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Dysregulation of intracellular calcium transporters in animal models of sepsis induced cardiomyopathy

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Abstract

Sepsis induced cardiomyopathy (SIC) develops as the result of myocardial calcium (Ca^{2+}) dysregulation. Here we reviewed all published studies that quantified the dysfunction of intracellular Ca^{2+} transporters and the myofilaments in animal models of SIC.

Cardiomyocytes isolated from septic animals showed, invariably, a decreased twitch amplitude, which is frequently caused by a decrease in the amplitude of cellular Ca^{2+} transients (Ca_i) and sarcoplasmic reticulum (SR) Ca^{2+} load (Ca_{SR}). Underlying these deficits, the L-type Ca^{2+} channel is downregulated, through mechanisms that may involve adrenomedullin-mediated redox signaling. SR Ca^{2+} pump (SERCA) is also inhibited, through oxidative modifications (sulphonylation) of one reactive thiol group (on Cys⁶⁷⁴), and/or modulation of phospholamban. Diastolic Ca^{2+} leak of ryanodine receptors is frequently increased. In contrast, $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibition may play a partially compensatory role by increasing Ca_{SR} and Ca_i . The action potential is usually shortened. Myofilaments show a bidirectional regulation, with decreased Ca^{2+} sensitivity in milder forms of disease (due to troponin I hyperphosphorylation) and a (redox mediated) increase in more severe forms. Most deficits occurred similarly in two different disease models, induced by either intraperitoneal administration of bacterial lipopolysaccharide (LPS) or cecal ligation and puncture (CLP).

In conclusion, substantial cumulative evidence implicates various Ca^{2+} transporters and the myofilaments in SIC pathology. What is less clear, however, is the identity and interplay of the signaling pathways that are responsible for Ca^{2+} transporters dysfunction. With few exceptions, all studies we found used solely male animals. Identifying sex differences in Ca^{2+} dysregulation in SIC becomes, therefore, another priority.

Keywords

L-type Ca^{2+} channel; SERCA; $\text{Na}^+/\text{Ca}^{2+}$ exchange; ryanodine receptor; myofilaments

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INTRODUCTION

Over 750,000 Americans (1) are admitted to Intensive Care Units (ICU) each year with sepsis and septic shock and 20% (2) to 50% (3) of them die in hospital. In the last 50 years, more than 100 clinical trials failed to improve survival, and sometimes worsened it (4). As such, treatment continues to rely on source control, antibiotics and supportive care, particularly early goal-directed therapy (5).

Certain septic patients develop cardiomyopathy, which complicates their management and worsens prognosis. In one study (6), the development of sepsis-induced cardiomyopathy (SIC) increased mortality to 91%, compared with 14% in septic patients without SIC. SIC usually develops within the first 48h after onset of sepsis, and, interestingly, in surviving patients, is fully reversible within 7–10 days (7, 8). A number of excellent reviews have been written about the clinical relevance and cellular pathophysiology of SIC (9–13). Continuing their efforts, we describe here a distinct component of the current view on the cellular pathology of SIC. Specifically, we focus on the dysregulation of myocardial calcium (Ca^{2+}) handling in SIC, which, in our opinion, represents the final integrating step of a myriad of signaling pathways which underlie the decrease in cardiac contractile force that defines SIC.

SIC develops as a result of the dysregulation of myocardial Ca^{2+} handling (Figure 1)

The importance of cellular Ca^{2+} handling in the patho-physiology of SIC becomes evident if one considers two axiomatic constraints:

First, from a mechanistic point of view, the depression of cardiac contractile force in SIC (as in any type of cardiomyopathy) can only be due to 2 factors: cardiomyocyte death (for example by apoptosis (14)) and the decrease in the contractile function of the surviving cardiomyocytes (Figure 1). The focus of this review is the latter. Without trying to minimize much previous work (14), the spontaneous resolution of SIC (7) argues against a primary role of cell death. In support of our focus, we will show below that, in all animal models of SIC, cardiomyocytes isolated from septic animals show, invariably, a decreased contractile function. The converse experiment (which will not be reviewed here) is also true: cardiac cells isolated from healthy animals can develop contractile dysfunction when exposed to either serum from septic patients (15) or directly to inflammatory mediators such as bacterial lipopolysaccharide (LPS) (16), tumor necrosis factor (TNF) (15), interleukine-1 (IL-1) (15), IL-6 (17) and others (18).

Second, at a cellular level, the decrease in contractile capacity can only be due to 2 factors (Figure 1): a decrease in the amplitude of transient rise in cellular Ca^{2+} (i.e. the Ca^{2+} transient, Ca_i) that is triggered by the action potential and activates the myofilaments, or to myofilament dysfunction (19). The generation of the Ca^{2+} transient and myofilament function are the main components of Ca^{2+} handling, and will be discussed here.

Upstream of Ca^{2+} handling, a number of signaling pathways are involved in SIC pathology, including nitric oxide (NO) (20) and radical oxygen / nitrogen species (RONS) (21) stress, activation of the cAMP (19) and cGMP (22) -dependent phosphorylation, transcriptional

(23, 24) and metabolic (25) changes, etc. However, it is essential to realize that all these signaling pathways can only induce a decrease in cardiac contractility through effects on Ca^{2+} handling, by inhibiting either Ca_i or the myofilament function.

Therefore, the focus of this review is to identify the various Ca^{2+} handling mechanisms (Ca^{2+} transporters and myofilaments) that have been implicated in animal models of SIC. First, we will briefly review the current view of myocardial Ca^{2+} handling, and specifically the process known as Excitation-Contraction Coupling (ECC), as well as the most commonly used animal models of SIC.

Excitation-Contraction Coupling (ECC) in the normal and septic heart (Figure 2)

The complex chain of events that leads to myocardial cell contraction during the action potential is called ECC. ECC is centered around Ca^{2+} release from the sarcoplasmic reticulum (SR), which, in the heart, plays the role of an intracellular Ca^{2+} store (26). SR Ca^{2+} release is triggered during the depolarization phase of the action potential by a small amount of Ca^{2+} ions that enter the cell via the membrane L-type Ca^{2+} channels (LTCC). This process is called Ca^{2+} -induced Ca^{2+} -release (CICR) (27, 28) (Figure 2) and lies at the core of ECC in the heart.

