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Modulation of sarcoplasmic reticulum Ca^{2+} cycling in systolic and diastolic heart failure associated with aging

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Abstract Hypertension, atherosclerosis, and resultant chronic heart failure (HF) reach epidemic proportions among older persons, and the clinical manifestations and the prognoses of these worsen with increasing age. Thus, age per se is the major risk factor for cardiovascular disease. Changes in cardiac cell phenotype that occur with normal aging, as well as in HF associated with aging, include deficits in ß-adrenergic receptor (ß-AR) signaling, increased generation of reactive oxygen species (ROS), and altered excitation–contraction (EC) coupling that involves prolongation of the action potential (AP), intracellular Ca^{2+} (Ca_i^{2+}) transient and contraction, and blunted force- and relaxation-frequency responses. Evidence suggests that altered sarcoplasmic reticulum (SR) Ca^{2+} uptake, storage, and release play central role in these changes, which also involve sarcolemmal L-type Ca^{2+} channel (LCC), Na^+–Ca^{2+} exchanger (NCX), and K^+ channels. We review the age-associated changes in the expression and function of Ca^{2+} transporting proteins, and functional consequences of these changes at the cardiac myocyte and organ levels. We also review sexual dimorphism and self-renewal of the heart in the context of cardiac aging and HF.

Keywords Heart · Aging · Heart failure · Cardiac myocyte · Sarcoplasmic reticulum · Calcium

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Introduction

It is estimated that by 2035, nearly one in four individuals in the United States will be 65 years of age or older. Hypertension, atherosclerosis, and resultant chronic HF reach epidemic proportions among older persons, and the clinical manifestations and the prognoses of these worsen with increasing age. The reason is that, in older individuals, specific pathophysiological mechanisms that underlie these diseases become superimposed on heart and vascular substrates that are modified by the process of aging. In other words, cardiovascular aging is "risky." An understanding of how age per se modifies cardiovascular structure and function is critical to the prevention or treatment of cardiovascular diseases in the older person.

Cardiac aging

Cellular and molecular mechanisms implicated in age-associated changes in myocardial structure and function in humans have also been studied largely in rodents (Table 1). The altered cardiac structural phenotype that evolves with aging in rodents includes an increase in LV mass due to an enlargement of myocyte size [17] and focal proliferation of the matrix in which the myocytes reside, which may be linked to an altered cardiac fibroblast number or function. The number of cardiac myocytes becomes reduced because of necrosis and apoptosis, with the former predominating [2]. Putative stimuli for cardiac cell enlargement with aging in rodents include an age-associated increase in vascular load due to arterial stiffening and stretching of cells caused by drop out of neighboring myocytes [39]. Stretch of cardiac myocytes and fibroblasts initiates growth factor signaling (e.g. angiotensin II/TGF-ß) that, in

Table 1 Myocardial changes with adult aging in rodents

Structural Δ	Functional Δ	Ionic, biophysical/biochemical mechanisms	Molecular mechanisms
↑ Myocyte size	Prolonged contraction	Prolonged cytosolic Ca^{2+} transient	
↓ Myocyte number		↓ SR Ca^{2+} pumping rate	↓ SERCA mRNA
		↓ Pump site density	No Δ calsequestrin mRNA
	Prolonged action potential	↓ I_{Ca} inactivation	↑ Na/CA exchanger mRNA
		↓ I_{TO} density	
	Diminished contraction velocity	↓ αMHC protein	↓ αMHC mRNA
		↑ βMHC protein	↑ βMHC mRNA
		↓ Myosin ATPase activity	Shift in myosin isoform mRNA
		↓ RxRβ1 and γ protein	↓ RxRβ1 and γ mRNA
		↓ Thyroid receptor protein	
	Diminished β-adrenergic contractile response	↓ Coupling βAR-acylase	↓ β1AR mRNA
		No Δ G_i activation	No Δ βARK mRNA
		No Δ βARK activity	
		↓ TNI phosphorylation	
		↓ Phospholamban phosphorylation	
		↓ I_{Ca} augmentation	
		↓ Ca_i transient augmentation	
		↑ Enkephalin peptides	
↑ Matrix connective tissue	↑ Myocardial stiffness	↑ Hydroxyline proline content	↑ Collagen mRNA
		↑ Activity of myocardial RAS	↑ Fibronectin mRNA
			↑ AT_1R mRNA
		↑ Atrial natriuretic peptide	↑ Atrial natriuretic peptide mRNA
	↓ Growth response		↓ Induction of immediate early genes
	↓ Heat shock response		↓ Activation of HSF
		↑ Opioid peptides	↑ Proenkephalin mRNA

SR, sarcoplasmic reticulum; *SERCA*, sarco/endoplasmic reticulum calcium ATPase; *Ca^{2+}*, calcium ions; *Na/Ca exchanger*, sarcolemmal sodium–calcium exchanger; *MHC*, myosin heavy chain; *mRNA*, messenger RNA; *RXR*, retinoid X-receptor; *βAR*, β-adrenergic receptor; *βARK*, β-adrenergic receptor kinase; *G_i*, inhibitory G protein; *TNI*, troponin-I; *I_{Ca}*, calcium influx via L-type Ca^{2+} channels; *Ca_i*, intracellular calcium concentrations; *HSF*, heat shock factor; AT_1R, angiotensin AR-1 receptor; *RAS*, renin–angiotensin system

addition to modulating cell growth and matrix production, leads to apoptosis [13]. The expression of atrial natriuretic [78] and opioid [11] peptides, molecules that are usually produced in response to chronic stress, is increased in the senescent rodent heart.

Reduced acute response to stress

Acute excess myocardial Ca^{2+} loading leads to dysregulation of Ca^{2+} homeostasis, impaired diastolic and systolic function, arrhythmias, and cell death [37]. The cell Ca^{2+} load is determined by membrane structure and permeability characteristics, the intensity of stimuli that modulate Ca^{2+} influx or efflux via their impact on regulatory function of proteins within membranes, and ROS, which affect both membrane structure and function. Excessive cytosolic Ca^{2+} loading occurs during physiological and pharmacological scenarios that increase Ca^{2+} influx (e.g. neurotransmitters, postischemic reperfusion, or oxidative stress) [23, 40]. In hearts or myocytes from the older heart, enhanced Ca^{2+} influx, impaired relaxation, and increased diastolic tone occur during pacing at an increased frequency [10, 45, 67, 79]. This is a "downside" of the aforementioned age-associated adaptation that occurs within the cells of senescent heart (and also of young animals chronically exposed to arterial pressure overload). Causes of reduced Ca^{2+} tolerance of the older heart include changes in the amounts of proteins that regulate Ca^{2+} handling, caused in part by altered gene expression (Tables 1 and 2), and an age-associated alteration in the composition of membranes in which Ca^{2+} regulatory

Table 2 Age-associated changes in the expression of Ca^{2+}-transporting proteins

Species	Rat	Rat	Rat	Rat	Rat	Mouse	Mouse	Mouse	Mouse
Breed	Fisher 344	Fisher 344	Wistar	Wistar-Kyoto	Fischer-Brown	B6C3F1	C57/BL6	C57B1/6	FVB
Sex	Male	(SU)	Male	Male	Norway	Male	(SU)	(M + F)	Male
					Male				
SERCA2	↔ [76]; ↓22% [65] ↓30% [58]	↓ [80]	↓32% [5]	↔ [35]		↔ [46]	↔ [27]	↔ [63]	↓31% [44]
PLB	↔ [58, 76]	↔ [80]				↑158% [46]		↔ [63]	↔ [44]
RYR	↔ [58, 76]	↔ [80]	↓39% [5]						
CSQ	↔ [76]	↔ [80]			↔ [49]			↔ [63]	↔ [44]
NCX	↔ [58]; ↓19% [28]		↓36% [5]		↔ [49]	↓37% [46]			↔ [44]

SERCA2, sarco/endoplasmic reticulum Ca^{2+}-ATPase 2; *PLB*, phospholamban; *RYR*, ryanodine receptor/sarcoplasmic reticulum Ca^{2+} release channel; *CSQ*, calsequestrin; *NCX*, sarcolemmal Na^+–Ca^{2+} exchanger; ↔, no change; ↓, reduced; ↑, increased; %, percent change in protein levels in hearts from aging (24–36 months) vs. young adult (2–5 months) or adult (6–15 months) animals. *SU*, sex unknown; *M + F*, male and female

proteins reside, which includes an increase in membrane ω_6:ω_3 polyunsaturated fatty acids (PUFAs) [55]. ω_3 PUFAs are protective of cardiac Ca^{2+} regulation. An additional potential cause of the reduced threshold of senescent myocytes for Ca^{2+} overload is an enhanced likelihood for intracellular generation of ROS [40, 48] in cells from the senescent versus the younger adult heart during stress. In this regard, the older cardiac myocyte and endothelial cells [69] share common "risks" with aging.

