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Increased Myofilament Ca^{2+} -Sensitivity and Arrhythmia Susceptibility

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Abstract

Increased myofilament Ca^{2+} sensitivity, a common attribute of inherited and acquired cardiomyopathies, is often associated with cardiac arrhythmias. Accumulating evidence supports that increased myofilament Ca^{2+} sensitivity is an independent risk factor for arrhythmias, but the underlying molecular mechanism remains unclear. This review focuses on potential mechanisms how myofilament Ca^{2+} sensitivity may affect cardiac excitation and leads to the generation of arrhythmias. We discuss in detail the downstream effects of increased myofilament Ca^{2+} sensitivity, i.e. altered Ca^{2+} buffering/handling, impaired energy metabolism and increased mechanical stretch, and how they may contribute to the proarrhythmic effect.

Keywords

sudden cardiac death; reentry arrhythmias; conduction velocity; Ca^{2+} handling; energetic deficit; mechanical stretch

1. Regulation of myofilament Ca^{2+} sensitivity

In heart muscle, cyclic interactions between thin (actin) filaments and thick (myosin) filaments result in muscle shortening and force production. Initiation of contraction occurs when Ca^{2+} binds to troponin C in the troponin complex on the thin filament [1]. The troponin complex is comprised of troponin C (TnC or the Ca^{2+} binding subunit), troponin I (TnI, involved in inhibition of actin–myosin interaction in the absence of Ca^{2+}), and troponin T (TnT which binds the troponin complex to tropomyosin, another thin filament protein) in a 1:1:1 stoichiometric ratio [2]. During diastole when cytosolic Ca^{2+} levels are low, tropomyosin is positioned such that actin and myosin cannot interact. Once Ca^{2+} binds to TnC, tropomyosin shifts and allows actin and myosin to form strong cross-bridges. The thin and thick filaments slide along each other driven by ATP hydrolysis and the heart contracts. Myofilament activation is directly dependent on the amount of activating Ca^{2+} , but is also determined by the Ca^{2+} dependence of force production (=myofilament Ca^{2+} sensitivity), which can be regulated within certain limits. The Ca^{2+} sensitivity of the myofilament is usually determined

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by the steady-state force-calcium relationship, which is well described by a Hill equation. The myofilament Ca^{2+} sensitivity is increased when the relationship is shifted to the left, towards lower concentrations on the $[\text{Ca}^{2+}]$ axis.

Myofilament Ca^{2+} sensitivity is dynamically regulated by a number of processes that relate calcium cycling to myofilament force production: Ca^{2+} binding to TnC, de-inhibition of actin-myosin interaction by the thin filament and actin-myosin cross-bridge properties [3]. For example, Ca sensitivity changes during each cardiac cycle with sarcomere length [4,5], an effect in part responsible for the immediate adaptation in cardiac output during beat-to-beat changes in ventricular filling (Frank-Starling response [6]). Longer lasting regulation of myofilament Ca^{2+} sensitivity is achieved by phosphorylation (for overview see [7]), most strikingly by phosphorylation of TnI [8]. The phosphorylation of two N-terminal serines by cAMP dependent protein kinase A (PKA) decreases myofilament Ca^{2+} sensitivity and contributes to the positive lusitropic effect of beta agonists [9,10]. The same serines are also phosphorylated by protein kinase D (PKD) [11] thereby allowing multiple signaling pathways to regulate Ca^{2+} dependence of force production via this route.

During acute myocardial ischemia, myofilament Ca^{2+} sensitivity decreases largely due to a combined effect of acidic pH and increased $[\text{PO}_4]$ (as a consequence of the decline in high energy phosphates) [12–14]. Myofilament Ca^{2+} sensitivity remains decreased in post-ischemic or "stunned" myocardium even once the intracellular milieu is restored, likely because of modification of or proteolytic injury to contractile proteins [15,16]. Myofilament Ca^{2+} sensitization has been proposed as an attractive drug target to increase pump function in failing hearts without deleterious effects on energetic efficiency [17,18]. In response several " Ca^{2+} sensitizers" were developed and studied in the 1990s [19–21], but often impaired cardiac relaxation [22,23] and only Levosimendan is currently in clinical use in Europe [24].

