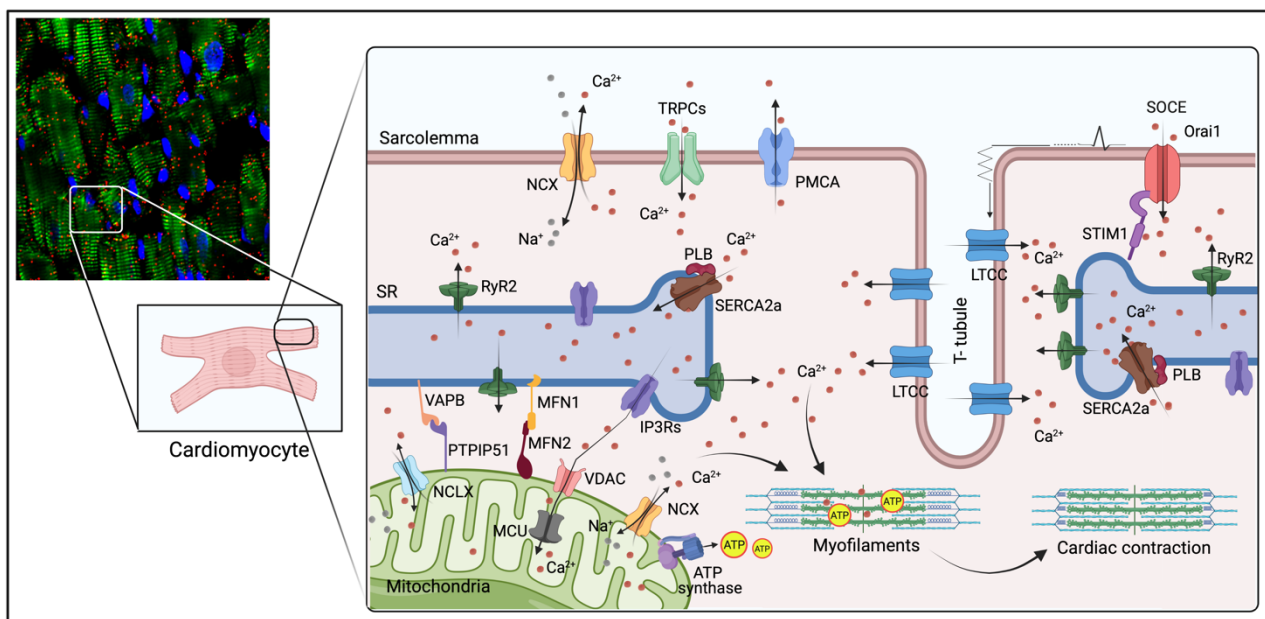


Figure 1. Calcium cycling and calcium handling in the heart

The figure depicts all channels, pumps and exchangers involved in cardiomyocyte calcium homeostasis. On the left, a transversal section of cardiac tissue was imaged in confocal microscopy which highlights in green the sarcomeric alpha actin, in blue the nuclei and in red the ATP synthase dimers. On the right, three “levels” of calcium regulation are showed: the sarcolemma and TT with NCX, TRPCs, PMCA, Orai1 and LTCCs; the SR with SERCA2a, RyRs and IP3Rs; the mitochondrial level with VDAC, MCU complex, NCX and NCLX. Calcium fluxes coupled with ATP consumption transduce electrical impulses in mechanical force to establish the ECC.

2. Calcium dysregulations in cardiac diseases

2.1 *The importance of the calcium cycling and the bioenergetics in the heart*

The heart is one of the highest energy-demanding organs as it beats continuously throughout the entire course of a lifetime and heavily reliant on mitochondria which provide the most energy to the heart [72]. This is evident as mitochondria occupy ~30% of the cardiomyocyte's volume and supply > 90% of the ATP required for cardiac contraction [73]. In addition, they are suited close proximity Ca^{2+} release, such as SR and SERCA2a pumps which actually requires an adequate ATP/ADP ratio in the cytoplasm [74]. Thus, the coupling of Ca^{2+} dynamics with mitochondrial bioenergetic is crucial for the functioning of cardiomyocytes both in health and disease conditions. The cardiac contraction (systole) is activated by an intracellular Ca^{2+} transient ($[\text{Ca}^{2+}]_i$) which triggers cross bridge cycling (binding of the protein complex to actin-binding sites followed by myosin head to bridge the gap) under load and ATP consumption by the myosin ATPases. Ca^{2+} , by traveling across the sarcolemma, TT, SR and cytosol, is thus an excellent signal for the cell to control contractile behavior of the heart. Also, it can enter the mitochondrial matrix and influence the behavior of key enzymes. Studies have shown that $[\text{Ca}^{2+}]_m$ is known to exert a complex activation of several dehydrogenases of tricarboxylic acid (TCA) cycle indirectly, increasing membrane potential [75,76] and to directly drive ATP production through an ATP synthase inhibitory factor, termed Ca^{2+} binding inhibitor which has been described to dissociate into monomers upon Ca^{2+} binding, leading to the activation of the ATP synthase [77]. Thus, rises in mitochondrial matrix Ca^{2+} regulates the respiration capacity [78]. A series of studies demonstrated Ca^{2+} sensitivities of the proteins and complexes associated with the ETC. Studies on skeletal muscle show the dose-response effect of Ca^{2+} sensitivity for all three proton pumps (Complexes I, III, and IV) in the ETC [79]. In addition to potentially regulating the ETC proton pumps, the effects of Ca^{2+} on oxidative phosphorylation were investigated in isolated porcine heart. Ca^{2+} has been indicated to critically regulate the F_1F_0 ATP synthase through increased ATP

production and adenylate translocase activity [80]. One novel protein, S100A1, a Ca^{2+} sensing protein of the EF-hand family and its interaction with mitochondrial $\text{F}_1\text{-ATPase}$, found to affect $\text{F}_1\text{-ATPase}$ activity and cellular ATP production. In particular, cardiomyocytes that overexpress S100A1 exhibited a higher ATP content than control cells, whereas knockdown of S100A1 expression decreased ATP levels. Consistently, ATP synthase activity is reduced in cardiomyocytes from S100A1 knockout mice [81]. Furthermore, $[\text{Ca}^{2+}]_c$ fluctuations are relayed to the mitochondria by MCU, closely coupling cytoplasm's Ca^{2+} dynamic to the cells energetic demands [82]. Moreover, the selection of energetic substrates in cardiomyocytes is a fundamental and fatty acids (FA) are known to be the main source of energy when the heart is at rest and during fasting periods. Most of the acetyl CoA that enters the TCA cycle comes from the β -oxidation of free FA within the mitochondria. Furthermore, Ca^{2+} activation of TCA cycle dehydrogenases controls NADH production, which in turn influences cellular detoxification (anti-oxidant) capacity and mitochondrial reactive oxygen species (ROS) production. In relation to energy production, ROS signaling triggers mitochondrial matrix Ca^{2+} sparks which also include MCU and NCX [83].

During diastole, to reach an optimal muscle relaxation, $[\text{Ca}^{2+}]_i$ should be removed and should return to resting levels. In this, proteins involved in Ca^{2+} removal from the cytosol are of fundamental importance and include in order of importance SERCA2a (ATP-dependent), NCX and PMCA (ATP-dependent) (Figure 1).

2.2 Calcium dysregulation in Myocardial Infarction

Myocardial Infarction (MI) is responsible for over 15% of mortality each year. The etiologies leading to the partial or complete block of the coronary circulation are manifold but they share the presence of common pathophysiological evolving features: the myocardial ischemia and the reperfusion injury (RI) [84,85].

Indeed, the occlusion of coronary vessels determines the arrest of either whole or partial blood flow to the heart inducing the ischemic condition, an imbalance of the ratio between oxygen demand and supply. After a brief period of ischemia (about 15-20 seconds), the only energy source of the heart becomes the anaerobic glycolysis. The limited amount of ATP produced by this process is not sufficient to satisfy the elevated ATP demand of the cardiac tissue [86]. Therefore, cardiomyocytes which do not adapt, die. The first line of clinical and mechanical intervention against ischemia is reperfusion, in which an early and fast restoration of the blood flow is induced [87]. However, this second phase can aggravate the damage of cardiac tissue, worsening the patient's condition as consequence [88]. During reperfusion, the oxidative phosphorylation of mitochondria reaches levels comparable to the pre-ischemic condition within seconds, meanwhile the myocardium does not reacquire the normal contractile functions immediately [89]. This postischemic condition is named stunned myocardium, in which the excessive H^+ accumulated during the aerobic glycolysis in the ischemic attack is transported outside the cardiac cell.

One of the most known Ca^{2+} dysregulated mechanisms in MI is the progressive intracellular Ca^{2+} overload priming during ischemia, and increasing with a more extent during the reperfusion phase [90]. In ischemia, to normalize the intracellular pH that has become acid, H^+ is inefficiently exchanged with Na^+ through the H^+/Na^+ exchanger (HNX). Again, intracellular Na^+ increases as the Na^+/K^+ is inhibited in the absence of ATP; the bidirectional NCX at the sarcolemma is active, but works in reverse mode causing cytosolic Ca^{2+} overload. At reperfusion, the sudden nutrient availability and oxygen restore the oxidative phosphorylation causing a burst in ROS generation and further Ca^{2+} accumulation due to the recovering of MMP.

Oxidative stress exacerbate Ca^{2+} fluxes by the hyperactivation of the opening of the RYR2 channels inducing Ca^{2+} leak from SR [91]. This pattern is dependent on the oxidation status of the RyR2 channel, this state would regulate the release threshold of Ca^{2+} [91].

Ca²⁺ released from the SR accumulates into the cytosol where can activate phospholipase A and protein kinase C, which mediate the destruction of cell membrane, with consequent release of toxic substance such as prostaglandins, ROS and leukotrienes. Additionally, [Ca²⁺]_c increase activates calpains, endonucleases, kinases and caspases, potentiating the activation of cell death events [90]. Among them, a particular role has been assigned for the CaM kinase type II (CaMKII) family. Indeed, it has been demonstrated that an increase in [Ca²⁺]_i may activate the isoform C, which is responsible to phosphorylate proteins of the apoptotic cascade [92] and those from which depend Ca²⁺ homeostasis and pathological consequences of MI (i.e., tissue remodeling, fibrosis, arrhythmia). An example is given by the impairment of the functioning of RyR2 channels, thereby provoking Ca²⁺ leakage from the SR that can contribute to cardiomyocyte contractile dysfunction and apoptosis [92]. At the same time, also the isoform B may be activated by Ca²⁺ overload. In this case, CaMKIIδB is responsible for maladaptive changes of the heart after MI, like hypertrophy that can lead to heart failure (HF) [93].

Intracellular Ca²⁺ during reperfusion also accumulates in the mitochondrial compartment via MCU. Consequently, mitochondria are subjected to an overload of Ca²⁺ that results in mPTP opening, mitochondrial dysfunctions and cardiomyocyte death [94] [95].

Indeed, it has been investigated that up-regulation of MCU promotes downregulation of mitochondrial functioning and activation of apoptotic process throughout activation of the Ca²⁺-dependent thiol-protease family calpains that, in turn, alter the functioning of the Optic Atrophy Protein 1 (OPA1), a master regulator of mitochondrial fusion [96]. Consistently, downregulation of expression of OPA1 was found in both humans with coronary disease and rat model of HF [97], and mice harboring OPA^{delTTAG} mutations showed altered Ca²⁺ dynamics and increased infarct size than WT littermates [98]. **In detail, cardiomyocytes from left ventricle with OPA1 deletion showed lower amplitude of Ca²⁺ transients, decreased SR Ca²⁺ uptake, increased cytosolic Ca²⁺ removal by NCX and**

impaired mitochondrial Ca^{2+} uptake. This has significant repercussion on the heart which results more sensitive to I/R and the number of the arrhythmias occurrence. Furthermore, genetic overexpression of OPA1 as well as the genetic ablation of its protease, OMA1, exert cardioprotective effects in *in vivo* ischemia reperfusion (I/R) model [99]. In contrast to OPA1, genetic ablation of mitofusins (MFNs), other regulator of mitochondrial fusion, have been shown to protective against I/R [100]. These unexpected effects may be explained by the fact that MFNs have pleiotropic non-fusion effects, including the preservation of ER/SR-mitochondria juxtaposition, which are fundamental for the Ca^{2+} transfer into mitochondria. Indeed, the ablation of MFNs reduced the proximity of mitochondria and SR protected from Ca^{2+} overload during I/R [100], thereby suggesting a close relationship between SR-mitochondria Ca^{2+} homeostasis and mitochondrial dynamics during I/R (Figure 2).

2.3 Calcium dysregulation in Arrhythmia

The regular heart contraction is permitted by a process in which the electrical activation (excitation) is converted into mechanical force (contraction) [101]. Ca^{2+} is an indispensable element for each ECC event. Substantially, arrhythmia (AT) is an irregular heartbeat; this pathological condition is caused by several factors, but in the middle lie the dysregulation of Ca^{2+} homeostasis. Although there are different methods to categorize ATs, we will refer to those related to ventricles because they are more lethal and differentiated by the mechanism of action in: reentry, triggered activity and automaticity [102].

Reentry ATs identifies cardiac area characterized by re-excitation after the initial depolarization event. They can be the consequence of predisposing genetic factors or can happen after cardiac remodeling that cause impaired refractoriness and conduction, thereby provoking the reentry event. Abnormal intracellular increase as well an enhanced SR Ca^{2+} release are fundamental factors to elicit ATs reentry.

Indeed, LTCCs downregulation induces shortening of the action potential duration, provoking reentry [103]. An opposite effect is obtained when small-conductance Ca^{2+} -dependent K^+ channels (SKc) are inhibited. SKc are sensitive to submicromolar $[\text{Ca}^{2+}]$ and play a role in atrial repolarization. It has been demonstrated that by using the specific SKc inhibitor, NS8593, canine ATs may be reduced [104]. However, a subsequent study performed in adult rat ventricular myocytes demonstrated not only that SR Ca^{2+} release is also determinant in SKc activation, but it suggests that SK up-regulation may have anti-arrhythmic effect [105].

Triggered activity are caused by early (E) or delayed (D) afterdepolarization (AD) events, which occur before and after full repolarization, respectively. DAD is mainly caused by spontaneous excessive Ca^{2+} increases arising from the Ca^{2+} -induced Ca^{2+} -release from SR, which activates the NCX. These spontaneous Ca^{2+} movements are the result of Ca^{2+} hot spots, which accumulate near Ca^{2+} releasing sites [106–108]. EAD are generally provoked by an excessive propagation of action potentials derived by NCX and LTCCs and by Na^+ and K^+ currents, which permit Ca^{2+} to enter [109,110]. Furthermore, EAD are also associated with an excessive phosphorylation activity of CaMKII and an over activation of LTCCs, thus accelerating the recovery after their shutdown [111].

Automaticity consists of spontaneous depolarizations during the diastolic phase.

At demonstration that intracellular Ca^{2+} handling can affect different cardiac ATs, mutations in genes encoding Ca^{2+} transporters, channels and regulatory proteins have been discovered in different inherited ATs syndromes. For example, catecholaminergic polymorphic ventricular tachycardia (CPVT), one of the most-deadly arrhythmias known, manifests mutations in RyR2 [112,113]. These mutations cause in increased SR Ca^{2+} leak, thereby causing frequent Ca^{2+} releasing events that activate NCX and, finally, induce ventricular ATs [114] (Figure 2). Mutations were also found for CaM. In this case, the mutations impair the Ca^{2+} binding of CaM and result in a compromised interaction

between the protein and RyR2 [115]. Since RyR2 exists as a macromolecular complex and interacts with other members including CaM, FK506-binding protein-12.6 (FKBP12.6 or Calstabin2), junctin (JCTN) and triadin (TRDN) [116], also the onset of mutations in each of these components, such as TRDN [117] carry out ATs phenotypes. All mutational profiles are already reviewed in [118].

Lastly, also TRPC proteins take part in Ca^{2+} dysregulation-mediated ATs; they have been reported as overexpressed in patients affected by atrial fibrillation, especially the first and third isoform [119], they also may contribute to DAD events [120].

2.4 Calcium dysregulation in Hypertrophy and Heart Failure

Cardiac hypertrophy (CH) is the process of heart remodeling in response to either increased workload (i.e., intended as physiological adaptation following chronic and sustained physical exercise or during pregnancy) or pressure overload (i.e., as consequence of hypertension and valvular diseases [121]). Macroscopically, physiologic CH differs from pathological hypertrophy because it induces changes that are reversible, and which involves also the ventricular chamber enlargement besides the walls thickness. Moreover, the cardiac structure remains normal and cardiac function is enhanced. From a molecular point of view, several pathways regulate CH and they, in part, differ between physiology and pathology as reviewed in [122]. Also, CH in pregnancy significantly differ from that occurs after exercise due to hormones contribution [123]. Regarding Ca^{2+} signaling, in physiologic CH there is an adaptation signaling pathway aimed to sustain enhanced cardiac function and workload; indeed, $[\text{Ca}^{2+}]_i$ are increased and SR Ca^{2+} uptake optimized [124]. A concomitant upregulation of RyR2 and SERCA2 proteins are reported in the mild and adaptative stages of CH.

Otherwise, physiologic CH can result in a diseased phenotype where the intracellular Ca^{2+} overload is reported to be involved in this transition; increased and prolonged $[\text{Ca}^{2+}]_i$ lead to the activation of pro-hypertrophic factors which, in turn, further stimulate CH. In this

scenario, CaM and calcineurin are the mainly involved Ca^{2+} signaling proteins and once they become activated, they trigger NFAT nuclear translocation and its activation [125,126]. Also their expression is reported to increase in CH pathological states [126–128]. These changes in Ca^{2+} homeostasis due to Ca^{2+} overload are responsible for mitochondrial dysfunctions, anomalies in cardiac contraction and cellular damage. In turn, developed pathologic CH supports VEGF secretion through mechanical stretch and in a NFkB-dependent manner [129].

After chronic insults, deep changes in thickness and elasticity of the walls of the heart degenerate in HF, a condition in which the heart fails to efficiently pump the blood to the systemic circulation. Indeed, CH is the main risk factor for HF and for these reasons they share also several similarities in terms of molecular pathways become impaired. In the literature are reported several dysregulations of Ca^{2+} signaling accompanying this process of maladaptation: they refer to several proteins involved and mainly focused on the impaired Ca^{2+} sequestration by SR.

The diastolic dysfunction and the correlated disturbance in ventricle relaxation are one aspect that characterize some types of HF [130]. As mentioned in the introductive paragraphs, the diastole phase which is responsible for a correct ventricle filling [131], is guaranteed by a rapid reduction of cytosolic Ca^{2+} channeled in the SR of the cardiomyocyte. In CH and HF this does not occur; the most accredited hypothesis is that reduced levels of SERCA2a and not functional ATPase activity of the remaining expressed protein, impair the correct Ca^{2+} homeostasis with consequent increased stasis of $[\text{Ca}^{2+}]_i$. Indeed, in failing hearts, severe metabolic derangements occur [72] and these prompt a real energy crisis. Since the functioning of some proteins involved in Ca^{2+} homeostasis is ATP-dependent, their activity result to be impaired and the contractile heart function as well. The decline of the respiratory chain function and ATP depletion prevent the above mentioned Ca^{2+} removal from the cytosol which is essential in diastole for

muscle relaxation before the next heartbeat [132]. A similar scenario is caused by the impaired action of PMCA and actomyosin, the other two ATP consuming proteins. It follows the absence of transients and a diminished contractility of the heart [133,134]. Nevertheless, other mechanistic insights are suggested in the literature. From the analysis of tissues of failing myocardium, it emerged a key role for an interactor of SERCA2a, phospholamban (PLB). PLB has the peculiarity to inhibit the Ca^{2+} transport through the pump by decreasing its affinity for the bivalent ion when it is dephosphorylated. On the contrary, when PLB is phosphorylated, it allows Ca^{2+} spread. In failing hearts, the phosphorylation status of PLB is significantly reduced as the kinetics of SERCA2a Ca^{2+} transport [135,136]. At the center of this regulatory pathway, the Serine (Ser) 16 and Threonine (Thr) 17, both phosphorylated by PKA and by CaMKII, respectively [137]. Further investigations on genetic profile in humans predisposing to HF phenotype, highlighted the importance of the mutational profile of PLB in the inhibition of SERCA2a and thus in adverse events accompanying HF. The most known are two missense variations R9C and the R14del [138,139]. The first one is able to block the PKA-mediated phosphorylation of the wild type PLB; the second one consisting in the deletion of arginine 14 on PLB failed the interaction PLB-SERCA2a. An additional mutation Leu39stop, identified in humans with a hereditary form of HF, entails severe HF with consequent need of heart transplant in young age when expressed in homozygosity [140]. If expressed in heterozygosity, signs of CH have been described. Here, low levels of PLB expressed were redirected to the PM causing Ca^{2+} dysregulation.

Histidine-rich calcium (HRC) binding protein is recognized as an additional negative regulator of SR Ca^{2+} uptake interacting with SERCA2a. This 170kDa protein is able to localize at SR thanks to its amino-terminal domain, while an histidine-rich sequence is supposed to have Ca^{2+} binding properties although there isn't a real Ca^{2+} binding motif

[141,142]. Its overexpression in the heart is reported to decrease Ca^{2+} uptake into SR with consequent impairment of muscle relaxation and contractility [143].

Restoring $[\text{Ca}^{2+}]_i$ for a correct ECC depends in a lower percentage also by NCX. However, literature investigating its expression in tissues from failing hearts, reported controversial results. On one hand, an increased expression seems act as compensatory mechanism to help the cardiac contractility during HF, but it inevitably results in Na^+ overload [144]; on the other hand, no appreciable differences have been detected in similar experiments [145]. In a scenario where $[\text{Ca}^{2+}]_i$ are increased and static and that SR is unable to accumulate Ca^{2+} , mitochondria may be in the position to buffer Ca^{2+} and suffer from these oscillations [146]. Indeed, Ca^{2+} overload prompts mPTP opening together to oxidative stress inducing apoptosis and cardiomyocytes loss [147] (Figure 2).

Increased activity of TRPCs is reported to additionally contribute to CH and its remodeling. They are able to inward great amount of Ca^{2+} inside cells and stimulate hypertrophy and fibrosis via the calcineurin-NFAT axis [148–150]. TRPC3 in particular, mediates CH also by modulating $\text{Ca}_{v1.2}$ activity and the reverse mode of action of NCX resulting in more Ca^{2+} entry [151].

2.5 Calcium dysregulation in drug-induced cardiomyopathies

In agreement with Elliot's classification established in 2008 [152], cardiomyopathies (CMs) are classified in dilated (DCM), hypertrophic (HCM), restrictive (RCM), arrhythmogenic (ACM) and those remained unclassified. It is clear CMs consist of a heterogenous group of disorders joined together by an improper functionality of the heart due to ultrastructural abnormalities. Despite some criteria in their classification have changed during last years, our priority is not the discussion of what differences exist among them but what types of Ca^{2+} signaling dysregulation occur and contribute to the maladapted phenotype of drug-induced CMs.

Indeed, it is known that CMs may be the side effect of chronic assumption of some classes of drugs and unhealthy lifestyle which cause a progressive and prolonged state of cardiotoxicity. Drug-induced CMs are potentially reversible but in most cases leads to HF. Unhealthy lifestyle, like the abuse of alcohol consumption, may induce adverse effects in the adult heart and it is considered a risk factor for CMs also in women during pregnancy. To unveil Ca^{2+} dysregulation, alcohol-dependent cardiac toxic effects have been studied in culture with cardiomyocytes treated with ethanol [153]. In the study, 69% of Ca^{2+} transients analyzed in cardiomyocytes treated with ethanol were abnormal. As cause of this phenotype, a deep change of protein expression profile has been observed; among the interested proteins, Annexin 6 was downregulated by 40% and ATP1A2 by 29% [153]. Both proteins are involved in Ca^{2+} transport: the first one modulating Ca^{2+} influx through the LTCCs and its release from SR by RyR2 and NCX [154]; the second protein is part of the Na^+ pump that can regulate intracellular Ca^{2+} and muscle contractility [155].

A problem currently considered of great importance is the cardiotoxicity of some classes of drugs in the long-term treatment of oncologic patients, such as anthracyclines. A general consensus is their use in therapy despite they are responsible for progressive accumulation of several mitochondrial dysfunctions [156], mostly mediated by a strong oxidant action. Although in principle they are potentially reversible changes, often they concur to the onset of severe CMs.

An example of cardiotoxicity from anthracyclines is given by doxorubicin. Patients experiencing chronic exposure to doxorubicin show the following dysregulations regarding Ca^{2+} signaling: i) increased susceptibility to Ca^{2+} -dependent mPTP opening and an altered Ca^{2+} transport: these effects are dose-dependent [157]; ii) inhibition of RyR2 and SERCA2a activities by a direct interaction of doxorubicin with the channels: this action is oxidative stress-mediated and already in the nanomolar range has a deleterious effect [158]; iii) ROS- and Mitogen Activated Protein Kinase (MAPK) axis- mediated

downregulation of SERCA2a mRNA in hearts treated with doxorubicin [159]; iv) a large-scale mRNA downregulation of Ca^{2+} -dependent proteins resident at SR, not only limited to SERCA2a but also including RyR2, PLB and CSQ have been found in a rabbit model of doxorubicin-induced CM [160] and v) $[\text{Ca}^{2+}]$ increase into the cytosol due to excessive RyR2 release [161]. All these changes prompt a severe Ca^{2+} homeostasis perturbation inside cells which are related in concurring several clinical symptoms, cell death (Figure 2) [162].

Other links between drug-induced CMs and Ca^{2+} dysregulation are described also for other drugs such as arsenic trioxide [163], mitoxantrone [164], abuse of cocaine [165], methamphetamine [166], despite mechanistic insights are not completely addressed.

2.6 References to the calcium-mediated PTPC

One of the best-characterized effectors of Ca^{2+} -mediated cardiac stress is the mPTP. This is a supramolecular entity assembled at the interface between the inner and outer mitochondrial membrane responsible for abrupt increase of permeability of the inner mitochondrial membrane, also known as mitochondrial permeability transition (MPT). In response to high $[\text{Ca}^{2+}]_m$, mPTP opens allowing the deregulated exchange of small solutes (up to 1.5 kDa) between the mitochondrial matrix and cytosol, along their electrochemical gradients. The mPTP is believed to open with 2 different configurations: one at low conductance, believed to operate mostly in physiological conditions to allow the equilibration of Ca^{2+} , mitochondrial pH and ROS; the second at high conductance, which causes immediate and complete dissipation of the $\Delta\Psi_m$, osmotic swelling of the mitochondrial matrix and inhibition of oxidative phosphorylation [167]. When a few mitochondria undergone MPT do not cause major cellular alterations [168] because they can be efficiently removed by the autophagy machinery and are proposed to participate in cell physiology (for a further discussion see [169]). On opposite, widespread MPT initiates cell death via regulated necrosis or apoptosis [170]. Regulated necrosis is characterized

by MPT-mediated block of mitochondrial energy production with the consequent arrest of its dependent activities [171]. Conversely, MPT-driven apoptosis mainly depends on the release of mitochondrial intermembrane proteins, especially cytochrome c, apoptosis-inducing factor, mitochondrion-associated, 1 (AIFM1, best known as AIF), and diablo, an IAP-binding mitochondrial protein (DIABLO, also known as Smac) [172].