Cellular ß-adrenergic signaling

Age-associated deficits in the myocardial ßAR signaling cascade also occur with aging (see [38] for review). A reduced myocardial contractile response to either $ß_1$AR or $ß_2$AR stimulation is observed with aging [57, 73, 74]. This is due to failure of ß-adrenergic stimulation to augment Ca_i^{2+} to the same extent in cells of senescent hearts that it does in those from younger adult hearts [73] an effect attributable to a deficient increase of L-type sarcolemmal Ca^{2+} channel availability, which leads to a lesser increase in Ca^{2+} influx [73]. The richly documented age-associated reduction in the postsynaptic response of myocardial cells to ß-adrenergic stimulation seems to be due to multiple changes in the molecular and biochemical steps that couple the receptor to postreceptor effectors. However, the major limiting modification of this signaling pathway that occurs with advancing age in rodents seems to be the coupling of the ß-AR to adenylyl cyclase via the Gs protein and changes in adenylyl cyclase protein, which lead to a reduction in the ability to sufficiently augment cell cAMP and to activate protein kinase A (PKA) to drive the phosphorylation of key proteins that are required to augment cardiac contractility [31, 74]. In contrast, the apparent desensitization of ß-adrenergic signaling that occurs with aging does not seem to be mediated via increased ß-AR kinase or increased G_i activity [74]. A blunted response to ß-adrenergic stimulation of the cells within older myocardium can, in one sense, be viewed as adaptive with respect to its effect to limit the risk of Ca^{2+} overload and cell death in these cells.

Reduced chronic adaptive capacity of the older heart

Many of the multiple changes in cardiac structure, excitation, myofilament activation, contraction mechanisms, Ca^{2+} dysregulation, deficient ß-AR signaling, and altered gene expression of proteins involved in EC coupling that occur with aging (Tables 1 and 2) also occur in the hypertrophied myocardium of younger animals with experimentally induced chronic hypertension [38, 77] and in failing animal or human hearts, in which they have been construed as an adaptive response to a chronic increase in

LV loading. When chronic mechanical stresses that evoke substantial myocardial hypertrophy (e.g. pressure or volume overload) are imposed on the older heart, the response in many instances is reduced. There is evidence that transcriptional events associated with hypertrophic stressors become altered with advancing age, e.g. the nuclear binding activity of the transcription factor nuclear factor-κB is increased and that of another transcription factor, Sp1, is diminished [24]. Because these transcription factors each influence expression of a number of genes, they may contribute to the pattern of gene expression observed in the hearts of senescent rodents and may also dictate the limits of adaptive responses to the imposition of additional chronic stress. The acute induction of both immediate early genes and later responding genes that are expressed during the hypertrophic response is blunted in hearts of aged rats after aortic constriction [60, 66]. Similarly, the acute induction of heat shock 70 protein genes in response to either ischemia or heat shock is reduced in hearts of senescent rats [9, 52]. A similar loss of adaptive capacity is observed in younger rats that have used a part of their reserve capacity before a growth factor challenge [61].

Molecular and functional changes of Ca^{2+}-handling proteins in the aging heart

The SR Ca^{2+} release and uptake play key role in the regulation of cardiac contraction and relaxation. The SR Ca^{2+}-transporting proteins include the sarcoplasmic reticular Ca^{2+}-ATPase (SERCA2), its inhibitory protein phospholamban (PLB), the Ca^{2+}-storage protein calsequestrin (CSQ), and the SR-Ca^{2+} release channel (ryanodine receptor; RYR). The SR Ca^{2+} cycling is further modulated by Ca^{2+} influx through LCC and by Ca^{2+} transport via NCX.

SR Ca^{2+} pump

Sequestration of Ca^{2+} by the SERCA2 pump serves a dual function: (1) to cause muscle relaxation by lowering the cytosolic Ca^{2+} and (2) to restore SR Ca^{2+} content necessary for muscle contraction. The age-associated reduction in SERCA2 mRNA levels is well documented (Table 1). As summarized in Table 2, the majority of studies in aging vs. younger rats have shown a significant reduction in protein levels of SERCA2 [5, 58, 65, 80]. On the other hand, most studies in aging mice have shown unchanged levels of SERCA2 [27, 46, 63]. Apart from the phosphorylation status of SERCA2-PLB complex, discussed later, SR Ca^{2+} uptake is dependent on the relative levels of both proteins, i.e. reduced at lower SERCA2/PLB ratio [50]. The majority of studies reporting expression levels of both SERCA2 and PLB showed reduced SERCA2/PLB ratios in aging rodent hearts (Table 2). Increasing SERCA/PLB ratio through in vivo gene transfer of SERCA2a markedly improved rate-dependent contractility and diastolic function in 26-months-old rat hearts [58]. Functional improvement consequent to increasing SERCA/PLB ratio by PLB suppression was also reported in human failing myocytes [14]. On the other hand, PLB ablation in transgenic mouse models of HF was beneficial only in some models (reviewed in [4] and [50]).

Age-associated decline in the Ca^{2+}-sequestering activity of SERCA2 in rodent myocardium has been well documented both by biochemical studies in isolated SR vesicles and by biophysical studies in cardiac preparations [18, 35, 58, 65, 76]. Apart from reduced content of SERCA2 or SERCA2/PLB ratio, discussed earlier, lower pumping activity of SERCA2 in aging myocardium may result from reduced phosphorylation of the SERCA2-PLB complex. Specifically, in its unphosphorylated state, PLB interacts with SERCA2 exerting an inhibitory effect manifested largely through a decrease in the enzyme's affinity for Ca^{2+}. Phosphorylation of PLB by PKA and/or Ca^{2+}/calmodulin-dependent protein kinase (CaMK) is thought to disrupt this interaction resulting in enhanced affinity of the ATPase for Ca^{2+} and stimulation of Ca^{2+} pump activity [50]. Besides PLB, CaMK has been suggested to modulate the SR Ca^{2+} uptake and release through direct phosphorylation of SERCA2 [75]. The well established deficits in ß-AR signaling that occur in aging humans and animals [38] include significantly lower PKA-dependent phosphorylation of PLB in aged vs. adult rat ventricular myocardium [31]. Recent studies have also shown that significant age-associated decrements occur in (1) the amount of CaMK (δ-isoform) in the rat heart, (2) the endogenous CaMK-mediated phosphorylation of SERCA and PLB, and (3) the phosphorylation-dependent stimulation of SR Ca^{2+} sequestration [76]. Increased activity of the SR-associated phosphatase PP1, which dephosphorylates PLB, had already been reported, and overexpression of PP1 in transgenic mice resulted in HF. PP1 activity was further shown to be regulated by the inhibitor I-1, and I-1 was found to be reduced in human HF (reviewed in [62]). However, potential age-related changes in the activity of cardiac phosphatases have yet to be examined.

SR Ca^{2+} release channel

Besides SERCA2, the RYR is a major determinant of the SR Ca^{2+} release, regulated by its protein expression and gating properties during Ca^{2+}-release triggered by Ca^{2+} influx through LCC, as well as during cardiac relaxation and diastole. Accordingly, alterations in the expression or function of RYR have been implicated in both systolic and diastolic dysfunction of the aging heart. Reduced protein

expression of cardiac RYR has been reported in aging Wistar rats [5], but not Fisher 344 rats [58, 76, 80] (Table 2). The RYR is phosphorylated by PKA and CaMK, and a significant reduction in the CaMK-mediated phosphorylation of the RYR has been shown to occur in the aged compared with adult Fisher 344 rats [76].

Single channel properties of RYR and unitary SR Ca^{2+} release events (Ca^{2+} sparks) in ventricular cardiomyocytes were recently examined in hearts from 6- to 24-month-old Fisher 344 rat [80]. Senescent myocytes displayed a decreased Ca_i^{2+} transient amplitude and an increased time constant of the Ca_i^{2+} transient decay, both of which correlated with a reduced Ca^{2+} content of the SR. Senescent cardiomyocytes also had an increased frequency of spontaneous Ca^{2+} sparks and a slight but statistically significant decrease in their average amplitude, full-width-at-half-maximum and full-duration-at-half-maximum. Single channel recordings of RYR demonstrated that in aging hearts, the open probability of RYR was increased but the mean open time was shorter, providing a molecular correlate for the increased frequency of Ca^{2+} sparks and decreased size of sparks, respectively [80]. These results suggest modifications of normal RYR gating properties associated with increased sensitivity of RYR to resting and activating Ca^{2+} that may play a role in the altered Ca^{2+} homeostasis observed in senescent myocytes. Another recent study [26] examined the effects of aging on whole cell electrically stimulated Ca^{2+} transients and Ca^{2+} sparks at 37°C in ventricular myocytes isolated from young adult (~5 mo) and aged (~24 mo) B6SJLF1/J mice of both sexes. The results showed reduced amplitude and abbreviated rise time of the Ca_i^{2+} transient in aged cells stimulated at 8 Hz and markedly higher incidence and frequency of spontaneous Ca^{2+} sparks in aged vs. young adult cells. Spark amplitudes and spatial widths were similar in both age groups. However, spark half-rise times and half-decay times were abbreviated in aged cells compared with younger cells. Neither resting Ca_i^{2+} levels nor SR Ca^{2+} content differed between young adult and aged cells, indicating that increased spark frequency in aging cells was not attributable to increased SR Ca^{2+} stores and that decrease in the Ca_i^{2+} transient amplitude was not due to a decrease in SR Ca^{2+} load. These results suggest that alterations in SR Ca^{2+} release units occur in aging ventricular myocytes and raise the possibility that alterations in Ca^{2+} release may reflect age-related changes in fundamental release events rather than changes in SR Ca^{2+} stores and/or diastolic Ca_i^{2+} levels.