2. Inherited and acquired conditions with increased myofilament Ca^{2+} sensitivity are often associated with arrhythmias

Mutations in genes encoding sarcomeric proteins have been identified in several inherited cardiomyopathies, one of which is Familial Hypertrophic Cardiomyopathy (FHC) [25]. FHC mutations are associated with an increased risk for cardiac arrhythmias leading to sudden cardiac death [26,27]. Especially several mutations in cardiac TnT are associated with a high incidence of sudden death, with the majority of deaths (75%) occurring in patients under the age of 45 [28–30], despite little or no cardiac hypertrophy [31]. Generally, the genotype/phenotype relationships of sarcomeric mutations are weak [32], suggesting that mechanisms in addition to hypertrophy, myofibrillar disarray and fibrosis play a significant role for the risk of ventricular arrhythmias and sudden cardiac death, especially in young patients with only minor structural remodeling of the heart.

FHC-mutations of the thin filament proteins almost universally increase myofilament Ca^{2+} sensitivity of force development, as we and others have reviewed [33–36]. This was studied *in vitro* [37–46] and in transgenic models expressing the mutant thin filament proteins [41, 42,47–54]. In contrast the effects of these sarcomeric mutations on other myofilament properties are rather diverse, i.e. differential effects on myofilament Ca^{2+} -ATPase activity, maximal force, troponin complex binding affinity to the thin filament and pH-regulation. While less well studied than the thin filament proteins, reports suggest that many FHC-linked mutations in myosin heavy chain (MHC) [55–57], in myosin binding protein C (MyBP-C) [58,59] and in regulatory light chain [60,61] can also increase Ca^{2+} sensitivity of force development. These data indicate that FHC-linked sarcomeric mutations disrupt the tight inhibitory regulation exerted by the thin filament, with a common downstream effect of increased Ca^{2+} sensitivity of force development.

In addition to inherited conditions, acquired diseases can also be associated with changes in myofilament Ca^{2+} sensitivity. Substantial evidence exists for increased Ca^{2+} sensitivity of force production in animals after myocardial infarction (MI) [62]. This disease is also characterized by a high incidence of ventricular tachycardia and sudden cardiac death. Interestingly, exercise training after MI, which normalizes the increased myofilament Ca^{2+} sensitivity [63], also has been shown to reduce mortality and the rate of ventricular arrhythmias in dog [64,65] and human studies [66](reviewed in [67]). Treatment with the Ca^{2+} sensitizer levosimendan significantly increased the incidence of ventricular tachycardia in one clinical study [68], further linking increased myofilament Ca^{2+} sensitivity with arrhythmias.

Myofilament Ca^{2+} sensitivity appears to be also increased in end-stage human heart failure [69–71], another condition associated with a high incidence of ventricular arrhythmia. The myofilament sensitization is at least partially due to decreased phosphorylation of troponin I [69,70,72–74]. While results from human tissues can be somewhat problematic as the tissue is not collected under controlled conditions and there are inherent differences between donor and recipient treatment [75], increased myofilament Ca^{2+} sensitivity has also been observed in experimental models where samples were obtained under controlled conditions [62,76,77].

3. Increased myofilament Ca^{2+} sensitivity – a novel mechanism of arrhythmogenesis?

The above observations support the hypothesis that Ca^{2+} sensitized myofilaments are a risk factor for developing ventricular tachyarrhythmias. We recently tested this hypothesis using different lines of transgenic mice where a range of myofilament Ca^{2+} sensitivity was produced by expressing different TnT mutants or TnI isoforms [78]. Compared to control mice with no change in myofilament Ca^{2+} sensitivity (non-transgenic littermates (NTG), human TnT-WT, TnT-R278C), mice with Ca^{2+} sensitized myofilaments (TnT-F110I, TnT-I79N or slow skeletal TnI expression) demonstrate an increase in the rate of premature ventricular complexes when injected with the β -agonist isoproterenol. The occurrence of ventricular tachycardia (VT) in the myofilament Ca^{2+} sensitized groups was directly proportional to the degree of shift in Ca^{2+} sensitivity, whereas VT did not occur in controls. Similarly, the average pacing frequency required to induce sustained VT in isolated perfused hearts was lower in Ca^{2+} sensitized TnT mutants. All transgenic mice used have been extensively phenotyped and do not exhibit cardiac hypertrophy, fibrosis, or myofibrillar disarray [41,49,79]. However, we cannot completely rule out that small defects in anatomy contribute to the arrhythmia susceptibility. Therefore we also investigated the effect of the Ca^{2+} sensitizing compound EMD 57033 at a concentration with minimal phosphodiesterase inhibition [19,80]. Presence of EMD rendered NTG hearts susceptible to VT induction, an effect that was completely reversible upon washout. These experiments suggest that myofilament Ca^{2+} sensitization *per se* is proarrhythmic and raise the question if a reduction in myofilament Ca^{2+} sensitivity to control level is anti-arrhythmic [78]. However, Ca^{2+} de-sensitizers are not readily available to test this hypothesis, as until now there was no known purpose for such a compound. Blebbistatin (BLEB), an actin-myosin uncoupler, has been shown to shift the Ca^{2+} dependence of force development to the right with only negligible effects on cardiac ion channels [81,82]. We reproduced this effect and showed that BLEB reduces myofilament Ca^{2+} sensitivity in TnT mutant mice and antagonizes the Ca^{2+} sensitizing effect of EMD [78]. In accordance with that result BLEB completely prevented the increased occurrence of VT in all Ca^{2+} sensitized groups (TnT-mutants and EMD-treated). This showed, for the first time, that a reduction of Ca^{2+} sensitivity in myofilaments is anti-arrhythmic, which may be beneficial for individuals with hypertrophic cardiomyopathy.