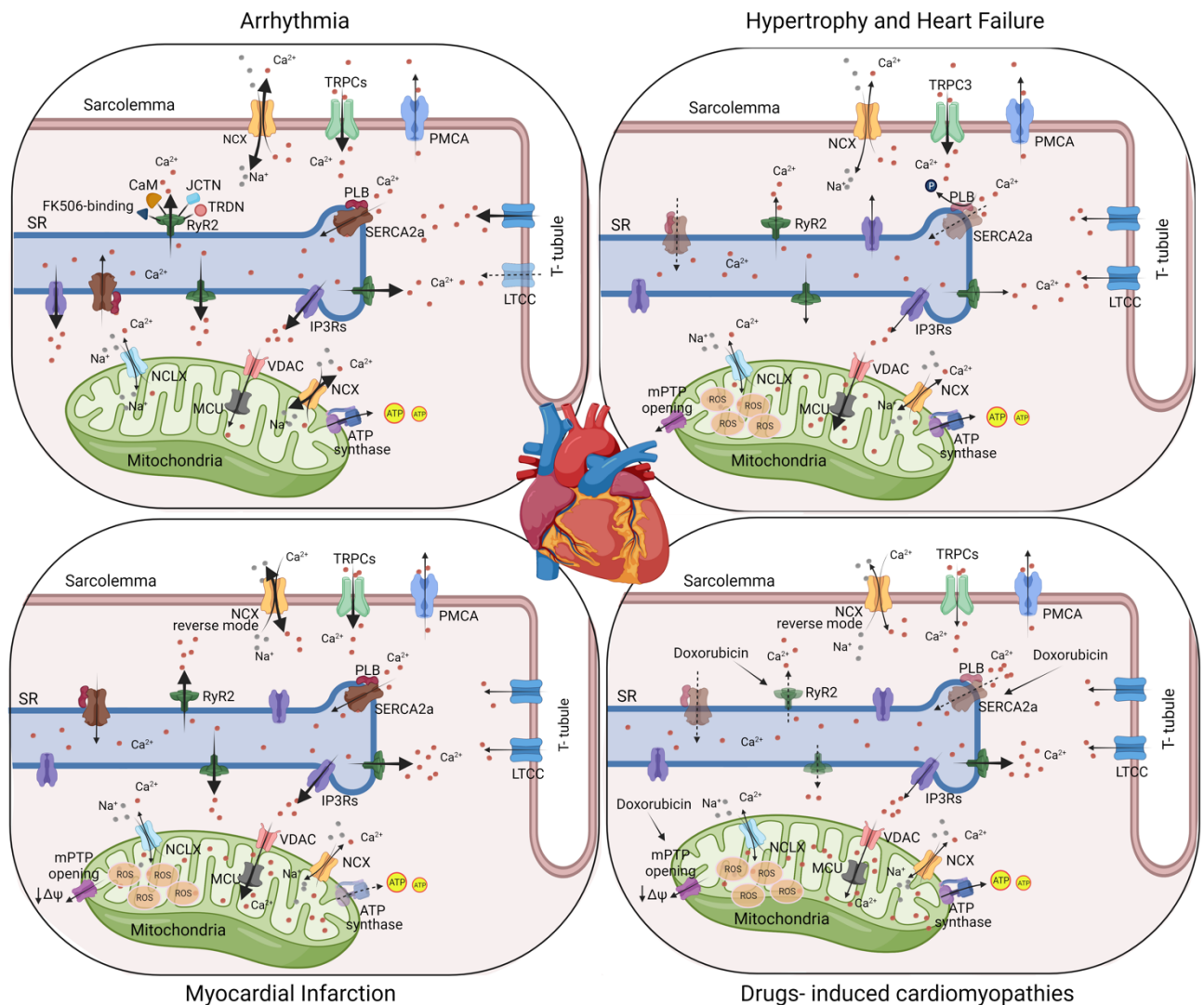
A role for mPTP has been demonstrated in several cardiac conditions, foremost reperfusion injury [94]. The most accepted model is that during cardiac ischemia mitochondria uptake Ca^{2+} and produces ROS (a strong sensitizer of mPTP) but MPT is blocked by the simultaneous accumulation of mPTP endogenous inhibitors as ADP and acidic matrix pH. At the reperfusion phase, the re-flow of oxygen favors a ROS burst and a mild restoration of pH and ADP levels which ultimately cause the massive opening of mPTP. Thanks to the identification of cyclosporine A (CsA) as a strong inhibitor of MPT, this mechanism has been proven as responsible for most of the necrotic area (and possibly a large fraction of apoptosis) in the myocardia undergoing reperfusion injury [95].

Pieces of evidence link mPTP also to reperfusion induced arrhythmia. Exposure to the mitochondrial uncoupler FCCP induces arrhythmias in explanted mouse hearts. FCCP causes dissipation of mitochondrial membrane potential which in turns favors the opening of the mPTP. It results the inability of IMM to hold a Ca^{2+} gradient between mitochondrial matrix and cytosol. The alteration of $[\text{Ca}^{2+}]_c$ in response to FCCP is believed a major determinant in FCCP-induced arrhythmias. Genetic inhibition of mPTP (via deletion of the gene *Ppif* coding for Cyclophilin D, CypD, the target of CsA) results in significant resistance of explanted heart to the insurgence of arrhythmias [173]. Contrasting investigation have tested the pharmacological inhibition of mPTP by CsA on reperfusion induced arrhythmias on different animal models. Arteaga and co-workers originally reported a significant effect of CsA on rat heart which were not confirmed by other investigation in rat [174] guinea pig [175] and rabbit [176]. Overall, these piece of

evidence calls for additional investigation, though should be noted that obtaining and experimental design able to discriminate the impact of mPTP in reperfusion damage or reperfusion-induced arrhythmia is challenging.

A similar mechanism is proposed for drug-induced cardiotoxicity. Compounds as doxorubicin [177,178], naproxen, diclofenac, celecoxib [179] and sorafenib [180] are proposed to elevate ROS production and affect Ca^{2+} homeostasis in cardiomyocytes, ultimately leading to mPTP opening and cell death. The exposure to mPTP inhibitors (as CsA) or antioxidants have been indeed shown to protect against drug-induced cardiotoxicity in different animal models. The MPT is also proposed to play a role in the necrotic cell death that is present in chronic HF. Cardiomyocytes isolated from dogs with chronic HF displayed increased opening of the mPTP, decreased mitochondrial membrane potential, and decreased mitochondrial cytochrome c oxidase and respiration. All the mitochondrial phenotypes were reverted by the administration of CsA [181,182].

However, a mechanism for mPTP opening in HF is not clear. Tamoxifen-dependent conditional deletion of the mitochondrial NCX cause the rapid onset on CH and HF in mouse heart by causing excessive accumulation of mitochondrial Ca^{2+} and mPTP opening [183]. In addition, the mitochondrial NAD^{+} -dependent deacetylase SIRT3, was demonstrated to target CypD - thus exerting a regulatory activity on mPTP and prevents age-related CH [184]. Despite this evidence, mice knock out for *Ppif* displayed propensity in develop HF, CH, fibrosis, and reduction in myocardial function in response to pressure overload stimulation [185]. This phenotype is proposed to be mediated by a chronic remodeling of energy metabolism (possibly mediated by mPTP) rather than a direct regulation of cell death.



689

690 **Figure 2. Calcium dysregulation in cardiac diseases**

691 The figure summarizes the main mechanisms known about calcium dysregulation occurring in
 692 cardiomyocytes in pathologies. From the diseased heart in the middle, four schematic views resume
 693 chapter 2 reviewed in the text. Clockwise, the first panel highlights the excessive propagation of
 694 action potentials derived by NCX and LTCCs and spontaneous Ca^{2+} increases in the cytosol arising
 695 from the Ca^{2+} -induced Ca^{2+} -release from SR. In the second, defects in SERCA2a expression or
 696 impaired function trigger increased and static intracellular calcium transients in heart failure. The
 697 third shows the significant cytosolic and mitochondrial calcium overload leading to mPTP opening
 698 and cell death following I/R. The last picture summarizes the increased susceptibility to calcium-
 699 dependent mPTP opening and an altered Ca^{2+} transport, the inhibition of RyR2 and SERCA2a
 700 activities by a direct interaction of doxorubicin with the channels, downregulation of SERCA2a and

other proteins resident at the SR and a significant increase of calcium into the cytosol due to excessive RyR2 release.

3. Approaches to correct calcium dysregulation: potentially many targets in cardiac diseases

Since the 90s, it is becoming increasingly clear that targeting intracellular Ca^{2+} can be a valid strategy for the treatment of several diseases, including in the heart. The knowledge of the previous mentioned studies has led to the development of regulators, more or less selective, of exchangers, pumps and channels with the aim to revert the pathologic phenotype and clinical symptoms of most cardiac diseases (Table 1). Moreover, the recent identification of the genetic component of the mitochondrial uniporter and other mechanistic insights about Ca^{2+} cycling allowed the better understanding the biological roles of Ca^{2+} regulation and to create more selective molecules that regulate its activity [186–192].

3.1 Targeting MCU

From the topics covered by this review it is clear that sustained mitochondrial Ca^{2+} overload is toxic for both tissues and cells in heart diseases, especially in I/R injury (IRI) where Ca^{2+} accumulation occur both in ischemia and reperfusion. Thus, although the inhibition of the MCU activity to treat this mitochondrial dysfunction may be considered an innovative therapeutic approach, it is necessary to take note of a series of apparent controversial results which don't always do theory the practice [193]. For example, it is reported that constitutive cardiac MCU-deficient animal models are not protected by IRI [188,192,194] despite mitochondria are protected from Ca^{2+} entry. These findings claim that the role of mitochondrial Ca^{2+} in IRI is extremely complex and whether on one hand sustained Ca^{2+} overload is harmful, also the complete and chronic inhibition of Ca^{2+} uptake may be deleterious or assumes compensative adaptations. In support with this

opinion, conditional knockout of the MCU or its transitory chemical inhibition, conferred the expected protective effects [195].

To date, several groups have developed molecules capable of reducing Ca^{2+} uptake into mitochondria through the inhibition of MCU. The most known and widely used is ruthenium red (RuRed), synthesized in 1892 [196]. RuRed is a substance that blocks Ca^{2+} uptake by MCU inhibition without affecting Ca^{2+} efflux and mitochondrial respiration [197–199]. The most important paper using RuRed against heart disease was by Grover et al. in which the treatment of perfused rat hearts with the compound in the micromolar range improved cardiac contractile function and oxygen efficiency at reperfusion after ischemia [200]. Despite its great potential, RuRed has shown low cellular permeability together with a poor selectivity for MCU and several off-target biological effects.

Subsequently, starting from RuRed another compound has been formulated and was called Ruthenium 360 (Ru360) [201,202]. The inhibition of MCU by Ru360 was highly selective and, unlike its predecessor, it did not induce changes in SR Ca^{2+} release, both actomyosin ATPase and NCX activity, LTCCs current and cytosolic Ca^{2+} dynamics [203]. The use of Ru360 as an intravenous single bolus (quantified in 15-50 nmol/kg) lasting 30 minutes before ischemia in rats, significantly reduced reperfusion-induced arrhythmias, mitochondrial damage and improved cardiac performance [201].

Recently, Woods et al. have synthesized and characterized the bioactivity of a new ruthenium-based MCU inhibitor, Ru265. This compound is structurally similar to Ru360 and would overcome it thanks to an increased selectivity, cell permeability associated to low toxicity detection [204]. It has been used as drug to prevent hypoxia/reoxygenation (H/R) in rat neonatal cardiomyocytes with a significant preservation from MMP lowering and mPTP opening [204].

Kon et al. have recently identified a novel cell permeable inhibitor of MCU, called DS16570511 [205]. Among the described effects ascribed to DS16570511 inhibition of mitochondrial Ca^{2+} uptake, the most common are increased heart contractility and reduction of RI when used in the range 3-30 μM . Its inhibition results reversible simply after washout.

However, two small compounds have been recently discovered through the screening of a library of about 44.000 substances for their capacity to regulate mitochondrial Ca^{2+} uptake. These compounds, called MCU-i4 and MCU-i11, directly bind a specific domain of MICU1, fundamental for the gating activity of MCU complex. Their interaction with MICU1 in muscle fibers has decreased Ca^{2+} influx altering the growth of myotubes [206]. MCU-i4, unlike -i11, impacts mitochondrial depolarization thus future investigations should be focused on the second one.

Santo-Domingo et al. have demonstrated that KB-R7943, a compound originally developed as inhibitor of the NCX, protects against myocardial I/R injury through the inhibition of MCU [207]. Inhibition of MCU by KB-R7943 may block the mitochondrial calcium uptake, the matrix overload and the subsequent opening of the mPTP, contributing to cardioprotective activity [207]. On the other hand, it has been seen that KB-R7943 inhibits Ca^{2+} -induced mPTP opening as primary and more direct function [208].

These data highlight that there is the need for new studies associated to the difficulty of finding permeable molecules that can inhibit the MCU, selectively, and without altering biological functions.

3.2 Targeting SERCA2a

As previously reviewed, SERCA2a is one of the main proteins to be dysregulated in CH and HF. Its function is impaired by an improper protein expression, an inhibitory action of molecular interactors and gene mutations. Malfunction of this protein is responsible for

non-physiological $[Ca^{2+}]_i$ transients which have repercussion in muscle relaxation during diastole and thus heart pumping. For these reasons, the recovery of their function may improve cardiac contraction and clinical conditions of patients affected.

According to experimental researches made in the field, the restoration of the functions of the SERCA2a can be carried out by either acting directly or indirectly, by targeting some of its regulators.

3.2.1 Direct ways

The most accredited way for the maintenance or the recovery of SERCA2a levels, especially for translational applications in humans, is gene therapy, which is applied from 25 years in the cardiovascular field and attracting interest for always new clinical trials. In the 90's, for the first time, an adenoviral expression (AV) system is used in ventricular cardiomyocytes of HF patients, to directly increase SERCA2a levels [209]. The effect has been replicated also in cardiomyocytes from different species [210]. By this method, Del Monte et al., have shown that the overexpression of SERCA2a in isolated failing human cardiomyocytes has restored the $[Ca^{2+}]_i$, muscle relaxation and increased cardiac contractility [209]. Moreover, several studies have further verified the beneficial effects of SERCA2a gene transfer on cardiac performance also in animal models of HF, improving myocardial excitability and preventing ventricular arrhythmia in pigs [211,212], diastolic dysfunction in swine [213] and dose-dependent functional benefit in ovine models [214]. It has been shown that Adeno-Associated Virus (AAV)-based vectors, compared to AV technique, are safer and more successfully alter gene expression in cardiac tissue, in addition to reducing inflammation [215,216]. Among more than 100 wild-type AAV serotypes, some have been demonstrated to have distinct features on cardiomyocyte transduction [215]. AAV-1 vector was the most used and studied. In several congestive heart failure (CHF) animal models it has been seen that the intracoronary injection of AAV carrying SERCA2a acutely improves vascular reactivity.

Also, long-term overexpression of SERCA2a by the same method, significantly restored cardiac ejection fraction, blood flow, eNOS expression in coronary arteries and decreased myocardial apoptosis [213,216–218]. Interestingly, SERCA2a gene transfer by improving Ca^{2+} signaling, directly influenced vascular endothelial and smooth muscle cell function [219]. Finally, AAVs/SERCA2a infusion in animal models of HF, have improved the myocardial and coronary artery Ca^{2+} handling and consequently the cardiac function.

On the basis the data obtained in preclinical models, Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease -Trial (CUPID) was established as first clinical trial in humans, to test the efficiency of recombinant AAV1/SERCA2a in HF [220,221]. The purpose of the clinical trial was intended of monitoring safety and efficacy of high doses of AAV1/SERCA2a administration as therapeutic approach. The result of the Phase I and Phase IIa trials showed reduced adverse cardiac events [220,221]. However, the double-blind randomized and placebo-controlled phase IIb clinical trial, named CUPID2, failed to show significant improvement in ventricular remodeling. Consequently, CUPID2 were terminated prematurely [222]. Despite the failure of these clinical trials, the field of cardiac gene therapy has gained valuable knowledge on AAV gene delivery in the heart and on safety in advanced HF population [223]. In recent years is emerging a new gene delivery method using other viral vectors mainly based on lentivirus. In a rat model of HF, it has been shown that lentivirus can integrate the gene of interest in the host genome with long-term effect of gene transduction [224–227]. In addition, in a model of I/R, the overexpression of SERCA2a reduced the incidence of the ventricular arrhythmia and improved hemodynamics [228–230].

3.2.2 Indirect ways

PLB is a SR membrane protein which modulates SR Ca^{2+} uptake, contractility and relaxation by SERCA2a inhibition [231]. In its dephosphorylated state, it lowers the

affinity of SERCA for Ca^{2+} , thereby inhibiting the uptake of the latter [231]. On the contrary, its phosphorylation relieves PLB-mediated inhibition of SERCA2a, thereby increasing the activity of the channel.

Several studies have demonstrated that to increase SERCA2a activity in treating HF, either increase PLB phosphorylation or its knockdown might be a promising therapeutic approach [232]. Indeed, all studies in large animals reported similar data output describing enhanced $[\text{Ca}^{2+}]_i$, improved systolic and diastolic LV function, the normalization of dilation and hypertrophy and reduced cardiac fibrosis; all clinical stages that act synergistically to reverse HF phenotype. Methods used are multiple and include the depletion of PLB through either AAV9-mediated shRNA [233] or antisense RNA directed to PLB mRNA [234], the use of an AV-mediated phosphorylated mutant of PLB which is constitutively active [235] and cell-permeable aptamers [236]. Nevertheless, attention should be maintained in the use of some methods due to the possible presence of side effects [237].

In addition to this, several drugs may act indirectly on SERCA2a activity. Some of these are: Hydralazine, glucocorticoids, Istaroxime and resveratrol. Hydralazine is a drug already used in the treatment of patients affected by HF, it acts indirectly being an inhibitor of DNA methylation [238]. Demethylation of the promoter region of SERCA2a increases its mRNA levels and leads to a correct Ca^{2+} homeostasis [238]. Glucocorticoids have been shown to restore the phosphorylation status of PLB together to SERCA2a when used as treatment in IRI or cardiac arrest [239].

Istaroxime is utilized for the treatment of acute HF syndromes, it increases function of SERCA2a by acting on PLB inhibition and decreases of $\text{Na}^{2+}/\text{K}^{+}$ -ATPase activity [240,241]. The results are clear and various; in principle administered in dogs, they have highlighted the absence of arrhythmic events associated with improved heart function [242]. In addition, also humans benefit from this [243] as evidenced by a clinical trial in

patients with HF which has shown that the administration of Istaroxime decreases heart rate, improves pulmonary capillary wedge pressure besides demonstrating safety and beneficial effects on cardiac contractility [244,245]. Likewise, other drugs like Losartan and Enalapril can prevent the downregulation of SERCA2a protein and the mRNA levels through blockage of the renin–angiotensin system (RAS) and an angiotensin II receptor blocker (ARBs) [246]. Moreover, the administration of Resveratrol in diabetic mice has shown an increase in SERCA2a expression and an improvement of the cardiac function through activation of SIRT1 [247,248]. In addition to this, in diabetic rat cardiomyocytes, the treatment with insulin-like growth factor 1 (IGF-1) through activation of the PI3 kinase-Akt-SERCA2a signaling pathway enables the increase the SERCA2a levels [249]. Lastly, in a rat model of chronic HF also the anti-inflammatory agent named Oxymatrine, improves the SERCA2a expression and cardiac function [250].

However, recent studies have focused the attention on possible SERCA2a post-translational modifications. Studies have shown that in cardiomyocytes the activity and levels of SERCA2a are modulated through SUMO1; SUMOylation of SERCA2a is reduced in HF and with it also the protein amount [251]. Kho et al., have developed a molecule that induced the activation of SUMO1, called N106, that leads to the SUMOylation of SERCA2a with consequent stimulation of the activity of the latter [252]. In addition to this, also the gene therapy through AAV9 gene transfer the SUMO1 levels are increased, leading to of SERCA2a normal levels. Consequently, induces an improved hemodynamic performance and a reduced mortality among the animals with HF. Likewise, it has been proven that increased expression of S100A1, a Ca^{2+} binding protein involved in intracellular Ca^{2+} regulation, improves the activity of both SERCA2a and RyR2, restoring the correct $[\text{Ca}^{2+}]_i$ transients and Ca^{2+} at the SR [253]. This is translated into a long-term reversal of LV dysfunction with improvements of contractile function in a pre-

clinical model of ischemic CM. These data indicate that S100A1 can be another target for therapy of the human HF [254,255].

Furthermore recent studies have discovered that many microRNAs (miRs) are dysregulated with HF [256,257]. In particular, experiments in cardiomyocytes of both mice and humans, have detected high levels of miR-25 that delays Ca^{2+} re-uptake kinetics with consequently increased HF phenotypes. Wahlquist et al. have demonstrated that the use of antagomiR-25 in a mouse model improves cardiac function and survival through restoring SERCA2a activity [258].

3.3 Targeting RyR2

RyR2 plays a central role in the dysregulation of Ca^{2+} homeostasis found mainly in ATs [259]. Indeed, the pathological leak of Ca^{2+} from RyR2 is at the basis of arrhythmogenesis. Several groups of research have developed compounds and drugs with the potential of regulating RyR2 channel activity, to maintain the normal SR Ca^{2+} release during systole and inhibit it during diastole [260].

From the study of the pluriannual use of Diltiazem in clinic, a 1,5-benzothiazepine-derivate and Ca^{2+} antagonist [261], led to the discovery of further additional benzothiazepine derivatives with potential therapeutic properties for the treatment of ATs. Among them, JTV519 is a 1,4-benzothiazepine compound that shows its cardioprotective effects against atrial fibrillation through the finely regulation of RyR2 properties without altering the capacity to the propagation of Ca^{2+} waves [262]. This piece of evidence on the molecular mechanism adopted by JTV519 in preventing the onset of ATs in cardiac diseases, seem not to be the only one existing. It has been seen that in dogs, after four weeks of chronic right ventricular pacing, the administration of JTV519 controlled RyR2 opening by restoring Calstabin2 levels and stabilized the RyR2/FKBP12.6 complex, improving cardiac function and decreasing SR Ca^{2+} with recorded reduced RyR2 hyperphosphorylation [263]. Subsequently, Wehrens et al. have shown that JTV519

906 stabilizes the closed state of RyR2 by promoting FKBP12.6 binding. The same group also
907 demonstrated that in Calstabin2 knockout mice, JTV519 had no effects, highlighting the
908 importance of Calstabin2 for the antiarrhythmic effect induced by this compound [264].
909 In recent years, in 2016, it has been reported an additional property for this drug, such as
910 its partial and mild block of SERCA2a just enough to prevent SR Ca²⁺ overload and related
911 harmful events in IRI; thus, as consequence, it has repercussion also on SR Ca²⁺ release
912 modulation [265].

913 A second 1,4-benzothiazepine derivative is S107; it has the same function of JTV519, or
914 preventing the Ca²⁺ leakage from the SR, by promoting the Calstabin2-RyR2 binding.
915 S107 has high specificity for RyR2 and no off-target activity [266]. But the real
916 innovation concerning this novel drug is the ability to create a complex RyR2-Calstabin2
917 and thus blocking pathological Ca²⁺ leakage and serious ATs also in the presence of a
918 mutated form of RyR2, which is the main responsible for CPVT [266,267]. These
919 compounds may have great promise for treating patients with CPVT and not only, indeed,
920 clinical trials studies for the treatment of HF and ATs are in phase 2. In addition to this,
921 S107 can also prevent muscle weakness in aging, stress-induced cognitive dysfunction,
922 Duchenne muscular dystrophy and the progression of HF [268–271].

923 Dantrolene is a hydantoin derivative that is clinically used for the treatment of malignant
924 hyperthermia, a condition caused by mutations in the skeletal RyR1 [272]. Its mechanism
925 of action involves CaM-dependent stabilization of the interaction between the amino
926 terminal and central domains of RyR1, required for the closed state of the RyR
927 Ca²⁺ channel [273]. Kobayashi et al. have shown that Dantrolene has a role against CPVT
928 by binding also the mutated form of RyR2, specifically to domain 601-620 and resulting
929 in the inhibition of cytosolic Ca²⁺ sparks in diastole and DAD [274]. However, it has been
930 demonstrated by using the knock-in mouse model with a human CPVT-associated RyR2
931 mutation (R2474S) that pretreatment with Dantrolene inhibited CPVT-related tachycardia,

induced by either epinephrine or exercise [275]. Subsequently, studies have been carried out on the therapeutic antiarrhythmic potential of Dantrolene in the treatment of CPVT. Dantrolene was administered intravenously and it reduced the number of premature ventricular complexes with antiarrhythmic effects in a subgroup of CPVT1 patients with specific RyR2 mutations [276]. These data showed comparable results even in experiments conducted in pluripotent stem cells (iPSC)-derived spontaneously beating cardiomyocytes generated from the same patients, proving once again the antiarrhythmic effects of Dantrolene [276]. Dantrolene has found useful also for the treatment of ATs following I/R in several animal models [277,278]. All these evidences underline that to attenuate Ca^{2+} -mediated arrhythmias associated with HF, this hydantoin derivative can be a possible therapy. Currently, a new clinical trial is in the recruitment phase to test the efficacy of Dantrolene in CPVT patients [279].

Flecainide is a trifluoroethoxybenzamide, an oral class Ic antiarrhythmic drug, already approved for medical use in the United States since 1984. Flecainide known as a Na^+ channel blocker, is used in patients who do not have structural heart disease, for the treatment of atrial fibrillation/atrial flutter, paroxysmal supraventricular tachycardia, atrioventricular nodal reentrant tachycardia, AV reentrant tachycardia [280,281]. Recent data have shown both in iPSC-cardiomyocytes and in mouse models of CPVT that Flecainide acts either reducing or removing aberrant RyR2 activity, blocking the open state of RyR2 and decreasing spontaneous Ca^{2+} leak, thus preventing ventricular tachycardia [282,283]. Confirming its efficacy in CPVT is not only due by the blockage of the Na^+ channel. In addition to this, treatment of two patients with Flecainide, who remained symptomatic with conventional drug therapy, has prevented CPVT with great success. Hence, these data provide clear evidence on the antiarrhythmic effectiveness of Flecainide in inhibiting defective RyR2 Ca^{2+} release channels in humans, identifying it as a possible currently available drug for fight CPVT [283].

A last class of drugs considered efficient in reducing spontaneous and pathological Ca^{2+} release are Tetracaine and derivatives (EL1-9) [284] for the treatment of ventricular tachycardia; they showed great affinity for RyR2 and few off-target side effects. The last one identified by Word et al., EL20, used at low nanomolar concentrations and also efficacy in cardiomyocytes harboring the R176Q variant from a CPVT patient [285].

3.4 Targeting LTCCs

LTCCs are the main mediators of VGCC-mediated Ca^{2+} influx into cardiac cells and are fundamental in certain mechanisms and electrical properties of the heart. These channels, thus are considered as an important target for the treatment of several diseases [286–288]. In the heart, the most expressed isoforms are $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ and their alteration is related to the onset of cardiac diseases related to the impairment of ECC and inotropy [289].

It is known that mutations in LTCC genes induce the dysfunctions of Ca^{2+} channels, resulting in the abnormal excitations of cardiomyocytes leading to the onset of cardiac ATs such as LQTS (Long QT syndrome) [290] and Brugada syndrome [291].

For decades antagonists of LTCCs have been used for the treatment of CH and I/R. However, in patients with HF, LTCC Ca^{2+} blockers are employed with great caution. In addition to this, the knockdown of LTCCs induces the secondary CH signaling through a compensatory increase in Ca^{2+} by RyR2 leak [292].