Differences in the characteristics of Ca^{2+} sparks (and the SR Ca^{2+} content) reported in these experiments [26, 80] might be partly related to differences in species and experimental conditions (e.g. temperature) employed. However, consistent with previous findings [23], both these studies [26, 80] have shown increased frequency of spontaneous Ca^{2+} sparks in aging ventricular myocytes. The resulting increased Ca^{2+} leak from the SR may reduce the *net* rate of SR Ca^{2+} sequestration. Functional consequences of the latter include (1) slower decline of the Ca_i^{2+} transient and increased diastolic Ca_i^{2+} (diastolic dysfunction), (2) reduced SR Ca^{2+} load available for release (systolic dysfunction), and (3) reduced threshold for myocardial cell Ca^{2+} intolerance [23, 37].

The PKA-mediated hyperphosphorylation of RYR, resulting in abnormal SR Ca^{2+} leak through the RYR, has been implicated in both diastolic and systolic dysfunction of the failing heart [51]. However, more recent evidence points to CaMKII site phosphorylation of RYR in normal cardiac tissue [22, 43], and a potential role and mechanism for PKA modulation of this process in the pathophysiology of HF associated with aging remains lacking.

Calsequestrin

Reports have consistently shown that aging does not alter CSQ expression at either the transcriptional (Table 1) or protein level (Table 2) [58, 65, 76, 80].

L-type Ca^{2+} channel

Ca^{2+} influx of via LCC has a dual role in cardiac EC coupling: peak L-type Ca^{2+} current (I_{CaL}) provides the primary "trigger" for SR Ca^{2+} release, while the integrated Ca^{2+} entry replenishes the SR Ca^{2+} content available for release. The effects of aging on protein expression of LCC have not been systematically studied. Electrophysiological examinations have shown that both the apparent number and the activity of individual cardiac LCCs increase with advanced age in the rat [32]. The age-associated changes in I_{CaL} are summarized in Table 3.

Maintained peak density of I_{CaL} has been shown in ventricular myocytes from senescent (21–25 months) vs. young (2–3 months) male Wistar rats [71, 73], and in 20–22 vs. 10- to 12-mo-old male FVB mice [68]. I_{CaL} inactivates more slowly in myocytes from older vs. younger Wistar rats [32, 71], and this, as well as reductions in peak transient outward K^+ current (I_{TO}) [71], might partially account for prolongation of the AP reported in senescent Wistar and Fisher 344 rat hearts [71, 72]. Experiments in ventricular myocytes isolated from young adult (6 mo) and aged (>27 mo) Fischer 344 and Long-Evans rats showed an age-associated decrease in peak density of I_{CaL} accompanied by a slower inactivation, and a greater amplitude of I_{TO} [47]. Compared to young myocytes, AP duration in aged myocytes was longer at 90% of repolarization but shorter 20 and 75% of repolarization [47]. In ventricular myocytes isolated from young (2 mo) and senescent (20–27 months) C57/BL6

Table 3 Age-associated changes in the L-type Ca^{2+} current

Peak I_{CaL} density (A/F)	Integrated I_{CaL} density (C/F)	Voltage-clamp protocol	Temperature (°C)	Model/Reference
↔		I–V relationship	24	Male Wistar rat 21–25 vs. 2–3 mo [71]
↔		I–V relationship	24	Male Wistar rat 24 vs. 2–4 mo [73]
↔		I–V relationship	26	Male FVB mouse 20–22 vs. 10–12 mo [68]
↓		I–V relationship	37	Male Fischer 344 rat 27 vs. 6 mo [47]
↓		I–V relationship	37	Male Long-Evans rat 27 vs. 6 mo; [47]
↓ (2 Hz)		−40 to 0 mV	37	Male B6SJLF1/J mouse 24 vs. 7 mo [21]
↔ (2 Hz)		−40 to 0 mV	37	Female B6SJLF1/J mouse 24 vs. 7 mo [21]
↑ (0.1–0.4 Hz)	↑ (0.4 Hz)	−45 to 0 mV	37	C57/BL6 mouse (sex not reported) 20–27 vs. 2 mo [27]
↔(1.0–8.0 Hz)	↔(1.0–6.0 Hz)			
	↓ (8.0 Hz)			
↑		I–V relationship	23	Female sheep > 8 yr vs. 18 mo [15]
↑ (0.5 Hz)	↑ (0.5 Hz)	−40 to 8 mV		

I_{CaL}, L-type Ca^{2+} current; values in parenthesis indicate rates of stimulation with voltage-clamp steps shown in the voltage-clamp protocol column; *I–V relationship*, current–voltage relationship of I_{CaL} over a range of step potentials

mice (sex unspecified), peak I_{CaL} density was similar at stimulation rates of 2–8 Hz, but higher in aged group at 0.4 and 1 Hz [27]. These experiments also showed that the I_{CaL} time integral (a function of peak amplitude and inactivation rate) normalized to cell capacitance was similar in both age groups under 2–6 Hz stimulation. Compared to young cells, I_{CaL} time integral in aged myocytes was significantly smaller at 8 Hz and larger at 0.4 Hz [27]. Experiments in ventricular myocytes isolated from young adult (~7 mo) and aged (~24 mo) male and female B6SJLF1/J mice [21] stimulated at 2 Hz, showed a significant reduction in peak I_{CaL} density, accompanied by a significantly slower inactivation, in aged vs. young adult myocytes from males. No age-related changes in I_{CaL} characteristics were found in the female group. In myocytes isolated from the hearts of young (18 months) and aged (>8 years) female sheep, the AP duration and both the peak I_{CaL} and integrated Ca^{2+} entry were significantly greater in aged cells [15].

Ca^{2+} influx via LCC is a complex function of several interdependent mechanisms, including (1) voltage-dependent modulation, (2) Ca^{2+}-dependent modulation via direct binding to LCC of Ca^{2+}-calmodulin and via CaMKII, and (3) β-adrenergic modulation via PKA. In aging myocardium, voltage-dependent changes may be consequent to prolongation of the AP duration and manifested by reduced peak amplitude accompanied by slower inactivation/larger time integral of I_{CaL} (e.g. [29]). Ca^{2+}-mediated effects may contribute to frequency-dependent reduction in the amplitude and time integral of I_{CaL} (e.g. [27]) due to rate-dependent diastolic Ca^{2+} accumulation, which has been shown to slow the rate of LCC recovery from inactivation in both normal and failing cardiac myocytes (reviewed in [7]). In addition, Ca^{2+}-mediated crosstalk between LCC and RYR, well established in normal myocardium [7], may facilitate Ca^{2+} influx via LCC in the presence of slower and/or smaller SR Ca^{2+} release in aging myocardium. For instance, buffering of Ca_i^{2+} with EGTA eliminated age-related differences in the AP configuration and the time course of I_{CaL} inactivation in myocytes from senescent and young rats [71]. As discussed earlier, aging is associated with deficits in the myocardial ß-AR signaling cascade [38] that includes reduced stimulation of the I_{CaL} [73] through PKA-mediated changes in the availability and gating properties of LCC.

Na^+–Ca^{2+} exchanger

The NCX serves as the main transsarcolemmal Ca^{2+} extrusion mechanism and is centrally involved in the beat-to-beat regulation of cellular Ca^{2+} content and cardiac contractile force, including regulation of the AP configuration in the late repolarization phase and the later Ca^{2+} clearance phase of the Ca_i^{2+} transient. Thus, alterations in NCX activity may contribute to the prolongation of both the AP duration and relaxation in aging myocardium (Lakatta, 1993). Age-related increase in the NCX expression has been demonstrated at the transcriptional level (Table 1). However, protein levels of NCX reported in aging rodent hearts were unchanged [44, 49, 58] or reduced [5, 28, 46] (Table 2).

The results of experiments using enriched sarcolemmal vesicles or muscle strips isolated from rats were also inconsistent, i.e. the NCX activity in aged myocardium was decreased [25, 28], increased [19], or unchanged [1]. More recent functional assessments of NCX activity in cardiac myocytes isolated from young (14–15 mo) and aged (27–31 mo) male Fischer Brown Norway rats [49] showed that under conditions where membrane potential and intracellular $[Na^+]$ and $[Ca^{2+}]$ could be controlled, "forward" NCX activity was increased in aged vs. young cells.

The increased "forward" NCX activity was interpreted as a factor contributing to the late AP prolongation in aging myocardium [49]. Such increased Ca^{2+} efflux via NCX would compensate for increased Ca^{2+} influx via LCC [15, 32, 71]. Prolongation of the AP consequent to reduced I_{TO} [71] may *temporarily* limit "forward" NCX during relaxation, allowing better SR Ca^{2+} reuptake by SERCA2 [29] and Fig. 2.