Therefore, at a first analysis, Ca_i depends on two related parameters: the amplitude of the trigger Ca^{2+} entry via LTCC (29) and the amount of Ca^{2+} stored in the SR (Ca_{SR}) and available for release. While the trigger Ca^{2+} entry is determined solely by the expression and function of LTCC, Ca_{SR} is regulated through a complex interaction between the 4 main transporters: LTCC, the SR ryanodine receptors (RyR), SR Ca^{2+} ATP-ase (SERCA) and the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchange (26). Downstream of CICR, the last determinants of cardiac contractile force are the myofilaments' sensitivity for Ca^{2+} and maximal developed tension (30) as well as the passive (viscous and elastic) resistance of the cell (31).

Here we will review the available evidence that implicates the dysregulation of cardiac Ca^{2+} handling in the development of SIC. We will first focus on global measurements, such as

Ca_i and Ca_{SR} and subsequently turn our attention to individual Ca^{2+} transporters, such as LTCC, SERCA, RyR and the NCX, as well as the myofilaments. By the nature of the required experiments, all these studies have been performed in animal models of sepsis.

Experimental models of SIC

Sepsis and SIC have been studied in a number of species, from the ubiquitous mice, guinea pigs, rats and rabbits, to pigs and monkeys (see Tables). The most commonly used model is administration of LPS, either intraperitoneally (ip) or intravenously (iv). LPS triggers an aseptic inflammatory shock syndrome, sometimes referred as “endotoxemic” shock, which is not identical to septic states (32) (see Discussion). A model that resembles more the clinical scenario is performing of cecal ligation and puncture (CLP), which induces fecal peritonitis (33). Other related methods include direct ip inoculum of cecal washing (30, 34).

METHODOLOGY

We searched the available literature for studies that investigated Ca^{2+} handling in animal models of sepsis, that used isolated cardiac cells, or measured the activity of individual Ca^{2+} transporters or the myofilaments function *in vitro*. We did not include studies that investigated the contractile function in whole hearts or multicellular preparations, because their usual lack of specificity with regard of Ca^{2+} handling mechanisms. Moreover, we did not include studies that investigated the effect of challenging isolated cardiac cells with inflammatory mediators *in vitro* (15–18, 35). We consider this is a different model of disease altogether, whose similarity to the *in vivo* models remains to be ascertained. Finally, we included all the papers we found and aimed to present an unbiased and comprehensive view of the state of the field.

In what regards data interpretation, we gave more weight to “positive” findings that showed a difference between the disease state and baseline, than to those showing no difference. In other words, we considered that, in order to be taken under consideration, any given finding has to be present in some, but not necessarily in *all* the studies published. In some instances, differences in experimental conditions may explain why certain modifications are present in some models but not in other. For example, the depression in myofilament Ca^{2+} sensitivity was reported in mice challenged with low doses of LPS (4–6 $\mu\text{g/g}$ weight (36)), but not after higher doses (37) (as discussed below). The timing of investigation is also important. Even within the same animal model, some Ca^{2+} handling modification, such as the decrease in Ca_{SR} (38) or SERCA function (39) occur with a specific timing, being present at some timepoints, but not others.

RESULTS AND DISCUSSION

Cardiomyocyte contractility is decreased in SIC (Table 1 and Figure 3A)

Invariably, cells isolated from the hearts of septic or endotoxemic animals show a decrease in contractile function, as measured by the amplitude of sarcomere shortening when stimulated by external pacing (Table 1 and Figure 3A). While undoubtedly also reflecting a publication bias towards positive findings, this common observation clearly indicates that animal models of SIC are associated with a contractile deficit at the level of the cardiac cells.

A decrease in Ca_i underlies most models of SIC (Table 2 and Figure 3B)

In most SIC models, the decrease in cardiomyocyte contractile function was associated with, and likely due to a decrease in Ca_i . In mice, ip administration of LPS induces a dose-dependent decrease in Ca_i consistently observed for doses between 25 and 50 $\mu\text{g/g}$ (37, 40) $\mu\text{g/g}$. Challenging mice with lower doses of LPS (6 $\mu\text{g/g}$) gave conflicting results, being associated with a decrease in Ca_i in one study (21) but not in another (36). In terms of timing, the decrease in Ca_i in this model appears as early as 4h after LPS ip administration (21), and has been documented for as long as 14–16h (37).

In rat models, results are more controversial. In one study, Ca_i was decreased after administration of 10 $\mu\text{g/g}$ LPS (41), however, another study found no change 20 $\mu\text{g/g}$ (42).

In rats, Ca_i were decreased 48 h after CLP (38) and also at 48h (but not 24h) after ip inoculum of cecal washings (34). Intravenous administration of 5 μ g/g LPS in rats did not change Ca_i (19). Finally, LPS administration to guinea pigs, either iv (43, 44) or ip (45) also decreased Ca_i .

Therefore, the emerging picture is that, generally, the decrease in cardiac contractility in SIC is due to a decrease in Ca_i . As an exception to this rule, mice and rats challenged with low doses of LPS may show solely a myofilament dysfunction, that decreases cell contractions even in the presence of a normal Ca_i (see below).

Diastolic Ca_i levels in SIC (Table 3 and Figure 3C)

The level of diastolic Ca^{2+} is one of the main factors that determine diastolic cardiac stiffness. As such, an increase in diastolic Ca^{2+} could potentially slow cardiac relaxation and thus induce a degree of cardiac diastolic dysfunction, which is known to be present in septic patients and independently associated with mortality (46).

In animal models of sepsis, the level of diastolic Ca^{2+} is variable. In LPS-challenged mice, diastolic Ca^{2+} was unchanged at 4h (21) and 18h (36) after a dose of 6 μ g/g, but decreased 14h after 25 μ g/g LPS (37). In rat models, diastolic Ca_i was unchanged after a dose of 5 μ g/g LPS (19), but increased after 10 (47) or 20 μ g/g LPS (42), or after CLP (38). In contrast, diastolic Ca^{2+} was decreased after ip administration of cecal washings in rat (34). In guinea pigs, diastolic Ca^{2+} was decreased after LPS administration, by either ip (45) or iv (43) route.

Thus, the available data indicate that, in some conditions, an increase in diastolic Ca^{2+} may be present in septic hearts, and thus represent the mechanism underlying the increase in diastolic stiffness seen clinically (46).

Ca^{2+} load of the SR is decreased in SIC (Table 4 and Figure 4D)

A decrease in Ca_{SR} has been demonstrated in mice challenged with 25 μ g/g (37) and 50 μ g/g LPS (40) as well as 48 h after induction of CLP in rats (38). In contrast, the decrease in cell twitch occurred with preserved Ca_{SR} in rabbits challenged with LPS iv (48).