4. How does myofilament Ca^{2+} sensitivity affect cardiac electrical activity?

Our experimental results clearly demonstrate that increasing Ca^{2+} sensitivity of myofilaments alters the electrical activity of the heart [78]. However, the underlying mechanisms are much less clear. In optical recordings using a voltage sensitive dye, we typically found long lasting rotors forming repetitive activation patterns [78]. These results suggest that Ca^{2+} sensitization causes a substrate for reentrant activation. The functional reentry appears to be caused by the regional slowing of impulse propagation (increased spatial dispersion of conduction velocity (CV)) during rapid pacing. This was observed in myofilament Ca^{2+} sensitized transgenic hearts, but also during treatment of control hearts with the Ca^{2+} sensitizer EMD, clearly demonstrating that this effect does not require an anatomical substrate. How can increased myofilament Ca^{2+} sensitivity cause the observed electrical heterogeneities? Myofilament Ca^{2+} sensitization may mediate the effect on cardiac excitation via three principal pathways [83]: (1) through an effect on the intracellular Ca^{2+} homeostasis (e.g. decreased Ca^{2+} transient amplitude and slowed Ca^{2+} transient decline [84]), (2) energy metabolism (energy depletion due to increased energy demand combined with decreased energy utilization, as described in FHC [47,85,86]) or (3) increased mechanical stress (hearts are hypercontractile [49], but fail under conditions of increased demand).

(1) Ca^{2+} -handling

In intact myocytes, free $[\text{Ca}^{2+}]$ is determined by the rate of sarcolemmal and trans-sarcoplasmic reticulum Ca^{2+} fluxes and the Ca^{2+} buffering properties of the cytosol (reviewed in detail in [87]). TnC represents a substantial portion of cytoplasmic Ca^{2+} buffering, binding in the order of 50% of Ca^{2+} released from the sarcoplasmic reticulum during a typical heart beat [88]. Hence, an increase in myofilament Ca^{2+} sensitivity, if mediated by increasing the Ca^{2+} affinity of TnC (see above), can be predicted to decrease peak free $[\text{Ca}^{2+}]$ [50,83]. This prediction has been confirmed experimentally by introducing exogenous Ca^{2+} buffers into ventricular myocytes: Two Ca^{2+} buffers both decreased peak systolic $[\text{Ca}^{2+}]$ and also slowed Ca^{2+} transient decline (while the exact kinetic effects were dependent on each buffers properties) [89]. Recently, we directly measured cytosolic Ca^{2+} buffering in myocytes from TnT mutant mice using two established techniques [90,91]. The increase in free $[\text{Ca}^{2+}]$ in response to a matched total Ca^{2+} influx was significantly lower in TnT-I79N myocytes compared to control (Fig. 1, published in abstract form [92]). Consistent with this finding, in field-stimulated myocytes from TnT-I79N mice Ca^{2+} transient amplitude is reduced and decay is prolonged, while the diastolic $[\text{Ca}^{2+}]$ was increased in the presence of isoproterenol or when the cells were rapidly paced [84]. This result supports the idea that at peak $[\text{Ca}^{2+}]$ levels more Ca^{2+} is bound to the myofilaments, but as $[\text{Ca}^{2+}]$ decreases the Ca^{2+} dissociation from the myofilaments becomes the rate limiting step for Ca^{2+} transient decline. Fig. 1 illustrates this concept of how increased myofilament Ca^{2+} sensitivity may change cytosolic $[\text{Ca}^{2+}]$ during a typical heart beat. Slow Ca^{2+} transient decay kinetics were also found in papillary muscles from transgenic mice expressing a mutant regulatory light chain (R58Q) with increased myofilament Ca^{2+} sensitivity [61].