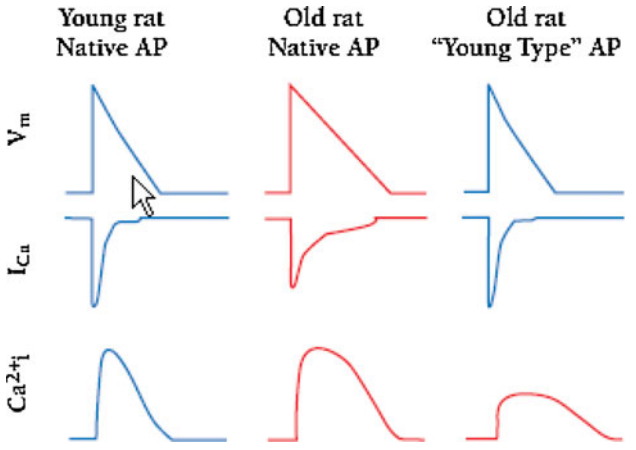

Fig. 2 Schematic representation of the role of age-dependent action potential (AP) prolongation on L-type Ca^{2+} current (I_{Ca}) and intracellular Ca^{2+} (Ca_i^{2+}) regulation in rat ventricular myocytes. Compared to young rats (6 mo; *left*) old rats (24 mo; *middle*) display a similar amplitude of the Ca_i^{2+} transient triggered by a markedly prolonged AP. Relative to stimulation with their long, native AP, stimulation of old rat ventricular myocytes with a short, "young type" AP (*right*) increases the amplitude of L-type Ca^{2+} current (I_{Ca}) but reduces its time integral and diminishes the amplitude and rate of relaxation of the Ca_i^{2+} transient. Thus, it appears that ventricular myocytes of old rats utilize AP prolongation to sustain youthful Ca_i^{2+} regulation. (From [29])

Excitation–contraction coupling in the aging heart

Coordinated changes in the expression and function of proteins that regulate several key steps of the cardiac EC coupling process occur in the rodent heart with aging and typically result in a prolonged AP, a prolonged Ca_i^{2+} transient, a prolonged contraction, and blunted inotropic and lusitropic responses to increased pacing rate (Figs. 1, 3 and 4).

Age-related prolongation of the AP [15, 49, 71, 72] is thought to stem, in part at least, from increased number and the activity of individual cardiac L-type Ca^{2+} channels [32], slower inactivation of I_{CaL} [15, 32, 71], and reductions in outward K^+ currents [71]. Additionally, the late repolarization phase of the AP may be prolonged by increased Ca^{2+} efflux via NCX [49]. Of particular interest is the role of I_{TO} as an indirect modulator of EC coupling in cardiac cells (reviewed by Bassani [6]). Specifically, recent studies have provided evidence that the early repolarization phase may considerably influence the entire AP waveform and that I_{TO} is the main current responsible for this phase. Decreased I_{TO} density is observed in immature and aging myocardium, as well as during several types of

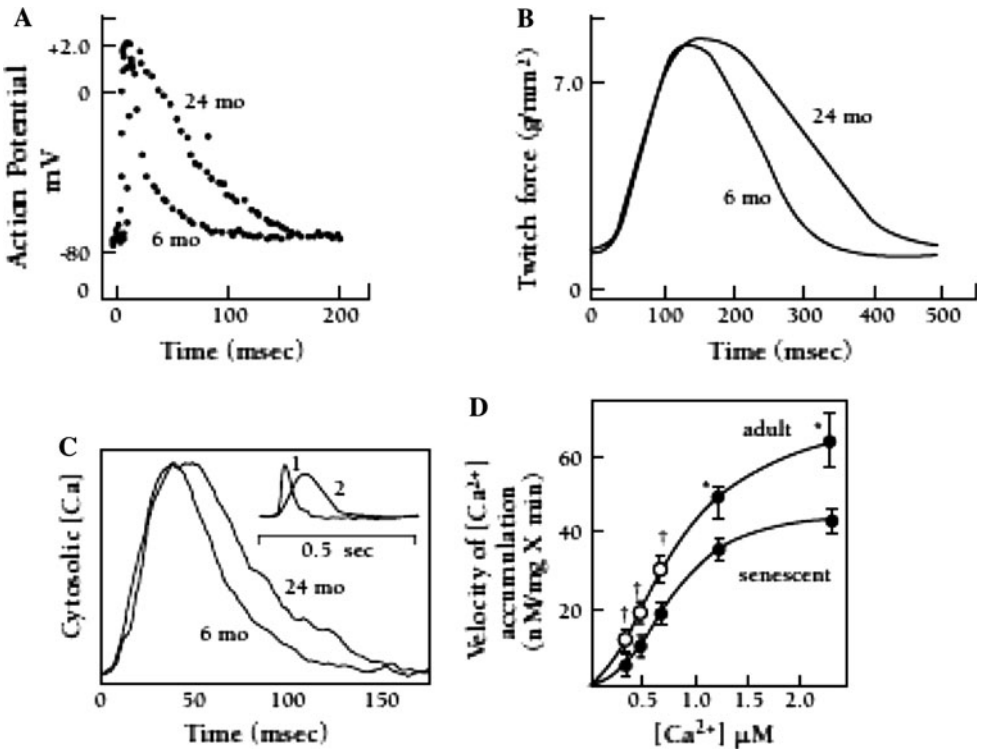

Fig. 1 Differences in various aspects of excitation–contraction coupling mechanisms measured between adult (6–9 months) and senescent (24–26 months) rat hearts. **a** Transmembrane action potential and **b** isometric contraction (from [72]). **c** Cytosolic calcium transient, measured by a change in the luminescence of aequorin injected into several cells comprising the muscle preparation (from [54]). **d** Sarcoplasmic reticulum Ca^{2+} uptake rate. (From [18].)

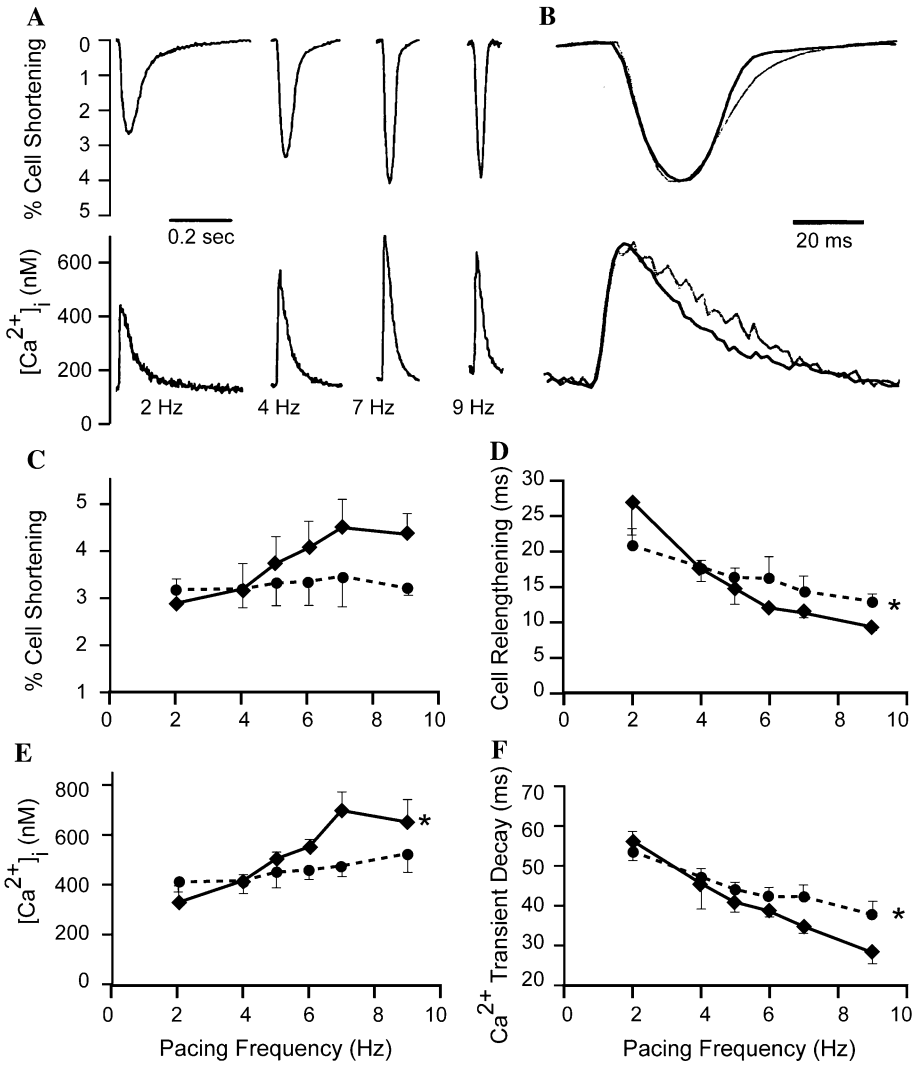

Fig. 3 *Top panels*: **a** Averaged tracings of percentage cell shortening and $[Ca^{2+}]_i$ recorded at 37°C from a representative adult myocyte paced at 2, 4, 7, and 9 Hz. B Normalized tracings of cell shortening (top panel) and $[Ca^{2+}]_i$ (*bottom panel*) from a representative adult (*black line*) and senescent (*gray line*) myocyte paced at 9 Hz; tracings were normalized to peak value to show changes in time course. Cell shortening and $[Ca^{2+}]_i$ were acquired at 4.2 and 2 ms sampling rates, respectively. To reduce the signal-to-noise ratio, each tracing was depicted as an average of ten original tracings. Percentage cell shortening (C), time constant (τ) of cell relengthening (D), calcium transient amplitude (E), and time constant (τ) of the intracellular calcium transient decay (F) in adult (*solid line*; $n = 4$) and senescent (*dashed line*; $n = 5$) myocytes at increasing pacing rates. Myocyte data from four adult and five senescent hearts were averaged and expressed as mean ± SEM (~4–5 myocytes were obtained from each heart). * $P < 0.05$ between adult and senescent curves (two-factor ANOVA). (From [45] with permission.)

cardiomyopathy and HF, i.e. under conditions in which SR function is depressed. The resulting AP prolongation favors Ca^{2+} influx during the depolarization and limits voltage-dependent Ca^{2+} efflux via NCX and thus may be adaptive since it provides partial compensation for SR deficiency, although possibly at the cost of asynchronous SR Ca^{2+} release and greater propensity to triggered arrhythmias [6]. The imposition of a shorter AP to myocytes from the old rat heart reduces the amplitude and the rate of decline of the Ca_i^{2+} transient [29]. This is attributable to a reduction in the SR Ca^{2+} uptake and loading which, in the presence of a reduced rate of Ca^{2+} sequestration by SERCA2, is presumably due to a reduced I_{CaL} time integral and likely also to an increased net Ca^{2+} extrusion via NCX (Fig. 2) [29].