However, over the years, several organic molecules have been identified as modulators of LTCCs by blocking the inward Ca^{2+} current. These modulators bind to three separate receptor sites on LTCCs and can be grouped into three groups: benzothiazepines, dihydropyridines (DHP) and phenylalkylamines [286,293,294]. All binding sites are situated within the IIIS5, IIIS6, and IVS6 transmembrane segments and near to the pore [295]. These substances are used to treat several diseases, including HCM, CHF, CH, angina pectoris and cardiac ATs [296–299]. In particular, the non-dihydropyridines

Ca²⁺ channel blockers that includes benzothiazepines and phenylalkylamines are used also for the treatment of ATs, but should not be used in patients with HF and atrial fibrillation united with preexcitation. Several studies have demonstrated that the use of non-dihydropyridines increase mortality and incidence of HF, due by their negative inotropic effects [300,301]. Indeed, to prevent further depression of cardiac function the non-dihydropyridines, are used only when β -blockers alone are unsuccessful [302].

In addition to this, several studies have shown that in arrhythmic conditions the combination of different Ca²⁺ channel blockers can be a valid therapeutic treatment. Interestingly, in patients affected by CPVT, it has been reported that by adding verapamil to existing β -blocker therapy, the incidence of exercise-induced arrhythmias was reduced when compared to β -blocker therapy alone [303].

Despite the physiological role of the T-type Ca²⁺ channel in the human heart is still little documented, it is known they are present in the sinoatrial node, atrioventricular node and Purkinje fibers and are involved in the pacemaker activity. Mangoni et al, have highlighting the importance of the channel in cardiac rhythm, through Cav_{3.1} knockout mice [304]. In this animal, the genetic inactivation of the Cav_{3.1} result in a significant slowing of the heart rate and atrioventricular conduction [304].

3.5 Targeting NCX and PMCA

In addition to SERCA2a, both PMCA and NCX are important regulators of [Ca²⁺]_i intervening in Ca²⁺ removal when it exceeds, for instance after contraction. NCX has the adjunctive peculiarity to work in a bidirectional way creating inward and outward currents of Ca²⁺ and Na⁺, depending on the intracellular concentrations of the ions exchanged and on membrane potential. Thus, both activators and inhibitors of this channel may result in cardioprotective effects. During I/R when it functions in reverse mode by increasing Ca²⁺ influx, its inhibition may result useful; otherwise, in HF Ca²⁺ extrusion may result

impaired caused by high $[Na^+]$, in this scenario, stimulators of its activity may provide benefit.

There exist many NCX inhibitors, but most of them are not selective (i.e., Amiodarone, Bepridil, Aprindine) as they modulate also Na^+/K^+ and LTCCs currents and they are already reviewed in [305], although they retain good antiarrhythmic properties.

The first, but less selective inhibitor, is KB-R7943 developed by Watano T. [306]. Its administration has showed protective effects following I/R by improving LV function, by reducing ventricular fibrillation and hypercontracture of cardiomyocytes [307,308]. KB-R7943 is not considered too selective because it results also a potent MCU inhibitor; thus, it is difficult to discriminate if its cardioprotection following I/R (at a concentration of 10 μM) is dependent on the inhibition of mitochondrial Ca^{2+} uptake or on the modulation of NCX, or both; although that dose should not influence the exchanger as claim some reports [207].

To date, the most potent and selective compounds targeting NCX are named as SEA0400, SN-6 and YM-244769. Overall, in the heart, SEA0400 is considered to have a higher potency in the inhibition of NCX compared to SN-6, which result similar to KB-R7943 [305]. At the same time, also its selectivity is greater than SN-6 which in turn, is more selective of KB-R7943. Cardioprotective effects of SA0400 are multiple and range from cell cultures to large animals. Generally, its treatment led to Ca^{2+} -induced cell death inhibition [309], recovery of LV function after reperfusion and a decrease of the infarct size [310], and the incidence of ventricular fibrillations. To conclude, the newest YM-244769, firstly described in the brain and targeting preferentially NCX3 [311], showed protective action under H/R both in neuronal diseases and in the heart [312].

On the other hand, although NCX stimulators own cardioprotective features, they share many molecular mechanisms of protection which often fall back so far into the modulation of Ca^{2+} homeostasis [313].

Concerning PMCAs, PMCA1 and PMCA4 are primarily reported to be expressed in the heart. These proteins are considered minor contributors for Ca^{2+} efflux from the cell, thus they have always been of secondary importance to study in diseases and as target of cardioprotection. Nevertheless, it is reported the existence of interesting mutational profiles of the PMCA1 gene concerning cardiac disease. Genome wide association studies (GWAS) performed in several cohorts of the whole world population and taking into consideration different ethnicities, identified at least 10 SNPs in correspondence of intronic regions of the gene encoding for PMCA1. As consequence, they are significantly associated to hypertension, pre-eclampsia, salt sensitivity and leading to coronary artery diseases, myocardial infarction and also calcification. These SNPs are the following: rs1401982 [314–316] associated to artery stenosis, hypertension and arterial stiffness; rs2070759 [317,318] associated to hypertension in both Chinese and Japanese population; rs2681472 [319–321] responsible for hypertension and arterial stiffness; rs2681492 [322,323] involved in high blood pressure in people from Africa; rs7136259 [324–326] associated to premature coronary disease and atherosclerosis in multi-ethnic populations; rs10858911 [327], rs11105354 [328–331] with increased risk of cardiovascular disease; rs11105378 [317,332] with increased susceptibility to hypertension; the same for both rs12817819 [333] and rs17249754 [319,320,333]. Besides what reported above, more than 50 additional papers reported same findings on the association of PMCA1 to hypertension and coronary artery disease like MI. This evidence involve loss of function of PMCA1 or reduced amount of the protein at the PM that has, as direct effect, the increase in the expression of LTCCs and blood pressure, triggering CH and heart remodeling [334,335]. Overall, no reports classify these mutational profiles as deadly themselves, but it is clear that they significantly increase the onset of hypertension and premature coronary artery disease with all related consequences. Also PMCA4 plays key roles in heart physiology by controlling cardiac contractility [336]. In a scenario of established I/R, its

overexpression alleviates all maladaptation of the heart included CH and HF [337].
However, to date there are no drugs able to induce PMCA overexpression.

3.6 Targeting TRP channels

Each of TRPCs has an individual role in cardiovascular pathologies, being differentially expressed in the diseased heart. On the basis of recent considerations made on TRPC targeting to prevent CH, fibrosis, heart remodeling and HF, there exist several pitfalls that obstacles the use of this target in human clinical trials, when compared to other strategies for other proteins. In particular, still very few drugs targeting these channels have been discovered where the majority are not selective, they do not exist cardiac-specific isoforms of these channels thus their targeting may involve severe off-target effects [8].
However, here we report what is known about advanced studies in cells. Deletion of the first isoform of TRCPs is able to deactivate the calcineurin-NFAT signaling axis in the progression of CH [338]. About TRCP3 there exists few inhibitors like GSK255B [339] which blocks the channel but does not led to any protective effect when administered alone. While, the synergistic inhibition with TRPC6 with GSK503A, antagonize the signaling inducing CH or reduced the phosphorylation status of many proteins involved in Ca^{2+} handling [340]. The second inhibitor Pyr-3, a pyrazole compound, mediate a plethora of effects ranging from Ca^{2+} influx, modulation of NFAT to reduce hypertrophy; Pyr-3 seems to acquire more selective skills [341]. Recently, it has been developed a selective compound 31, c31, that working in the picomolar range of concentrations it inhibits the assembly of TRPC1/4/5 complex, but no cardioprotective effects have yet been investigated [342].

3.7 Targeting CAMKII

CaMKII is a kinase reported as mainly involved in intracellular Ca^{2+} -dependent changes occurring during HF, CH and ATs and caused by its intense activity of phosphorylation towards proteins involved in Ca^{2+} handling. It has many targets (i.e., PLB, RyR2,

SERCA2a) and, overall, it triggers SR Ca^{2+} leaking and the opening of LTCCs. It is clear that its inhibition constitutes the way to preserve physiological functions.

There are two types of CaMKII inhibitors: those grouped in the pharmacological area and those based on peptides structures. In the 90's, one of the first CaMKII pharmacological inhibitor was KN-93 [343]; if its use in basic research was spread, it had no future in clinical practice. Indeed, side effects targeting multiple proteins as LTCCs, IP3Rs and calmodulin have been described several times [344–346].

The second known pharmacological inhibitor is AS105 [347]; by working 1000-fold less concentrated than the first one, it successfully reduced SR Ca^{2+} leak in cardiomyocytes from mice with HF. Given the very low dose of use, no negative effects on ECC and SR Ca^{2+} loading were recorded.

Again, another similar binding molecule (which results in a ATP-competitive binding) is GS-680 [348]. It has been improved by Wagner S et al. with a strong selection against cardiac CaMKII and with beneficial effects reducing arrhythmia and increasing contractility in failing myocardium. Also, this compound worked efficiently in the low nanomolar range. Of the same class, RA306 was described for the first time by Beauverger P. and colleagues in 2020 [349]; by treating diseased mice with oral administrations, after few weeks it improved cardiac parameters linked to contractility.

Among the peptide-based inhibitors, autocamtide-2-related inhibitory peptide (AIP) [350] and autocamtide-3 derived inhibitory peptide (AC3-I) [351] interact with CaMKII and blocked its canonical activation. This resulted in a strong enzyme inhibition in several animal models providing evidence once again on the importance to target this protein to counteract pathological hypertrophy and lethal arrhythmias [352].

Across the end of 90's and the early 2000's, by using the catalytic domain of CaMKII as bait [353,354], proteins with inhibitory function against the enzyme have been unveiled; they were resident in the brain, but absent in the heart. For this reason, the first effort was

an efficient delivery in the heart by the use of AAV vectors and by targeting the CaMKII once active and limiting I/R consequences and ATs [111,355]. Taking advantage from growing biomolecular methods, it was created a mitochondria-targeted inhibitory protein [356]. Its administration at reperfusion relieved CaMKII activation and thus its positive loop on MCU-mediated mitochondrial Ca^{2+} load and mPTP opening. Further studies in improving these peptides achieved a great result by creating the smallest sequence of 19 aa (named as CN19o) sufficient to exert all cardioprotective effects in the picomolar range [357].

Table 1. Existing modulators of intracellular calcium to counteract cardiac diseases

Modulators	Protein target	Main experimental model	Molecular effects	Physiologic effects
Ruthenium Red	MCU	Cells, perfused animal hearts of I/R	Inhibition of mitochondrial Ca^{2+} load, changes in SR Ca^{2+} release;	Improved cardiac contractility
Ru360		Cells, whole body laboratory animals of I/R	Inhibition of mitochondrial Ca^{2+} load	Reduced RI, prevention of arrhythmias and mitochondrial damage (MMP lowering and mPTP opening), improved cardiac performance, increased heart contractility
Ru265				
DS16570511				
MCU-i4	MICU1	Cells and muscle tissues	Inhibition of mitochondrial Ca^{2+} load, impairment of MMP	N/A
MCU-i11			Inhibition of mitochondrial Ca^{2+} load	
KB-R7943	MCU NCX	Cells, perfused animal hearts of I/R	Inhibition of mitochondrial Ca^{2+} load, inhibition of Ca^{2+} -induced mPTP opening Inhibition of the reverse mode of NCX	Protective effects following I/R by improving LV function, by reducing ventricular fibrillation and hypercontracture

				of cardiomyocytes
AV/SERCA2a	SERCA2a	Cells, large animal models of HF	Restore SERCA2a expression, restore of Ca ²⁺ transients	Muscle relaxation, increased cardiac contractility, improved myocardial excitability, prevention of arrhythmia
AAV/SERCA2a	SERCA2a	Cells, large animal models of HF, humans		Improved vascular reactivity, restored cardiac ejection fraction, blood flow, eNOS expression, decreased apoptosis
AAV9/PLB shRNA	PLB	Cells, large animal models of HF	PLB inhibition/silencing	Improved systolic and diastolic function, normalization of dilation and hypertrophy, reduced fibrosis
Antisense RNA			PLB phosphorylation	
AV/S16E				
Aptamers				
Hydralazine	SERCA2a	Patients with HF	Demethylation of the promoter region of SERCA2a	Ca ²⁺ homeostasis correction
Glucocorticoids	PLB	Patients with I/R	PLB phosphorylation, Unaltered Ca ²⁺ transients, reduced calpain activation	Improved myocardial function after cardiopulmonary bypass
Istaroxime	PLB Na ²⁺ /K ⁺	Large animal models of HF, patients with HF	PLB inhibition, decrease of Na ²⁺ /K ⁺ -ATPase activity	Decreased heart rate, cardiac contractility, improved pulmonary capillary wedge pressure
Resveratrol	SIRT1 PI3 kinase-Akt-SERCA2a	Laboratory animals	Increased SERCA2a activity and Ca ²⁺ transient	Improved contractile amplitude, reduced relengthening time and superoxide generation
Losartan/Enalapril	SERCA2a RAS	Patients	Prevented downregulation of SERCA2a, RyR2, PLB and CSQ	Partial improvement in LV function
N106	SUMO1	Cells, mouse model of HF	SUMOylation of SERCA2a	Improved hemodynamic performance and

				reduced mortality among the animals with HF
antagomiR-25	miR-25	Mice models of HF	SERCA2a restoring	Improved cardiac function and survival
Diltiazem	Calcium antagonist	Patients		
JTV519	RyR2	Large animals with AT	Decreased Ca ²⁺ leak from SR	Improved cardiac function
	RyR2/Calstabin2 complex		Stabilization of the RyR2/Calstabin2 complex to prevent Ca ²⁺ release	
S107	RyR2/Calstabin2 complex	Humans with AT, HF and CPVT	Prevention of the Ca ²⁺ leakage from the SR	
Dantrolene	CaM/RyR2 complex	Humans with CPVT, treatment of ATs following I/R in large animal models	Stabilization of CaM/RyR2 complex, inhibition of cytosolic Ca ²⁺ sparks in diastole	Prevention of DAD, antiarrhythmic
Flecainide	RyR2	Patients	Correction of aberrant RyR2 activity, blocking the open state of RyR2, decreased spontaneous Ca ²⁺ leak	Completely prevention of CPVT
Tetracaine and derivatives (EL1-9), EL20	RyR2	Laboratory animals	Inhibition of RyR2 and Ca ²⁺ leak	Prevented the induction of ventricular tachycardia without affecting heart rate or cardiac contractility
Benzothiazepines	LTCCs	Patients with HCM, CHF, CH, angina pectoris and cardiac ATs	Ca ²⁺ channel blockers	Decreased traits of hypertrophy, AT and improved contractility
Dihydropyridines				
Phenylalkylamines				
SEA0400	NCX	Cells, large animal models of I/R	Inhibition of NCX and Ca ²⁺ -induced cell death	Recovery of LV function after reperfusion and decreased of infarct size and incidence of ventricular fibrillations.
SN-6				
YM-244769				
GSK255B + GSK503A	TRCP3 TRCP6	Cell models of CH	TRCPs inhibitors, antagonize either the molecular signaling inducing CH or reduced the phosphorylation status of many	N/A

			proteins involved in Ca ²⁺ handling	
Pyr-3	TRCP3	Cells	TRCP3 inhibitor, inhibition of Ca ²⁺ influx, modulation of NFAT	Reduced hypertrophy
C31	TRPC1/4/5 complex	Cells	N/A	N/A
KN-93	CaMKII LTCCs IP3Rs CaM	Cells	Inhibition of Ca ²⁺ currents	N/A
AS105	CaMKII	Cells, laboratory animals	Inhibition of CaMKII, reduced SR Ca ²⁺ leak	Reduced arrhythmia, increased contractility in failing myocardium, improved cardiac parameters linked to contractility
GS-680				
RA306				
AIP				
AC3-I		Large animal with CH and ATs	Interaction and block of CaMKII	N/A
CN19o		Cells		

4. Conclusions

From the literature reviewed above, it emerges that the knowledge of the tasks accomplished by each of Ca²⁺-dependent proteins in the heart physiology and how they become differentially impaired in diseases is essential to draw a successfully targeted therapy. Indeed, Ca²⁺ cycling involves a finely tuned and dynamic spatio-temporal waves to achieve a proper contraction-relaxation with high frequency, thousands of times, every day. Overall, there is a good understanding about Ca²⁺ dysregulation in cardiac diseases; now, the efforts should be more directed towards drugs developing and in translating results from preclinical models to patients.

Also, proper drug delivery methods are needed to increase bioavailability, to better target only specific cardiac protein isoforms and minimize off-target effects.

Acknowledgements

1138 The Signal Transduction Laboratory is supported by the Italian Association for Cancer Research (IG-
 1139 23670 to P.P. and IG-19803 to C.G.), A-ROSE, Progetti di Rilevante Interesse Nazionale
 1140 (PRIN2017E5L5P3 to P.P. and PRIN20177E9EPY to C.G.), the Italian Ministry of Health (GR-2013-
 1141 02356747 to C.G. and GR-2019-12369862 to G.M.), the European Research Council (853057-
 1142 InflaPML to C.G.) and local funds from the University of Ferrara to C.G. and P.P. P.P. is grateful to
 1143 C. degli Scrovegni for her continuous support. M.R.W. was supported by the Polish National Science
 1144 Centre (Grant: UMO-2014/15/B/NZ1/00490).

1145

1146 References

- 1147 [1] W.A. Catterall, Voltage-gated calcium channels, *Cold Spring Harb Perspect Biol.* 3 (2011)
 1148 a003947. <https://doi.org/10.1101/cshperspect.a003947>.
- 1149 [2] F. Hofmann, V. Flockerzi, S. Kahl, J.W. Wegener, L-type CaV1.2 calcium channels: from in
 1150 vitro findings to in vivo function, *Physiol Rev.* 94 (2014) 303–326.
 1151 <https://doi.org/10.1152/physrev.00016.2013>.
- 1152 [3] M. Prakriya, R.S. Lewis, Store-Operated Calcium Channels, *Physiol Rev.* 95 (2015) 1383–
 1153 1436. <https://doi.org/10.1152/physrev.00020.2014>.
- 1154 [4] M.D. Bootman, K. Rietdorf, Tissue Specificity: Store-Operated Ca²⁺ Entry in Cardiac
 1155 Myocytes, *Adv Exp Med Biol.* 993 (2017) 363–387. [https://doi.org/10.1007/978-3-319-57732-](https://doi.org/10.1007/978-3-319-57732-6_19)
 1156 [6_19](https://doi.org/10.1007/978-3-319-57732-6_19).
- 1157 [5] P. Rosenberg, H. Zhang, V.G. Bryson, C. Wang, SOCE in the cardiomyocyte: the secret is
 1158 in the chambers, *Pflugers Arch.* 473 (2021) 417–434. <https://doi.org/10.1007/s00424-021-02540-3>.
- 1159 [6] G.M. Salido, I. Jardín, J.A. Rosado, The TRPC ion channels: association with Orai1 and
 1160 STIM1 proteins and participation in capacitative and non-capacitative calcium entry, *Adv Exp Med*
 1161 *Biol.* 704 (2011) 413–433. https://doi.org/10.1007/978-94-007-0265-3_23.
- 1162 [7] M.Y. Asghar, K. Törnquist, Transient Receptor Potential Canonical (TRPC) Channels as
 1163 Modulators of Migration and Invasion, *Int J Mol Sci.* 21 (2020) E1739.
 1164 <https://doi.org/10.3390/ijms21051739>.
- 1165 [8] T. Hof, S. Chaigne, A. Récalde, L. Sallé, F. Brette, R. Guinamard, Transient receptor
 1166 potential channels in cardiac health and disease, *Nat Rev Cardiol.* 16 (2019) 344–360.
 1167 <https://doi.org/10.1038/s41569-018-0145-2>.
- 1168 [9] N.L. Jernigan, M.L. Paffett, B.R. Walker, T.C. Resta, ASIC1 contributes to pulmonary
 1169 vascular smooth muscle store-operated Ca(2+) entry, *Am J Physiol Lung Cell Mol Physiol.* 297
 1170 (2009) L271–285. <https://doi.org/10.1152/ajplung.00020.2009>.
- 1171 [10] X. Liu, C. Liu, J. Ye, S. Zhang, K. Wang, R. Su, Distribution of Acid Sensing Ion Channels
 1172 in Axonal Growth Cones and Presynaptic Membrane of Cultured Hippocampal Neurons, *Front Cell*
 1173 *Neurosci.* 14 (2020) 205. <https://doi.org/10.3389/fncel.2020.00205>.
- 1174 [11] X.-H. Zhang, T. Šarić, N.Z. Mehrjardi, S. Hamad, M. Morad, Acid-Sensitive Ion Channels
 1175 Are Expressed in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes, *Stem Cells Dev.*
 1176 28 (2019) 920–932. <https://doi.org/10.1089/scd.2018.0234>.
- 1177 [12] M.A. Redd, S.E. Scheuer, N.J. Saez, Y. Yoshikawa, H.S. Chiu, L. Gao, M. Hicks, J.E.
 1178 Villanueva, Y. Joshi, C.Y. Chow, G. Cuellar-Partida, J.N. Peart, L.E. See Hoe, X. Chen, Y. Sun,
 1179 J.Y. Suen, R.J. Hatch, B. Rollo, D. Xia, M.A.H. Alzubaidi, S. Maljevic, G.A. Quaife-Ryan, J.E.

1180 Hudson, E.R. Porrello, M.Y. White, S.J. Cordwell, J.F. Fraser, S. Petrou, M.E. Reichelt, W.G.
 1181 Thomas, G.F. King, P.S. Macdonald, N.J. Palpant, Therapeutic Inhibition of Acid-Sensing Ion
 1182 Channel 1a Recovers Heart Function After Ischemia-Reperfusion Injury, *Circulation*. 144 (2021)
 1183 947–960. <https://doi.org/10.1161/CIRCULATIONAHA.121.054360>.
 1184 [13] F. Di Virgilio, P2X receptors and inflammation, *Curr Med Chem*. 22 (2015) 866–877.
 1185 <https://doi.org/10.2174/0929867322666141210155311>.
 1186 [14] Q. Mei, B.T. Liang, P2 purinergic receptor activation enhances cardiac contractility in
 1187 isolated rat and mouse hearts, *Am J Physiol Heart Circ Physiol*. 281 (2001) H334–341.
 1188 <https://doi.org/10.1152/ajpheart.2001.281.1.H334>.
 1189 [15] I.S. Gurung, A. Kalin, A.A. Grace, C.L.-H. Huang, Activation of purinergic receptors by
 1190 ATP induces ventricular tachycardia by membrane depolarization and modifications of Ca²⁺
 1191 homeostasis, *J Mol Cell Cardiol*. 47 (2009) 622–633. <https://doi.org/10.1016/j.yjmcc.2009.08.001>.
 1192 [16] G.J. Jeffs, B.P. Meloni, A.J. Bakker, N.W. Knuckey, The role of the Na⁽⁺⁾/Ca⁽²⁺⁾
 1193 exchanger (NCX) in neurons following ischaemia, *J Clin Neurosci*. 14 (2007) 507–514.
 1194 <https://doi.org/10.1016/j.jocn.2006.07.013>.
 1195 [17] J. Krebs, The Plasma Membrane Calcium Pump (PMCA): Regulation of Cytosolic Ca²⁺,
 1196 Genetic Diversities and Its Role in Sub-plasma Membrane Microdomains, *Adv Exp Med Biol*. 981
 1197 (2017) 3–21. https://doi.org/10.1007/978-3-319-55858-5_1.
 1198 [18] X. Wu, B. Chang, N.S. Blair, M. Sargent, A.J. York, J. Robbins, G.E. Shull, J.D. Molkentin,
 1199 Plasma membrane Ca²⁺-ATPase isoform 4 antagonizes cardiac hypertrophy in association with
 1200 calcineurin inhibition in rodents, *J Clin Invest*. 119 (2009) 976–985.
 1201 <https://doi.org/10.1172/JCI36693>.
 1202 [19] Y. Wang, C. Wilson, E.J. Cartwright, M. Lei, Plasma membrane Ca²⁺ -ATPase 1 is
 1203 required for maintaining atrial Ca²⁺ homeostasis and electrophysiological stability in the mouse, *J*
 1204 *Physiol*. 595 (2017) 7383–7398. <https://doi.org/10.1113/JP274110>.
 1205 [20] N.A. Beard, D.R. Laver, A.F. Dulhunty, Calsequestrin and the calcium release channel of
 1206 skeletal and cardiac muscle, *Prog Biophys Mol Biol*. 85 (2004) 33–69.
 1207 <https://doi.org/10.1016/j.pbiomolbio.2003.07.001>.
 1208 [21] A. Danese, S. Marchi, V.A.M. Vitto, L. Modesti, S. Leo, M.R. Wieckowski, C. Giorgi, P.
 1209 Pinton, Cancer-Related Increases and Decreases in Calcium Signaling at the Endoplasmic
 1210 Reticulum-Mitochondria Interface (MAMs), *Rev Physiol Biochem Pharmacol*. (2020).
 1211 https://doi.org/10.1007/112_2020_43.
 1212 [22] G. Santulli, G. Monaco, V. Parra, G. Morciano, Editorial: Mitochondrial Remodeling and
 1213 Dynamic Inter-Organellar Contacts in Cardiovascular Physiopathology, *Front Cell Dev Biol*. 9
 1214 (2021) 679725. <https://doi.org/10.3389/fcell.2021.679725>.
 1215 [23] A. Bartok, D. Weaver, T. Golenár, Z. Nichtova, M. Katona, S. Bánsághi, K.J. Alzayady,
 1216 V.K. Thomas, H. Ando, K. Mikoshiba, S.K. Joseph, D.I. Yule, G. Csordás, G. Hajnóczky, IP3
 1217 receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer, *Nat*
 1218 *Commun*. 10 (2019) 3726. <https://doi.org/10.1038/s41467-019-11646-3>.
 1219 [24] M.C. Moschella, A.R. Marks, Inositol 1,4,5-trisphosphate receptor expression in cardiac
 1220 myocytes, *J Cell Biol*. 120 (1993) 1137–1146. <https://doi.org/10.1083/jcb.120.5.1137>.
 1221 [25] J.T. Lanner, D.K. Georgiou, A.D. Joshi, S.L. Hamilton, Ryanodine receptors: structure,
 1222 expression, molecular details, and function in calcium release, *Cold Spring Harb Perspect Biol*. 2
 1223 (2010) a003996. <https://doi.org/10.1101/cshperspect.a003996>.
 1224 [26] R. Lewis, J. Li, P.J. McCormick, C. L-H Huang, K. Jeevaratnam, Is the sigma-1 receptor a
 1225 potential pharmacological target for cardiac pathologies? A systematic review, *Int J Cardiol Heart*
 1226 *Vasc*. 26 (2020) 100449. <https://doi.org/10.1016/j.ijcha.2019.100449>.
 1227 [27] I. Bezprozvanny, B.E. Ehrlich, ATP modulates the function of inositol 1,4,5-trisphosphate-
 1228 gated channels at two sites, *Neuron*. 10 (1993) 1175–1184. [https://doi.org/10.1016/0896-6273\(93\)90065-y](https://doi.org/10.1016/0896-6273(93)90065-y).
 1229

1230 [28] G. Csordás, C. Renken, P. Várnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A.
1231 Mannella, G. Hajnóczky, Structural and functional features and significance of the physical linkage
1232 between ER and mitochondria, *J Cell Biol.* 174 (2006) 915–921.
1233 <https://doi.org/10.1083/jcb.200604016>.

1234 [29] R. Stoica, K.J. De Vos, S. Paillusson, S. Mueller, R.M. Sancho, K.-F. Lau, G. Vizcay-
1235 Barrena, W.-L. Lin, Y.-F. Xu, J. Lewis, D.W. Dickson, L. Petrucelli, J.C. Mitchell, C.E. Shaw,
1236 C.C.J. Miller, ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and
1237 are disrupted by ALS/FTD-associated TDP-43, *Nat Commun.* 5 (2014) 3996.
1238 <https://doi.org/10.1038/ncomms4996>.

1239 [30] S. Marchi, C. Giorgi, L. Galluzzi, P. Pinton, Ca²⁺ Fluxes and Cancer, *Mol Cell.* 78 (2020)
1240 1055–1069. <https://doi.org/10.1016/j.molcel.2020.04.017>.