Myofilament Ca^{2+} sensitizing drugs can be expected to affect Ca^{2+} homeostasis similarly to some sarcomeric mutations, that is if they indeed increase Ca^{2+} binding affinity of TnC. Ca^{2+} sensitizers may also stabilize the Ca^{2+} -induced conformation of TnC or have other downstream effects [93], e.g. direct effects on cross-bridge interaction as for example caffeine [94]. The mechanism of Ca^{2+} sensitization for each drug cannot be simply determined by measuring peak $[\text{Ca}^{2+}]$, but often requires direct assessment of Ca^{2+} binding to TnC. This is necessary because most Ca^{2+} sensitizers inhibit to some degree cyclic nucleotide phosphodiesterases (PDE), which in parallel enhances Ca^{2+} cycling through activation of PKA, like e.g. pimobendan [95]. However, EMD57033, together with CGP-48506 [96], seems to be

a Ca^{2+} sensitizer with only negligible PDE inhibition at effective concentrations [80]. In ferret cardiac muscle, EMD57033 produced an enhanced and prolonged contraction, while it decreased Ca^{2+} transient amplitude, consistent with increased Ca^{2+} binding to TnC [80]. In the same study it appeared to shorten the Ca^{2+} transient at higher concentrations (20 μM), while 50 μM prolonged time to 80% Ca^{2+} decay and increased diastolic $[\text{Ca}^{2+}]$ without an effect on peak $[\text{Ca}^{2+}]$ in human ventricular muscle [22]. Thus, while it is unclear if EMD57033 increases TnC Ca^{2+} buffering, it likely has some effect on Ca^{2+} homeostasis in cardiomyocytes, while the exact effects still need to be determined.

Ca^{2+} handling and action potential (AP)—There is a clear interdependence between intracellular Ca^{2+} signaling and membrane potential. Ca^{2+} ion movements can affect the AP during the plateau phase (phase 2) in ventricular myocytes. L-type Ca^{2+} channel current is the major inward current during that phase and the Ca^{2+} that enters triggers Ca^{2+} induced Ca^{2+} release (CICR) from the SR. The inactivation of the L-type Ca^{2+} current greatly depends on Ca^{2+} induced inactivation, i.e. the higher the amplitude of the Ca^{2+} transient the more accelerated is the inactivation, creating a negative feedback loop (when the Ca^{2+} release is large Ca^{2+} entry is reduced) [97]. Therefore, if peak $[\text{Ca}^{2+}]$ is blunted due to increased myofilament Ca^{2+} sensitivity, the negative feedback is reduced and more Ca^{2+} enters during each AP. In TnT-I79N myocytes we failed to observe any changes in L-type Ca^{2+} current magnitude or inactivation, despite lower peak $[\text{Ca}^{2+}]$ [84]. However, a decrease in whole cell peak $[\text{Ca}^{2+}]$ due to increased myofilament Ca^{2+} buffering may not affect Ca^{2+} levels in the junctional cleft, where RyRs and L-type Ca^{2+} channels communicate.

Another opportunity for Ca^{2+} handling to affect the AP is through the activity of the Na/Ca exchanger (NCX). NCX is also active during the plateau phase producing a net-inward current by transporting Ca^{2+} out of the cell. Large NCX inward currents will therefore prolong the plateau phase. Since the Ca^{2+} transients are prolonged in myofilament Ca^{2+} sensitized myocytes, Ca^{2+} comes off more slowly, therefore potentially resulting in decreased NCX peak activity and slower activity decay. The result would be that the plateau phase is shorter, but overall AP duration may not necessarily be affected. This is exactly what we found before in TnT-I79N: the AP duration at 70% repolarization levels (APD_{70}) is reduced at 1 and 5 Hz, while AP duration at 90% repolarization levels (APD_{90}) is reduced at 1 Hz, but not at 5 Hz [84]. The AP differences were abolished by perfusion with Li^+ , which can enter though Na^+ channels, but cannot be transported by the NCX, confirming the role of NCX activity. Similarly, we found a decreased APD_{70} after perfusion with EMD57033, consistent with the TnT mutant results, which was reversible upon washout of EMD57033. Thus it appears that APs are shorter and assume a more triangular shape (AP “triangulation”) when myofilaments are Ca^{2+} sensitized, due to a different time-course of NCX activity. It should be noted that APDs of higher mammals are less dependent on NCX activity and the application of the above mechanism may be limited [98,99]. However, we also confirmed APD shortening of APD_{30} , APD_{50} and APD_{70} in cat myocytes, which have AP shapes closely resembling human APs.