A slower decay of the Ca_i^{2+} transient is a hallmark of aged cardiac myocyte (Figs. 1, 2, 3, 4, and Table 1). Reduction in the amplitude of the Ca_i^{2+} transient in myocytes from aged hearts, compared to younger counterparts, has been reported in some studies already at low (≤2 Hz) stimulation rates [21, 80]. Examinations using a range of stimulation rates [27, 45] typically showed blunted force- and relaxation-frequency responses in myocytes from old

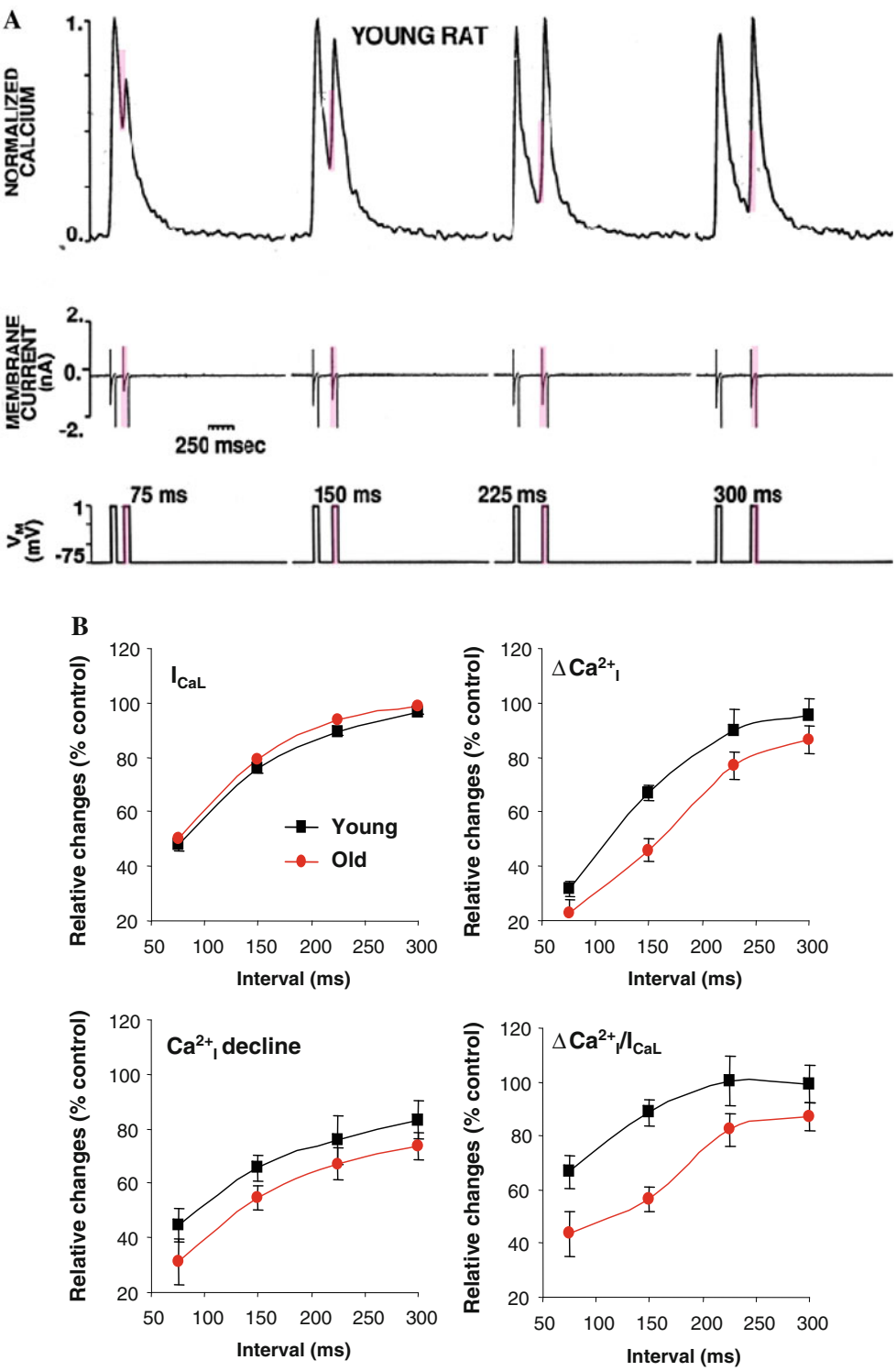

Fig. 4 Recovery of the L-type Ca^{2+} current (I_{CaL}) and the intracellular Ca^{2+} (Ca_i^{2+}) transient following a prior depolarization. A, Recordings of Ca_i^{2+} transients (top) and I_{CaL} (middle), induced by voltage-clamp depolarizations (bottom) from −75 to 0 mV (with Na^+ current, K^+ currents and "reverse" Na^+–Ca^{2+} exchange blocked) in a representative ventricular myocyte isolated from young adult (6 mo) Wistar rat. Test pulse intervals of 75–300 ms duration were applied following a train of 9 conditioning voltage pulses (50 ms, from −75 to 0 mV at 0.5 Hz). B, Averaged data from these experiments in myocytes from young ($n = 5$) and old (24 mo; $n = 7$) rats, show slower rate of Ca_i^{2+} decline during the last conditioning pulse, and similar kinetics of I_{CaL} recovery but prolonged recovery time of the Ca_i^{2+} transient and "gain" of I_{CaL}-dependent Ca^{2+} release (Ca_i^{2+}/I_{CaL}) during premature depolarizations in old vs. young myocytes. (From AM Janczewski and EG Lakatta, unpublished results.)

vs. young hearts. Thus, while the age-related differences in the amplitude and the rate of decay of the Ca_i^{2+} transients (and diastolic Ca_i^{2+} levels) were small or absent at low stimulation rates, they became apparent and progressively larger at pacing rates approximating those in vivo (Fig. 3) [27, 45]. Likewise, abrupt changes in the stimulation rate reveal an impaired SR Ca^{2+} release in ventricular myocytes isolated from senescent vs. young rats (Fig. 4). Specifically, in the presence of similar kinetics of I_{CaL} recovery, reduction in the amplitude of the Ca_i^{2+} transients and the gain of I_{CaL}-dependent Ca^{2+} release during premature depolarizations are attributable to a slower rate of

SR Ca^{2+} reuptake in older myocytes (Fig. 4). Taken together, these results are consistent with a reduced adaptive capacity of the older heart to physiological stress, i.e. pacing rate and β-adrenergic stimulation (see [38] for review), and underscore the value of appropriate experimental design in examining potential age-related differences in cardiac phenotype. Another characteristic of age-related changes in the configuration of the Ca_i^{2+} transient, reported in several (Fig. 1) and [27] but not all [21, 45] studies, is a prolonged time to peak Ca_i^{2+}.

In cardiac myocytes, the development of the Ca_i^{2+} transient is dependent primarily on the amount and the rate of Ca^{2+} release from the SR, and the decline of the Ca_i^{2+} transient and the amount of Ca^{2+} available for subsequent release are dependent primarily on Ca^{2+} sequestration by the SR. Age-related reduction in the rate of rise and the amplitude of the Ca_i^{2+} transient (systolic dysfunction), as well as the rate of decline of the Ca_i^{2+} transient (diastolic dysfunction), appear to result, in large part, from impaired Ca^{2+} pumping by SERCA2. These changes have been extensively documented in biochemical and functional studies [18, 35, 58, 65, 76]. At the molecular level, they are attributable to a reduced protein expression of SERCA2 or its ratio to PLB (Table 2) and/or reduced phosphorylation of the SERCA2-PLB complex by PKA and CaMK [31, 75, 76]. A shift of SERCA2b distribution to the subsarcolemmal space has also been suggested [27]. Age-related alterations in the gating properties of RYR [26, 80], resulting in an increased SR Ca^{2+} leak, may also contribute to both diastolic and systolic dysfunction of the aging myocardium by limiting the *net* rate of SR Ca^{2+} sequestration and SR Ca^{2+} loading, respectively. Finally, a slower rate of development/longer time to peak of the Ca_i^{2+} transient in aging myocytes is likely to result from reduced SR Ca^{2+} loading but may be also consequent to a longer time to peak I_{CaL} [71], which synchronizes SR Ca^{2+} release.