1241 [31] A. Rimessi, G. Pedriali, B. Vezzani, A. Tarocco, S. Marchi, M.R. Wieckowski, C. Giorgi, P.
1242 Pinton, Interorganellar calcium signaling in the regulation of cell metabolism: A cancer perspective,
1243 *Semin Cell Dev Biol.* 98 (2020) 167–180. <https://doi.org/10.1016/j.semcdb.2019.05.015>.

1244 [32] R. Rizzuto, A.W. Simpson, M. Brini, T. Pozzan, Rapid changes of mitochondrial Ca²⁺
1245 revealed by specifically targeted recombinant aequorin, *Nature.* 358 (1992) 325–327.
1246 <https://doi.org/10.1038/358325a0>.

1247 [33] T. Rostovtseva, M. Colombini, ATP flux is controlled by a voltage-gated channel from the
1248 mitochondrial outer membrane, *J Biol Chem.* 271 (1996) 28006–28008.
1249 <https://doi.org/10.1074/jbc.271.45.28006>.

1250 [34] M.Y. Liu, M. Colombini, Regulation of mitochondrial respiration by controlling the
1251 permeability of the outer membrane through the mitochondrial channel, VDAC, *Biochim Biophys*
1252 *Acta.* 1098 (1992) 255–260. [https://doi.org/10.1016/s0005-2728\(05\)80344-5](https://doi.org/10.1016/s0005-2728(05)80344-5).

1253 [35] M. Zizi, M. Forte, E. Blachly-Dyson, M. Colombini, NADH regulates the gating of VDAC,
1254 the mitochondrial outer membrane channel, *J Biol Chem.* 269 (1994) 1614–1616.

1255 [36] E. Pavlov, S.M. Grigoriev, L.M. Dejean, C.L. Zweihorn, C.A. Mannella, K.W. Kinnally,
1256 The mitochondrial channel VDAC has a cation-selective open state, *Biochim Biophys Acta.* 1710
1257 (2005) 96–102. <https://doi.org/10.1016/j.bbabbio.2005.09.006>.

1258 [37] A. Messina, S. Reina, F. Guarino, V. De Pinto, VDAC isoforms in mammals, *Biochim*
1259 *Biophys Acta.* 1818 (2012) 1466–1476. <https://doi.org/10.1016/j.bbammem.2011.10.005>.

1260 [38] E.H.Y. Cheng, T.V. Sheiko, J.K. Fisher, W.J. Craigen, S.J. Korsmeyer, VDAC2 inhibits
1261 BAK activation and mitochondrial apoptosis, *Science.* 301 (2003) 513–517.
1262 <https://doi.org/10.1126/science.1083995>.

1263 [39] C.P. Baines, R.A. Kaiser, T. Sheiko, W.J. Craigen, J.D. Molkenin, Voltage-dependent anion
1264 channels are dispensable for mitochondrial-dependent cell death, *Nat Cell Biol.* 9 (2007) 550–555.
1265 <https://doi.org/10.1038/ncb1575>.

1266 [40] M.J. Sampson, W.K. Decker, A.L. Beaudet, W. Ruitenbeek, D. Armstrong, M.J. Hicks, W.J.
1267 Craigen, Immobile sperm and infertility in mice lacking mitochondrial voltage-dependent anion
1268 channel type 3, *J Biol Chem.* 276 (2001) 39206–39212. <https://doi.org/10.1074/jbc.M104724200>.

1269 [41] K. Hill, K. Model, M.T. Ryan, K. Dietmeier, F. Martin, R. Wagner, N. Pfanner, Tom40
1270 forms the hydrophilic channel of the mitochondrial import pore for preproteins [see comment],
1271 *Nature.* 395 (1998) 516–521. <https://doi.org/10.1038/26780>.

1272 [42] G. Szabadkai, K. Bianchi, P. Várnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I.
1273 Nagy, T. Balla, R. Rizzuto, Chaperone-mediated coupling of endoplasmic reticulum and
1274 mitochondrial Ca²⁺ channels, *J Cell Biol.* 175 (2006) 901–911.
1275 <https://doi.org/10.1083/jcb.200608073>.

1276 [43] D. De Stefani, A. Bononi, A. Romagnoli, A. Messina, V. De Pinto, P. Pinton, R. Rizzuto,
1277 VDAC1 selectively transfers apoptotic Ca²⁺ signals to mitochondria, *Cell Death Differ.* 19 (2012)
1278 267–273. <https://doi.org/10.1038/cdd.2011.92>.

1279 [44] W.M. Rosencrans, V.M. Aguilera, T.K. Rostovtseva, S.M. Bezrukov, α -Synuclein emerges

as a potent regulator of VDAC-facilitated calcium transport, *Cell Calcium*. 95 (2021) 102355.
<https://doi.org/10.1016/j.ceca.2021.102355>.

[45] W. Peng, Y.C. Wong, D. Krainc, Mitochondria-lysosome contacts regulate mitochondrial Ca^{2+} dynamics via lysosomal TRPML1, *Proc Natl Acad Sci U S A*. 117 (2020) 19266–19275.
<https://doi.org/10.1073/pnas.2003236117>.

[46] P.A. Gurnev, T.K. Rostovtseva, S.M. Bezrukov, Tubulin-blocked state of VDAC studied by polymer and ATP partitioning, *FEBS Lett*. 585 (2011) 2363–2366.
<https://doi.org/10.1016/j.febslet.2011.06.008>.

[47] M.W. McEnery, A.M. Snowman, R.R. Trifiletti, S.H. Snyder, Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier, *Proc Natl Acad Sci U S A*. 89 (1992) 3170–3174.
<https://doi.org/10.1073/pnas.89.8.3170>.

[48] M. Fan, J. Zhang, C.-W. Tsai, B.J. Orlando, M. Rodriguez, Y. Xu, M. Liao, M.-F. Tsai, L. Feng, Structure and mechanism of the mitochondrial Ca^{2+} uniporter holocomplex, *Nature*. 582 (2020) 129–133. <https://doi.org/10.1038/s41586-020-2309-6>.

[49] K.J. Kamer, Z. Grabarek, V.K. Mootha, High-affinity cooperative Ca^{2+} binding by MICU1-MICU2 serves as an on-off switch for the uniporter, *EMBO Rep*. 18 (2017) 1397–1411.
<https://doi.org/10.15252/embr.201643748>.

[50] H. Vais, R. Payne, U. Paudel, C. Li, J.K. Foskett, Coupled transmembrane mechanisms control MCU-mediated mitochondrial Ca^{2+} uptake, *Proc Natl Acad Sci U S A*. 117 (2020) 21731–21739. <https://doi.org/10.1073/pnas.2005976117>.

[51] A.P. Wescott, J.P.Y. Kao, W.J. Lederer, L. Boyman, Voltage-energized Calcium-sensitive ATP Production by Mitochondria, *Nat Metab*. 1 (2019) 975–984. <https://doi.org/10.1038/s42255-019-0126-8>.

[52] Y. Sancak, A.L. Markhard, T. Kitami, E. Kovács-Bogdán, K.J. Kamer, N.D. Udeshi, S.A. Carr, D. Chaudhuri, D.E. Clapham, A.A. Li, S.E. Calvo, O. Goldberger, V.K. Mootha, EMRE is an essential component of the mitochondrial calcium uniporter complex, *Science*. 342 (2013) 1379–1382. <https://doi.org/10.1126/science.1242993>.

[53] S. Magi, S. Piccirillo, A. Preziuso, S. Amoroso, V. Lariccia, Mitochondrial localization of NCXs: Balancing calcium and energy homeostasis, *Cell Calcium*. 86 (2020) 102162.
<https://doi.org/10.1016/j.ceca.2020.102162>.

[54] P. Altmann, P. Wagenbichler, A. Schaller, A casuistic report on the Gruber or Meckel syndrome, *Hum Genet*. 38 (1977) 357–362. <https://doi.org/10.1007/BF00402165>.

[55] M.P. Blaustein, W.J. Lederer, Sodium/calcium exchange: its physiological implications, *Physiol Rev*. 79 (1999) 763–854. <https://doi.org/10.1152/physrev.1999.79.3.763>.

[56] E. Carafoli, R. Tiozzo, G. Lugli, F. Crovetto, C. Kratzing, The release of calcium from heart mitochondria by sodium, *J Mol Cell Cardiol*. 6 (1974) 361–371. [https://doi.org/10.1016/0022-2828\(74\)90077-7](https://doi.org/10.1016/0022-2828(74)90077-7).

[57] E. Carafoli, K. Malmström, E. Sigel, M. Crompton, The regulation of intracellular calcium, *Clin Endocrinol (Oxf)*. 5 Suppl (1976) 49S–59S. <https://doi.org/10.1111/j.1365-2265.1976.tb03815.x>.

[58] A. Minelli, P. Castaldo, P. Gobbi, S. Salucci, S. Magi, S. Amoroso, Cellular and subcellular localization of Na^{+} - Ca^{2+} exchanger protein isoforms, NCX1, NCX2, and NCX3 in cerebral cortex and hippocampus of adult rat, *Cell Calcium*. 41 (2007) 221–234.
<https://doi.org/10.1016/j.ceca.2006.06.004>.

[59] R. Palty, W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, D. Khananshvil, I. Sekler, NCLX is an essential component of mitochondrial Na^{+} / Ca^{2+} exchange, *Proc Natl Acad Sci U S A*. 107 (2010) 436–441.
<https://doi.org/10.1073/pnas.0908099107>.

[60] W. Li, Z. Shariat-Madar, M. Powers, X. Sun, R.D. Lane, K.D. Garlid, Reconstitution,

1330 identification, purification, and immunological characterization of the 110-kDa Na⁺/Ca²⁺
 1331 antiporter from beef heart mitochondria, *J Biol Chem.* 267 (1992) 17983–17989.
 1332 [61] P. Jadiya, D.W. Kolmetzky, D. Tomar, A. Di Meco, A.A. Lombardi, J.P. Lambert, T.S.
 1333 Luongo, M.H. Ludtmann, D. Praticò, J.W. Elrod, Impaired mitochondrial calcium efflux
 1334 contributes to disease progression in models of Alzheimer's disease, *Nat Commun.* 10 (2019) 3885.
 1335 <https://doi.org/10.1038/s41467-019-11813-6>.
 1336 [62] X. Ren, D.A. Nicoll, G. Galang, K.D. Philipson, Intermolecular cross-linking of Na⁺-Ca²⁺
 1337 exchanger proteins: evidence for dimer formation, *Biochemistry.* 47 (2008) 6081–6087.
 1338 <https://doi.org/10.1021/bi800177t>.
 1339 [63] L.H. Hayat, M. Crompton, The effects of Mg²⁺ and adenine nucleotides on the sensitivity of
 1340 the heart mitochondrial Na⁺-Ca²⁺ carrier to extramitochondrial Ca²⁺. A study using arsenazo III-
 1341 loaded mitochondria, *Biochem J.* 244 (1987) 533–538. <https://doi.org/10.1042/bj2440533>.
 1342 [64] E. Ligeti, J. Bodnar, E. Karoly, E. Lindner, Ni²⁺, a new inhibitor of mitochondrial calcium
 1343 transport, *Biochim Biophys Acta.* 656 (1981) 177–182. [https://doi.org/10.1016/0005-](https://doi.org/10.1016/0005-2787(81)90084-8)
 1344 [2787\(81\)90084-8](https://doi.org/10.1016/0005-2787(81)90084-8).
 1345 [65] G.L. Lukács, A. Fonyó, The Ba²⁺ sensitivity of the Na⁺-induced Ca²⁺ efflux in heart
 1346 mitochondria: the site of inhibitory action, *Biochim Biophys Acta.* 858 (1986) 125–134.
 1347 [https://doi.org/10.1016/0005-2736\(86\)90298-1](https://doi.org/10.1016/0005-2736(86)90298-1).
 1348 [66] M. Kostic, I. Sekler, Functional properties and mode of regulation of the mitochondrial
 1349 Na⁺/Ca²⁺ exchanger, NCLX, *Semin Cell Dev Biol.* 94 (2019) 59–65.
 1350 <https://doi.org/10.1016/j.semcdb.2019.01.009>.
 1351 [67] L. Annunziato, G. Pignataro, G.F. Di Renzo, Pharmacology of brain Na⁺/Ca²⁺ exchanger:
 1352 from molecular biology to therapeutic perspectives, *Pharmacol Rev.* 56 (2004) 633–654.
 1353 <https://doi.org/10.1124/pr.56.4.5>.
 1354 [68] X. Cai, J. Lytton, The cation/Ca(2+) exchanger superfamily: phylogenetic analysis and
 1355 structural implications, *Mol Biol Evol.* 21 (2004) 1692–1703.
 1356 <https://doi.org/10.1093/molbev/msh177>.
 1357 [69] S. Roy, K. Dey, M. Hershfinkel, E. Ohana, I. Sekler, Identification of residues that control
 1358 Li⁺ versus Na⁺ dependent Ca²⁺ exchange at the transport site of the mitochondrial NCLX,
 1359 *Biochim Biophys Acta Mol Cell Res.* 1864 (2017) 997–1008.
 1360 <https://doi.org/10.1016/j.bbamcr.2017.01.011>.
 1361 [70] L. Reilly, J. Howie, K. Wypijewski, M.L.J. Ashford, D.W. Hilgemann, W. Fuller,
 1362 Palmitoylation of the Na/Ca exchanger cytoplasmic loop controls its inactivation and internalization
 1363 during stress signaling, *FASEB J.* 29 (2015) 4532–4543. <https://doi.org/10.1096/fj.15-276493>.
 1364 [71] A. Yaradanakul, S. Feng, C. Shen, V. Lariccia, M.-J. Lin, J. Yang, null Kang T M, P. Dong,
 1365 H.L. Yin, J.P. Albanesi, D.W. Hilgemann, Dual control of cardiac Na⁺ Ca²⁺ exchange by PIP(2):
 1366 electrophysiological analysis of direct and indirect mechanisms, *J Physiol.* 582 (2007) 991–1010.
 1367 <https://doi.org/10.1113/jphysiol.2007.132712>.
 1368 [72] G. Morciano, V.A.M. Vitto, E. Bouhamida, C. Giorgi, P. Pinton, Mitochondrial
 1369 Bioenergetics and Dynamism in the Failing Heart, *Life (Basel).* 11 (2021) 436.
 1370 <https://doi.org/10.3390/life11050436>.
 1371 [73] I. Fernández-Ruiz, Imaging: Mitochondria shine light on heart function, *Nat Rev Cardiol.* 14
 1372 (2017) 633. <https://doi.org/10.1038/nrcardio.2017.158>.
 1373 [74] J. Piquereau, M. Novotova, D. Fortin, A. Garnier, R. Ventura-Clapier, V. Veksler, F.
 1374 Joubert, Postnatal development of mouse heart: formation of energetic microdomains, *J Physiol.*
 1375 588 (2010) 2443–2454. <https://doi.org/10.1113/jphysiol.2010.189670>.
 1376 [75] J. Gambardella, B. Trimarco, G. Iaccarino, G. Santulli, New Insights in Cardiac Calcium
 1377 Handling and Excitation-Contraction Coupling, *Adv Exp Med Biol.* 1067 (2018) 373–385.
 1378 https://doi.org/10.1007/5584_2017_106.
 1379 [76] N. Traaseth, S. Elfering, J. Solien, V. Haynes, C. Giulivi, Role of calcium signaling in the

1380 activation of mitochondrial nitric oxide synthase and citric acid cycle, *Biochim Biophys Acta*. 1658
1381 (2004) 64–71. <https://doi.org/10.1016/j.bbabbio.2004.04.015>.

1382 [77] L.S. Jouaville, P. Pinton, C. Bastianutto, G.A. Rutter, R. Rizzuto, Regulation of
1383 mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming, *Proc Natl*
1384 *Acad Sci U S A*. 96 (1999) 13807–13812. <https://doi.org/10.1073/pnas.96.24.13807>.

1385 [78] R. Moreno-Sánchez, B.A. Hogue, R.G. Hansford, Influence of NAD-linked dehydrogenase
1386 activity on flux through oxidative phosphorylation, *Biochem J*. 268 (1990) 421–428.
1387 <https://doi.org/10.1042/bj2680421>.

1388 [79] B. Glancy, W.T. Willis, D.J. Chess, R.S. Balaban, Effect of calcium on the oxidative
1389 phosphorylation cascade in skeletal muscle mitochondria, *Biochemistry*. 52 (2013) 2793–2809.
1390 <https://doi.org/10.1021/bi3015983>.

1391 [80] P.R. Territo, V.K. Mootha, S.A. French, R.S. Balaban, Ca(2+) activation of heart
1392 mitochondrial oxidative phosphorylation: role of the F(0)/F(1)-ATPase, *Am J Physiol Cell Physiol*.
1393 278 (2000) C423–435. <https://doi.org/10.1152/ajpcell.2000.278.2.C423>.

1394 [81] M. Boerries, P. Most, J.R. Gledhill, J.E. Walker, H.A. Katus, W.J. Koch, U. Aebi, C.-A.
1395 Schoenenberger, Ca²⁺-dependent interaction of S100A1 with F1-ATPase leads to an increased
1396 ATP content in cardiomyocytes, *Mol Cell Biol*. 27 (2007) 4365–4373.
1397 <https://doi.org/10.1128/MCB.02045-06>.

1398 [82] Y. Kirichok, G. Krapivinsky, D.E. Clapham, The mitochondrial calcium uniporter is a
1399 highly selective ion channel, *Nature*. 427 (2004) 360–364. <https://doi.org/10.1038/nature02246>.

1400 [83] Y. Yan, J. Liu, C. Wei, K. Li, W. Xie, Y. Wang, H. Cheng, Bidirectional regulation of Ca²⁺
1401 sparks by mitochondria-derived reactive oxygen species in cardiac myocytes, *Cardiovasc Res*. 77
1402 (2008) 432–441. <https://doi.org/10.1093/cvr/cvm047>.

1403 [84] R. Pavasini, F. D’Ascenzo, G. Campo, Moving forward from statistical to clinical
1404 considerations regarding complete revascularization, *Eur Heart J*. 41 (2020) 2225.
1405 <https://doi.org/10.1093/eurheartj/ehaa351>.

1406 [85] S. Biscaglia, V. Guiducci, A. Santarelli, I. Amat Santos, F. Fernandez-Aviles, V. Lanzilotti,
1407 F. Varbella, L. Fileti, R. Moreno, F. Giannini, I. Colaiori, M. Menozzi, A. Redondo, M. Ruozzi, E.
1408 Gutiérrez Ibañes, J.L. Díez Gil, E. Maietti, G. Biondi Zoccai, J. Escaned, M. Tebaldi, E. Barbato, D.
1409 Dudek, A. Colombo, G. Campo, Physiology-guided revascularization versus optimal medical
1410 therapy of nonculprit lesions in elderly patients with myocardial infarction: Rationale and design of
1411 the FIRE trial, *Am Heart J*. 229 (2020) 100–109. <https://doi.org/10.1016/j.ahj.2020.08.007>.

1412 [86] C.J.A. Ramachandra, S. Hernandez-Resendiz, G.E. Crespo-Avilan, Y.-H. Lin, D.J.
1413 Hausenloy, Mitochondria in acute myocardial infarction and cardioprotection, *EBioMedicine*. 57
1414 (2020) 102884. <https://doi.org/10.1016/j.ebiom.2020.102884>.

1415 [87] S. Leonardi, C. Montalto, G. Casella, D. Grosseto, A. Repetto, M. Portolan, F. Fortuni, F.
1416 Ottani, M. Galvani, L.S. Cardelli, S. De Servi, A. Rubboli, G.M. De Ferrari, L. Oltrona Visconti, G.
1417 Campo, Clinical governance programme in patients with acute coronary syndrome: design and
1418 methodology of a quality improvement initiative, *Open Heart*. 7 (2020) e001415.
1419 <https://doi.org/10.1136/openhrt-2020-001415>.

1420 [88] E. Tonet, D. Bernucci, G. Morciano, G. Campo, Pharmacological protection of reperfusion
1421 injury in ST-segment elevation myocardial infarction. Gone with the wind?, *Postepy Kardiol*
1422 *Interwencyjne*. 14 (2018) 5–8. <https://doi.org/10.5114/aic.2018.74349>.

1423 [89] M. Dambrova, C.J. Zuurbier, V. Borutaite, E. Liepinsh, M. Makrecka-Kuka, Energy
1424 substrate metabolism and mitochondrial oxidative stress in cardiac ischemia/reperfusion injury,
1425 *Free Radic Biol Med*. 165 (2021) 24–37. <https://doi.org/10.1016/j.freeradbiomed.2021.01.036>.

1426 [90] G. Morciano, C. Giorgi, M. Bonora, S. Punzetti, R. Pavasini, M.R. Wieckowski, G. Campo,
1427 P. Pinton, Molecular identity of the mitochondrial permeability transition pore and its role in
1428 ischemia-reperfusion injury, *J Mol Cell Cardiol*. 78 (2015) 142–153.
1429 <https://doi.org/10.1016/j.yjmcc.2014.08.015>.

1430 [91] H.M.M. Waddell, J.Z. Zhang, K.J. Hoeksema, J.J. McLachlan, J.C. McLay, P.P. Jones,
1431 Oxidation of RyR2 Has a Biphasic Effect on the Threshold for Store Overload-Induced Calcium
1432 Release, *Biophys J.* 110 (2016) 2386–2396. <https://doi.org/10.1016/j.bpj.2016.04.036>.

1433 [92] M.A. Salas, C.A. Valverde, G. Sánchez, M. Said, J.S. Rodriguez, E.L. Portiansky, M.A.
1434 Kaetzel, J.R. Dedman, P. Donoso, E.G. Kranias, A. Mattiazzi, The signalling pathway of CaMKII-
1435 mediated apoptosis and necrosis in the ischemia/reperfusion injury, *J Mol Cell Cardiol.* 48 (2010)
1436 1298–1306. <https://doi.org/10.1016/j.yjmcc.2009.12.015>.

1437 [93] M. Weinreuter, M.M. Kreusser, J. Beckendorf, F.C. Schreiter, F. Leuschner, L.H. Lehmann,
1438 K.P. Hofmann, J.S. Rostovsky, N. Diemert, C. Xu, H.C. Volz, A. Jungmann, A. Nickel, C. Sticht, N.
1439 Gretz, C. Maack, M.D. Schneider, H.-J. Gröne, O.J. Müller, H.A. Katus, J. Backs, CaM Kinase II
1440 mediates maladaptive post-infarct remodeling and pro-inflammatory chemoattractant signaling but
1441 not acute myocardial ischemia/reperfusion injury, *EMBO Mol Med.* 6 (2014) 1231–1245.
1442 <https://doi.org/10.15252/emmm.201403848>.

1443 [94] M. Bonora, M.R. Wieckowski, D.A. Sinclair, G. Kroemer, P. Pinton, L. Galluzzi, Targeting
1444 mitochondria for cardiovascular disorders: therapeutic potential and obstacles, *Nat Rev Cardiol.* 16
1445 (2019) 33–55. <https://doi.org/10.1038/s41569-018-0074-0>.

1446 [95] G. Morciano, G. Pedriali, M. Bonora, R. Pavasini, E. Mikus, S. Calvi, M. Bovolenta, M.
1447 Lebedzinska-Arciszewska, M. Pinotti, A. Albertini, M.R. Wieckowski, C. Giorgi, R. Ferrari, L.
1448 Galluzzi, G. Campo, P. Pinton, A naturally occurring mutation in ATP synthase subunit c is
1449 associated with increased damage following hypoxia/reoxygenation in STEMI patients, *Cell Rep.*
1450 35 (2021) 108983. <https://doi.org/10.1016/j.celrep.2021.108983>.

1451 [96] L. Guan, Z. Che, X. Meng, Y. Yu, M. Li, Z. Yu, H. Shi, D. Yang, M. Yu, MCU Up-
1452 regulation contributes to myocardial ischemia-reperfusion Injury through calpain/OPA-1-mediated
1453 mitochondrial fusion/mitophagy Inhibition, *J Cell Mol Med.* 23 (2019) 7830–7843.
1454 <https://doi.org/10.1111/jcmm.14662>.

1455 [97] L. Chen, Q. Gong, J.P. Stice, A.A. Knowlton, Mitochondrial OPA1, apoptosis, and heart
1456 failure, *Cardiovasc Res.* 84 (2009) 91–99. <https://doi.org/10.1093/cvr/cvp181>.

1457 [98] S. Le Page, M. Niro, J. Fauconnier, L. Cellier, S. Tamareille, A. Gharib, A. Chevrollier, L.
1458 Loufrani, C. Grenier, R. Kamel, E. Sarzi, A. Lacampagne, M. Ovize, D. Henrion, P. Reynier, G.
1459 Lenaers, D. Mirebeau-Prunier, F. Prunier, Increase in Cardiac Ischemia-Reperfusion Injuries in
1460 Opa1^{+/-} Mouse Model, *PLoS One.* 11 (2016) e0164066.
1461 <https://doi.org/10.1371/journal.pone.0164066>.

1462 [99] X. Xiao, Y. Hu, P.M. Quirós, Q. Wei, C. López-Otín, Z. Dong, OMA1 mediates OPA1
1463 proteolysis and mitochondrial fragmentation in experimental models of ischemic kidney injury, *Am*
1464 *J Physiol Renal Physiol.* 306 (2014) F1318–1326. <https://doi.org/10.1152/ajprenal.00036.2014>.

1465 [100] A.R. Hall, N. Burke, R.K. Dongworth, S.B. Kalkhoran, A. Dyson, J.M. Vicencio, G.W.
1466 Dorn, D.M. Yellon, D.J. Hausenloy, Hearts deficient in both Mfn1 and Mfn2 are protected against
1467 acute myocardial infarction, *Cell Death Dis.* 7 (2016) e2238.
1468 <https://doi.org/10.1038/cddis.2016.139>.

1469 [101] D.M. Bers, Cardiac excitation-contraction coupling, *Nature.* 415 (2002) 198–205.
1470 <https://doi.org/10.1038/415198a>.

1471 [102] J.N. Weiss, A. Garfinkel, H.S. Karagueuzian, T.P. Nguyen, R. Olcese, P.-S. Chen, Z. Qu,
1472 Perspective: a dynamics-based classification of ventricular arrhythmias, *J Mol Cell Cardiol.* 82
1473 (2015) 136–152. <https://doi.org/10.1016/j.yjmcc.2015.02.017>.

1474 [103] U. Schotten, M. Duytschaever, J. Ausma, S. Eijssbouts, H.-R. Neuberger, M. Allessie,
1475 Electrical and contractile remodeling during the first days of atrial fibrillation go hand in hand,
1476 *Circulation.* 107 (2003) 1433–1439. <https://doi.org/10.1161/01.cir.0000055314.10801.4f>.

1477 [104] X.-Y. Qi, J.G. Diness, B.J.J.M. Brundel, X.-B. Zhou, P. Naud, C.-T. Wu, H. Huang, M.
1478 Harada, M. Aflaki, D. Dobrev, M. Grunnet, S. Nattel, Role of small-conductance calcium-activated
1479 potassium channels in atrial electrophysiology and fibrillation in the dog, *Circulation.* 129 (2014)

430–440. <https://doi.org/10.1161/CIRCULATIONAHA.113.003019>.

[105] D. Terentyev, J.A. Rochira, R. Terentyeva, K. Roder, G. Koren, W. Li, Sarcoplasmic reticulum Ca^{2+} release is both necessary and sufficient for SK channel activation in ventricular myocytes, *Am J Physiol Heart Circ Physiol*. 306 (2014) H738–746. <https://doi.org/10.1152/ajpheart.00621.2013>.