A decrease in steady state Ca_{SR} is one of the main determinants of the decreased Ca_i , by reducing the amount of Ca^{2+} available for release during CICR. Mechanistically, the decrease in Ca_{SR} in SIC models can be explained by both an inhibition of SERCA activity and by an increase in RyR diastolic leak (see below).

L-type Ca^{2+} channels are dysfunctional in SIC (Table 5 and Figure 3A)

A decrease in LTCC current ($I_{Ca,L}$) has been consistently demonstrated in endotoxemic rats (49), guinea pigs (44) and mice (37) as well as in pigs challenged with live bacteria iv (50). The decrease in $I_{Ca,L}$ is likely one of the principal factors that explain the decrease in Ca_i , by decreasing the amount of Ca^{2+} that enters the cell and triggers CICR.

Mechanistically, two different studies showed that the decrease in $I_{Ca,L}$ is caused by a decrease in LTCC channel expression, both in mice after LPS administration (51) and in rats

after CLP (23). It is possible that LTCC expression may exhibit a bi-modal evolution. One study reported that LTCC are overexpressed early after induction of CLP in rats, in association with a hyperdynamic circulatory septic state, which was followed by LTCC underexpression during the late hypodynamic phase (23).

In what regards the underlying mechanisms of LTCC downregulation, two main theories, not mutually exclusive, have been postulated. One laboratory (49, 52) implicated adrenomedullin, a 52 aminoacid hormone first identified in human pheochromocytoma cells (53), and later demonstrated also in the heart and other tissues, and which can thus act both as an endocrine and/or paracrine factor (54). Adrenomedullin is overexpressed in septic states (55), but it is still controversial whether it has a predominantly protective (56, 57) or pathologic (49, 52) role. As far as the latter is concerned, a number of studies have demonstrated that, *in vitro*, adrenomedullin can exert negative inotropic effects by inhibiting LTCC current and Ca_i (49, 52). The signaling pathway that mediates LTCC inhibition is incompletely understood, but may involve G_i protein, phosphokinase A (PKA), cyclooxygenase 2 and ultimately nitric oxide synthase 2 (NOS2) activation (52).

Another theory (51) implicates cellular oxidative stress, although the available evidence is still indirect only. Increased production of RONS, such as superoxide anion and peroxynitrite, is a hallmark of sepsis and septic shock (58) and has been demonstrated in septic hearts (40). Moreover, exposure of normal mouse cardiomyocytes to peroxynitrite *in vitro* is able to induce LTCC downregulation (51). In principle, oxidative stress is able to accelerate protein turnover (59), and induce the downregulation of constitutive proteins (59). This has been demonstrated for certain ion channels, such as the amiloride sensitive Na^+ channel (ENaC) in the kidney (60) and the cystic fibrosis transmembrane conductance regulator (CFTR) channel in the airway epithelia (61), but whether the same mechanism is active in septic hearts is currently unknown.

SERCA is inhibited in SIC (Table 6 and Figure 4B)

SERCA inhibition has been demonstrated in numerous models of sepsis and septic shock (Table 6). As early as 1977, a first study showed a deficit in SR Ca^{2+} uptake (62) in LPS-challenged dogs, which was later demonstrated to be specifically due to SERCA inhibition (63). Since then, SERCA inhibition has also been demonstrated in LPS challenged mice (21, 37, 40) and rats (42), as well as rats after CLP (34, 38, 39). In one study in mice, SERCA inhibition after LPS occurred in a dose dependent fashion (37), further confirming a direct causative relation.

In some rat models, SERCA inhibition may occur with a biphasic time dependence. For example, in 4 studies in rats after CLP (34, 38, 39, 64), SERCA function was found to be unchanged at early stages but decreased in the late phases of the disease. Conversely, in rats challenged with LPS *ip*, SERCA activity was decreased early (at 6h) but not later (20h after LPS administration) (42). This dichotomy represents one of the few consistent differences between the LPS and CLP models of SIC (see Discussion, below).

Mechanistically, the changes in SERCA function have been attributed to changes in the phosphorylation levels of phospholamban (PLB, SERCA main associated regulatory

protein) (64), and/or redox modifications (37). In one study in rats, PLB phosphorylation was increased 9h after CLP (associated with an increased SERCA function) and decreased at 18h (which was associated with a decrease in SERCA function) (64). In contrast, in LPS-challenged mice, SERCA inhibition occurred in the absence of any changes in PLB phosphorylation, and in association with oxidative modifications (sulphonylation) of one reactive thiol, at Cys⁶⁷⁴ (Cys⁶⁷⁴-S₀3H) (37) a modification previously shown to be able to exert inhibitory effects (65). Consistent with this hypothesis, in another study in rats (39) SERCA inhibition occurred through a decrease in the maximal rate of transport, which is compatible with the effects of Cys⁶⁷⁴-S₀3H.

Defective ryanodine receptors show increased diastolic leak in SIC (Table 7 and Figure 3C)

In pathological states, RyR dysfunction is manifested as an increase in the diastolic SR leak flux, leading to a decrease in Ca_{SR} and Ca_i (66). An increase in RyR leak has been reported for mice challenged with high dose LPS (50 µg/g) (40), but was not present for lower doses of 25 µg/g LPS (37). RyR leak was also increased in rats after CLP (38).

The mechanisms responsible for the increase in RyR leak are yet unknown. In principle, RyR leak could be induced by both RyR hyperphosphorylation (67) or redox modifications (68), but no direct evidence for their involvement in SIC is yet available.

One other study showed a decreased RyR density (with an unchanged affinity) 2h after iv administration of LPS (2.8 mg/kg) in monkeys (24), without elaborating on the functional consequences of this finding. A decrease in RyR density may lead to a decrease in CICR efficiency (or “gain”, i.e. the amount of SR Ca²⁺ that is released for a given trigger Ca²⁺ entry (69)), however (despite initial claims otherwise (70)), such a mechanism is unlikely to be responsible for the decrease in Ca_i, because of compensatory changes in Ca_{SR} (71).

Na⁺/Ca²⁺ exchange is inhibited in SIC (Table 8 and Figure 3D)

Na⁺/Ca²⁺ exchange function was decreased in mice challenged with 50 µg/g LPS ip (40), but not after a dose of 25 µg/g (37). Similarly, Na⁺/Ca²⁺ exchange inhibition has been demonstrated in septic rats, 18h (but not at 9h) after CLP (72).