Prolonged time to peak and slower relaxation of contraction, typical for aged myocardium (Figs. 1 and 3) [21, 45, 46, 64, 67, 80] are attributable to both changes in the αMHC and βMHC protein ratio (Table 1) [38] and in the configuration of the Ca^{2+} transient. Clearly, the latter underlies impaired frequency-dependent inotropic and lusitropic responses [21, 45, 46, 58, 59] that largely contribute to the systolic and diastolic dysfunction of the aging heart. Consistent with the major role of SERCA2 in these effects, studies in rat isolated cardiac muscle preparations have shown that exercise training reverses age-associated slowing of contraction and relaxation [64, 67]. This was associated with increased Ca^{2+} transport by SERCA2 but not myosin ATPase activity in cardiac homogenates [67]. Likewise, overexpression of SERCA2 markedly improved rate-dependent contractility and contractile function in senescent rat hearts [58].

Sex-related differences

Biological sex is a well-recognized factor in the physiology and pathophysiology of the cardiovascular system, including the aging heart (reviewed in [41] and [36]). However, despite the growing number of reports in the literature identifying sex-related differences in cardiac function in both humans and rodents, the underlying mechanisms remain incompletely understood.

New insights into the molecular bases of sexual dimorphism in the context of aging and cardiovascular disease have been recently afforded by large-scale analyses of gene expression at the transcriptional level using microarrays. Specifically, Boheler and colleagues [8] have identified several HF-dependent gene products that may act as potential regulators for transducing mechanical, stress and neurohormonal stimuli into changes in gene expression. Most HF-candidate genes demonstrated significant changes in gene expression; however, the majority of differences among samples depended on variables such as sex and age, and not on HF alone. Some HF-responsive gene products also demonstrated highly significant changes in expression as a function of age and/or sex, but independent of HF. These results emphasize the need to account for biological variables (HF, sex, and age interactions) to elucidate genomic correlates that trigger molecular pathways responsible for the progression of HF syndromes. Subsequent analysis of gene expression differences by sex and age in left ventricular samples from patients with dilated cardiomyopathy has identified more than 1,800 genes displaying sexual dimorphism in the heart. A significant number of these genes were highly represented in gene ontology pathways involved in ion transport and G protein-coupled receptor signaling [16].

Postmortem morphometric assessments in non-failing human hearts have shown extensive age-related myocyte loss and hypertrophy of the surviving myocytes in males, but preserved ventricular myocardial mass, average cell diameter, and volume in aging female hearts [53]. These sex differences may stem, in part, from differences in the replicative potential of cardiac myocytes. For instance, the activity of telomerase, an enzyme present only during cell replication, was decreased 31% in aging male rat myocytes, but increased 72% in female counterparts [42]. Premature development of HF or death in males compared to matching females has been documented in rat models of pressure overload and/or myocardial infarction (reviewed in [36]). Sexually dimorphic cardiac phenotypes have been also discovered in some studies in genetically engineered mice (Table 4) [36, 41]. In general, transgenic models of HF present a more rapid onset and/or a greater severity of cardiac dysfunction in male vs. female hearts. For instance, mice with cardiac-specific overexpression of tumor

Table 4 Summary of male- and female-related differences in genetically manipulated mice (From [41] with permission.)

Cardiovascular component or characteristic	Observed effect		Reference (publication listed in [41])
	Males	Females	
α_{1A}- and $\alpha_{1\beta}$-adrenergic receptor null	Small heart: ↓ exercise performance	↓ Aortic restriction response; heart normal following ovariectomy	[3]
Constitutively active Akt	Heart size ↑ compared to wild type	Heart size ↑ compared to wild type; heart size ↑ compared to transgenic males	[44]
Mutant α-MyHC	Cardiac chamber dilatation; heart failure	Hypertrophy; preserved function	[58]
Mutant cardiac troponin T (R92Q)	Heart size ↓ compared to wild type	No change in heart size when compared to wild type	[59] (L. Leinwand, unpublished observations)
Superinhibition of phospholamban	Progress to dilated cardiomyopathy at 6 months of age	Hypertrophy; normal cardiac function	[60]
Phospholamban overexpression	Hypertrophy; mortality: 15 months of age	Delayed hypertrophy; mortality: 22 months of age despite similar dysfunction in males	[61]
ADH overexpression	No phenotype	Myocytes more sensitive to ethanol; depression of contractions	[62]
TNF-α overexpression	Cardiac chamber dilatation; impaired Ca^{2+} handling	Hypertrophy; normal Ca^{2+} handling	[63]
Lipoprotein lipase I overexpression/PPAR-null	Die at 4 months of age	Live	[64]
Relaxin null	Cardiomyopathy	No altered phenotype	[65]
β2-adrenergic receptor overexpression	↑ Contractility; ↑ ischemia reperfusion injury	↑ Contractility; normal response to injury	[66]

Reference numbers in parenthesis indicate publications listed in [41].

necrosis factor-α (TNF-α) exhibit HF and increased mortality that is markedly higher in young males than females (~50% and 4%, respectively, by 20 weeks of age) [33]. At 12 weeks of age, female mice displayed LV hypertrophy without dilatation and only a small reduction of basal LV fractional shortening and response to isoproterenol (Iso), while male mice showed a large LV dilatation, reduced fractional shortening relative to both wild-type littermates and transgenic females, and minimal response to Iso [30]. Cardiac myocyte hypertrophy was similar in male and female transgenic mice. Compared to wild-type mice, myocytes from female TNF-α transgenic mice displayed a slower decline of the Ca_i^{2+} transient, but similar amplitudes of Ca_i^{2+} transients and contractions and the inotropic response to Iso. In contrast, the amplitude and the rate of decline of Ca_i^{2+} transients and contractions, and the response to Iso were significantly reduced in myocytes from male transgenic TNF-α mice [30].

These studies underscore the value of considering biological sex and age in the assessment of cardiac phenotypes. However, inspection of the literature shows that the majority of studies in animal models of human cardiac disease has been carried out only in males or do not indicate which sex was studied. The same applies to studies of myocardial changes with adult aging, including molecular changes (Table 2). Remarkably, the available examinations of cardiac EC coupling in rodents of both sexes [21] or females only [70] show a lack of age-related changes in the configuration of the Ca_i^{2+} transient in myocytes from female hearts. Specifically, Grandy and Howlett [21] have reported a significant reduction in the I_{CaL} density, amplitudes of the Ca_i^{2+} transients, fractional cell shortening, and the SR Ca^{2+} content in ventricular myocytes isolated from the hearts of aged (~24 mo) vs. young adult (~7 mo) male mice. In contrast, I_{CaL} density, the amplitude of Ca_i^{2+} transients and fractional cell shortening were similar in young adult and aged myocytes from female hearts, while the SR Ca^{2+} content was increased in the aged female group. Accordingly, fractional SR Ca^{2+} release was similar in both age groups of male myocytes but reduced in aged vs. young adult female myocytes. The gain of EC coupling was similar in young adult and aged myocytes, regardless of the sex of the animal. The somewhat unexpected findings of this study in aged female myocytes include (a) an apparent increase in the SR Ca^{2+} content, (b) the lack of the effect of the latter on the configuration of the Ca_i^{2+} transients and contraction, and (c) a reduction of fractional SR Ca^{2+} release, in the presence of unchanged $I_{CaL.}$ Nevertheless, these results provide an initial direct demonstration of sexually dimorphic changes in cardiac EC coupling associated with normal adult aging.

Interestingly, the I_{CaL} and the amplitude and the rate of decline of the Ca_i^{2+} transients were significantly increased in cardiac myocytes isolated from aged (>8 years) sheep vs. young (18 months) female sheep [15]. However, a potential sex aspect of this finding has not been explored.

Myocyte progenitors in the aging heart

Studies by Anversa and colleagues (reviewed in [3, 34]) have demonstrated that the heart is a self-renewing organ containing a pool of progenitor cells (PCs) that dictate cell turnover, organ homeostasis and myocardial aging.

Observations in humans and animals suggest that myocyte maturation and aging are characterized by loss of replicative potential, telomeric shortening and the expression of the senescence-associated protein/cell cycle inhibitor $p16^{INK4a}$ [12, 20, 56]. Telomeric shortening in PCs leads to generation of progeny that rapidly acquires the senescent phenotype. As discussed earlier, the latter involves progressive increase in the size of the cell (up to a critical volume beyond which myocyte hypertrophy is no longer possible), deficits in the electrical, Ca^{2+} cycling and mechanical properties, and cell death. Cardiac myocytes with senescent and non-senescent phenotypes already coexist at young age [56]. However, aging limits the growth and differentiation potential of PCs, thus interfering not only with their ability to sustain physiological cell turnover but also with their capacity to adapt to increases in pressure and volume loads [3, 34].