[106] K. Acsai, G. Antoons, L. Livshitz, Y. Rudy, K.R. Sipido, Microdomain $[\text{Ca}^{2+}]$ near ryanodine receptors as reported by L-type Ca^{2+} and $\text{Na}^+/\text{Ca}^{2+}$ exchange currents, *J Physiol*. 589 (2011) 2569–2583. <https://doi.org/10.1113/jphysiol.2010.202663>.

[107] C.R. Weber, K.S. Ginsburg, K.D. Philipson, T.R. Shannon, D.M. Bers, Allosteric regulation of Na/Ca exchange current by cytosolic Ca in intact cardiac myocytes, *J Gen Physiol*. 117 (2001) 119–131. <https://doi.org/10.1085/jgp.117.2.119>.

[108] C.R. Weber, V. Piacentino, K.S. Ginsburg, S.R. Houser, D.M. Bers, $\text{Na}(+)-\text{Ca}(2+)$ exchange current and submembrane $[\text{Ca}(2+)]$ during the cardiac action potential, *Circ Res*. 90 (2002) 182–189. <https://doi.org/10.1161/hh0202.103940>.

[109] N. Chiamvimonvat, Y. Chen-Izu, C.E. Clancy, I. Deschenes, D. Dobrev, J. Heijman, L. Izu, Z. Qu, C.M. Ripplinger, J.I. Vandenberg, J.N. Weiss, G. Koren, T. Banyasz, E. Grandi, M.C. Sanguinetti, D.M. Bers, J.M. Nerbonne, Potassium currents in the heart: functional roles in repolarization, arrhythmia and therapeutics, *J Physiol*. 595 (2017) 2229–2252. <https://doi.org/10.1113/JP272883>.

[110] H.S. Karagueuzian, A. Pezhouman, M. Angelini, R. Olcese, Enhanced Late Na and Ca Currents as Effective Antiarrhythmic Drug Targets, *Front Pharmacol*. 8 (2017) 36. <https://doi.org/10.3389/fphar.2017.00036>.

[111] O.M. Koval, X. Guan, Y. Wu, M.-L. Joiner, Z. Gao, B. Chen, I.M. Grumbach, E.D. Luczak, R.J. Colbran, L.-S. Song, T.J. Hund, P.J. Mohler, M.E. Anderson, $\text{CaV}1.2$ beta-subunit coordinates CaMKII -triggered cardiomyocyte death and afterdepolarizations, *Proc Natl Acad Sci U S A*. 107 (2010) 4996–5000. <https://doi.org/10.1073/pnas.0913760107>.

[112] S.G. Priori, C. Napolitano, N. Tiso, M. Memmi, G. Vignati, R. Bloise, V. Sorrentino, G.A. Danielli, Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia, *Circulation*. 103 (2001) 196–200. <https://doi.org/10.1161/01.cir.103.2.196>.

[113] T.M. Bauer, E. Murphy, Role of Mitochondrial Calcium and the Permeability Transition Pore in Regulating Cell Death, *Circ Res*. 126 (2020) 280–293. <https://doi.org/10.1161/CIRCRESAHA.119.316306>.

[114] K. Kujala, J. Paavola, A. Lahti, K. Larsson, M. Pekkanen-Mattila, M. Viitasalo, A.M. Lahtinen, L. Toivonen, K. Kontula, H. Swan, M. Laine, O. Silvennoinen, K. Aalto-Setälä, Cell model of catecholaminergic polymorphic ventricular tachycardia reveals early and delayed afterdepolarizations, *PLoS One*. 7 (2012) e44660. <https://doi.org/10.1371/journal.pone.0044660>.

[115] M. Nyegaard, M.T. Overgaard, M.T. Søndergaard, M. Vranas, E.R. Behr, L.L. Hildebrandt, J. Lund, P.L. Hedley, A.J. Camm, G. Wettrell, I. Fosdal, M. Christiansen, A.D. Børghlum, Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death, *Am J Hum Genet*. 91 (2012) 703–712. <https://doi.org/10.1016/j.ajhg.2012.08.015>.

[116] H. Chen, G. Valle, S. Furlan, A. Nani, S. Gyorke, M. Fill, P. Volpe, Mechanism of calsequestrin regulation of single cardiac ryanodine receptor in normal and pathological conditions, *J Gen Physiol*. 142 (2013) 127–136. <https://doi.org/10.1085/jgp.201311022>.

[117] N. Chopra, T. Yang, P. Asghari, E.D. Moore, S. Huke, B. Akin, R.A. Cattolica, C.F. Perez, T. Hlaing, B.E.C. Knollmann-Ritschel, L.R. Jones, I.N. Pessah, P.D. Allen, C. Franzini-Armstrong, B.C. Knollmann, Ablation of triadin causes loss of cardiac Ca^{2+} release units, impaired excitation-contraction coupling, and cardiac arrhythmias, *Proc Natl Acad Sci U S A*. 106 (2009) 7636–7641. <https://doi.org/10.1073/pnas.0902919106>.

[118] A.P. Landstrom, D. Dobrev, X.H.T. Wehrens, Calcium Signaling and Cardiac Arrhythmias,

Circ Res. 120 (2017) 1969–1993. <https://doi.org/10.1161/CIRCRESAHA.117.310083>.

[119] Y.-H. Zhang, H.-J. Wu, H. Che, H.-Y. Sun, L.-C. Cheng, X. Li, W.-K. Au, H.-F. Tse, G.-R. Li, Functional transient receptor potential canonical type 1 channels in human atrial myocytes, *Pflugers Arch.* 465 (2013) 1439–1449. <https://doi.org/10.1007/s00424-013-1291-3>.

[120] Y.-H. Zhang, H.-Y. Sun, K.-H. Chen, X.-L. Du, B. Liu, L.-C. Cheng, X. Li, M.-W. Jin, G.-R. Li, Evidence for functional expression of TRPM7 channels in human atrial myocytes, *Basic Res Cardiol.* 107 (2012) 282. <https://doi.org/10.1007/s00395-012-0282-4>.

[121] G. Morciano, S. Patergnani, G. Pedriali, P. Cimaglia, E. Mikus, S. Calvi, A. Albertini, C. Giorgi, G. Campo, R. Ferrari, P. Pinton, Impairment of mitophagy and autophagy accompanies calcific aortic valve stenosis favoring cell death and the severity of disease, *Cardiovasc Res.* (2021) cvab267. <https://doi.org/10.1093/cvr/cvab267>.

[122] J.R. McMullen, G.L. Jennings, Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure, *Clin Exp Pharmacol Physiol.* 34 (2007) 255–262. <https://doi.org/10.1111/j.1440-1681.2007.04585.x>.

[123] E. Chung, J. Heimiller, L.A. Leinwand, Distinct cardiac transcriptional profiles defining pregnancy and exercise, *PLoS One.* 7 (2012) e42297. <https://doi.org/10.1371/journal.pone.0042297>.

[124] B.M.R. Carvalho, R.A. Bassani, K.G. Franchini, J.W.M. Bassani, Enhanced calcium mobilization in rat ventricular myocytes during the onset of pressure overload-induced hypertrophy, *Am J Physiol Heart Circ Physiol.* 291 (2006) H1803–1813. <https://doi.org/10.1152/ajpheart.01345.2005>.

[125] J. Nie, Q. Duan, M. He, X. Li, B. Wang, C. Zhou, L. Wu, Z. Wen, C. Chen, D.W. Wang, K.M. Alsina, X.H.T. Wehrens, D.W. Wang, L. Ni, Ranolazine prevents pressure overload-induced cardiac hypertrophy and heart failure by restoring aberrant Na⁺ and Ca²⁺ handling, *J Cell Physiol.* 234 (2019) 11587–11601. <https://doi.org/10.1002/jcp.27791>.

[126] T. Zhang, L.S. Maier, N.D. Dalton, S. Miyamoto, J. Ross, D.M. Bers, J.H. Brown, The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure, *Circ Res.* 92 (2003) 912–919. <https://doi.org/10.1161/01.RES.0000069686.31472.C5>.

[127] S. Haq, G. Choukroun, H. Lim, K.M. Tymitz, F. del Monte, J. Gwathmey, L. Grazette, A. Michael, R. Hajjar, T. Force, J.D. Molkentin, Differential activation of signal transduction pathways in human hearts with hypertrophy versus advanced heart failure, *Circulation.* 103 (2001) 670–677. <https://doi.org/10.1161/01.cir.103.5.670>.

[128] J.D. Molkentin, J.R. Lu, C.L. Antos, B. Markham, J. Richardson, J. Robbins, S.R. Grant, E.N. Olson, A calcineurin-dependent transcriptional pathway for cardiac hypertrophy, *Cell.* 93 (1998) 215–228. [https://doi.org/10.1016/s0092-8674\(00\)81573-1](https://doi.org/10.1016/s0092-8674(00)81573-1).

[129] A. Leychenko, E. Konorev, M. Jijiwa, M.L. Matter, Stretch-induced hypertrophy activates NFkB-mediated VEGF secretion in adult cardiomyocytes, *PLoS One.* 6 (2011) e29055. <https://doi.org/10.1371/journal.pone.0029055>.

[130] A.F. Leite-Moreira, Current perspectives in diastolic dysfunction and diastolic heart failure, *Heart.* 92 (2006) 712–718. <https://doi.org/10.1136/hrt.2005.062950>.

[131] I. Hay, J. Rich, P. Ferber, D. Burkhoff, M.S. Maurer, Role of impaired myocardial relaxation in the production of elevated left ventricular filling pressure, *Am J Physiol Heart Circ Physiol.* 288 (2005) H1203–1208. <https://doi.org/10.1152/ajpheart.00681.2004>.

[132] P.A. Gorski, D.K. Ceholski, R.J. Hajjar, Altered myocardial calcium cycling and energetics in heart failure--a rational approach for disease treatment, *Cell Metab.* 21 (2015) 183–194. <https://doi.org/10.1016/j.cmet.2015.01.005>.

[133] M. Arai, T. Suzuki, R. Nagai, Sarcoplasmic reticulum genes are upregulated in mild cardiac hypertrophy but downregulated in severe cardiac hypertrophy induced by pressure overload, *J Mol Cell Cardiol.* 28 (1996) 1583–1590. <https://doi.org/10.1006/jmcc.1996.0149>.

[134] R. Stüdeli, S. Jung, P. Mohacsi, S. Perruchoud, P. Castiglioni, P. Wenaweser, G. Heimbeck, M. Feller, R. Hullin, Diastolic dysfunction in human cardiac allografts is related with reduced

SERCA2a gene expression, *Am J Transplant.* 6 (2006) 775–782. <https://doi.org/10.1111/j.1600-6143.2006.01241.x>.

[135] U. Schmidt, R.J. Hajjar, C.S. Kim, D. Lebeche, A.A. Doye, J.K. Gwathmey, Human heart failure: cAMP stimulation of SR Ca(2+)-ATPase activity and phosphorylation level of phospholamban, *Am J Physiol.* 277 (1999) H474–480. <https://doi.org/10.1152/ajpheart.1999.277.2.H474>.

[136] R.H. Schwinger, G. Münch, B. Bölk, P. Karczewski, E.G. Krause, E. Erdmann, Reduced Ca(2+)-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation, *J Mol Cell Cardiol.* 31 (1999) 479–491. <https://doi.org/10.1006/jmcc.1998.0897>.

[137] E.G. Kranias, R.J. Hajjar, Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome, *Circ Res.* 110 (2012) 1646–1660. <https://doi.org/10.1161/CIRCRESAHA.111.259754>.

[138] J.P. Schmitt, M. Kamisago, M. Asahi, G.H. Li, F. Ahmad, U. Mende, E.G. Kranias, D.H. MacLennan, J.G. Seidman, C.E. Seidman, Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban, *Science.* 299 (2003) 1410–1413. <https://doi.org/10.1126/science.1081578>.

[139] K. Haghighi, T. Pritchard, J. Bossuyt, J.R. Waggoner, Q. Yuan, G.-C. Fan, H. Osinska, A. Anjak, J. Rubinstein, J. Robbins, D.M. Bers, E.G. Kranias, The human phospholamban Arg14-deletion mutant localizes to plasma membrane and interacts with the Na/K-ATPase, *J Mol Cell Cardiol.* 52 (2012) 773–782. <https://doi.org/10.1016/j.yjmcc.2011.11.012>.

[140] K. Haghighi, F. Kolokathis, L. Pater, R.A. Lynch, M. Asahi, A.O. Gramolini, G.-C. Fan, D. Tsiapras, H.S. Hahn, S. Adamopoulos, S.B. Liggett, G.W. Dorn, D.H. MacLennan, D.T. Kremastinos, E.G. Kranias, Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human, *J Clin Invest.* 111 (2003) 869–876. <https://doi.org/10.1172/JCI17892>.

[141] S.L. Hofmann, J.L. Goldstein, K. Orth, C.R. Moomaw, C.A. Slaughter, M.S. Brown, Molecular cloning of a histidine-rich Ca²⁺-binding protein of sarcoplasmic reticulum that contains highly conserved repeated elements, *J Biol Chem.* 264 (1989) 18083–18090.

[142] S.L. Hofmann, M.S. Brown, E. Lee, R.K. Pathak, R.G. Anderson, J.L. Goldstein, Purification of a sarcoplasmic reticulum protein that binds Ca²⁺ and plasma lipoproteins, *J Biol Chem.* 264 (1989) 8260–8270.

[143] C.S. Park, S. Chen, H. Lee, H. Cha, J.G. Oh, S. Hong, P. Han, K.S. Ginsburg, S. Jin, I. Park, V.P. Singh, H.-S. Wang, C. Franzini-Armstrong, W.J. Park, D.M. Bers, E.G. Kranias, C. Cho, D.H. Kim, Targeted ablation of the histidine-rich Ca(2+)-binding protein (HRC) gene is associated with abnormal SR Ca(2+)-cycling and severe pathology under pressure-overload stress, *Basic Res Cardiol.* 108 (2013) 344. <https://doi.org/10.1007/s00395-013-0344-2>.

[144] M. Flesch, R.H. Schwinger, F. Schiffer, K. Frank, M. Südkamp, F. Kuhn-Regnier, G. Arnold, M. Böhm, Evidence for functional relevance of an enhanced expression of the Na(+)-Ca²⁺ exchanger in failing human myocardium, *Circulation.* 94 (1996) 992–1002. <https://doi.org/10.1161/01.cir.94.5.992>.

[145] R.H. Schwinger, J. Wang, K. Frank, J. Müller-Ehmsen, K. Brixius, A.A. McDonough, E. Erdmann, Reduced sodium pump alpha1, alpha3, and beta1-isoform protein levels and Na⁺,K⁺-ATPase activity but unchanged Na⁺-Ca²⁺ exchanger protein levels in human heart failure, *Circulation.* 99 (1999) 2105–2112. <https://doi.org/10.1161/01.cir.99.16.2105>.

[146] V. Robert, P. Gurlini, V. Tosello, T. Nagai, A. Miyawaki, F. Di Lisa, T. Pozzan, Beat-to-beat oscillations of mitochondrial [Ca²⁺] in cardiac cells, *EMBO J.* 20 (2001) 4998–5007. <https://doi.org/10.1093/emboj/20.17.4998>.

[147] H. Tsutsui, S. Kinugawa, S. Matsushima, Oxidative stress and mitochondrial DNA damage in heart failure, *Circ J.* 72 Suppl A (2008) A31–37. <https://doi.org/10.1253/circj.cj-08-0014>.

1631 [148] T. Ohba, H. Watanabe, M. Murakami, Y. Takahashi, K. Iino, S. Kuromitsu, Y. Mori, K.
 1632 Ono, T. Iijima, H. Ito, Upregulation of TRPC1 in the development of cardiac hypertrophy, *J Mol*
 1633 *Cell Cardiol.* 42 (2007) 498–507. <https://doi.org/10.1016/j.yjmcc.2006.10.020>.
 1634 [149] E.W. Bush, D.B. Hood, P.J. Papst, J.A. Chapo, W. Minobe, M.R. Bristow, E.N. Olson, T.A.
 1635 McKinsey, Canonical transient receptor potential channels promote cardiomyocyte hypertrophy
 1636 through activation of calcineurin signaling, *J Biol Chem.* 281 (2006) 33487–33496.
 1637 <https://doi.org/10.1074/jbc.M605536200>.
 1638 [150] G. Oguri, T. Nakajima, Y. Yamamoto, N. Takano, T. Tanaka, H. Kikuchi, T. Morita, F.
 1639 Nakamura, T. Yamasoba, I. Komuro, Effects of methylglyoxal on human cardiac fibroblast: roles of
 1640 transient receptor potential ankyrin 1 (TRPA1) channels, *Am J Physiol Heart Circ Physiol.* 307
 1641 (2014) H1339–1352. <https://doi.org/10.1152/ajpheart.01021.2013>.
 1642 [151] M. Goel, C.-D. Zuo, W.G. Sinkins, W.P. Schilling, TRPC3 channels colocalize with
 1643 Na⁺/Ca²⁺ exchanger and Na⁺ pump in axial component of transverse-axial tubular system of rat
 1644 ventricle, *Am J Physiol Heart Circ Physiol.* 292 (2007) H874–883.
 1645 <https://doi.org/10.1152/ajpheart.00785.2006>.
 1646 [152] P. Elliott, B. Andersson, E. Arbustini, Z. Bilinska, F. Cecchi, P. Charron, O. Dubourg, U.
 1647 Köhl, B. Maisch, W.J. McKenna, L. Monserrat, S. Pankuweit, C. Rapezzi, P. Seferovic, L. Tavazzi,
 1648 A. Keren, Classification of the cardiomyopathies: a position statement from the European Society
 1649 Of Cardiology Working Group on Myocardial and Pericardial Diseases, *Eur Heart J.* 29 (2008)
 1650 270–276. <https://doi.org/10.1093/eurheartj/ehm342>.
 1651 [153] R. Liu, F. Sun, P. Forghani, L.C. Armand, A. Rampoldi, D. Li, R. Wu, C. Xu, Proteomic
 1652 Profiling Reveals Roles of Stress Response, Ca²⁺ Transient Dysregulation, and Novel Signaling
 1653 Pathways in Alcohol-Induced Cardiotoxicity, *Alcohol Clin Exp Res.* 44 (2020) 2187–2199.
 1654 <https://doi.org/10.1111/acer.14471>.
 1655 [154] R.G. Matteo, C.S. Moravec, Immunolocalization of annexins IV, V and VI in the failing and
 1656 non-failing human heart, *Cardiovasc Res.* 45 (2000) 961–970. [https://doi.org/10.1016/s0008-6363\(99\)00409-5](https://doi.org/10.1016/s0008-6363(99)00409-5).
 1657 [155] L. Liu, J. Wu, D.J. Kennedy, Regulation of Cardiac Remodeling by Cardiac Na⁺/K⁺-
 1658 ATPase Isoforms, *Front Physiol.* 7 (2016) 382. <https://doi.org/10.3389/fphys.2016.00382>.
 1659 [156] J. Alexandre, J.J. Moslehi, K.R. Bersell, C. Funck-Brentano, D.M. Roden, J.-E. Salem,
 1660 Anticancer drug-induced cardiac rhythm disorders: Current knowledge and basic underlying
 1661 mechanisms, *Pharmacol Ther.* 189 (2018) 89–103.
 1662 <https://doi.org/10.1016/j.pharmthera.2018.04.009>.
 1663 [157] L.E. Solem, L.J. Heller, K.B. Wallace, Dose-dependent increase in sensitivity to calcium-
 1664 induced mitochondrial dysfunction and cardiomyocyte cell injury by doxorubicin, *J Mol Cell*
 1665 *Cardiol.* 28 (1996) 1023–1032. <https://doi.org/10.1006/jmcc.1996.0095>.
 1666 [158] A.D. Hanna, A. Lam, S. Tham, A.F. Dulhunty, N.A. Beard, Adverse effects of doxorubicin
 1667 and its metabolic product on cardiac RyR2 and SERCA2A, *Mol Pharmacol.* 86 (2014) 438–449.
 1668 <https://doi.org/10.1124/mol.114.093849>.
 1669 [159] M. Arai, A. Yoguchi, T. Takizawa, T. Yokoyama, T. Kanda, M. Kurabayashi, R. Nagai,
 1670 Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca²⁺-ATPase gene
 1671 transcription, *Circ Res.* 86 (2000) 8–14. <https://doi.org/10.1161/01.res.86.1.8>.
 1672 [160] M. Arai, K. Tomaru, T. Takizawa, K. Sekiguchi, T. Yokoyama, T. Suzuki, R. Nagai,
 1673 Sarcoplasmic reticulum genes are selectively down-regulated in cardiomyopathy produced by
 1674 doxorubicin in rabbits, *J Mol Cell Cardiol.* 30 (1998) 243–254.
 1675 <https://doi.org/10.1006/jmcc.1997.0588>.
 1676 [161] S.-Y. Kim, S.-J. Kim, B.-J. Kim, S.-Y. Rah, S.M. Chung, M.-J. Im, U.-H. Kim,
 1677 Doxorubicin-induced reactive oxygen species generation and intracellular Ca²⁺ increase are
 1678 reciprocally modulated in rat cardiomyocytes, *Exp Mol Med.* 38 (2006) 535–545.
 1679 <https://doi.org/10.1038/emm.2006.63>.
 1680 [162] D. Ramaccini, V. Montoya-Urbe, F.J. Aan, L. Modesti, Y. Potes, M.R. Wieckowski, I.
 1681

Krga, M. Glibetić, P. Pinton, C. Giorgi, M.L. Matter, Mitochondrial Function and Dysfunction in Dilated Cardiomyopathy, *Front Cell Dev Biol.* 8 (2020) 624216. <https://doi.org/10.3389/fcell.2020.624216>.

[163] X. Zhao, T. Feng, H. Chen, H. Shan, Y. Zhang, Y. Lu, B. Yang, Arsenic trioxide-induced apoptosis in H9c2 cardiomyocytes: implications in cardiotoxicity, *Basic Clin Pharmacol Toxicol.* 102 (2008) 419–425. <https://doi.org/10.1111/j.1742-7843.2007.00150.x>.

[164] L.G. Rossato, V.M. Costa, V. Vilas-Boas, M. de Lourdes Bastos, A. Rolo, C. Palmeira, F. Remião, Therapeutic concentrations of mitoxantrone elicit energetic imbalance in H9c2 cells as an earlier event, *Cardiovasc Toxicol.* 13 (2013) 413–425. <https://doi.org/10.1007/s12012-013-9224-0>.

[165] F.A. Lattanzio, D. Tiangco, C. Osgood, S. Beebe, J. Kerry, B.Y. Hargrave, Cocaine increases intracellular calcium and reactive oxygen species, depolarizes mitochondria, and activates genes associated with heart failure and remodeling, *Cardiovasc Toxicol.* 5 (2005) 377–390. <https://doi.org/10.1385/ct:5:4:377>.

[166] S.K. Shenouda, K.C. Lord, E. McIlwain, P.A. Lucchesi, K.J. Varner, Ecstasy produces left ventricular dysfunction and oxidative stress in rats, *Cardiovasc Res.* 79 (2008) 662–670. <https://doi.org/10.1093/cvr/cvn129>.

[167] M. Bonora, S. Patergnani, D. Ramaccini, G. Morciano, G. Pedriali, A.E. Kahsay, E. Bouhamida, C. Giorgi, M.R. Wieckowski, P. Pinton, Physiopathology of the Permeability Transition Pore: Molecular Mechanisms in Human Pathology, *Biomolecules.* 10 (2020) E998. <https://doi.org/10.3390/biom10070998>.

[168] X. Lu, J.Q. Kwong, J.D. Molkentin, D.M. Bers, Individual Cardiac Mitochondria Undergo Rare Transient Permeability Transition Pore Openings, *Circ Res.* 118 (2016) 834–841. <https://doi.org/10.1161/CIRCRESAHA.115.308093>.

[169] M. Bonora, C. Giorgi, P. Pinton, Molecular mechanisms and consequences of mitochondrial permeability transition, *Nat Rev Mol Cell Biol.* (2021). <https://doi.org/10.1038/s41580-021-00433-y>.

[170] G. Morciano, M. Bonora, G. Campo, G. Aquila, P. Rizzo, C. Giorgi, M.R. Wieckowski, P. Pinton, Mechanistic Role of mPTP in Ischemia-Reperfusion Injury, *Adv Exp Med Biol.* 982 (2017) 169–189. https://doi.org/10.1007/978-3-319-55330-6_9.

[171] L. Galluzzi, I. Vitale, S.A. Aaronson, J.M. Abrams, D. Adam, P. Agostinis, E.S. Alnemri, L. Altucci, I. Amelio, D.W. Andrews, M. Annicchiarico-Petruzzelli, A.V. Antonov, E. Arama, E.H. Baehrecke, N.A. Barlev, N.G. Bazan, F. Bernassola, M.J.M. Bertrand, K. Bianchi, M.V. Blagosklonny, K. Blomgren, C. Borner, P. Boya, C. Brenner, M. Campanella, E. Candi, D. Carmona-Gutierrez, F. Cecconi, F.K.-M. Chan, N.S. Chandel, E.H. Cheng, J.E. Chipuk, J.A. Cidlowski, A. Ciechanover, G.M. Cohen, M. Conrad, J.R. Cubillos-Ruiz, P.E. Czabotar, V. D'Angiolella, T.M. Dawson, V.L. Dawson, V. De Laurenzi, R. De Maria, K.-M. Debatin, R.J. DeBerardinis, M. Deshmukh, N. Di Daniele, F. Di Virgilio, V.M. Dixit, S.J. Dixon, C.S. Duckett, B.D. Dynlacht, W.S. El-Deiry, J.W. Elrod, G.M. Fimia, S. Fulda, A.J. García-Sáez, A.D. Garg, C. Garrido, E. Gavathiotis, P. Golstein, E. Gottlieb, D.R. Green, L.A. Greene, H. Gronemeyer, A. Gross, G. Hajnoczky, J.M. Hardwick, I.S. Harris, M.O. Hengartner, C. Hetz, H. Ichijo, M. Jäättelä, B. Joseph, P.J. Jost, P.P. Juin, W.J. Kaiser, M. Karin, T. Kaufmann, O. Kepp, A. Kimchi, R.N. Kitsis, D.J. Klionsky, R.A. Knight, S. Kumar, S.W. Lee, J.J. Lemasters, B. Levine, A. Linkermann, S.A. Lipton, R.A. Lockshin, C. López-Otín, S.W. Lowe, T. Luedde, E. Lugli, M. MacFarlane, F. Madeo, M. Malewicz, W. Malorni, G. Manic, J.-C. Marine, S.J. Martin, J.-C. Martinou, J.P. Medema, P. Mehlen, P. Meier, S. Melino, E.A. Miao, J.D. Molkentin, U.M. Moll, C. Muñoz-Pinedo, S. Nagata, G. Nuñez, A. Oberst, M. Oren, M. Overholtzer, M. Pagano, T. Panaretakis, M. Pasparakis, J.M. Penninger, D.M. Pereira, S. Pervaiz, M.E. Peter, M. Piacentini, P. Pinton, J.H.M. Prehn, H. Puthalakath, G.A. Rabinovich, M. Rehm, R. Rizzuto, C.M.P. Rodrigues, D.C. Rubinsztein, T. Rudel, K.M. Ryan, E. Sayan, L. Scorrano, F. Shao, Y. Shi, J. Silke, H.-U. Simon, A. Sistigu, B.R. Stockwell, A. Strasser, G. Szabadkai, S.W.G. Tait, D. Tang, N. Tavernarakis, A.