Importantly, in diseased myocardium, a decrease in Na⁺/Ca²⁺ exchange activity would be expected to exert a partially compensatory effect, by partially opposing SERCA inhibition and increasing Ca_{SR} and Ca_i (73). In fact, Na⁺/Ca²⁺ exchange inhibitors have been proposed as a therapeutic strategy to improve cellular Ca²⁺ handling and contractile function in patients with congestive heart failure (74). It is interesting therefore, that such a beneficial phenomenon occurs spontaneously in septic hearts, where it may contribute to the spontaneous resolution of the disease (75).

Action potential (AP) is shortened in SIC (Table 9 and Figure 3E)

The duration of the cardiac action potential has been found to be reduced after LPS challenge in rats (49) and guinea pigs (44) (ip) and rabbits (iv) (48). However, this was not the case after induction of CLP in rats (76). Mechanistically, the shortening of the AP duration could be explained by the decrease *I*_{Ca,L} amplitude, that will shorten especially the

plateau phase of the AP. Downstream, a shortened AP will further decrease the time during which Ca^{2+} enters the cell through LTCC and reverse $\text{Na}^+/\text{Ca}^{2+}$ exchange, and thus exert a further negative inotropic effect.

Myofilament dysfunction in SIC (Table 10)

The dysfunction of myofilaments is one of the first mechanistic theories proposed in SIC. As early as 1978, Bruni and Hess (77, 78) reported a decrease in ATP hydrolysis rate in dogs, 5h after challenge with 4 mg/kg LPS iv. Subsequent studies confirmed the myofilament dysfunction in animal models of SIC (Table 10), although the methodology used varied.

In isolated cells, the simplest way to assess myofilament function is to compare the amplitude of cell shortening obtained at similar Ca_i (Table 10). As such, a number of studies showed that cells isolated from LPS-challenged mice (36) and rats (19) showed decreased cell shortening for similar Ca_i , either during paced contractions (19, 36), or, in other experiments, during tetanic contractions elicited by prolonged membrane depolarizations (19, 47). In other words, these studies showed a deficit in “ Ca^{2+} -contraction coupling” in SIC models. Although it is tempting to attribute this to a decrease in myofilament sensitivity (79) for Ca^{2+} , it is important to realize that this is not necessarily so. Other mechanisms could explain this observation, such as a decrease in myofilament maximal tension (45) or to an increase in the passive resistance of the cell, such as internal cell viscosity (80) or the myofilament elastic resistance (81).

In the future, it will be important to differentiate between these possible mechanisms, especially since the deficit in Ca^{2+} -contraction coupling may be only part of the whole story. More recent data showed that, in mice, the effectiveness of Ca^{2+} -contraction coupling may be decreased only in early or mild disease states, such as those induced by a low dose (6 $\mu\text{g/g}$) of LPS (36). In contrast, Ca^{2+} -contraction coupling was unchanged in cells isolated from mice challenged with 25 $\mu\text{g/g}$ LPS (37), and was actually increased after a dose of 50 $\mu\text{g/g}$ LPS (40). If one compares data from these three laboratories, with the associated caveats, what emerges is that LPS administration can induce a bidirectional modulation of the effectiveness of Ca^{2+} -contraction coupling. Low doses of LPS may inhibit myofilament sensitivity (36), while higher doses may increase it (40). Whether this is the case for other models of SIC (such as CLP), is unknown.

Myofilament desensitization in SIC (Table 11 and Figure 4A)

Notwithstanding these theoretical concerns and necessary qualifiers, it is important to emphasize that a decrease in myofilament sensitivity has been demonstrated in a number of studies across species (Table 11 and Figure 4A). Mechanistically, the decrease in myofilament sensitivity has been shown to be the result of troponin I (Tn I) hyperphosphorylation (19, 36), since it was prevented in transgenic mice with a cardiac specific overexpression of the skeletal-muscle isoform of Tn I (which is insensitive to PKA-dependent phosphorylation) (36). On the other end of the spectrum, reversible oxidative modifications likely mediate the increased myofilament sensitivity for Ca^{2+} in mice challenged with higher doses (50 $\mu\text{g/g}$) of LPS, since it could be reversed by addition of reducing agents (40).

The Tn I hyperphosphorylation theory (19, 36) gives rise, however, to an internal inconsistency that is yet to be resolved. It is well known that PKA-dependent phosphorylation is one of the most potent activators of cardiac Ca^{2+} handling (82). Why are these stimulating effects not seen in SIC models? More specifically, PKA-dependent phosphorylation increases SERCA and LTCC function (83). As detailed above, in most SIC models SERCA and LTCC function is actually depressed, which, in the case of SERCA has been associated specifically with a decrease (64) or no change (37) in PLB phosphorylation. If myofilaments are hyperphosphorylated, why isn't PLB hyperphosphorylated? Are these two mechanisms (myofilament hyperphosphorylation vs. SERCA and LTCC inhibition) occurring separately, in different models, or at different timepoints? Or could they occur simultaneously, as a result of intracellular compartmentation of PKA signaling (84)? The answer to these questions must remain for the future.

Myofilament maximal tension is usually unchanged in SIC (Table 12 and Figure 4B)

In an interesting study, Rigby, et al, (45) found that the maximal developed tension of the myofilaments is decreased in cells isolated from LPS-challenged guinea pigs. However, this was only the case if the experimental solution used to isolate the cells did not contain protease inhibitors. The authors considered thus that the observed decrease in myofilament maximal tension was an experimental artifact. It may be due to degradation of the myofilaments during the isolation procedure, as a result of the release of proteolytic enzymes from inflammatory cells that may be present in the myocardium of LPS-challenged (but not control) animals (45).

Other studies have confirmed that maximal developed tension is also unchanged in LPS challenged rats (79), dogs (78) and rabbits (85). In rats after CLP, maximal tension was also unchanged at 24h, and it was actually increased in the later phases of the disease (3–7 days) (30).

The available data indicates, therefore, that a decrease in the myofilaments maximal developed tension does not contribute to the contractile deficit in SIC models.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, our literature review showed that, in animal models, the development of SIC can be attributed to the dysfunction of a number of intracellular Ca^{2+} transporters, including: 1) inhibition of LTCC (44, 49); 2) inhibition of SERCA (38–40, 64), 3), an increase in RyR Ca^{2+} leak (38, 40) and 4) a decrease in myofilament Ca^{2+} sensitivity (19, 36, 79, 85).

Putting these data together, a few general considerations can be made:

SIC vs. congestive cardiomyopathy

A number of interesting conclusions can be reached by comparing SIC with animal models of chronic cardiomyopathy, usually due to pressure- or volume- overload, or metabolic causes (86). A number of Ca^{2+} transporters play similar roles in the patho-physiology of both diseases, whereas others are relatively specific to SIC. SERCA (87) and RyR (66) dysfunction, for example, are common mechanisms for models of chronic heart failure and

SIC. In contrast, LTCC dysfunction (69) and myofilament desensitization are uncommon findings in chronic cardiomyopathy models and are, therefore, relatively specific for SIC.