The loss of PC function with aging is mediated partly by an imbalance between factors enhancing oxidative stress, telomere attrition and death, and factors promoting growth, migration, and survival. Recent findings suggest a preeminent position of insulin-like growth factor-1 (IGF-1) among factors that interfere with cardiac cellular senescence. Specifically, cardiac-restricted overexpression of IGF-1 in transgenic mice has been shown to delay the aging myopathy and the manifestations of HF [68] and to restore SERCA2a expression and rescue age-associated impairment of cardiac myocyte contractile function [44]. The latter effect was also partly mimicked by short-term in vitro treatment with recombinant IGF-1 [44]. Furthermore, intramyocardial delivery of IGF-1 improved senescent heart phenotype in male Fisher 344 rats [20], including increased proliferation of functionally competent PCs and diminished angiotensin II-induced apoptosis. Myocardial regeneration mediated by PC activation attenuated ventricular dilation and the decrease in ventricular mass-to-chamber volume ratio, resulting in improvement of in vivo cardiac function in animals at 28 to 29 months of age [20].

Thus, understanding the biology of cardiac PCs, including factors enhancing the activation of the PC pool, their mobilization, and translocation may facilitate the development of novel strategies to prevent or reverse the diminished adaptive capacity to increases in pressure and volume loads (and perhaps HF) in the old population.

Summary

The occurrence, clinical manifestations and prognoses of HF worsen with increasing age because the underlying pathophysiological mechanisms become superimposed on heart and vascular substrates that are modified by the process of aging. Changes in the cardiac cell phenotype associated with aging include (1) enlargement of the myocyte size, consequent to increase in vascular load and loss of neighboring myocytes, (2) reduced acute response to stress, consequent to deficits in ß-AR signaling, changes in the composition of the cell membranes and enhanced intracellular generation of ROS, and (3) altered EC coupling process. The latter involves prolongation of the AP, Ca_i^{2+} transient and contraction, and blunted force- and relaxation-frequency responses, consequent to changes in the expression and function of Ca^{2+} transport proteins involved in SR Ca^{2+} cycling, and in sarcolemmal K^+ currents. Studies of sexual dimorphism in the context of cardiac aging and HF and the expansion of the concept of the heart as a continuously self-renewing organ may assist in our understanding the pathophysiology of cardiovascular aging and in the development of effective therapeutic strategies.

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References

1. Abete P, Ferrara N, Cioppa A, Ferrara P, Bianco S, Calabrese C, Napoli C, Rengo F (1996) The role of aging on the control of contractile force by Na^+–Ca^{2+} exchange in rat papillary muscle. J Gerontol A Biol Sci Med Sci 51:M251–M259
2. Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Meggs LG, Capasso JM (1990) Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. Circ Res 67:871–885
3. Anversa P, Rota M, Urbanek K, Hosoda T, Sonnenblick EH, Leri A, Kajstura J, Bolli R (2005) Myocardial aging-a stem cell problem. Basic Res Cardiol 100:482–493
4. Armand A-S, De Windt LJ (2004) Calcium cycling in heart failure: how the fast became too furious. Cardiovasc Res 62: 439–441
5. Assayag P, Charlemagne D, Marty I, de Leiris J, Lompre AM, Boucher F, Valere PE, Lortet S, Swynghedauw B, Besse S (1998)

Effects of sustained low-flow ischemia on myocardial function and calcium-regulating proteins in adult and senescent rat hearts. Cardiovasc Res 38:169–180
6. Bassani RA (2006) Transient outward potassium current and Ca^{2+} homeostasis in the heart: beyond the action potential. Braz J Med Biol Res 39:393–403
7. Bito V, Heinzel FR, Biesmans L, Antoons G, Sipido KR (2008) Crosstalk between L-type Ca^{2+} channels and the sarcoplasmic reticulum: alterations during cardiac remodeling. Cardiovasc Res 77:315–324
8. Boheler KR, Volkova M, Morrell C, Garg R, Zhu Y, Marguelis K, Seymour AM, Lakatta EG (2003) Sex- and age-dependent human transcriptome variability: implications for chronic heart failure. Proc Natl Acad Sci USA 100:2754–2759
9. Boluyt MO, Lakatta EG (1998) Cardiovascular aging in health. In: Altschuld RA, Haworth RA (eds) Heart metabolism in failure, vol 4B. Connecticut JAI Press Inc, Greenwich, pp 257–303
10. Brenner DA, Apstein CS, Saupe KW (2001) Exercise training attenuates age-associated diastolic dysfunction in rats. Circulation 104:221–226
11. Caffrey JL, Boluyt MO, Younes A, Barron BA, O'Neill L, Crow MT, Lakatta EG (1994) Aging, cardiac proenkephalin mRNA and enkephalin peptides in the Fisher 344 rat. J Mol Cell Cardiol 26:701–711
12. Chimenti C, Kajstura J, Torella D, Urbanek K, Heleniak H, Colussi C, Di Meglio F, Nadal-Ginard B, Frustaci A, Leri A, Maseri A, Anversa P (2003) Senescence and death of primitive cells and myocytes leads to premature cardiac aging and heart failure. Circ Res 93:604–613
13. Cigola E, Kastura J, Li B, Meggs LG, Anversa P (1997) Angiotensin II activates programmed myocyte cell death in vitro. Exp Cell Res 231:363–371
14. del Monte F, Harding SE, Dec GW, Gwathmey JK, Hajjar RJ (2002) Targeting phospholamban by gene transfer in human heart failure. Circulation 105:904–907
15. Dibb K, Rueckschloss U, Eisner D, Insberg G, Trafford A (2004) Mechanisms underlying enhanced excitation contraction coupling observed in the senescent sheep myocardium. J Mol Cell Cardiol 37:1171–1181
16. Fermin DR, Barac A, Lee S, Polster SP, Hannenhalli S, Bergemann TL, Grindle S, Dyke DB, Pagani F, Miller LW, Tan S, dos Remedios C, Cappola TP, Margulies KB, Hall JL (2008) Sex and age dimorphism of myocardial gene expression in nonischemic human heart failure. Circulation 1:117–125
17. Fraticelli A, Josephson R, Danziger R, Lakatta E, Spurgeon H (1989) Morphological and contractile characteristics of rat cardiac myocytes from maturation to senescence. Am J Physiol 257:H259–H265
18. Froehlich JP, Lakatta EG, Beard E, Spurgeon HA, Weisfeldt ML, Gerstenblith G (1978) Studies of sarcoplasmic reticulum function and contraction duration in young and aged rat myocardium. J Mol Cell Cardiol 10:427–438
19. Frolkis VV, Frolkis RA, Mkhitarian LS, Shevchuk VG, Fraifeld VE, Vakulenko LG, Syrovy I (1988) Contractile function and Ca^{2+} transport system of myocardium in ageing. Gerontology 34:64–74
20. Gonzalez A, Rota M, Nurzynska D, Misao Y, Tillmanns J, Ojaimi C, Padin-Iruegas ME, Müller P, Esposito G, Bearzi C, Vitale S, Dawn B, Anganalmath SK, Baker M, Hintze TH, Bolli R, Urbanek K, Hosoda T, Anversa P, Kajstura J, Leri A (2008) Activation of cardiac progenitor cells reverses the failing heart senescent phenotype and prolongs lifespan. Circ Res 102:597–606
21. Grandy SA, Howlett SE (2006) Cardiac excitation-contraction coupling is altered in myocytes from aged male mice but not in cells from aged female mice. Am J Physiol 291:H2362–H2370
22. Guo T, Zhang T, Mestril R, Bers DM (2006) Ca/calmodulin-dependent protein kinase II phosphorylation of ryanodine receptor does affect calcium sparks in mouse ventricular myocytes. Circ Res 99:398–406
23. Hano O, Bogdanov KY, Sakai M, Danziger RG, Spurgeon HA, Lakatta EG (1995) Reduced threshold for myocardial cell calcium intolerance in the rat heart with aging. Am J Physiol 269:H1607–H1612
24. Helenius M, Hänninen M, Lehtinen SK, Salminen A (1996) Aging-induced up-regulation of nuclear binding activities of oxidative stress responsive NF-β transcription factor in mouse cardiac muscle. J Mol Cell Cardiol 28:487–498
25. Heyliger C, Prakash A, McNeill J (1988) Alterations in membrane Na^{+}–Ca^{2+} exchange in the aging myocardium. Age 1988:1–6
26. Howlett SE, Grandy SA, Ferrier GR (2006) Calcium spark properties in ventricular myocytes are altered in aged mice. Am J Physiol 290:H1566–H1574
27. Isenberg G, Borschke B, Rueckschloss U (2003) Ca^{2+} transients in cardiomyocytes from senescent mice peak late and decay slowly. Cell Calcium 34:271–280
28. Janapati V, Wu A, Davis N, Derrico CA, Levengood J, Schummers J, Colvin RA (1995) Post-transcriptional regulation of the Na^{+}/Ca^{2+} exchanger in aging rat heart. Mech Ageing Dev 84: 195–208
29. Janczewski AM, Spurgeon HA, Lakatta EG (2002) Action potential prolongation in cardiac myocytes of old rats is an adaptation to sustain youthful intracellular Ca^{2+} regulation. J Mol Cell Cardiol 34:641–648
30. Janczewski AM, Kadokami T, Lemster B, Frye CS, McTiernan CF, Feldman AM (2003) Morphological and functional changes in cardiac myocytes isolated from mice overexpressing TNF-α. Am J Physiol 284:H960–H969
31. Jiang MT, Moffat MP, Narayanan N (1993) Age-related alterations in the phosphorylation of sarcoplasmic reticulum and myofibrillar proteins and diminished contractile response to isoproterenol in intact rat ventricle. Circ Res 72:102–111
32. Josephson IR, Guia A, Stern MD, Lakatta EG (2002) Alterations in properties of L-type Ca channels in aging rat heart. J Mol Cell Cardiol 34:297–308
33. Kadokami T, McTiernan CF, Kubota T, Frye CS, Feldman AM (2000) Sex-related survival differences in murine cardiomyopathy are associated with differences in TNF-receptor expression. J Clin Invest 106:589–597
34. Kajstura J, Urbanek K, Rota M, Bearzi C, Hosoda T, Bolli R, Anversa P, Leri A (2008) Cardiac stem cells and myocardial disease. J Mol Cell Cardiol 45:505–513
35. Kaplan P, Jurkovicova D, Babusikova E, Hudecova S, Racay P, Sirova M, Lehotsky J, Drgova A, Dobrota D, Krizanova O (2007) Effect of aging on the expression of intracellular Ca^{2+} transport proteins in a rat heart. Mol Cell Biochem 301:219–226
36. Konhilas JP, Leinwand LA (2007) The effects of biological sex and diet on the development of heart failure. Circulation 116: 2747–2759
37. Lakatta EG (1992) Functional implications of spontaneous sarcoplasmic reticulum Ca^{2+} release in the heart. Cardiovasc Res 26:193–214
38. Lakatta EG (1993) Cardiovascular regulatory mechanisms in advanced age. Physiol Rev 73:413–465
39. Lakatta EG (1999) Cardiovascular aging research: the next horizons. J Am Geriatr Soc 47:613–625
40. Lakatta EG, Sollott SJ, Pepe S (2001) The old heart: operating on the edge. In: Ageing vulnerability: causes and interventions. Novartis foundation symposium 235. Wiley, Ltd, pp 172–201
41. Leinwand LA (2003) Sex is a potent modifier of the cardiovascular system. J Clin Invest 112:302–307