Ca^{2+} handling and ventricular arrhythmias—In order to discuss how Ca^{2+} handling may contribute to ventricular arrhythmia susceptibility, we briefly review several characteristics of arrhythmias found in association with increased myofilament Ca^{2+} sensitivity. First, arrhythmias can be initiated by fast pacing rates (hearts with highest degree of Ca^{2+} sensitization required the lowest pacing frequencies for induction) [78], which is consistent with the observation that fast heart rates often precede arrhythmic events in patients with hypertrophic cardiomyopathy [100,101]. Second, optical mapping studies revealed that the spatial dispersion of ventricular activation (determined by standard deviation of conduction velocity (CV) at 10 different angles) is increased in myofilament Ca^{2+} sensitized TnT mutants at higher pacing frequencies. This regional slowing of CV was similarly observed after perfusion of control hearts with EMD57033, confirming that this is not due to intrinsic

structural abnormalities [78]. Third, the repetitive activation patterns observed during VT, e.g. stationary rotors, are indicative of reentry-type arrhythmia [78].

These findings support the notion that the observed regional abnormalities of conduction provide the substrate for the arrhythmia [102], possibly forming zones of cells that are refractory and temporarily unexcitable (conduction block). Once initiated, the arrhythmia is then maintained by a regenerative circuit of electrical activity around relatively inexcitable tissue (re-entry). Local alterations in channel function or passive tissue properties can also disrupt the normal activation pattern and contribute to altered conduction patterns and reentrant arrhythmias.

One of the best supported possibilities how altered intracellular Ca^{2+} handling may support reentrant arrhythmias is the occurrence of Ca^{2+} transient alternans [103–105]. Briefly, myocytes with prolonged Ca^{2+} transients (e.g. induced by myofilament Ca^{2+} sensitization) are more likely to develop Ca^{2+} transient alternans at fast heart rates [106]. As discussed earlier, Ca^{2+} handling will affect APD via Ca^{2+} -sensitive membrane currents and APD will also begin to alternate (also *visa versa*). This bidirectional coupling between Ca^{2+} homeostasis and APD can drive the Ca^{2+} transient of two neighboring cells to be out-of-phase, which will result in spatially discordant alternans on the tissue level (independent of CV restitution) [107]. The spatial gradients in APD or Ca^{2+} transient amplitude are very steep at the nodal line, which separates the out-of-phase regions, predisposing to localized conduction block [108–111]. Under these conditions, ectopic beats have a high probability of inducing re-entry [111].

Another possibility is based on the shortening of APD as a consequence of lower peak $[\text{Ca}^{2+}]$ observed in TnT mutants myocytes. Shorter APD, or AP “triangulation”, if associated with beat-to-beat instability (as demonstrated in cat heart after EMD57033 perfusion [78]), has been shown to be a strong predictor of drug-induced proarrhythmia *in vivo* [112]. Mechanistically, the AP “triangulation” may be associated with a shallow terminal AP repolarization slope, which could lead to incomplete and varying recovery of Na^+ channels and subsequently to APD alternans during fast pacing.

Together, these two mechanisms may be responsible to provide a substrate that is able to sustain VT, but in most cases an additional triggering mechanism is still required. A possible hypothesis is that the increased diastolic $[\text{Ca}^{2+}]$ at fast pacing rates shifts into the SR during pauses and causes greater post-rest Ca^{2+} transient potentiation. This in turn will lead to considerable post-pause APD prolongation and, if long enough for the L-type Ca^{2+} current to recover from inactivation, the generation of early afterdepolarizations (EADs) [113]. In addition to a mechanism based on reactivation of L-type current, early or delayed afterdepolarizations can be induced by spontaneous Ca^{2+} release from the above steady-state filled SR after a pause and activate the NCX inward current (reviewed in [114]). However, post-rest increase of the SR load is species dependent and both mechanisms may only apply to rodents [115]. Another consideration is that the slow Ca^{2+} transient decay and/or increased diastolic $[\text{Ca}^{2+}]$ activates calmodulin-dependent kinase 2 (CaMKII), which has been shown to cause EADs in mice [116] and in a dog model [117].

(2) energy metabolism

There are several reports documenting an energetic deficits in FHC and, just like myofilament Ca^{2+} sensitization, it appears to be a common feature (reviewed in [85,118]). The positive inotropic effect associated with myofilament Ca^{2+} sensitization, expected when Ca^{2+} cycling remains unaltered, can be predicted to proportionally increase ATP utilization as a direct consequence of increased myosin ATPase activity [83]. In addition, inefficient energy utilization, i.e. increased Ca^{2+} activated ATPase rate divided by tension (increased “tension cost” [119]), or simply put, more ATP is hydrolyzed to generate the same amount of force,

may further raise the energy demand. This particular feature does not appear to require an increase in myofilament Ca^{2+} sensitivity, considering that an increased tension cost was demonstrated in some FHC models with increased [51] as well as decreased [120,121] myofilament Ca^{2+} sensitivity. However, in animals [86,122,123] and humans alike [124–126], the increased energy use leads to a decrease in high-energy phosphates in the FHC heart (predominantly phosphocreatine). This demonstrates that the increased energy utilization creates an energetic deficit and possible metabolic remodeling *in vivo*, without a primary defect in energy supply.