1732 Thorburn, Y. Tsujimoto, B. Turk, T. Vanden Berghe, P. Vandenabeele, M.G. Vander Heiden, A.
 1733 Villunger, H.W. Virgin, K.H. Vousden, D. Vucic, E.F. Wagner, H. Walczak, D. Wallach, Y. Wang,
 1734 J.A. Wells, W. Wood, J. Yuan, Z. Zakeri, B. Zhivotovsky, L. Zitvogel, G. Melino, G. Kroemer,
 1735 Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell
 1736 Death 2018, *Cell Death Differ.* 25 (2018) 486–541. <https://doi.org/10.1038/s41418-017-0012-4>.
 1737 [172] S.W.G. Tait, D.R. Green, Mitochondria and cell death: outer membrane permeabilization
 1738 and beyond, *Nat Rev Mol Cell Biol.* 11 (2010) 621–632. <https://doi.org/10.1038/nrm2952>.
 1739 [173] R. Gordan, N. Fefelova, J.K. Gwathmey, L.-H. Xie, Involvement of mitochondrial
 1740 permeability transition pore (mPTP) in cardiac arrhythmias: Evidence from cyclophilin D knockout
 1741 mice, *Cell Calcium.* 60 (2016) 363–372. <https://doi.org/10.1016/j.ceca.2016.09.001>.
 1742 [174] J. Dow, A. Bhandari, R.A. Kloner, The mechanism by which ischemic postconditioning
 1743 reduces reperfusion arrhythmias in rats remains elusive, *J Cardiovasc Pharmacol Ther.* 14 (2009)
 1744 99–103. <https://doi.org/10.1177/1074248408329606>.
 1745 [175] F.G. Akar, M.A. Aon, G.F. Tomaselli, B. O'Rourke, The mitochondrial origin of
 1746 postischemic arrhythmias, *J Clin Invest.* 115 (2005) 3527–3535. <https://doi.org/10.1172/JCI25371>.
 1747 [176] D.A. Brown, M.A. Aon, F.G. Akar, T. Liu, N. Sorraín, B. O'Rourke, Effects of 4'-
 1748 chlorodiazepam on cellular excitation-contraction coupling and ischaemia-reperfusion injury in
 1749 rabbit heart, *Cardiovasc Res.* 79 (2008) 141–149. <https://doi.org/10.1093/cvr/cvn053>.
 1750 [177] R. Dhingra, M. Guberman, I. Rabinovich-Nikitin, J. Gerstein, V. Margulets, H. Gang, N.
 1751 Madden, J. Thliveris, L.A. Kirshenbaum, Impaired NF- κ B signalling underlies cyclophilin D-
 1752 mediated mitochondrial permeability transition pore opening in doxorubicin cardiomyopathy,
 1753 *Cardiovasc Res.* 116 (2020) 1161–1174. <https://doi.org/10.1093/cvr/cvz240>.
 1754 [178] K.B. Wallace, V.A. Sardão, P.J. Oliveira, Mitochondrial Determinants of Doxorubicin-
 1755 Induced Cardiomyopathy, *Circ Res.* 126 (2020) 926–941.
 1756 <https://doi.org/10.1161/CIRCRESAHA.119.314681>.
 1757 [179] A. Salimi, M.R. Neshat, P. Naserzadeh, J. Pourahmad, Mitochondrial Permeability
 1758 Transition Pore Sealing Agents and Antioxidants Protect Oxidative Stress and Mitochondrial
 1759 Dysfunction Induced by Naproxen, Diclofenac and Celecoxib, *Drug Res (Stuttg).* 69 (2019) 598–
 1760 605. <https://doi.org/10.1055/a-0866-9356>.
 1761 [180] W. Ma, M. Liu, F. Liang, L. Zhao, C. Gao, X. Jiang, X. Zhang, H. Zhan, H. Hu, Z. Zhao,
 1762 Cardiotoxicity of sorafenib is mediated through elevation of ROS level and CaMKII activity and
 1763 dysregulation of calcium homeostasis, *Basic Clin Pharmacol Toxicol.* 126 (2020) 166–180.
 1764 <https://doi.org/10.1111/bcpt.13318>.
 1765 [181] V.G. Sharov, A. Todor, S. Khanal, M. Imai, H.N. Sabbah, Cyclosporine A attenuates
 1766 mitochondrial permeability transition and improves mitochondrial respiratory function in
 1767 cardiomyocytes isolated from dogs with heart failure, *J Mol Cell Cardiol.* 42 (2007) 150–158.
 1768 <https://doi.org/10.1016/j.yjmcc.2006.09.013>.
 1769 [182] V.G. Sharov, A.V. Todor, M. Imai, H.N. Sabbah, Inhibition of mitochondrial permeability
 1770 transition pores by cyclosporine A improves cytochrome C oxidase function and increases rate of
 1771 ATP synthesis in failing cardiomyocytes, *Heart Fail Rev.* 10 (2005) 305–310.
 1772 <https://doi.org/10.1007/s10741-005-7545-1>.
 1773 [183] T.S. Luongo, J.P. Lambert, P. Gross, M. Nwokedi, A.A. Lombardi, S. Shanmughapriya,
 1774 A.C. Carpenter, D. Kolmetzky, E. Gao, J.H. van Berlo, E.J. Tsai, J.D. Molkentin, X. Chen, M.
 1775 Madesh, S.R. Houser, J.W. Elrod, The mitochondrial Na⁺/Ca²⁺ exchanger is essential for Ca²⁺-
 1776 homeostasis and viability, *Nature.* 545 (2017) 93–97. <https://doi.org/10.1038/nature22082>.
 1777 [184] A.V. Hafner, J. Dai, A.P. Gomes, C.-Y. Xiao, C.M. Palmeira, A. Rosenzweig, D.A. Sinclair,
 1778 Regulation of the mPTP by SIRT3-mediated deacetylation of CypD at lysine 166 suppresses age-
 1779 related cardiac hypertrophy, *Aging (Albany NY).* 2 (2010) 914–923.
 1780 <https://doi.org/10.18632/aging.100252>.
 1781 [185] J.W. Elrod, R. Wong, S. Mishra, R.J. Vagnozzi, B. Sakthivel, S.A. Goonasekera, J. Karch,

1782 S. Gabel, J. Farber, T. Force, J.H. Brown, E. Murphy, J.D. Molkentin, Cyclophilin D controls
1783 mitochondrial pore-dependent Ca^{2+} exchange, metabolic flexibility, and propensity for heart
1784 failure in mice, *J Clin Invest*. 120 (2010) 3680–3687. <https://doi.org/10.1172/JCI43171>.
1785 [186] R. Rizzuto, D. De Stefani, A. Raffaello, C. Mammucari, Mitochondria as sensors and
1786 regulators of calcium signalling, *Nat Rev Mol Cell Biol*. 13 (2012) 566–578.
1787 <https://doi.org/10.1038/nrm3412>.
1788 [187] J.K. Foskett, B. Philipson, The mitochondrial Ca^{2+} uniporter complex, *J Mol Cell Cardiol*.
1789 78 (2015) 3–8. <https://doi.org/10.1016/j.yjmcc.2014.11.015>.
1790 [188] X. Pan, J. Liu, T. Nguyen, C. Liu, J. Sun, Y. Teng, M.M. Fergusson, I.I. Rovira, M. Allen,
1791 D.A. Springer, A.M. Aponte, M. Gucek, R.S. Balaban, E. Murphy, T. Finkel, The physiological
1792 role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter, *Nat*
1793 *Cell Biol*. 15 (2013) 1464–1472. <https://doi.org/10.1038/ncb2868>.
1794 [189] Y. Wu, T.P. Rasmussen, O.M. Koval, M.-L.A. Joiner, D.D. Hall, B. Chen, E.D. Luczak, Q.
1795 Wang, A.G. Rokita, X.H.T. Wehrens, L.-S. Song, M.E. Anderson, The mitochondrial uniporter
1796 controls fight or flight heart rate increases, *Nat Commun*. 6 (2015) 6081.
1797 <https://doi.org/10.1038/ncomms7081>.
1798 [190] K.M. Holmström, X. Pan, J.C. Liu, S. Menazza, J. Liu, T.T. Nguyen, H. Pan, R.J. Parks, S.
1799 Anderson, A. Noguchi, D. Springer, E. Murphy, T. Finkel, Assessment of cardiac function in mice
1800 lacking the mitochondrial calcium uniporter, *J Mol Cell Cardiol*. 85 (2015) 178–182.
1801 <https://doi.org/10.1016/j.yjmcc.2015.05.022>.
1802 [191] J.Q. Kwong, J.D. Molkentin, Physiological and pathological roles of the mitochondrial
1803 permeability transition pore in the heart, *Cell Metab*. 21 (2015) 206–214.
1804 <https://doi.org/10.1016/j.cmet.2014.12.001>.
1805 [192] T.P. Rasmussen, Y. Wu, M.A. Joiner, O.M. Koval, N.R. Wilson, E.D. Luczak, Q. Wang, B.
1806 Chen, Z. Gao, Z. Zhu, B.A. Wagner, J. Soto, M.L. McCormick, W. Kutschke, R.M. Weiss, L. Yu,
1807 R.L. Boudreau, E.D. Abel, F. Zhan, D.R. Spitz, G.R. Buettner, L.-S. Song, L.V. Zingman, M.E.
1808 Anderson, Inhibition of MCU forces extramitochondrial adaptations governing physiological and
1809 pathological stress responses in heart, *Proc Natl Acad Sci U S A*. 112 (2015) 9129–9134.
1810 <https://doi.org/10.1073/pnas.1504705112>.
1811 [193] K. Márta, P. Hasan, M. Rodríguez-Prados, M. Paillard, G. Hajnóczy, Pharmacological
1812 inhibition of the mitochondrial Ca^{2+} uniporter: Relevance for pathophysiology and human therapy,
1813 *J Mol Cell Cardiol*. 151 (2021) 135–144. <https://doi.org/10.1016/j.yjmcc.2020.09.014>.
1814 [194] M. Nichols, P.A. Elustondo, J. Warford, A. Thirumaran, E.V. Pavlov, G.S. Robertson,
1815 Global ablation of the mitochondrial calcium uniporter increases glycolysis in cortical neurons
1816 subjected to energetic stressors, *J Cereb Blood Flow Metab*. 37 (2017) 3027–3041.
1817 <https://doi.org/10.1177/0271678X16682250>.
1818 [195] Z. Yu, R. Chen, M. Li, Y. Yu, Y. Liang, F. Han, S. Qin, X. Chen, Y. Su, J. Ge,
1819 Mitochondrial calcium uniporter inhibition provides cardioprotection in pressure overload-induced
1820 heart failure through autophagy enhancement, *Int J Cardiol*. 271 (2018) 161–168.
1821 <https://doi.org/10.1016/j.ijcard.2018.05.054>.
1822 [196] J.H. Luft, Ruthenium red and violet. I. Chemistry, purification, methods of use for electron
1823 microscopy and mechanism of action, *Anat Rec*. 171 (1971) 347–368.
1824 <https://doi.org/10.1002/ar.1091710302>.
1825 [197] C.L. Moore, Specific inhibition of mitochondrial Ca^{++} transport by ruthenium red, *Biochem*
1826 *Biophys Res Commun*. 42 (1971) 298–305. [https://doi.org/10.1016/0006-291x\(71\)90102-1](https://doi.org/10.1016/0006-291x(71)90102-1).
1827 [198] F.D. Vasington, P. Gazzotti, R. Tiozzo, E. Carafoli, The effect of ruthenium red on Ca^{2+}
1828 transport and respiration in rat liver mitochondria, *Biochim Biophys Acta*. 256 (1972) 43–54.
1829 [https://doi.org/10.1016/0005-2728\(72\)90161-2](https://doi.org/10.1016/0005-2728(72)90161-2).
1830 [199] C.S. Rossi, F.D. Vasington, E. Carafoli, The effect of ruthenium red on the uptake and
1831 release of Ca^{2+} by mitochondria, *Biochem Biophys Res Commun*. 50 (1973) 846–852.

[https://doi.org/10.1016/0006-291x\(73\)91322-3](https://doi.org/10.1016/0006-291x(73)91322-3).
 [200] G.J. Grover, S. Dzwonczyk, P.G. Sleph, Ruthenium red improves postischemic contractile function in isolated rat hearts, *J Cardiovasc Pharmacol.* 16 (1990) 783–789.
<https://doi.org/10.1097/00005344-199011000-00014>.
 [201] G. de J. García-Rivas, K. Carvajal, F. Correa, C. Zazueta, Ru360, a specific mitochondrial calcium uptake inhibitor, improves cardiac post-ischaemic functional recovery in rats in vivo, *Br J Pharmacol.* 149 (2006) 829–837. <https://doi.org/10.1038/sj.bjp.0706932>.
 [202] G. de Jesús García-Rivas, A. Guerrero-Hernández, G. Guerrero-Serna, J.S. Rodríguez-Zavala, C. Zazueta, Inhibition of the mitochondrial calcium uniporter by the oxo-bridged dinuclear ruthenium amine complex (Ru360) prevents from irreversible injury in postischemic rat heart, *FEBS J.* 272 (2005) 3477–3488. <https://doi.org/10.1111/j.1742-4658.2005.04771.x>.
 [203] M.A. Matlib, Z. Zhou, S. Knight, S. Ahmed, K.M. Choi, J. Krause-Bauer, R. Phillips, R. Altschuld, Y. Katsube, N. Sperelakis, D.M. Bers, Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca²⁺ uptake into mitochondria in vitro and in situ in single cardiac myocytes, *J Biol Chem.* 273 (1998) 10223–10231. <https://doi.org/10.1074/jbc.273.17.10223>.
 [204] J.J. Woods, N. Nemani, S. Shanmughapriya, A. Kumar, M. Zhang, S.R. Nathan, M. Thomas, E. Carvalho, K. Ramachandran, S. Srikantan, P.B. Stathopulos, J.J. Wilson, M. Madesh, A Selective and Cell-Permeable Mitochondrial Calcium Uniporter (MCU) Inhibitor Preserves Mitochondrial Bioenergetics after Hypoxia/Reoxygenation Injury, *ACS Cent Sci.* 5 (2019) 153–166. <https://doi.org/10.1021/acscentsci.8b00773>.
 [205] N. Kon, M. Murakoshi, A. Isobe, K. Kagechika, N. Miyoshi, T. Nagayama, DS16570511 is a small-molecule inhibitor of the mitochondrial calcium uniporter, *Cell Death Discov.* 3 (2017) 17045. <https://doi.org/10.1038/cddiscovery.2017.45>.
 [206] G. Di Marco, F. Vallese, B. Jourde, C. Bergsdorf, M. Sturlese, A. De Mario, V. Techer-Etienne, D. Haasen, B. Oberhauser, S. Schleegeer, G. Minetti, S. Moro, R. Rizzuto, D. De Stefani, M. Fornaro, C. Mammucari, A High-Throughput Screening Identifies MICU1 Targeting Compounds, *Cell Rep.* 30 (2020) 2321–2331.e6. <https://doi.org/10.1016/j.celrep.2020.01.081>.
 [207] J. Santo-Domingo, L. Vay, E. Hernández-Sanmiguel, C.D. Lobatón, A. Moreno, M. Montero, J. Alvarez, The plasma membrane Na⁺/Ca²⁺ exchange inhibitor KB-R7943 is also a potent inhibitor of the mitochondrial Ca²⁺ uniporter, *Br J Pharmacol.* 151 (2007) 647–654. <https://doi.org/10.1038/sj.bjp.0707260>.
 [208] B.M. Wiczer, R. Marcu, B.J. Hawkins, KB-R7943, a plasma membrane Na⁽⁺⁾/Ca⁽²⁺⁾ exchanger inhibitor, blocks opening of the mitochondrial permeability transition pore, *Biochem Biophys Res Commun.* 444 (2014) 44–49. <https://doi.org/10.1016/j.bbrc.2014.01.009>.
 [209] null del MonteF, S.E. Harding, U. Schmidt, T. Matsui, Z.B. Kang, G.W. Dec, J.K. Gwathmey, A. Rosenzweig, R.J. Hajjar, Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a, *Circulation.* 100 (1999) 2308–2311. <https://doi.org/10.1161/01.cir.100.23.2308>.
 [210] R.J. Hajjar, J.X. Kang, J.K. Gwathmey, A. Rosenzweig, Physiological effects of adenoviral gene transfer of sarcoplasmic reticulum calcium ATPase in isolated rat myocytes, *Circulation.* 95 (1997) 423–429. <https://doi.org/10.1161/01.cir.95.2.423>.
 [211] L.J. Motloch, M. Cacheux, K. Ishikawa, C. Xie, J. Hu, J. Agüero, K.M. Fish, R.J. Hajjar, F.G. Akar, Primary Effect of SERCA 2a Gene Transfer on Conduction Reserve in Chronic Myocardial Infarction, *J Am Heart Assoc.* 7 (2018) e009598. <https://doi.org/10.1161/JAHA.118.009598>.
 [212] F. Prunier, Y. Kawase, D. Gianni, C. Scapin, S.B. Danik, P.T. Ellinor, R.J. Hajjar, F. Del Monte, Prevention of ventricular arrhythmias with sarcoplasmic reticulum Ca²⁺ ATPase pump overexpression in a porcine model of ischemia reperfusion, *Circulation.* 118 (2008) 614–624. <https://doi.org/10.1161/CIRCULATIONAHA.108.770883>.
 [213] Y. Kawase, H.Q. Ly, F. Prunier, D. Lebeche, Y. Shi, H. Jin, L. Hadri, R. Yoneyama, K.

1882 Hoshino, Y. Takewa, S. Sakata, R. Peluso, K. Zsebo, J.K. Gwathmey, J.-C. Tardif, J.-F. Tanguay,
 1883 R.J. Hajjar, Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene
 1884 transfer in a pre-clinical model of heart failure, *J Am Coll Cardiol.* 51 (2008) 1112–1119.
 1885 <https://doi.org/10.1016/j.jacc.2007.12.014>.
 1886 [214] M.J. Byrne, J.M. Power, A. Prevolos, J.A. Mariani, R.J. Hajjar, D.M. Kaye, Recirculating
 1887 cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of
 1888 heart failure in large animals, *Gene Ther.* 15 (2008) 1550–1557.
 1889 <https://doi.org/10.1038/gt.2008.120>.
 1890 [215] M.Y. Rincon, T. VandenDriessche, M.K. Chuah, Gene therapy for cardiovascular disease:
 1891 advances in vector development, targeting, and delivery for clinical translation, *Cardiovasc Res.*
 1892 108 (2015) 4–20. <https://doi.org/10.1093/cvr/cvv205>.
 1893 [216] T.J. Samuel, R.P. Rosenberry, S. Lee, Z. Pan, Correcting Calcium Dysregulation in Chronic
 1894 Heart Failure Using SERCA2a Gene Therapy, *Int J Mol Sci.* 19 (2018) E1086.
 1895 <https://doi.org/10.3390/ijms19041086>.
 1896 [217] W. Xin, X. Lu, X. Li, K. Niu, J. Cai, Attenuation of endoplasmic reticulum stress-related
 1897 myocardial apoptosis by SERCA2a gene delivery in ischemic heart disease, *Mol Med.* 17 (2011)
 1898 201–210. <https://doi.org/10.2119/molmed.2010.00197>.
 1899 [218] L. Hadri, R. Bobe, Y. Kawase, D. Ladage, K. Ishikawa, F. Atassi, D. Lebeche, E.G. Kranias,
 1900 J.A. Leopold, A.-M. Lompré, L. Lipskaia, R.J. Hajjar, SERCA2a gene transfer enhances eNOS
 1901 expression and activity in endothelial cells, *Mol Ther.* 18 (2010) 1284–1292.
 1902 <https://doi.org/10.1038/mt.2010.77>.
 1903 [219] L. Lipskaia, L. Hadri, J.J. Lopez, R.J. Hajjar, R. Bobe, Benefit of SERCA2a gene transfer to
 1904 vascular endothelial and smooth muscle cells: a new aspect in therapy of cardiovascular diseases,
 1905 *Curr Vasc Pharmacol.* 11 (2013) 465–479. <https://doi.org/10.2174/1570161111311040010>.
 1906 [220] B.E. Jaski, M.L. Jessup, D.M. Mancini, T.P. Cappola, D.F. Pauly, B. Greenberg, K. Borow,
 1907 H. Dittrich, K.M. Zsebo, R.J. Hajjar, Calcium Up-Regulation by Percutaneous Administration of
 1908 Gene Therapy In Cardiac Disease (CUPID) Trial Investigators, Calcium upregulation by
 1909 percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human
 1910 phase 1/2 clinical trial, *J Card Fail.* 15 (2009) 171–181.
 1911 <https://doi.org/10.1016/j.cardfail.2009.01.013>.
 1912 [221] M. Jessup, B. Greenberg, D. Mancini, T. Cappola, D.F. Pauly, B. Jaski, A. Yaroshinsky,
 1913 K.M. Zsebo, H. Dittrich, R.J. Hajjar, Calcium Upregulation by Percutaneous Administration of
 1914 Gene Therapy in Cardiac Disease (CUPID) Investigators, Calcium Upregulation by Percutaneous
 1915 Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene
 1916 therapy of sarcoplasmic reticulum Ca²⁺-ATPase in patients with advanced heart failure,
 1917 *Circulation.* 124 (2011) 304–313. <https://doi.org/10.1161/CIRCULATIONAHA.111.022889>.
 1918 [222] J.-S. Hulot, J.-E. Salem, A. Redheuil, J.-P. Collet, S. Varnous, P. Jourdain, D. Logeart, E.
 1919 Gandjbakhch, C. Bernard, S.N. Hatem, R. Isnard, P. Cluzel, C. Le Feuvre, P. Leprince, N.
 1920 Hammoudi, F.M. Lemoine, D. Klatzmann, E. Vicaud, M. Komajda, G. Montalescot, A.-M. Lompré,
 1921 R.J. Hajjar, AGENT-HF Investigators, Effect of intracoronary administration of AAV1/SERCA2a
 1922 on ventricular remodelling in patients with advanced systolic heart failure: results from the
 1923 AGENT-HF randomized phase 2 trial, *Eur J Heart Fail.* 19 (2017) 1534–1541.
 1924 <https://doi.org/10.1002/ehhf.826>.
 1925 [223] J.-S. Hulot, K. Ishikawa, R.J. Hajjar, Gene therapy for the treatment of heart failure: promise
 1926 postponed, *Eur Heart J.* 37 (2016) 1651–1658. <https://doi.org/10.1093/eurheartj/ehw019>.
 1927 [224] K. Niwano, M. Arai, N. Koitabashi, A. Watanabe, Y. Ikeda, H. Miyoshi, M. Kurabayashi,
 1928 Lentiviral vector-mediated SERCA2 gene transfer protects against heart failure and left ventricular
 1929 remodeling after myocardial infarction in rats, *Mol Ther.* 16 (2008) 1026–1032.
 1930 <https://doi.org/10.1038/mt.2008.61>.
 1931 [225] S. Fleury, E. Simeoni, C. Zuppinger, N. Déglon, L.K. von Segesser, L. Kappenberger, G.

1932 Vassalli, Multiply attenuated, self-inactivating lentiviral vectors efficiently deliver and express
1933 genes for extended periods of time in adult rat cardiomyocytes in vivo, *Circulation*. 107 (2003)
1934 2375–2382. <https://doi.org/10.1161/01.CIR.0000065598.46411.EF>.

1935 [226] L. Naldini, U. Blömer, P. Gallay, D. Ory, R. Mulligan, F.H. Gage, I.M. Verma, D. Trono, In
1936 vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector, *Science*. 272
1937 (1996) 263–267. <https://doi.org/10.1126/science.272.5259.263>.

1938 [227] H. Miyoshi, M. Takahashi, F.H. Gage, I.M. Verma, Stable and efficient gene transfer into
1939 the retina using an HIV-based lentiviral vector, *Proc Natl Acad Sci U S A*. 94 (1997) 10319–10323.
1940 <https://doi.org/10.1073/pnas.94.19.10319>.

1941 [228] S. Sakata, D. Lebeche, N. Sakata, Y. Sakata, E.R. Chemaly, L.F. Liang, T. Tsuji, Y.
1942 Takewa, F. del Monte, R. Peluso, K. Zsebo, D. Jeong, W.J. Park, Y. Kawase, R.J. Hajjar,
1943 Restoration of mechanical and energetic function in failing aortic-banded rat hearts by gene transfer
1944 of calcium cycling proteins, *J Mol Cell Cardiol*. 42 (2007) 852–861.
1945 <https://doi.org/10.1016/j.yjmcc.2007.01.003>.

1946 [229] S. Sakata, D. Lebeche, Y. Sakata, N. Sakata, E.R. Chemaly, L.F. Liang, P. Padmanabhan, N.
1947 Konishi, M. Takaki, F. del Monte, R.J. Hajjar, Mechanical and metabolic rescue in a type II
1948 diabetes model of cardiomyopathy by targeted gene transfer, *Mol Ther*. 13 (2006) 987–996.
1949 <https://doi.org/10.1016/j.ymthe.2006.01.002>.

1950 [230] L. Lipskaia, E.R. Chemaly, L. Hadri, A.-M. Lompre, R.J. Hajjar, Sarcoplasmic reticulum
1951 Ca(2+) ATPase as a therapeutic target for heart failure, *Expert Opin Biol Ther*. 10 (2010) 29–41.
1952 <https://doi.org/10.1517/14712590903321462>.

1953 [231] D.H. MacLennan, E.G. Kranias, Phospholamban: a crucial regulator of cardiac contractility,
1954 *Nat Rev Mol Cell Biol*. 4 (2003) 566–577. <https://doi.org/10.1038/nrm1151>.

1955 [232] N.-A.D. Abloh, D.D. Thomas, Phospholamban phosphorylation, mutation, and structural
1956 dynamics: a biophysical approach to understanding and treating cardiomyopathy, *Biophys Rev*. 7
1957 (2015) 63–76. <https://doi.org/10.1007/s12551-014-0157-z>.

1958 [233] L. Suckau, H. Fechner, E. Chemaly, S. Krohn, L. Hadri, J. Kocksämper, D. Westermann,
1959 E. Bisping, H. Ly, X. Wang, Y. Kawase, J. Chen, L. Liang, I. Sipo, R. Vetter, S. Weger, J. Kurreck,
1960 V. Erdmann, C. Tschope, B. Pieske, D. Lebeche, H.-P. Schultheiss, R.J. Hajjar, W.C. Poller, Long-
1961 term cardiac-targeted RNA interference for the treatment of heart failure restores cardiac function
1962 and reduces pathological hypertrophy, *Circulation*. 119 (2009) 1241–1252.
1963 <https://doi.org/10.1161/CIRCULATIONAHA.108.783852>.

1964 [234] J. Li, B.H. Jia, J. Sun, X.L. Lou, S.J. Hu, Phospholamban antisense RNA improves SR
1965 Ca²⁺-ATPase activity and left ventricular function in STZ-induced diabetic rats, *Biomed Environ*
1966 *Sci*. 26 (2013) 577–583. <https://doi.org/10.3967/0895-3988.2013.07.009>.

1967 [235] D.M. Kaye, A. Prevolos, T. Marshall, M. Byrne, M. Hoshijima, R. Hajjar, J.A. Mariani, S.
1968 Pepe, K.R. Chien, J.M. Power, Percutaneous cardiac recirculation-mediated gene transfer of an
1969 inhibitory phospholamban peptide reverses advanced heart failure in large animals, *J Am Coll*
1970 *Cardiol*. 50 (2007) 253–260. <https://doi.org/10.1016/j.jacc.2007.03.047>.

1971 [236] H. Sakai, Y. Ikeda, T. Honda, Y. Tanaka, K. Shiraishi, M. Inui, A cell-penetrating
1972 phospholamban-specific RNA aptamer enhances Ca²⁺ transients and contractile function in
1973 cardiomyocytes, *J Mol Cell Cardiol*. 76 (2014) 177–185.
1974 <https://doi.org/10.1016/j.yjmcc.2014.09.006>.