As far as the latter is concerned, it is interesting to note that only one other form of cardiomyopathy is known to be primarily due myofilament dysfunction: cardiac stunning (88). This interesting parallel evokes old theories, now largely forgotten, that considered SIC as a form of cardiac stunning (77). It also indicates that a similar signaling pathway may act upstream of Ca^{2+} handling in the two disease processes.

One major difference between SIC and congestive cardiomyopathy is the regulation of the $\text{Na}^+/\text{Ca}^{2+}$ exchange, which is inhibited in SIC (40, 72, 89) and upregulated in congestive cardiomyopathy (90). $\text{Na}^+/\text{Ca}^{2+}$ exchange upregulation in chronic heart failure models plays a critical determinant role in the decrease in Ca_{SR} and Ca_i (73). Conversely, a reduced $\text{Na}^+/\text{Ca}^{2+}$ exchange is able to induce an compensatory increase in Ca_{SR} and Ca_i , and thus to limit the deleterious effects of SERCA and LTCC inhibition (73). The naturally occurring inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange can, therefore, be considered a beneficial influence in SIC (75).

Endotoxemia- vs. sepsis-induced cardiomyopathy

The two most common animal models of sepsis and septic shock are the administration of LPS and performing CLP. Since the pathological trigger, as well as many inflammatory features are not identical between these models, justified concerns exist whether these models could be expected to lead to the same patho-physiological insights and indicate the same therapeutic strategies (32, 33, 91).

From the data reviewed here, we conclude that, in what regards cardiac Ca^{2+} handling, both LPS- and CLP-challenge are associated with largely similar modifications.

As detailed above, cardiomyocyte shortening is similarly decreased in both models, both in mice (37, 92) and rats (38, 52). Ca_i is also similarly decreased in both models (37, 38, 47, 92). In rats, SERCA is inhibited (42) and diastolic Ca_i levels are increased (42) similarly after both LPS- and CLP- challenge. Ca_{SR} was decreased (37, 38), RyR leak increased (37, 38) and the $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibited (40, 72) in both LPS-challenged mice and CLP-challenged rats. Myofilament desensitization was also decreased in rats both after LPS challenge (79) and after ip cecal inoculum (30). Moreover, in one of the few studies (40) that compared the two models head to head, one therapeutic interventions (NOS3 overexpression) prevented cardiomyocyte Ca^{2+} handling dysfunction similarly after LPS and CLP challenge in mice.

However, some consistent differences could be found between the two models. First, action potential was shortened in rats challenged with LPS (49) but not after induction of CLP (76). Second, the timing of Ca^{2+} transporter dysregulation could be different between the two models. For example, in CLP-challenged rats, SERCA function was initially unchanged and only decreased at later stages of the disease (34, 38, 39, 64). The opposite pattern was reported for LPS challenge, where SERCA dysfunction was evident early and followed by subsequent normalization (42). The temporal pattern of Ca_i decrease in rats is another

example. Ca_i was initially (24h) unchanged and subsequently (48h) decreased after either CLP (38) or ip administration of cecal washings (34). However, LPS challenge was associated with the reverse pattern, in which Ca_i was initially (4–6h) decreased (41, 47, 52) and subsequently (12–20h) normalized (19, 42).

Therefore, the emerging picture is that the LPS and CLP models induce similar changes at the level of Ca^{2+} transporters, although the timing may be different between the two models.

Moreover, like for any animal models of human disease, controversy exists on the extent to which both these models (and others) can reproduce clinical sepsis. Significant scientific effort has been devoted continuously to developing more and more sophisticated experimental models (32, 33, 91) of sepsis and septic shock, a detailed discussion of which is beyond the scope of this article. While controversy and room for improvement will always exist, it is obvious to us that animal models remain essential for providing mechanistic insights into the disease patho-physiology, which can form the basis for new therapies. The insights obtained need, of course, be subsequently confirmed in clinical studies in humans. However, in our opinion, this first step of medical research cannot be bypassed, and it is evident that the power of hypothesis-driven experimental research cannot be replaced by clinical observational studies alone, despite the advantage of being performed in humans, and not in animals.

What are the signaling pathways that underlie the dysfunction of Ca^{2+} transporters in SIC?

Sufficient cumulative evidence exists on which Ca^{2+} transporters are implicated in SIC pathology, and which are not. However, what is largely still unknown is what are the signaling pathways that generate this dysfunction.

We know that SERCA inhibition may be due to $\text{Cys}^{674}\text{-S}_0\text{H}$ (37), and/or to a decrease in PLB phosphorylation (64). We also know that, in mice challenged with low dose LPS, myofilament desensitization is due to hyperphosphorylation of TnI (36), whereas the increased myofilament sensitivity after higher doses of LPS is probably redox mediated (40). Fragmented evidence exists about the pathways implicated in LTCC downregulation, although we are far from having a complete picture. Practically nothing is known about what causes RyR dysfunction, as well as $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibition. Moreover, with a few exceptions (36), it still remains to be proven that preventing these modifications is able to prevent SIC development, to demonstrate causality.

What about females?

Even a cursory examination of the Tables reveals a shocking reality: with 3 exceptions (77, 78, 89) (and one unspecified (48)) all the studies we found have used only male animals. There are numerous known sex differences in sepsis (93), which, in experimental models, usually confer a certain degree of protection to females (93). However, this protection is only partially seen in humans, in whom sepsis is indeed less prevalent in women, but is associated with higher mortality (94).

It is therefore reasonable to assume that sex differences may exist in what concerns the regulation of cardiac Ca^{2+} handling. A deliberate effort to elucidate these differences is

critically necessary. Since the ultimate goal of our research efforts is to deliver new drugs and therapeutic modalities, it is important to ensure that our insights and achievements do not target pathological phenomena that occur only in males. This is also in agreement with the recent NIH call to action (95).

Future directions

What is the future likely to bring? As detailed above, the most important area is the identification of signaling pathways responsible for the dysfunction of LTCC, SERCA, RyR and the myofilaments. Is PKA-dependent phosphorylation increased (36) or decreased (64) in SIC? More work also needs to be done in order to understand sex differences, and perhaps the difference between various experimental models (LPS administration vs. CLP).