Ca^{2+} sensitizers are expected to improve the energetic economy of contraction when compared to other positive inotropic agents such as catecholamines [127–132]. Nevertheless, any intervention that increases contractile force by increasing myosin ATPase activity will consume more energy and increase energy demand (unless an agent actually decreases tension cost [133]). Thus, increased myofilament Ca^{2+} sensitivity is expected to increase myosin ATPase activity and the increased ATP utilization may cause deficits in energy supply. The effect of energy depletion on cardiac electrical activity has been intensively studied in the context of hypoxia and ischemia, but it is less clear to which extent these results are applicable to a potentially chronic energetic deficit induced by increased myofilament Ca^{2+} sensitivity.

Energetic deficit and AP—It is easily conceivable that increased ATP consumption by myosin ATPase subsequently affects other high energy consuming processes, e.g. the SR Ca^{2+} ATPase (SERCA) responsible for Ca^{2+} uptake into the SR. SERCA operates close to its theoretical thermodynamic limit and therefore is most vulnerable to decreases in free energy released from ATP hydrolysis (ΔG_{ATP} ↓; that is expected if e.g. [ADP] increases) [134]. Decreased SERCA function would enhance the direct effect of increased myofilament Ca^{2+} sensitivity on Ca^{2+} homeostasis, by further decreasing Ca^{2+} transient amplitude and exacerbating transient prolongation as shown in Fig. 1 [135]. This may affect the AP via NCX as outlined above (prolonged Ca^{2+} transients).

The NCX activity is directly and indirectly dependent on $[\text{Ca}^{2+}]$, but also depends on $[\text{Na}^+]$ [136]. Thus, the effect of energy depletion on NCX becomes more complex if also Na^+/K^+ ATPase (NKA) activity, responsible for maintaining the Na^+ and K^+ gradient across the sarcolemma, becomes limited by insufficient energy supply. The primary consequence of NKA inhibition will be a rise in intracellular $[\text{Na}^+]$, which favors Ca^{2+} influx through the NCX during an AP, in contrast to the almost exclusive Ca^{2+} extrusion under physiological conditions [87]. This therefore would then generate first a transient outward current and the additional Ca^{2+} influx later enhances the transient inward current during the plateau phase, both resulting in altered AP shape.

A decrease in ΔG_{ATP} can also activate ATP-dependent K^+ channels (K_{ATP}), which are present in high density in cardiac myocytes [137]. The single channel conductance is large and therefore the opening of very few channels (<1%) appears to be sufficient to shorten APD by as much as 50% [138,139]. The opening of these channels may also contribute to the loss of intracellular $[\text{K}^+]$ and subsequent increase in extracellular $[\text{K}^+]$ during ischemia, which is an important factor for the formation of lethal ventricular arrhythmias (see below). Secondary to an elevation in extracellular $[\text{K}^+]$, the resting membrane potential increases (due to an increase in E_{K}) and proportionally inactivates the inward Na^+ current, causing slowing of AP upstroke and a decrease in AP amplitude [140]. The role of K_{ATP} channels in the net-loss of intracellular K^+ however is controversial, and may depend more on an increase in intracellular $[\text{Na}^+]$ [141].

Recent findings also put forward a role of unpaired connexin43 (Cx43) hemichannels in disturbing ionic balances in the heart during conditions when energy supply is limited, e.g.

ischemia and metabolic inhibition [142–145]. Hemichannels (or connexons) are inserted into the membrane to partner with other hemichannels from neighboring cells to form functional gap junction channels [146]. Based on theoretical estimates several thousand hemichannels exist in cardiac myocytes at any given time [143] and it has been demonstrated that metabolically sensitive hemichannels exist in cultured neonatal and isolated adult myocytes [144,147]. Similar to the K_{ATP} channels, their unitary conductance for cations is high and the opening of just a few channels can be predicted to cause significant loss of intracellular K^+ and intracellular loading of Na^+ and Ca^{2+} , with profound consequences for excitability and action potential shape. Hemichannel opening has been demonstrated during ischemia in neurons and likely contributes to neuronal death during stroke [148].