1975 [237] L.T. Bish, M.M. Sleeper, C. Reynolds, J. Gazzara, E. Withnall, G.E. Singletary, G. Buchlis,
1976 D. Hui, K.A. High, G. Gao, J.M. Wilson, H.L. Sweeney, Cardiac gene transfer of short hairpin
1977 RNA directed against phospholamban effectively knocks down gene expression but causes cellular
1978 toxicity in canines, *Hum Gene Ther*. 22 (2011) 969–977. <https://doi.org/10.1089/hum.2011.035>.

1979 [238] Y.-H. Kao, C.-C. Cheng, Y.-C. Chen, C.-C. Chung, T.-I. Lee, S.-A. Chen, Y.-J. Chen,
1980 Hydralazine-induced promoter demethylation enhances sarcoplasmic reticulum Ca²⁺ -ATPase and
1981 calcium homeostasis in cardiac myocytes, *Lab Invest*. 91 (2011) 1291–1297.

1982 <https://doi.org/10.1038/labinvest.2011.92>.

1983 [239] J.M. Pearl, D.M. Plank, K.M. McLean, C.J. Wagner, J.Y. Duffy, Glucocorticoids improve

1984 calcium cycling in cardiac myocytes after cardiopulmonary bypass, *J Surg Res.* 167 (2011) 279–

1985 286. <https://doi.org/10.1016/j.jss.2009.05.001>.

1986 [240] G.G. Mattera, P. Lo Giudice, F.M.P. Loi, E. Vanoli, J.-P. Gagnol, F. Borsini, P. Carminati,

1987 Istaroxime: a new luso-inotropic agent for heart failure, *Am J Cardiol.* 99 (2007) 33A-40A.

1988 <https://doi.org/10.1016/j.amjcard.2006.09.004>.

1989 [241] M. Ferrandi, P. Barassi, F. Tadini-Buoninsegni, G. Bartolommei, I. Molinari, M.G. Tripodi,

1990 C. Reina, M.R. Moncelli, G. Bianchi, P. Ferrari, Istaroxime stimulates SERCA2a and accelerates

1991 calcium cycling in heart failure by relieving phospholamban inhibition, *Br J Pharmacol.* 169 (2013)

1992 1849–1861. <https://doi.org/10.1111/bph.12278>.

1993 [242] H.N. Sabbah, M. Imai, D. Cowart, A. Amato, P. Carminati, M. Gheorghiade, Hemodynamic

1994 properties of a new-generation positive luso-inotropic agent for the acute treatment of advanced

1995 heart failure, *Am J Cardiol.* 99 (2007) 41A-46A. <https://doi.org/10.1016/j.amjcard.2006.09.005>.

1996 [243] R. Micheletti, F. Palazzo, P. Barassi, G. Giacalone, M. Ferrandi, A. Schiavone, B. Moro, O.

1997 Parodi, P. Ferrari, G. Bianchi, Istaroxime, a stimulator of sarcoplasmic reticulum calcium adenosine

1998 triphosphatase isoform 2a activity, as a novel therapeutic approach to heart failure, *Am J Cardiol.*

1999 99 (2007) 24A-32A. <https://doi.org/10.1016/j.amjcard.2006.09.003>.

2000 [244] M. Gheorghiade, J.E.A. Blair, G.S. Filippatos, C. Macarie, W. Ruzyllo, J. Korewicki, S.I.

2001 Bubenek-Turconi, M. Ceracchi, M. Bianchetti, P. Carminati, D. Kremastinos, G. Valentini, H.N.

2002 Sabbah, HORIZON-HF Investigators, Hemodynamic, echocardiographic, and neurohormonal

2003 effects of istaroxime, a novel intravenous inotropic and lusitropic agent: a randomized controlled

2004 trial in patients hospitalized with heart failure, *J Am Coll Cardiol.* 51 (2008) 2276–2285.

2005 <https://doi.org/10.1016/j.jacc.2008.03.015>.

2006 [245] J.K. Ghali, W.B. Smith, G. Torre-Amione, W. Haynos, B.K. Rayburn, A. Amato, D. Zhang,

2007 D. Cowart, G. Valentini, P. Carminati, M. Gheorghiade, A phase 1-2 dose-escalating study

2008 evaluating the safety and tolerability of istaroxime and specific effects on electrocardiographic and

2009 hemodynamic parameters in patients with chronic heart failure with reduced systolic function, *Am J*

2010 *Cardiol.* 99 (2007) 47A-56A. <https://doi.org/10.1016/j.amjcard.2006.09.006>.

2011 [246] X. Guo, D. Chapman, N.S. Dhalla, Partial prevention of changes in SR gene expression in

2012 congestive heart failure due to myocardial infarction by enalapril or losartan, *Mol Cell Biochem.*

2013 254 (2003) 163–172. <https://doi.org/10.1023/a:1027321130997>.

2014 [247] T. Bai, X. Hu, Y. Zheng, S. Wang, J. Kong, L. Cai, Resveratrol protects against

2015 lipopolysaccharide-induced cardiac dysfunction by enhancing SERCA2a activity through

2016 promoting the phospholamban oligomerization, *Am J Physiol Heart Circ Physiol.* 311 (2016)

2017 H1051–H1062. <https://doi.org/10.1152/ajpheart.00296.2016>.

2018 [248] M. Sulaiman, M.J. Matta, N.R. Sunderesan, M.P. Gupta, M. Periasamy, M. Gupta,

2019 Resveratrol, an activator of SIRT1, upregulates sarcoplasmic calcium ATPase and improves cardiac

2020 function in diabetic cardiomyopathy, *Am J Physiol Heart Circ Physiol.* 298 (2010) H833-843.

2021 <https://doi.org/10.1152/ajpheart.00418.2009>.

2022 [249] F.L. Norby, L.E. Wold, J. Duan, K.K. Hintz, J. Ren, IGF-I attenuates diabetes-induced

2023 cardiac contractile dysfunction in ventricular myocytes, *Am J Physiol Endocrinol Metab.* 283

2024 (2002) E658-666. <https://doi.org/10.1152/ajpendo.00003.2002>.

2025 [250] S.-T. Hu, Y. Tang, Y.-F. Shen, H.-H. Ao, J. Bai, Y.-L. Wang, Y.-J. Yang, Protective effect

2026 of oxymatrine on chronic rat heart failure, *J Physiol Sci.* 61 (2011) 363–372.

2027 <https://doi.org/10.1007/s12576-011-0154-y>.

2028 [251] C. Kho, A. Lee, D. Jeong, J.G. Oh, A.H. Chaanine, E. Kizana, W.J. Park, R.J. Hajjar,

2029 SUMO1-dependent modulation of SERCA2a in heart failure, *Nature.* 477 (2011) 601–605.

2030 <https://doi.org/10.1038/nature10407>.

2031 [252] C. Kho, A. Lee, D. Jeong, J.G. Oh, P.A. Gorski, K. Fish, R. Sanchez, R.J. DeVita, G.

2032 Christensen, R. Dahl, R.J. Hajjar, Small-molecule activation of SERCA2a SUMOylation for the

2033 treatment of heart failure, *Nat Commun.* 6 (2015) 7229. <https://doi.org/10.1038/ncomms8229>.

2034 [253] P. Most, S.T. Pleger, M. Völkers, B. Heidt, M. Boerries, D. Weichenhan, E. Löffler, P.M.L.

2035 Janssen, A.D. Eckhart, J. Martini, M.L. Williams, H.A. Katus, A. Remppis, W.J. Koch, Cardiac

2036 adenoviral S100A1 gene delivery rescues failing myocardium, *J Clin Invest.* 114 (2004) 1550–

2037 1563. <https://doi.org/10.1172/JCI21454>.

2038 [254] S.T. Pleger, P. Most, M. Boucher, S. Soltys, J.K. Chuprun, W. Pleger, E. Gao, A. Dasgupta,

2039 G. Rengo, A. Remppis, H.A. Katus, A.D. Eckhart, J.E. Rabinowitz, W.J. Koch, Stable myocardial-

2040 specific AAV6-S100A1 gene therapy results in chronic functional heart failure rescue, *Circulation.*

2041 115 (2007) 2506–2515. <https://doi.org/10.1161/CIRCULATIONAHA.106.671701>.

2042 [255] P. Most, W.J. Koch, S100A1: a calcium-modulating inotropic prototype for future clinical

2043 heart failure therapy, *Future Cardiol.* 3 (2007) 5–11. <https://doi.org/10.2217/14796678.3.1.5>.

2044 [256] S. Ikeda, S.W. Kong, J. Lu, E. Bisping, H. Zhang, P.D. Allen, T.R. Golub, B. Pieske, W.T.

2045 Pu, Altered microRNA expression in human heart disease, *Physiol Genomics.* 31 (2007) 367–373.

2046 <https://doi.org/10.1152/physiolgenomics.00144.2007>.

2047 [257] S. Leptidis, H. El Azzouzi, S.I. Lok, R. de Weger, S. Olieslagers, S. Olieslagers, N. Kisters,

2048 G.J. Silva, S. Heymans, E. Cuppen, E. Berezikov, L.J. De Windt, P. da Costa Martins, A deep

2049 sequencing approach to uncover the miRNOME in the human heart, *PLoS One.* 8 (2013) e57800.

2050 <https://doi.org/10.1371/journal.pone.0057800>.

2051 [258] C. Wahlquist, D. Jeong, A. Rojas-Muñoz, C. Kho, A. Lee, S. Mitsuyama, A. van Mil, W.J.

2052 Park, J.P.G. Sluijter, P.A.F. Doevendans, R.J. Hajjar, M. Mercola, Inhibition of miR-25 improves

2053 cardiac contractility in the failing heart, *Nature.* 508 (2014) 531–535.

2054 <https://doi.org/10.1038/nature13073>.

2055 [259] M.D. McCauley, X.H.T. Wehrens, Targeting ryanodine receptors for anti-arrhythmic

2056 therapy, *Acta Pharmacol Sin.* 32 (2011) 749–757. <https://doi.org/10.1038/aps.2011.44>.

2057 [260] P. Connell, T.A. Word, X.H.T. Wehrens, Targeting pathological leak of ryanodine

2058 receptors: preclinical progress and the potential impact on treatments for cardiac arrhythmias and

2059 heart failure, *Expert Opin Ther Targets.* 24 (2020) 25–36.

2060 <https://doi.org/10.1080/14728222.2020.1708326>.

2061 [261] M. Chaffman, R.N. Brogden, Diltiazem. A review of its pharmacological properties and

2062 therapeutic efficacy, *Drugs.* 29 (1985) 387–454. [https://doi.org/10.2165/00003495-198529050-](https://doi.org/10.2165/00003495-198529050-00001)

2063 [00001](https://doi.org/10.2165/00003495-198529050-00001).

2064 [262] M. Sacherer, S. Sedej, P. Wakuła, M. Wallner, M.A. Vos, J. Kockskämper, P. Stiegler, M.

2065 Sereinigg, D. von Lewinski, G. Antoons, B.M. Pieske, F.R. Heinzel, CONTICA investigators,

2066 JTV519 (K201) reduces sarcoplasmic reticulum Ca²⁺ leak and improves diastolic function in vitro

2067 in murine and human non-failing myocardium, *Br J Pharmacol.* 167 (2012) 493–504.

2068 <https://doi.org/10.1111/j.1476-5381.2012.01995.x>.

2069 [263] M. Yano, S. Kobayashi, M. Kohno, M. Doi, T. Tokuhisa, S. Okuda, M. Suetsugu, T.

2070 Hisaoka, M. Obayashi, T. Ohkusa, M. Kohno, M. Matsuzaki, FKBP12.6-mediated stabilization of

2071 calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure,

2072 *Circulation.* 107 (2003) 477–484. <https://doi.org/10.1161/01.cir.0000044917.74408.be>.

2073 [264] X.H.T. Wehrens, S.E. Lehnart, S.R. Reiken, S.-X. Deng, J.A. Vest, D. Cervantes, J.

2074 Coromilas, D.W. Landry, A.R. Marks, Protection from cardiac arrhythmia through ryanodine

2075 receptor-stabilizing protein calstabin2, *Science.* 304 (2004) 292–296.

2076 <https://doi.org/10.1126/science.1094301>.

2077 [265] Y.L. Darcy, P.L. Diaz-Sylvester, J.A. Copello, K201 (JTV519) is a Ca²⁺-Dependent

2078 Blocker of SERCA and a Partial Agonist of Ryanodine Receptors in Striated Muscle, *Mol*

2079 *Pharmacol.* 90 (2016) 106–115. <https://doi.org/10.1124/mol.115.102277>.

2080 [266] S.E. Lehnart, M. Mongillo, A. Bellinger, N. Lindegger, B.-X. Chen, W. Hsueh, S. Reiken,

2081 A. Wronska, L.J. Drew, C.W. Ward, W.J. Lederer, R.S. Kass, G. Morley, A.R. Marks, Leaky Ca²⁺

2082 release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice, *J Clin*

Invest. 118 (2008) 2230–2245. <https://doi.org/10.1172/JCI35346>.

[267] K. Sasaki, T. Makiyama, Y. Yoshida, Y. Wuriyanghai, T. Kamakura, S. Nishiuchi, M. Hayano, T. Harita, Y. Yamamoto, H. Kohjitani, S. Hirose, J. Chen, M. Kawamura, S. Ohno, H. Itoh, A. Takeuchi, S. Matsuoka, M. Miura, N. Sumitomo, M. Horie, S. Yamanaka, T. Kimura, Patient-Specific Human Induced Pluripotent Stem Cell Model Assessed with Electrical Pacing Validates S107 as a Potential Therapeutic Agent for Catecholaminergic Polymorphic Ventricular Tachycardia, *PLoS One*. 11 (2016) e0164795. <https://doi.org/10.1371/journal.pone.0164795>.

[268] J. Shan, M.J. Betzenhauser, A. Kushnir, S. Reiken, A.C. Meli, A. Wronska, M. Dura, B.-X. Chen, A.R. Marks, Role of chronic ryanodine receptor phosphorylation in heart failure and β -adrenergic receptor blockade in mice, *J Clin Invest*. 120 (2010) 4375–4387. <https://doi.org/10.1172/JCI37649>.

[269] D.C. Andersson, M.J. Betzenhauser, S. Reiken, A.C. Meli, A. Umanskaya, W. Xie, T. Shiomi, R. Zalk, A. Lacampagne, A.R. Marks, Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging, *Cell Metab*. 14 (2011) 196–207. <https://doi.org/10.1016/j.cmet.2011.05.014>.

[270] A.M. Bellinger, S. Reiken, C. Carlson, M. Mongillo, X. Liu, L. Rothman, S. Matecki, A. Lacampagne, A.R. Marks, Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle, *Nat Med*. 15 (2009) 325–330. <https://doi.org/10.1038/nm.1916>.

[271] X. Liu, M.J. Betzenhauser, S. Reiken, A.C. Meli, W. Xie, B.-X. Chen, O. Arancio, A.R. Marks, Role of leaky neuronal ryanodine receptors in stress-induced cognitive dysfunction, *Cell*. 150 (2012) 1055–1067. <https://doi.org/10.1016/j.cell.2012.06.052>.

[272] T. Krause, M.U. Gerbershagen, M. Fiege, R. Weissborn, F. Wappler, Dantrolene--a review of its pharmacology, therapeutic use and new developments, *Anaesthesia*. 59 (2004) 364–373. <https://doi.org/10.1111/j.1365-2044.2004.03658.x>.

[273] S. Kobayashi, M.L. Bannister, J.P. Gangopadhyay, T. Hamada, J. Parness, N. Ikemoto, Dantrolene stabilizes domain interactions within the ryanodine receptor, *J Biol Chem*. 280 (2005) 6580–6587. <https://doi.org/10.1074/jbc.M408375200>.

[274] S. Kobayashi, M. Yano, T. Suetomi, M. Ono, H. Tateishi, M. Mochizuki, X. Xu, H. Uchinoumi, S. Okuda, T. Yamamoto, N. Koseki, H. Kyushiki, N. Ikemoto, M. Matsuzaki, Dantrolene, a therapeutic agent for malignant hyperthermia, markedly improves the function of failing cardiomyocytes by stabilizing interdomain interactions within the ryanodine receptor, *J Am Coll Cardiol*. 53 (2009) 1993–2005. <https://doi.org/10.1016/j.jacc.2009.01.065>.

[275] S. Kobayashi, M. Yano, H. Uchinoumi, T. Suetomi, T. Susa, M. Ono, X. Xu, H. Tateishi, T. Oda, S. Okuda, M. Doi, T. Yamamoto, M. Matsuzaki, Dantrolene, a therapeutic agent for malignant hyperthermia, inhibits catecholaminergic polymorphic ventricular tachycardia in a RyR2(R2474S/+) knock-in mouse model, *Circ J*. 74 (2010) 2579–2584. <https://doi.org/10.1253/circj.cj-10-0680>.

[276] K. Penttinen, H. Swan, S. Vanninen, J. Paavola, A.M. Lahtinen, K. Kontula, K. Aalto-Setälä, Antiarrhythmic Effects of Dantrolene in Patients with Catecholaminergic Polymorphic Ventricular Tachycardia and Replication of the Responses Using iPSC Models, *PLoS One*. 10 (2015) e0125366. <https://doi.org/10.1371/journal.pone.0125366>.

[277] E.O. Balam Ortiz, K. Carvajal, D. Cruz, [Protective effect of dantrolene in post-ischemic reperfusion myocardial damage], *Arch Inst Cardiol Mex*. 69 (1999) 311–319.

[278] J.A. Boys, A.H. Toledo, R. Anaya-Prado, F. Lopez-Neblina, L.H. Toledo-Pereyra, Effects of dantrolene on ischemia-reperfusion injury in animal models: a review of outcomes in heart, brain, liver, and kidney, *J Invest Med*. 58 (2010) 875–882. <https://doi.org/10.231/JIM.0b013e3181e5d719>.

[279] S. Kobayashi, T. Wakeyama, S. Ono, Y. Ikeda, M. Omura, T. Oda, Y. Hisamatsu, K. Seki, A. Satoh, M. Hiromoto, S. Akashi, K. Uchida, M. Harada, Y. Furutani, Y. Nakamura, M. Kohno, S. Kawamura, M. Obayashi, H. Michishige, M. Yano, A multicenter, randomized, double-blind,

controlled study to evaluate the efficacy and safety of dantrolene on ventricular arrhythmia as well as mortality and morbidity in patients with chronic heart failure (SHO-IN trial): rationale and design, *J Cardiol.* 75 (2020) 454–461. <https://doi.org/10.1016/j.jjcc.2019.08.020>.

[280] G.K. Andrikopoulos, S. Pastromas, S. Tzeis, Flecainide: Current status and perspectives in arrhythmia management, *World J Cardiol.* 7 (2015) 76–85. <https://doi.org/10.4330/wjc.v7.i2.76>.

[281] K. Arunachalam, T. Alzahrani, Flecaïnide, in: *StatPearls*, StatPearls Publishing, Treasure Island (FL), 2021. <http://www.ncbi.nlm.nih.gov/books/NBK542291/> (accessed December 10, 2021).

[282] F.A. Hilliard, D.S. Steele, D. Laver, Z. Yang, S.J. Le Marchand, N. Chopra, D.W. Piston, S. Huke, B.C. Knollmann, Flecaïnide inhibits arrhythmogenic Ca²⁺ waves by open state block of ryanodine receptor Ca²⁺ release channels and reduction of Ca²⁺ spark mass, *J Mol Cell Cardiol.* 48 (2010) 293–301. <https://doi.org/10.1016/j.yjmcc.2009.10.005>.

[283] H. Watanabe, N. Chopra, D. Laver, H.S. Hwang, S.S. Davies, D.E. Roach, H.J. Duff, D.M. Roden, A.A.M. Wilde, B.C. Knollmann, Flecaïnide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans, *Nat Med.* 15 (2009) 380–383. <https://doi.org/10.1038/nm.1942>.

[284] N. Li, Q. Wang, M. Sibrian-Vazquez, R.C. Klipp, J.O. Reynolds, T.A. Word, L. Scott, G. Salama, R.M. Strongin, J.J. Abramson, X.H.T. Wehrens, Treatment of catecholaminergic polymorphic ventricular tachycardia in mice using novel RyR2-modifying drugs, *Int J Cardiol.* 227 (2017) 668–673. <https://doi.org/10.1016/j.ijcard.2016.10.078>.

[285] T.A. Word, A.P. Quick, C.Y. Miyake, M.K. Shak, X. Pan, J.J. Kim, H.D. Allen, M. Sibrian-Vazquez, R.M. Strongin, A.P. Landstrom, X.H.T. Wehrens, Efficacy of RyR2 inhibitor EL20 in induced pluripotent stem cell-derived cardiomyocytes from a patient with catecholaminergic polymorphic ventricular tachycardia, *J Cell Mol Med.* (2021). <https://doi.org/10.1111/jcmm.16521>.

[286] G.W. Zamponi, J. Striessnig, A. Koschak, A.C. Dolphin, The Physiology, Pathology, and Pharmacology of Voltage-Gated Calcium Channels and Their Future Therapeutic Potential, *Pharmacol Rev.* 67 (2015) 821–870. <https://doi.org/10.1124/pr.114.009654>.

[287] D.M. Bers, Calcium cycling and signaling in cardiac myocytes, *Annu Rev Physiol.* 70 (2008) 23–49. <https://doi.org/10.1146/annurev.physiol.70.113006.100455>.

[288] I. Bidaud, A. Mezghrani, L.A. Swayne, A. Monteil, P. Lory, Voltage-gated calcium channels in genetic diseases, *Biochim Biophys Acta.* 1763 (2006) 1169–1174. <https://doi.org/10.1016/j.bbamcr.2006.08.049>.

[289] L. Tang, T.M. Gamal El-Din, T.M. Swanson, D.C. Pryde, T. Scheuer, N. Zheng, W.A. Catterall, Structural basis for inhibition of a voltage-gated Ca²⁺ channel by Ca²⁺ antagonist drugs, *Nature.* 537 (2016) 117–121. <https://doi.org/10.1038/nature19102>.

[290] D.J. Tester, M.J. Ackerman, Genetics of long QT syndrome, *Methodist Debaque Cardiovasc J.* 10 (2014) 29–33. <https://doi.org/10.14797/mdcj-10-1-29>.

[291] E. Burashnikov, R. Pfeiffer, H. Barajas-Martinez, E. Delpón, D. Hu, M. Desai, M. Borggrefe, M. Häissaguerre, R. Kanter, G.D. Pollevick, A. Guerchicoff, R. Laiño, M. Marieb, K. Nademanee, G.-B. Nam, R. Robles, R. Schimpf, D.D. Stapleton, S. Viskin, S. Winters, C. Wolpert, S. Zimmern, C. Veltmann, C. Antzelevitch, Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death, *Heart Rhythm.* 7 (2010) 1872–1882. <https://doi.org/10.1016/j.hrthm.2010.08.026>.

[292] S.A. Goonasekera, K. Hammer, M. Auger-Messier, I. Bodi, X. Chen, H. Zhang, S. Reiken, J.W. Elrod, R.N. Correll, A.J. York, M.A. Sargent, F. Hofmann, S. Moosmang, A.R. Marks, S.R. Houser, D.M. Bers, J.D. Molkentin, Decreased cardiac L-type Ca²⁺ channel activity induces hypertrophy and heart failure in mice, *J Clin Invest.* 122 (2012) 280–290. <https://doi.org/10.1172/JCI58227>.

[293] D.B. Tikhonov, B.S. Zhorov, Structural model for dihydropyridine binding to L-type calcium channels, *J Biol Chem.* 284 (2009) 19006–19017.

<https://doi.org/10.1074/jbc.M109.011296>.
 [294] J. Striessnig, M. Grabner, J. Mitterdorfer, S. Hering, M.J. Sinnegger, H. Glossmann, Structural basis of drug binding to L Ca²⁺ channels, *Trends Pharmacol Sci.* 19 (1998) 108–115. [https://doi.org/10.1016/s0165-6147\(98\)01171-7](https://doi.org/10.1016/s0165-6147(98)01171-7).
 [295] G.H. Hockerman, B.Z. Peterson, B.D. Johnson, W.A. Catterall, Molecular determinants of drug binding and action on L-type calcium channels, *Annu Rev Pharmacol Toxicol.* 37 (1997) 361–396. <https://doi.org/10.1146/annurev.pharmtox.37.1.361>.
 [296] C.T. January, L.S. Wann, J.S. Alpert, H. Calkins, J.E. Cigarroa, J.C. Cleveland, J.B. Conti, P.T. Ellinor, M.D. Ezekowitz, M.E. Field, K.T. Murray, R.L. Sacco, W.G. Stevenson, P.J. Tchou, C.M. Tracy, C.W. Yancy, American College of Cardiology/American Heart Association Task Force on Practice Guidelines, 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society, *J Am Coll Cardiol.* 64 (2014) e1–76. <https://doi.org/10.1016/j.jacc.2014.03.022>.
 [297] M. Lei, L. Wu, D.A. Terrar, C.L.-H. Huang, Modernized Classification of Cardiac Antiarrhythmic Drugs, *Circulation.* 138 (2018) 1879–1896. <https://doi.org/10.1161/CIRCULATIONAHA.118.035455>.
 [298] Y. Mori, G. Mikala, G. Varadi, T. Kobayashi, S. Koch, M. Wakamori, A. Schwartz, Molecular pharmacology of voltage-dependent calcium channels, *Jpn J Pharmacol.* 72 (1996) 83–109. <https://doi.org/10.1254/jjp.72.83>.
 [299] W.J. Elliott, C.V.S. Ram, Calcium channel blockers, *J Clin Hypertens (Greenwich).* 13 (2011) 687–689. <https://doi.org/10.1111/j.1751-7176.2011.00513.x>.
 [300] Effect of verapamil on mortality and major events after acute myocardial infarction (the Danish Verapamil Infarction Trial II--DAVIT II), *Am J Cardiol.* 66 (1990) 779–785. [https://doi.org/10.1016/0002-9149\(90\)90351-z](https://doi.org/10.1016/0002-9149(90)90351-z).
 [301] R.E. Goldstein, S.J. Boccuzzi, D. Cruess, S. Nattel, Diltiazem increases late-onset congestive heart failure in postinfarction patients with early reduction in ejection fraction. The Adverse Experience Committee; and the Multicenter Diltiazem Postinfarction Research Group, *Circulation.* 83 (1991) 52–60. <https://doi.org/10.1161/01.cir.83.1.52>.
 [302] T. Godfraind, Discovery and Development of Calcium Channel Blockers, *Front Pharmacol.* 8 (2017) 286. <https://doi.org/10.3389/fphar.2017.00286>.
 [303] R. Rosso, J.M. Kalman, O. Rogowski, S. Diamant, A. Birger, S. Biner, B. Belhassen, S. Viskin, Calcium channel blockers and beta-blockers versus beta-blockers alone for preventing exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia, *Heart Rhythm.* 4 (2007) 1149–1154. <https://doi.org/10.1016/j.hrthm.2007.05.017>.
 [304] M.E. Mangoni, A. Traboulsie, A.-L. Leoni, B. Couette, L. Marger, K. Le Quang, E. Kupfer, A. Cohen-Solal, J. Vilar, H.-S. Shin, D. Escande, F. Charpentier, J. Nargeot, P. Lory, Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/alpha1G T-type calcium channels, *Circ Res.* 98 (2006) 1422–1430. <https://doi.org/10.1161/01.RES.0000225862.14314.49>.
 [305] Y. Watanabe, Y. Koide, J. Kimura, Topics on the Na⁺/Ca²⁺ exchanger: pharmacological characterization of Na⁺/Ca²⁺ exchanger inhibitors, *J Pharmacol Sci.* 102 (2006) 7–16. <https://doi.org/10.1254/jphs.fmj06002x2>.
 [306] T. Iwamoto, T. Watano, M. Shigekawa, A novel isothioureia derivative selectively inhibits the reverse mode of Na⁺/Ca²⁺ exchange in cells expressing NCX1, *J Biol Chem.* 271 (1996) 22391–22397. <https://doi.org/10.1074/jbc.271.37.22391>.
 [307] A. Nakamura, K. Harada, H. Sugimoto, F. Nakajima, N. Nishimura, [Effects of KB-R7943, a novel Na⁺/Ca²⁺ exchange inhibitor, on myocardial ischemia/reperfusion injury], *Nihon Yakurigaku Zasshi.* 111 (1998) 105–115. <https://doi.org/10.1254/fpj.111.105>.
 [308] Y. Ladilov, S. Haffner, C. Balser-Schäfer, H. Maxeiner, H.M. Piper, Cardioprotective effects of KB-R7943: a novel inhibitor of the reverse mode of Na⁺/Ca²⁺ exchanger, *Am J Physiol.*

2233 276 (1999) H1868-1876. <https://doi.org/10.1152/ajpheart.1999.276.6.H1868>.