However, without this knowledge, it would be difficult, if not impossible, to be able to devise novel drugs and therapies that could counteract the development of SIC, and that of circulatory failure in sepsis in general. Apart from hoping that an effective drug will be discovered serendipitously, committing to a concentrated effort to elucidate the cellular mechanisms that underlie SIC is the only way forward.

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List of abbreviations

AP	action potential
Ca²⁺	calcium
Ca_{SR}	sarcoplasmic reticulum calcium load
CLP	cecal ligation and puncture
Cys⁶⁷⁴-S0₃H	sulphonylation of cysteine 674 of SERCA
Ca_i	calcium transient amplitude
ECC	excitation contraction coupling
IL-1	interleukine-1
ip	intraperitoneally
iv	intravenously
LPS	lipopolysaccharide
LTCC	L-type Ca ²⁺ channels
PKA	phosphokinase A
PLB	phospholamban
RyR	ryanodine receptors

SERCA	sarcoplasmic reticulum calcium pump
SR	sarcoplasmic reticulum
SIC	sepsis induced cardiomyopathy
TNF	tumor necrosis factor
Tn I	troponin I

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Figure 1.

The only mechanisms that could explain the decrease in cardiac contractile force that defines SIC are either cell death or a deficit in myocyte contractility. Various Ca^{2+} handling mechanisms, as well as upstream signaling pathways can induce cardiomyocyte contractile dysfunction. For the purpose of this review, it is important to realize that modulatory changes in signaling pathways can only cause a decrease in cardiac contractility if they induce the dysregulation of one or more Ca^{2+} transporters and/or the cardiac myofilaments. See text for more details.

Figure 2. Diagram of the cardiac excitation contraction coupling

The first step in CICR is the opening of the voltage-sensitive sarcolemmal L-type Ca^{2+} channels, which is triggered by membrane depolarization in the phase 1 of the action potential. When LTCC open, they allow a small amount of Ca^{2+} to enter the cell, which, in turn, activates SR Ca^{2+} - sensitive release channels, the ryanodine receptors (which are juxtaposed to the LTCC, forming structures called “dyads”). Ca^{2+} released from the SR generates a transient rise in cytosolic Ca^{2+} which activates the myofilament contraction. In diastole, the cell relaxes because Ca^{2+} is removed from cytosol, both by re-uptake into the SR, via SERCA (tightly regulated by its main regulatory subunit, phospholamban, PLB) and by extrusion from the cell through the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchange.

Figure 3. Cell shortening (A), Ca_i (B), diastolic Ca^{2+} levels (C) and Ca_{SR} (D) in various studies of SIC

In all published studies, cell shortening was found to be decreased in animal models of SIC (A). Ca_i (B) and Ca_{SR} (D) were decreased in the majority (but not in all) studies, whereas diastolic Ca_i level was reported to be either increased, decreased or unchanged (C).

In this and the following figures, data is shown as average and SEM, as obtained from the text or Tables of original studies, or, if not explicitly stated, by extrapolating the data in the graphs and bar diagrams. Closed symbols represent data that was statistically significant from control. Open symbols show data that was not statistically significant from control.

Studies are shown on the y axis as first author and year of publication, in the same order as in the respective Tables. Studies that compared more than one experimental condition (such as 25 vs. 50 µg/g LPS in ref. (37)), or different time points after induction of SIC (24 vs. 48h in ref. (34)), are shown twice, with these different conditions given in brackets. To differentiate between 2 studies published by Zhong, et al, in 1997, the journal name is given in brackets (“Shock”(43) vs. American Journal of Physiology, AJP(44)).

Figure 4. The function of LTCC (as $I_{Ca,L}$, A), SERCA (B), ryanodine receptors (C) and the Na^+/Ca^{2+} exchange (D), as well as APD (E) in various models of SIC

In all 3 published studies LTCC current was found to be decreased in animal models of SIC (A). Similarly, SERCA function was inhibited in all 9 studies found (at least at some timepoints, B). RyR leak was found to be increased (C), and NCX function decreased (D) in some (but not all) studies found. In 3 published studies, APD was found to be shortened in SIC (E).

Figure 5. Myofilament function in various models of SIC

A: Myofilament sensitivity for Ca^{2+} was commonly decreased in models of SIC, evidenced as a rightward shift in the half maximal Ca^{2+} concentration (K_m), i.e. a positive value for the difference between K_m in SIC vs. baseline groups (ΔK_m).

B. Maximal developed tension in various SIC models. PI: protease inhibitors added to the cell isolation solution.

Table 1

Myocyte unloaded shortening (twitch) in various SIC models

In all studies, unless stated otherwise, all LPS was E coli derived, with the serotype shown in brackets. ?: not stated.

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Turdi, 2012	(21)	Mouse (FVB)	M	12–16	LPS (O55:B5), 6 mg/kg, ip	4h	Decreased cell twitch
Layland, 2005	(36)	Mouse (CD1)	M	8–10	LPS (O11:B4), 6 mg/kg, ip	18 h	Decreased cell twitch
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 25 mg/kg, ip	14h	Decreased cell twitch
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 50 mg/kg, ip	7h	Decreased cell twitch
Ichinose, 2007	(40)	Mouse (C57BL/6J)	M	8–16	LPS (O111:B4), 50 mg/kg, ip	7h	Decreased cell twitch
Zou, 2010	(92)	Mouse (? strain)	M	8–12	CLP	24h	Decreased cell twitch
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing ip,	24h	Decreased cell twitch
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing ip,	48h	Decreased cell twitch
Vona-Davis, 2002	(96)	Rat (Sprague-Dawley)	M	?	LPS (055:B5), 4 mg/kg, ip	4h	Decreased cell twitch
Mitra, 2004	(52)	Rat (Sprague-Dawley)	M	10–15	LPS (? serotype), 10 mg/kg, ip	4h	Decreased cell twitch
Mitra, 2006	(41)	Rat (Sprague-Dawley)	M	10–15	LPS (? serotype), 10 mg/kg, ip	4h	Decreased cell twitch
Lancel, 2005	(47)	Rat (Sprague-Dawley)	M	?	LPS (055:B5) , 10 mg/kg, ip	4h	Decreased cell twitch
Tavernier, 2001	(19)	Rat (Sprague-Dawley)	M	?	LPS (0111:B4), 5 mg/kg, iv	12h	Decreased cell twitch

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	24h	Unchanged cell twitch
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	48h	Decreased cell twitch
Zhong, 1997 (Shock)	(43)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 1 mg/kg, ip	4h	Decreased cell twitch
Zhong, 1997 (AJP)	(44)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 4 mg/kg, iv	4h	Decreased cell twitch
Rigby, 1998	(45)	Guinea pig (Hartley)	M	?	LPS (0127:B8), 4 mg/kg, ip	4h	Decreased cell twitch
Hung, 1993	(48)	Rabbits (New Zealand White)	?	?	LPS (O55:B5), 0.174 mg/kg, iv	4h	Decreased cell twitch

Table 2

Cai in various SIC models

In this and the following, the use of commas (i.e. “unchanged”, “increased” or “decreased”) signifies it was so stated in the text, but no data was actually showed.