Energetic deficit and arrhythmias—In the case of compromised SERCA function the generation of arrhythmias is triggered by disturbances in Ca^{2+} homeostasis (see under Ca^{2+} handling and arrhythmias), but in the other cases discussed above it is due to primary disturbances in Na^+/K^+ homeostasis (inhibition of NKA, opening of K_{ATP} or Cx43 hemichannels). These later possibilities would all require a decrease in subsarcolemmal [ATP], but it is currently unclear which target would be most sensitive under conditions of a rising energetic deficit elicited by increased energy demand.

The consequences of intracellular Na^+ overload, as a consequence of NKA inhibition and/or Cx43 hemichannel opening, has been recently reviewed [149]. Increased intracellular $[Na^+]$ is a solid predictor of ventricular fibrillation during hypoxia and therefore seems to contribute to electrical instability [150]. The increase in $[Na^+]$ secondarily promotes passive net K^+ loss, which may be necessary to maintain electroneutrality and osmotic balance (sum of cations $[K^+] + [Na^+]$ inside and outside remain unchanged) [141]. Extracellular K^+ accumulation depolarizes the membrane, slows conduction and alters refractoriness and altogether these factors increase the probability of reentrant arrhythmias [151]. K_{ATP} channels contribute to the K^+ loss, but their activation may also render a cell non-excitable (at membrane potentials above -70 mV sodium channels do not recover from inactivation [152]). This may be generally a protective mechanism that allows an energetically compromised cell to conserve energy. For example, K_{ATP} (Kir 6.2) is required for cellular adaptation during periods of stress [153] and may have a protective role during ischemia/reperfusion [154]. However, once non-excitable cells accumulate in one region this can cause conduction block and promote reentrant arrhythmias. Furthermore, epicardial cells may be more susceptible to K_{ATP} opening and APD shortening during metabolic inhibition than endocardial cells, i.e. epicardial APDs shortens more drastically than endocardial APDs [155]. This may further increase heterogeneity of repolarization, which has long been recognized to play a crucial role in the induction or maintenance of ventricular tachyarrhythmias [156,157].

In addition to the role of Cx43 hemichannels in the formation of arrhythmias, alteration of Cx43 gap junction function may also contribute by influencing the precisely orchestrated patterns of impulse propagation. Gap junctions are intercellular channels that permit the transfer of electrical current and small molecules between directly adjacent cells [158]. Essentially all forms of cardiomyopathy are associated with changes in the expression and distribution of Cx43, e.g. in human hypertrophic cardiomyopathy a redistribution from the intercalated disks to the sides of the myocytes (lateralization) has been described [159]. Under control conditions myocardial Cx43 is highly phosphorylated at multiple carboxyterminal residues [160], but during global ischemia rapid Cx43 dephosphorylation paralleled the loss of gap junctional coupling within 30 min [161]. This dephosphorylation of Cx43 is reversible and tightly linked to cellular levels of ATP in neonatal rat cardiomyocytes [162]. This result is consistent with the observation that gap junctional current rapidly declined in adult rat cardiomyocytes due to dephosphorylation when no ATP was added to the patch pipette solution [163]. Thus, cellular uncoupling is a potential mechanism that contributes to abnormal impulse

conduction under conditions of increased energy demand. It may not be in itself sufficient to initiate arrhythmias, as coupling has to be reduced to very low levels to slow conduction [164], but it may be a contributing factor by enhancing APD heterogeneity [165].

(3) mechanical stress/strain

Ca^{2+} sensitized myofilaments will by definition produce more force for a given $[\text{Ca}^{2+}]$. Therefore, as expected, systolic function was enhanced in myofilament Ca^{2+} sensitized TnT-I79N mice under baseline conditions *in vivo* [166] and *ex vivo* (physiological $[\text{Ca}^{2+}]$) and in control hearts after treatment with EMD57033 [167]. However, at fast heart rates and/or in the presence of β -adrenergic stimulation myofilament Ca^{2+} sensitized hearts show impaired systolic and diastolic performance [167]. The diastolic failure can be directly explained by the increased myofilament Ca^{2+} sensitivity induced by the TnT-I79N mutation, causing delayed diastolic separation of Ca^{2+} from the troponin complex and therefore slowing of relaxation. *In vivo* the pressure-volume relationship is shifted to the right (towards larger volume) and upwards (towards higher diastolic pressure) during isoproterenol stress, indicating increased mechanical stretch of the ventricular wall under those conditions [166].