2234 [309] K. Takahashi, T. Takahashi, T. Suzuki, M. Onishi, Y. Tanaka, A. Hamano-Takahashi, T.

2235 Ota, K. Kameo, T. Matsuda, A. Baba, Protective effects of SEA0400, a novel and selective inhibitor

2236 of the Na⁺/Ca²⁺ exchanger, on myocardial ischemia-reperfusion injuries, *Eur J Pharmacol.* 458

2237 (2003) 155–162. [https://doi.org/10.1016/s0014-2999\(02\)02732-2](https://doi.org/10.1016/s0014-2999(02)02732-2).

2238 [310] M. Yoshiyama, Y. Nakamura, T. Omura, T. Hayashi, Y. Takagi, T. Hasegawa, H. Nishioka,

2239 K. Takeuchi, H. Iwao, J. Yoshikawa, Cardioprotective effect of SEA0400, a selective inhibitor of

2240 the Na⁽⁺⁾/Ca⁽²⁺⁾ exchanger, on myocardial ischemia-reperfusion injury in rats, *J Pharmacol Sci.* 95

2241 (2004) 196–202. <https://doi.org/10.1254/jphs.fpj03101x>.

2242 [311] T. Iwamoto, S. Kita, YM-244769, a novel Na⁺/Ca²⁺ exchange inhibitor that preferentially

2243 inhibits NCX3, efficiently protects against hypoxia/reoxygenation-induced SH-SY5Y neuronal cell

2244 damage, *Mol Pharmacol.* 70 (2006) 2075–2083. <https://doi.org/10.1124/mol.106.028464>.

2245 [312] K. Yamashita, Y. Watanabe, S. Kita, T. Iwamoto, J. Kimura, Inhibitory effect of YM-

2246 244769, a novel Na⁺/Ca²⁺ exchanger inhibitor on Na⁺/Ca²⁺ exchange current in guinea pig

2247 cardiac ventricular myocytes, *Naunyn Schmiedebergs Arch Pharmacol.* 389 (2016) 1205–1214.

2248 <https://doi.org/10.1007/s00210-016-1282-y>.

2249 [313] Y. Watanabe, Cardiac Na⁺/Ca²⁺ exchange stimulators among cardioprotective drugs, *J*

2250 *Physiol Sci.* 69 (2019) 837–849. <https://doi.org/10.1007/s12576-019-00721-5>.

2251 [314] D. An, J. Zhang, X. Tang, P. Gao, Y. Li, Y. Wang, D. Zhu, Association of ATP2B1

2252 common variants with asymptomatic intracranial and extracranial large artery stenosis in

2253 hypertension patients, *Clin Exp Hypertens.* 41 (2019) 323–329.

2254 <https://doi.org/10.1080/10641963.2018.1481421>.

2255 [315] Y. Wang, Y. Zhang, Y. Li, X. Zhou, X. Wang, P. Gao, L. Jin, X. Zhang, D. Zhu, Common

2256 variants in the ATP2B1 gene are associated with hypertension and arterial stiffness in Chinese

2257 population, *Mol Biol Rep.* 40 (2013) 1867–1873. <https://doi.org/10.1007/s11033-012-2242-3>.

2258 [316] H.-J. Lee, J.-O. Kang, S.-M. Kim, S.-M. Ji, S.-Y. Park, M.E. Kim, B. Jigden, J.E. Lim, S.-Y.

2259 Hwang, Y.-H. Lee, B. Oh, Gene Silencing and Haploinsufficiency of Csk Increase Blood Pressure,

2260 *PLoS One.* 11 (2016) e0146841. <https://doi.org/10.1371/journal.pone.0146841>.

2261 [317] J. Xu, H. Qian, S. Hu, L. Liu, M. Zhou, M. Feng, J. Su, L. Ji, Gender-Specific Association

2262 of ATP2B1 Variants with Susceptibility to Essential Hypertension in the Han Chinese Population,

2263 *Biomed Res Int.* 2016 (2016) 1910565. <https://doi.org/10.1155/2016/1910565>.

2264 [318] Y. Tabara, K. Kohara, Y. Kita, N. Hirawa, T. Katsuya, T. Ohkubo, Y. Hiura, A. Tajima, T.

2265 Morisaki, T. Miyata, T. Nakayama, N. Takashima, J. Nakura, R. Kawamoto, N. Takahashi, A. Hata,

2266 M. Soma, Y. Imai, Y. Kokubo, T. Okamura, H. Tomoike, N. Iwai, T. Ogiwara, I. Inoue, K.

2267 Tokunaga, T. Johnson, M. Caulfield, P. Munroe, Global Blood Pressure Genetics Consortium, S.

2268 Umemura, H. Ueshima, T. Miki, Common variants in the ATP2B1 gene are associated with

2269 susceptibility to hypertension: the Japanese Millennium Genome Project, *Hypertension.* 56 (2010)

2270 973–980. <https://doi.org/10.1161/HYPERTENSIONAHA.110.153429>.

2271 [319] H.K. Sombié, J.K. Kologo, D. Tchelougou, S.Y. Ouédraogo, A.K. Ouattara, T.R.

2272 Compaoré, B.M. Nagalo, A.P. Sorgho, I. Nagabila, S.T. Soubeïga, F.W. Djigma, A.T. Yonli, P.

2273 Zabsonré, H. Millogo, J. Simporté, Positive association between ATP2B1 rs17249754 and essential

2274 hypertension: a case-control study in Burkina Faso, West Africa, *BMC Cardiovasc Disord.* 19

2275 (2019) 155. <https://doi.org/10.1186/s12872-019-1136-x>.

2276 [320] X.-M. Sun, M. Yang, C.-X. Jiang, Association of ATP2B1 gene polymorphism with

2277 incidence of eclampsia, *Eur Rev Med Pharmacol Sci.* 23 (2019) 10609–10616.

2278 https://doi.org/10.26355/eurrev_201912_19756.

2279 [321] S.T. Nurnberg, H. Zhang, N.J. Hand, R.C. Bauer, D. Saleheen, M.P. Reilly, D.J. Rader,

2280 From Loci to Biology: Functional Genomics of Genome-Wide Association for Coronary Disease,

2281 *Circ Res.* 118 (2016) 586–606. <https://doi.org/10.1161/CIRCRESAHA.115.306464>.

2282 [322] S.A. Lule, A.J. Mentzer, B. Namara, A.G. Muwenz, B. Nassanga, D. Kizito, H. Akurut, L.

2283 Lubyayi, J. Tumusiime, C. Zziwa, F. Akello, D. Gurdasani, M. Sandhu, L. Smeeth, A.M. Elliott,
 2284 E.L. Webb, A genome-wide association and replication study of blood pressure in Ugandan early
 2285 adolescents, *Mol Genet Genomic Med.* 7 (2019) e00950. <https://doi.org/10.1002/mgg3.950>.
 2286 [323] J. Kayima, J. Liang, Y. Natanzon, J. Nankabirwa, I. Ssinabulya, J. Nakibuuka, A. Katamba,
 2287 H. Mayanja-Kizza, A. Miron, C. Li, X. Zhu, Association of genetic variation with blood pressure
 2288 traits among East Africans, *Clin Genet.* 92 (2017) 487–494. <https://doi.org/10.1111/cge.12974>.
 2289 [324] Y. Guo, M.T. Garcia-Barrio, L. Wang, Y.E. Chen, Experimental Biology for the
 2290 Identification of Causal Pathways in Atherosclerosis, *Cardiovasc Drugs Ther.* 30 (2016) 1–11.
 2291 <https://doi.org/10.1007/s10557-016-6644-7>.
 2292 [325] J.D. Vargas, A. Manichaikul, X.-Q. Wang, S.S. Rich, J.I. Rotter, W.S. Post, J.F. Polak, M.J.
 2293 Budoff, D.A. Bluemke, Detailed analysis of association between common single nucleotide
 2294 polymorphisms and subclinical atherosclerosis: The Multi-ethnic Study of Atherosclerosis, *Data*
 2295 *Brief.* 7 (2016) 229–242. <https://doi.org/10.1016/j.dib.2016.01.048>.
 2296 [326] X. Liu, M. Zhang, H.-W. Shan, X.-T. Song, S.-Z. Lyu, Association of single nucleotide
 2297 polymorphism rs2076185 in chromosome 6P24.1 with premature coronary artery diseases in
 2298 Chinese Han population, *J Geriatr Cardiol.* 13 (2016) 138–144.
 2299 <https://doi.org/10.11909/j.issn.1671-5411.2016.02.008>.
 2300 [327] Y. Wang, C. Huang, Y. Fang, Q. Yang, R. Li, Flexible semiparametric analysis of
 2301 longitudinal genetic studies by reduced rank smoothing, *J R Stat Soc Ser C Appl Stat.* 61 (2012) 1–
 2302 24. <https://doi.org/10.1111/j.1467-9876.2011.01016.x>.
 2303 [328] S. Kulkarni, M. Lenin, R. Ramesh, S.C.W. Delphine, K. Velu, Evaluation of Single-
 2304 Nucleotide Polymorphisms of Transcription Factor 7-Like 2 and ATP2B1 Genes as Cardiovascular
 2305 Risk Predictors in Chronic Kidney Disease, *Int J Appl Basic Med Res.* 9 (2019) 221–225.
 2306 https://doi.org/10.4103/ijabmr.IJABMR_92_19.
 2307 [329] G. Shi, E. Boerwinkle, A.C. Morrison, C.C. Gu, A. Chakravarti, D.C. Rao, Mining gold dust
 2308 under the genome wide significance level: a two-stage approach to analysis of GWAS, *Genet*
 2309 *Epidemiol.* 35 (2011) 111–118. <https://doi.org/10.1002/gepi.20556>.
 2310 [330] T. Johnson, T.R. Gaunt, S.J. Newhouse, S. Padmanabhan, M. Tomaszewski, M. Kumari,
 2311 R.W. Morris, I. Tzoulaki, E.T. O'Brien, N.R. Poulter, P. Sever, D.C. Shields, S. Thom, S.G.
 2312 Wannamethee, P.H. Whincup, M.J. Brown, J.M. Connell, R.J. Dobson, P.J. Howard, C.A. Mein, A.
 2313 Onipinla, S. Shaw-Hawkins, Y. Zhang, G. Davey Smith, I.N.M. Day, D.A. Lawlor, A.H. Goodall,
 2314 Cardiogenics Consortium, F.G. Fowkes, G.R. Abecasis, P. Elliott, V. Gateva, Global BPgen
 2315 Consortium, P.S. Braund, P.R. Burton, C.P. Nelson, M.D. Tobin, P. van der Harst, N. Glorioso, H.
 2316 Neuvirth, E. Salvi, J.A. Staessen, A. Stucchi, N. Devos, X. Jeunemaitre, P.-F. Plouin, J. Tichet, P.
 2317 Juhanson, E. Org, M. Putku, S. Söber, G. Veldre, M. Viigimaa, A. Levinsson, A. Rosengren, D.S.
 2318 Thelle, C.E. Hastie, T. Hedner, W.K. Lee, O. Melander, B. Wahlstrand, R. Hardy, A. Wong, J.A.
 2319 Cooper, J. Palmen, L. Chen, A.F.R. Stewart, G.A. Wells, H.-J. Westra, M.G.M. Wolfs, R. Clarke,
 2320 M.G. Franzosi, A. Goel, A. Hamsten, M. Lathrop, J.F. Peden, U. Seedorf, H. Watkins, W.H.
 2321 Ouwehand, J. Sambrook, J. Stephens, J.-P. Casas, F. Drenos, M.V. Holmes, M. Kivimaki, S. Shah,
 2322 T. Shah, P.J. Talmud, J. Whittaker, C. Wallace, C. Delles, M. Laan, D. Kuh, S.E. Humphries, F.
 2323 Nyberg, D. Cusi, R. Roberts, C. Newton-Cheh, L. Franke, A.V. Stanton, A.F. Dominiczak, M.
 2324 Farrall, A.D. Hingorani, N.J. Samani, M.J. Caulfield, P.B. Munroe, Blood pressure loci identified
 2325 with a gene-centric array, *Am J Hum Genet.* 89 (2011) 688–700.
 2326 <https://doi.org/10.1016/j.ajhg.2011.10.013>.
 2327 [331] D. Levy, G.B. Ehret, K. Rice, G.C. Verwoert, L.J. Launer, A. Dehghan, N.L. Glazer, A.C.
 2328 Morrison, A.D. Johnson, T. Aspelund, Y. Aulchenko, T. Lumley, A. Köttgen, R.S. Vasan, F.
 2329 Rivadeneira, G. Eiriksdottir, X. Guo, D.E. Arking, G.F. Mitchell, F.U.S. Mattace-Raso, A.V. Smith,
 2330 K. Taylor, R.B. Scharpf, S.-J. Hwang, E.J.G. Sijbrands, J. Bis, T.B. Harris, S.K. Ganesh, C.J.
 2331 O'Donnell, A. Hofman, J.I. Rotter, J. Coresh, E.J. Benjamin, A.G. Uitterlinden, G. Heiss, C.S. Fox,
 2332 J.C.M. Witteman, E. Boerwinkle, T.J. Wang, V. Gudnason, M.G. Larson, A. Chakravarti, B.M.
 2333 Psaty, C.M. van Duijn, Genome-wide association study of blood pressure and hypertension, *Nat*

Genet. 41 (2009) 677–687. <https://doi.org/10.1038/ng.384>.

[332] J. Li, J. Shi, W. Huang, J. Sun, Y. Wu, Q. Duan, J. Luo, L.A. Lange, P. Gordon-Larsen, S.L. Zheng, W. Yuan, Y. Wang, B.M. Popkin, Z. Mo, J. Xu, S. Du, K.L. Mohlke, E.M. Lange, Variant Near FGF5 Has Stronger Effects on Blood Pressure in Chinese With a Higher Body Mass Index, *Am J Hypertens.* 28 (2015) 1031–1037. <https://doi.org/10.1093/ajh/hpu263>.

[333] V. Fontana, C.W. McDonough, Y. Gong, N.M. El Rouby, A.C.C. Sá, K.D. Taylor, Y.-D.I. Chen, J.G. Gums, A.B. Chapman, S.T. Turner, C.J. Pepine, J.A. Johnson, R.M. Cooper-DeHoff, Large-scale gene-centric analysis identifies polymorphisms for resistant hypertension, *J Am Heart Assoc.* 3 (2014) e001398. <https://doi.org/10.1161/JAHA.114.001398>.

[334] Y. Okuyama, N. Hirawa, M. Fujita, A. Fujiwara, Y. Ehara, K. Yatsu, K. Sumida, M. Kagimoto, M. Katsumata, Y. Kobayashi, S. Saka, S. Umemura, K. Tamura, The effects of anti-hypertensive drugs and the mechanism of hypertension in vascular smooth muscle cell-specific ATP2B1 knockout mice, *Hypertens Res.* 41 (2018) 80–87. <https://doi.org/10.1038/hr.2017.92>.

[335] R. Little, M. Zi, S.K. Hammad, L. Nguyen, A. Njegic, S. Kurusamy, S. Prehar, A.L. Armesilla, L. Neyses, C. Austin, E.J. Cartwright, Reduced expression of PMCA1 is associated with increased blood pressure with age which is preceded by remodelling of resistance arteries, *Aging Cell.* 16 (2017) 1104–1113. <https://doi.org/10.1111/acer.12637>.

[336] T.M.A. Mohamed, D. Oceandy, M. Zi, S. Prehar, N. Alatwi, Y. Wang, M.A. Shaheen, R. Abou-Leisa, C. Schelcher, Z. Hegab, F. Baudoin, M. Emerson, M. Mamas, G. Di Benedetto, M. Zaccolo, M. Lei, E.J. Cartwright, L. Neyses, Plasma membrane calcium pump (PMCA4)-neuronal nitric-oxide synthase complex regulates cardiac contractility through modulation of a compartmentalized cyclic nucleotide microdomain, *J Biol Chem.* 286 (2011) 41520–41529. <https://doi.org/10.1074/jbc.M111.290411>.

[337] A.M. Sadi, T. Afroze, M.A. Siraj, A. Momen, C. White-Dzuro, D. Zarrin-Khat, S. Handa, K. Ban, M.G. Kabir, M.G. Trivieri, R. Gros, P. Backx, M. Husain, Cardiac-specific inducible overexpression of human plasma membrane Ca²⁺ ATPase 4b is cardioprotective and improves survival in mice following ischemic injury, *Clin Sci (Lond).* 132 (2018) 641–654. <https://doi.org/10.1042/CS20171337>.

[338] M. Seth, Z.-S. Zhang, L. Mao, V. Graham, J. Burch, J. Stiber, L. Tsiokas, M. Winn, J. Abramowitz, H.A. Rockman, L. Birnbaumer, P. Rosenberg, TRPC1 channels are critical for hypertrophic signaling in the heart, *Circ Res.* 105 (2009) 1023–1030. <https://doi.org/10.1161/CIRCRESAHA.109.206581>.

[339] D.G. Washburn, D.A. Holt, J. Dodson, J.J. McAtee, L.R. Terrell, L. Barton, S. Manns, A. Waszkiewicz, C. Pritchard, D.J. Gillie, D.M. Morrow, E.A. Davenport, I.M. Lozinskaya, J. Guss, J.B. Basilla, L.K. Negron, M. Klein, R.N. Willette, R.E. Fries, T.C. Jensen, X. Xu, C.G. Schnackenberg, J.P. Marino, The discovery of potent blockers of the canonical transient receptor channels, TRPC3 and TRPC6, based on an anilino-thiazole pharmacophore, *Bioorg Med Chem Lett.* 23 (2013) 4979–4984. <https://doi.org/10.1016/j.bmcl.2013.06.047>.

[340] K. Seo, P.P. Rainer, V. Shalkey Hahn, D.-I. Lee, S.-H. Jo, A. Andersen, T. Liu, X. Xu, R.N. Willette, J.J. Lepore, J.P. Marino, L. Birnbaumer, C.G. Schnackenberg, D.A. Kass, Combined TRPC3 and TRPC6 blockade by selective small-molecule or genetic deletion inhibits pathological cardiac hypertrophy, *Proc Natl Acad Sci U S A.* 111 (2014) 1551–1556. <https://doi.org/10.1073/pnas.1308963111>.

[341] S. Kiyonaka, K. Kato, M. Nishida, K. Mio, T. Numaga, Y. Sawaguchi, T. Yoshida, M. Wakamori, E. Mori, T. Numata, M. Ishii, H. Takemoto, A. Ojida, K. Watanabe, A. Uemura, H. Kurose, T. Morii, T. Kobayashi, Y. Sato, C. Sato, I. Hamachi, Y. Mori, Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound, *Proc Natl Acad Sci U S A.* 106 (2009) 5400–5405. <https://doi.org/10.1073/pnas.0808793106>.

[342] H.N. Rubaiy, M.J. Ludlow, M. Henrot, H.J. Gaunt, K. Miteva, S.Y. Cheung, Y. Tanahashi, N. Hamzah, K.E. Musialowski, N.M. Blythe, H.L. Appleby, M.A. Bailey, L. McKeown, R. Taylor, R. Foster, H. Waldmann, P. Nussbaumer, M. Christmann, R.S. Bon, K. Muraki, D.J. Beech,

2385 Picomolar, selective, and subtype-specific small-molecule inhibition of TRPC1/4/5 channels, *J Biol*
 2386 *Chem.* 292 (2017) 8158–8173. <https://doi.org/10.1074/jbc.M116.773556>.
 2387 [343] M. Sumi, K. Kiuchi, T. Ishikawa, A. Ishii, M. Hagiwara, T. Nagatsu, H. Hidaka, The newly
 2388 synthesized selective Ca²⁺/calmodulin dependent protein kinase II inhibitor KN-93 reduces
 2389 dopamine contents in PC12h cells, *Biochem Biophys Res Commun.* 181 (1991) 968–975.
 2390 [https://doi.org/10.1016/0006-291x\(91\)92031-e](https://doi.org/10.1016/0006-291x(91)92031-e).
 2391 [344] M.E. Anderson, A.P. Braun, Y. Wu, T. Lu, Y. Wu, H. Schulman, R.J. Sung, KN-93, an
 2392 inhibitor of multifunctional Ca⁺⁺/calmodulin-dependent protein kinase, decreases early
 2393 afterdepolarizations in rabbit heart, *J Pharmacol Exp Ther.* 287 (1998) 996–1006.
 2394 [345] C.N. Johnson, R. Pattanayek, F. Potet, R.T. Rebbeck, D.J. Blackwell, R. Nikolaienko, V.
 2395 Sequeira, R. Le Meur, P.B. Radwański, J.P. Davis, A.V. Zima, R.L. Cornea, S.M. Damo, S.
 2396 Györke, A.L. George, B.C. Knollmann, The CaMKII inhibitor KN93-calmodulin interaction and
 2397 implications for calmodulin tuning of NaV1.5 and RyR2 function, *Cell Calcium.* 82 (2019) 102063.
 2398 <https://doi.org/10.1016/j.ceca.2019.102063>.
 2399 [346] J.T. Smyth, A.L. Abbott, B. Lee, I. Sienaert, N.N. Kasri, H. De Smedt, T. Ducibella, L.
 2400 Missiaen, J.B. Parys, R.A. Fissore, Inhibition of the inositol trisphosphate receptor of mouse eggs
 2401 and A7r5 cells by KN-93 via a mechanism unrelated to Ca²⁺/calmodulin-dependent protein kinase
 2402 II antagonism, *J Biol Chem.* 277 (2002) 35061–35070. <https://doi.org/10.1074/jbc.M202928200>.
 2403 [347] S. Neef, A. Steffens, P. Pellicena, J. Mustroph, S. Lebek, K.R. Ort, H. Schulman, L.S.
 2404 Maier, Improvement of cardiomyocyte function by a novel pyrimidine-based CaMKII-inhibitor, *J*
 2405 *Mol Cell Cardiol.* 115 (2018) 73–81. <https://doi.org/10.1016/j.yjmcc.2017.12.015>.
 2406 [348] S. Lebek, A. Plöchl, M. Baier, J. Mustroph, D. Tarnowski, C.M. Lucht, S. Schopka, B.
 2407 Flörchinger, C. Schmid, Y. Zausig, N. Pagratis, B. Marchand, D.O. Koltun, W.K. Hung, S.
 2408 Ahmadyar, L. Belardinelli, L.S. Maier, S. Wagner, The novel CaMKII inhibitor GS-680 reduces
 2409 diastolic SR Ca leak and prevents CaMKII-dependent pro-arrhythmic activity, *J Mol Cell Cardiol.*
 2410 118 (2018) 159–168. <https://doi.org/10.1016/j.yjmcc.2018.03.020>.
 2411 [349] P. Beauverger, M.-L. Ozoux, G. Bégis, V. Glénat, V. Briand, M.-C. Philippo, C. Daveu, G.
 2412 Tavares, S. Roy, A. Corbier, P. Briand, O. Dorchies, A.-L. Bauchet, E. Nicolai, O. Duclos, D.
 2413 Tamarelle, M.-P. Pruniaux, A.J. Muslin, P. Janiak, Reversion of cardiac dysfunction by a novel
 2414 orally available calcium/calmodulin-dependent protein kinase II inhibitor, RA306, in a genetic
 2415 model of dilated cardiomyopathy, *Cardiovasc Res.* 116 (2020) 329–338.
 2416 <https://doi.org/10.1093/cvr/cvz097>.
 2417 [350] A. Ishida, I. Kameshita, S. Okuno, T. Kitani, H. Fujisawa, A novel highly specific and
 2418 potent inhibitor of calmodulin-dependent protein kinase II, *Biochem Biophys Res Commun.* 212
 2419 (1995) 806–812. <https://doi.org/10.1006/bbrc.1995.2040>.
 2420 [351] A.P. Braun, H. Schulman, A non-selective cation current activated via the multifunctional
 2421 Ca(2+)-calmodulin-dependent protein kinase in human epithelial cells, *J Physiol.* 488 (Pt 1) (1995)
 2422 37–55. <https://doi.org/10.1113/jphysiol.1995.sp020944>.
 2423 [352] R. Zhang, M.S.C. Khoo, Y. Wu, Y. Yang, C.E. Grueter, G. Ni, E.E. Price, W. Thiel, S.
 2424 Guatimosim, L.-S. Song, E.C. Madu, A.N. Shah, T.A. Vishnivetskaya, J.B. Atkinson, V.V.
 2425 Gurevich, G. Salama, W.J. Lederer, R.J. Colbran, M.E. Anderson, Calmodulin kinase II inhibition
 2426 protects against structural heart disease, *Nat Med.* 11 (2005) 409–417.
 2427 <https://doi.org/10.1038/nm1215>.
 2428 [353] B.H. Chang, S. Mukherji, T.R. Soderling, Characterization of a calmodulin kinase II
 2429 inhibitor protein in brain, *Proc Natl Acad Sci U S A.* 95 (1998) 10890–10895.
 2430 <https://doi.org/10.1073/pnas.95.18.10890>.
 2431 [354] B.H. Chang, S. Mukherji, T.R. Soderling, Calcium/calmodulin-dependent protein kinase II
 2432 inhibitor protein: localization of isoforms in rat brain, *Neuroscience.* 102 (2001) 767–777.
 2433 [https://doi.org/10.1016/s0306-4522\(00\)00520-0](https://doi.org/10.1016/s0306-4522(00)00520-0).
 2434 [355] P.D. Swaminathan, A. Purohit, S. Soni, N. Voigt, M.V. Singh, A.V. Glukhov, Z. Gao, B.J.

2435 He, E.D. Luczak, M.A. Joiner, W. Kutschke, J. Yang, J.K. Donahue, R.M. Weiss, I.M. Grumbach,
2436 M. Ogawa, P.-S. Chen, I. Efimov, D. Dobrev, P.J. Mohler, T.J. Hund, M.E. Anderson, Oxidized
2437 CaMKII causes cardiac sinus node dysfunction in mice, *J Clin Invest.* 121 (2011) 3277–3288.
2438 <https://doi.org/10.1172/JCI57833>.
2439 [356] M.-L.A. Joiner, O.M. Koval, J. Li, B.J. He, C. Allamargot, Z. Gao, E.D. Luczak, D.D. Hall,
2440 B.D. Fink, B. Chen, J. Yang, S.A. Moore, T.D. Scholz, S. Strack, P.J. Mohler, W.I. Sivitz, L.-S.
2441 Song, M.E. Anderson, CaMKII determines mitochondrial stress responses in heart, *Nature.* 491
2442 (2012) 269–273. <https://doi.org/10.1038/nature11444>.
2443 [357] S.J. Coultrap, K.U. Bayer, Improving a natural CaMKII inhibitor by random and rational
2444 design, *PLoS One.* 6 (2011) e25245. <https://doi.org/10.1371/journal.pone.0025245>.
2445