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Turdi, 2012	(21)	Mouse (FVB)	M	12–16	LPS (O55:B5), 6 mg/kg, ip	4h	Decreased Ca_i
Layland, 2005	(36)	Mouse (CD1)	M	8–10	LPS (O11:B4), 6 mg/kg, ip	18 h	Unchanged Ca_i
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 25 mg/kg, ip	14h	Decreased Ca_i
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 25 mg/kg, ip	7h	Decreased Ca_i
Ichinose, 2007	(40)	Mouse (C57Bl/6)	M	8–16	LPS (O111:B4), 50 mg/kg, ip	7h	Decreased Ca_i
Zou, 2010	(92)	Mouse (? strain)	M	8–12	CLP	24h	Decreased Ca_i
Tavemier, 2001	(19)	Rat (Sprague-Dawley)	M	?	LPS (O111:B, 5 mg/kg, iv	12h	Unchanged Ca_i
Mitra, 2004	(52)	Rat (Sprague-Dawley)	M	10–15	LPS (serotype ?), 10 mg/kg, ip	4h	Decreased Ca_i
Mitra, 2006	(41)	Rat (Sprague-Dawley)	M	10–15	LPS (serotype ?), 10 mg/kg, ip	4h	Decreased Ca_i
Lancel, 2005	(47)	Rat (Sprague-Dawley)	M	?	LPS (055:B5) , 10 mg/kg, ip	4h	Decreased Ca_i
Comini, 2005	(42)	Rat (Sprague-Dawley)	M	?	LPS (0127:B8), 20 mg/kg, ip	6h	“Unchanged” Ca_i
Comini, 2005	(42)	Rat (Sprague-Dawley)	M	?	LPS (0127:B8), 20 mg/kg, ip	20h	“Unchanged” Ca_i
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing ip,	24h	Unchanged Ca_i

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing ip,	48h	Decreased Ca_i
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	24h	Unchanged Ca_i
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	48h	Decreased Ca_i
Zhong, 1997 (Shock)	(43)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 1 mg/kg, ip	4h	Decreased Ca_i
Zhong, 1997 (AJP)	(44)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 4 mg/kg, iv	4h	Decreased Ca_i
Rigby, 1998	(45)	Guinea Pig (Hartley)	M	?	LPS (0127:B8), 4 mg/kg, ip	4h	Decreased Ca_i

Table 3**Diastolic Ca_i levels in various SIC models**

Author, year	Ref:	Species (strain)	Sex	Age (week s)	Model	Time	Finding
Turdi, 2012	(21)	Mouse (FVB)	M	12–16	LPS (O55:B5), 6 mg/kg, ip	4h	Diastolic Ca_i unchanged
Layland, 2005	(36)	Mouse (CD1)	M	8–10	LPS (O11:B4), 6 mg/kg, ip	18 h	Diastolic Ca_i unchanged
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O11:B4), 25 mg/kg, ip	14h	Diastolic Ca_i decreased
Tavernier, 2001	(19)	Rat (Sprague-Dawley)	M	?	LPS (O111:B4), 5 mg/kg, iv	12 h	Diastolic Ca_i unchanged
Lancel, 2005	(47)	Rat (Sprague-Dawley)	M	?	LPS (O55:B5), 10 mg/kg, ip	4h	Diastolic Ca_i increased
Comini, 2005	(42)	Rat (Sprague-Dawley)	M	?	LPS (O127:B8), 20 mg/kg, ip	6h	Diastolic Ca_i increased
Comini, 2005	(42)	Rat (Sprague-Dawley)	M	?	LPS (O127:B8), 20 mg/kg, ip	20h	Diastolic Ca_i increased
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	48h	Diastolic Ca_i “increased”
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing, ip	24h	Diastolic Ca_i unchanged
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing, ip	48h	Diastolic Ca_i decreased
Zhong, 1997 (Shock)	(43)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 1 mg/kg, ip	4h	Diastolic Ca_i decreased
Rigby, 1998	(45)	Guinea pig (Hartley)	M	?	LPS (O127:B8), 4 mg/kg, ip	4h	Diastolic Ca_i decreased

Table 4**CasR in various SIC models**

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Hobai, 2013	(37)	Mouse (C57BL/6)	M	12–15	LPS (O111:B4), 25 mg/kg, ip	14h	Decreased CasR
Ichinose, 2007	(40)	Mouse (C57BL/6J)	M	8–16	LPS (O111:B4), 50 mg/kg, ip	7h	Decreased CasR
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	24h	“Unchanged” CasR
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	48h	Decreased CasR
Hung, 1995	(48)	Rabbits (New Zealand White)	?	?	LPS (O55:B5), 0.174 mg/kg, iv	4h	Unchanged CasR

Table 5**LTCC function in various SIC models**

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O11:B4), 25 mg/kg, ip	14h	Decreased I_{CaL}
Zhong, 1997 (AJP)	(44)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 4 mg/kg, iv	4h	Decreased I_{CaL}
Zhang, 2007	(49)	Rat (Sprague-Dawley)	M	10–15	LPS (serotype ?), 10 mg/kg, ip	4h	Decreased I_{CaL}
Stengl, 2010	(50)	Pigs (domestic)	either	?	IV infusion of Pseudomonas Aeruginosa (strain O1), 10^9 colony-forming units/mL) for 22h	22h	Decreased I_{CaL}