Mechanical stress/strain and AP/arrhythmias—The crosstalk between mechanical stretch and AP is well established and has been demonstrated in various mammalian hearts and human (reviewed in [168,169]). This relationship is often referred to as mechano-electric feedback (MEF) [170]. Generally an inverse relationship is found, such that increased intraventricular pressure causes shorter APDs. In addition to altering AP shape, increasing stretch raises the membrane potential until the depolarization reaches threshold and an action potential is triggered [170]. In intact canine hearts, both isolated and *in situ*, increases in ventricular volume and pressure resulted in a decrease in AP plateau duration (APD_{20}) and appearance of early afterdepolarizations [171]. In rabbit heart the occurrence of triggered premature ventricular excitations was dependent on the amplitude and velocity of stretch application [172]. Regions that experience greater relative stretch may be susceptible to focal excitation and trigger stretch-activated arrhythmias, a theory that was supported by the observed spatial variability of AP properties [172]. In particular, moderate stretch may induce heterogeneous strain and focal excitation rather than larger stretch that causes synchronous excitation [173]. In intact rabbit hearts these focal excitations developed into reentrant arrhythmias.

5. Conclusions

Accumulative evidence suggest that increased myofilament Ca^{2+} sensitivity contributes to the risk for ventricular arrhythmias, but the underlying molecular mechanism remains unclear. Direct consequences of increased myofilament Ca^{2+} sensitivity, i.e. altered Ca^{2+} buffering/handling, impaired energy metabolism and increased mechanical stretch, each by itself or together in varying degree may be responsible for the proarrhythmic effect. Blebbistatin prevents arrhythmias in Ca^{2+} sensitized hearts by reducing myofilament Ca^{2+} sensitivity to control level [78] and can be expected to simultaneously prevent all molecular consequences (it interferes with actin-myosin interaction downstream of Ca^{2+} binding [174], by inhibition of myosin ATPase it preserves cellular ATP [175] and it minimizes stretch by inhibiting contraction [81]). Therefore it does not provide additional insights into the signaling pathway responsible for ventricular arrhythmias and additional detailed experiments are required.

This is an exciting area of research that may lead to development of improved Ca^{2+} sensitizers for inotropic support of failing hearts, or may clarify that Ca^{2+} desensitization is a life prolonging treatment. However, since Ca^{2+} desensitization might decrease heart pump function, other downstream targets are of therapeutic interest. The successful dissection of the

signaling chain from myofilament Ca^{2+} sensitization to ventricular arrhythmias will allow the identification of new targets for the treatment of cardiac arrhythmias.

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Fig. 1.

The effect of myofilament Ca^{2+} sensitization on myocyte Ca^{2+} handling. Panel (A) shows the effect of three TnT mutations on myofilament Ca^{2+} sensitivity measured in skinned fibers of transgenic mice [78]. Panel (B) shows the effect myofilament Ca^{2+} sensitization on apparent Ca^{2+} buffering. The change in total Ca^{2+} was measured by the Na/Ca exchanger integral (bottom, shaded area) simultaneously with the change in $[\text{Ca}^{2+}]_{\text{free}}$ (top panel) in voltage clamped fluo-4 loaded myocytes [90,92]. Note that the peak $[\text{Ca}^{2+}]$ is lower in TnT-I79N despite a similar change in total Ca^{2+} , consistent with increased Ca^{2+} buffering. (C)+(D) Ca^{2+} transients simulated with LabHEART 5.0 [176] containing a newly integrated force response [177]. Ionic current conductances were adjusted to reproduce a typical mouse action potential. The myofilament Ca^{2+} sensitivity curves in (A) were reproduced by changing the TnC Ca^{2+} on/off rates and Ca^{2+} binding cooperativity. Ca^{2+} transients are simulated at 1 Hz (C) and 5 Hz (D) pacing frequency. Note that peak $[\text{Ca}^{2+}]$ is lower and Ca^{2+} transients are prolonged in Ca^{2+} sensitized mutants, while the diastolic $[\text{Ca}^{2+}]$ is additionally increased in TnT-I79N at 5 Hz. Panel (C)+(D) were kindly provided by Dr. J. Puglisi, UC Davis.

Fig. 2.

Mechanism of arrhythmogenesis caused by myofilament Ca^{2+} sensitization. The cartoon illustrates components likely involved in the signaling pathway from myofilament Ca^{2+} sensitization to sudden cardiac death. On the molecular level increased mechanical stress [166], increased Ca^{2+} buffering (published in abstract form [92]) and/or an energetic deficit (reduced free energy from ATP hydrolysis (ΔG_{ATP})) [83] may contribute to the observed cellular changes in Ca^{2+} handling [84] and AP repolarization and effective refractory period (ERP)[78,84]. On the organ level this results in stress-induced contractile dysfunction [49,166] and abnormalities in propagation of excitation (increased conduction velocity (CV) dispersion [78]), possibly supported by abnormal cell-to-cell coupling (published in abstract form [178]). Together, these factors contribute to ventricular tachyarrhythmias and sudden death.