Table 6

SERCA function in various SIC models

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Turdi, 2012	(21)	Mouse (FVB)	M	12–16	LPS (O55:B5), 6 mg/kg, ip	4h	Decreased SERCA
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 25 mg/kg, ip	14h	Decreased SERCA
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 50 mg/kg, ip	7h	Decreased SERCA
Ichinose, 2007	(40)	Mouse (C57Bl/6J)	M	8–16	LPS (O111:B4), 50 mg/kg, ip	7h	Decreased SERCA
Comini, 2005	(42)	Rat (Sprague-Dawley)	M	?	LPS (0127:B8), 20 mg/kg, ip	6h	Decreased SERCA
Comini, 2005	(42)	Rat (Sprague-Dawley)	M	?	LPS (0127:B8), 20 mg/kg, ip	20h	“Unchanged” SERCA
Wu, 2001	(39)	Rats (Sprague-Dawley)	M	?	CLP	9h	Unchanged SERCA
Wu, 2001	(39)	Rats (Sprague-Dawley)	M	?	CLP	18h	Decreased SERCA
Wu, 2002	(64)	Rats (Sprague-Dawley)	M	?	CLP	9h	Unchanged SERCA
Wu, 2002	(64)	Rats (Sprague-Dawley)	M	?	CLP	18h	Decreased SERCA
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing ip,	24h	Unchanged SERCA
Ren, 2002	(34)	Rat (Sprague-Dawley)	M	?	cecal washing ip,	48h	Decreased SERCA
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	24h	Decreased SERCA

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	48h	Decreased SERCA
Wu, 1991	(63)	Dog (mongrel)	M	?	LPS (serotype ?), 0.5 mg/kg, iv	4h	Decreased SERCA

Table 7

RyR function in various SIC models

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O111:B4), 50 mg/kg, ip	14h	Unchanged RyR leak
Ichinose, 2007	(40)	Mouse (C57BL/6J)	M	8–16	LPS (O111:B4), 50 mg/kg, ip	7h	Increased RyR leak
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	24	Increased spark frequency, increased RyR leak, unchanged RyR expression.
Zhu, 2005	(38)	Rat (Sprague-Dawley)	M	?	CLP	48	Increased spark frequency, increased RyR leak, unchanged RyR expression.

Table 8**Na⁺/Ca²⁺ exchange in various SIC models**

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS, 25 mg/kg, ip	14h	Unchanged Na ⁺ /Ca ²⁺ exchange
Ichinose, 2007	(40)	Mouse (C57BL/6J)	M	8–16	LPS, 50 mg/kg, ip	7h	Decreased Na ⁺ /Ca ²⁺ exchange
Wang, 2000	(72)	Rat (Sprague-Dawley)	M	?	CLP	9h	Unchanged Na ⁺ /Ca ²⁺ exchange
Wang, 2000	(72)	Rat (Sprague-Dawley)	M	?	CLP	18h	Decreased Na ⁺ /Ca ²⁺ exchange
Liu, 1986	(89)	Dog (mongrel)	either	?	LPS (serotype ?), 0.5 mg/kg, iv	4h	Decreased Na ⁺ /Ca ²⁺ exchange

Table 9**Action potential duration in various SIC models**

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Zhang, 2007	(49)	Rat (Sprague-Dawley)	M	10–15	LPS (serotype ?), 10 mg/kg, ip	4h	Decreased APD
Zhong, 1997	(44)	Guinea pig (strain ?)	M	?	LPS (serotype ?), 4 mg/kg, iv	4h	Decreased APD
Hung, 1993	(48)	Rabbits (New Zealand White)	?	?	LPS (O55:B5), 0.174 mg/kg, iv	4h	Decreased APD

Table 10

Cardiomyocyte shortening vs. Ca_i in various SIC models

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Paced Ca^{2+} transients	Tetanic contractions
Layland, 2005	(36)	Mouse (CD1)	M	8–10	LPS (O11:B4), 6 mg/kg, ip	18h	Decreased cell shortening, unchanged Ca_i	
Hobai, 2013	(37)	Mouse (C57Bl/6)	M	12–15	LPS (O11:B4), 50 mg/kg, ip	14h	Similar cell shortening vs. Ca_i	
Ichinose, 2007	(40)	Mouse (C57Bl/6J)	M	8–16	LPS (O11:B4), 50 mg/kg, ip	7h	Increased cell shortening for Ca_i	
Tavemier, 2001	(19)	Rat (Sprague-Dawley)	M	?	LPS (O111:B4), 5 mg/kg, iv	12h	Decreased cell shortening, unchanged Ca_i	Depressed SS for same Ca_i
Lancel, 2005	(47)	Rat (Sprague-Dawley)	M	?	LPS (055:B5), 10 mg/kg, ip	4h		Depressed SS for same Ca_i

Table 11
Myofilament Ca²⁺ sensitivity in various SIC models

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Hess, 1981–endocardium	(78)	Dogs (mongrel)	either	?	LPS (O260B6), 4 mg/kg, iv	5h	Decreased Ca ²⁺ sensitivity
Hess, 1981–epicardium	(78)	Dogs (mongrel)	either	?	LPS (O260B6), 4 mg/kg, iv	5h	Unchanged Ca ²⁺ sensitivity
Jozefowicz, 2007	(79)	Rat (Wistar)	M	12	LPS, 5mg/kg, iv	12h	Decreased Ca ²⁺ sensitivity
Powers, 1998	(30)	Rat (Sprague–Dawley)	M	?	cecal inoculum ip,	1 day	Unchanged Ca ²⁺ sensitivity
Powers, 1998	(30)	Rat (Sprague–Dawley)	M	?	cecal inoculum ip,	3–7 days	Decreased Ca ²⁺ sensitivity
Tavernier, 2001	(85)	Rabbit (New Zealand)	M	?	LPS (E coli, Salmonella enteridis, Salmonella minnesota), 0.6 mg/kg, iv	36h	Decreased Ca ²⁺ sensitivity
Rigby, 1998	(45)	Guinea pig (Hartley)	M	?	LPS (0127:B8), 4 mg/kg, ip	4h	Unchanged Ca ²⁺ sensitivity

Table 12**Myofilament maximal developed tension in various SIC models**

Author, year	Ref:	Species (strain)	Sex	Age (weeks)	Model	Time	Finding
Jozefowicz, 2007	(79)	Rat (Wistar)	M	12	LPS, 5mg/kg, iv	12h	Unchanged max. tension
Powers, 1998	(30)	Rat (Sprague-Dawley)	M	?	cecal inoculum ip.	1 day	Unchanged max. tension
Powers, 1998	(30)	Rat (Sprague-Dawley)	M	?	cecal inoculum ip.	3–7 days	Increased max. tension
Tavernier, 2001	(85)	Rabbit (New Zealand)	M	?	LPS (E coli, Salmonella enteridis, Salmonella minnesota), 0.6 mg/kg, iv	36h	Unchanged max. tension
Rigby, 1998 (with PIC added)	(45)	Guinea Pig (Hartley)	M	?	LPS (0127:B8), 4 mg/kg, ip	4h	Unchanged max. tension
Rigby, 1998	(45)	Guinea Pig (Hartley)	M	?	LPS (0127:B8), 4 mg/kg, ip	4h	Decreased max. tension