42. Leri A, Malhotra A, Liew CC, Kajstura J, Anversa P (2000) Telomerase activity in rat cardiac myocytes is age and gender dependent. J Mol Cell Cardiol 32:385–390
43. Li Y, Kranias EG, Mignery GA, Bers DM (2002) Protein kinase a phosphorylation of the ryanodine receptor does not affect calcium sparks in mouse ventricular myocytes. Circ Res 90:309–316
44. Li Q, Wu S, Li S-Y, Lopez FL, Du M, Kajstura J, Anversa P, Ren J (2007) Cardiac-specific overexpression of insulin-like growth factor 1 attenuates aging-associated cardiac diastolic contractile dysfunction and protein damage. Am J Physiol 292:H1398–H1403
45. Lim CC, Apstein CS, Colucci WS, Liao R (2000) Impaired cell shortening and relengthening with increased pacing frequency are intrinsic to the senescent mouse cardiomyocyte. J Mol Cell Cardiol 32:2075–2082
46. Lim CC, Liao R, Varma N, Apstein CS (1999) Impaired lusitropy-frequency in the aging mouse: role of Ca^{2+} handling proteins and effects of isoproterenol. Am J Physiol 277:H2083–H2090
47. Liu SJ, Wyeth RP, Melchert RB, Kennedy RH (2000) Aging-associated changes in whole cell K^+ and L-type Ca^{2+} currents in rat ventricular myocytes. Am J Physiol 279:H889–H900
48. Lucas D, Sweda L (1998) Cardiac reperfusion injury, aging, lipid peroxidation, and mitochondrial dysfunction. Proc Natl Acad Sci USA 95:510–514
49. Mace LC, Palmer BM, Brown DA, Jew KN, Lynch JM, Glunt JM, Parsons TA, Cheung JY, Moore RL (2003) Influence of age and run training on cardiac Na^+/Ca^{2+} exchange. J Appl Physiol 95:1994–2003
50. MacLennan DH, Kranias EG (2003) Phospholamban: a crucial regulator of cardiac contractility. Nat Rev Mol Cell Biol 4:566–577
51. Marks AR (2000) Cardiac intracellular calcium release channels: role in heart failure. Circ Res 87:8–11
52. Nitta Y, Abe K, Aoki M, Ohno I, Isoyama S (1994) Diminished heat shock protein 70 mRNA induction in aged rat hearts after ischemia. Am J Physiol 267:H1795–H1803
53. Olivetti G, Giordano G, Corradi D, Melissari M, Lagrasta C, Gambert SR, Anversa P (1995) Gender differences and aging: effects on the human heart. J Am Coll Cardiol 26:1068–1079
54. Orchard CH, Lakatta EG (1985) Intracellular calcium transients and developed tensions in rat heart muscle. A mechanism for the negative interval-strength relationship. J Gen Physiol 86:637–651
55. Pepe S, Tsuchiya N, Lakatta EG, Hansford RG (1999) PUFA and aging modulate cardiac mitochondrial membrane lipid composition and Ca^{2+} activation of PDH. Am J Physiol 276:H149–H158
56. Rota M, Hosoda T, De Angelis A, Arcarese ML, Esposito G, Rizzi R, Tillmanns J, Tugal D, Musso E, Rimoldi O, Bearzi C, Urbanek K, Anversa P, Leri A, Kajstura J (2007) The young mouse heart is composed of myocytes heterogeneous in age and function. Circ Res 101:387–399
57. Sakai M, Danziger RS, Staddon JM, Lakatta EG, Hansford RG (1989) Decrease with senescence in the norepinephrine-induced phosphorylation of myofilament proteins in isolated rat cardiac myocytes. J Mol Cell Cardiol 21:1327–1336
58. Schmidt U, del Monte F, Miyamoto MI, Matsui T, Gwathmey JK, Rosenzweig A, Hajjar RJ (2000) Restoration of diastolic function in senescent rat hearts through adenoviral gene transfer of sarcoplasmic reticulum Ca^{2+}-ATPase. Circulation 101:790–796
59. Schmidt U, Zhu X, Lebeche D, Huq F, Guerrero JL, Hajjar RJ (2005) In vivo gene transfer of parvalbumin improves diastolic function in aged rat hearts. Cardiovasc Res 66:318–323
60. Shida M, Isoyama S (1993) Effects of age on c-fos and c-myc gene expression in response to hemodynamic stress in isolated, perfused rat hearts. J Mol Cell Cardiol 25:1025–1035
61. Shunkert H, Weinberg E, Bruckschlegel G, Riegger AJ, Lorell BH (1995) Alteration of growth responses in established cardiac pressure overload hypertrophy in rats with aortic banding. J Clin Invest 96:2768–2774
62. Sipido KR, Eisner D (2005) Something old, something new: Changing views on the cellular mechanisms of heart failure. Cardiovasc Res 68:167–174
63. Slack JP, Grupp IL, Dash R, Holder D, Schmidt A, Gerst MJ, Tamura T, Tilgmann C, James PF, Johnson R, Gerdes AM, Kranias EG (2001) The enhanced contractility of the phospholamban-deficient mouse heart persists with aging. J Mol Cell Cardiol 33:1031–1040
64. Spurgeon HA, Steinbach MF, Lakatta EG (1983) Chronic exercise prevents characteristic age-related changes in rat cardiac contraction. Am J Physiol 244:H513–H518
65. Taffet GE, Tate CA (1993) CaATPase content is lower in cardiac sarcoplasmic reticulum isolated from old rats. Am J Physiol 264:H1609–H1614
66. Takahashi T, Schunkert H, Isoyama S, Wei JY, Nadal-Ginard B, Grossman W, Izumo S (1992) Age-related differences in the expression of proto-oncogene and contractile protein genes in response to pressure overload in the rat myocardium. J Clin Invest 89:939–946
67. Tate CA, Taffet GE, Hudson EK, Blaylock SL, McBride RP, Michael LH (1990) Enhanced calcium uptake of cardiac sarcoplasmic reticulum in exercise-trained old rats. Am J Physiol 258:H431–H435
68. Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, Zias E, Walsh K, Rosenzweig A, Sussman MA, Urbanek K, Nadal-Ginard B, Kajstura J, Anversa P, Leri A (2004) Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. Circ Res 94:514–524
69. Van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, Palacios-Callendere M, Erusalimsky JD, Quaschning T, Malinski T, Gygi D, Ullrich V, Lüscher TF (2000) Enhanced peroxynitrite formation is associated with vascular aging. J Exp Med 192:1731–1743
70. Wahr PA, Michele DE, Metzger JM (2000) Effects of aging on single cardiac myocyte function in Fischer 344 x Brown Norway rats. Am J Physiol 279:H559–H565
71. Walker KE, Lakatta EG, Houser SR (1993) Age associated changes in membrane currents in rat ventricular myocytes. Cardiovasc Res 27:1968–1977
72. Wei JY, Spurgeon A, Lakatta EG (1984) Excitation-contraction in rat myocardium: alterations with adult aging. Am J Physiol 246:H784–H791
73. Xiao RP, Spurgeon HA, O'Connor F, Lakatta EG (1994) Age-associated changes in beta-adrenergic modulation on rat cardiac excitation-contraction coupling. J Clin Invest 94:2051–2059
74. Xiao R-P, Tomhave ED, Wang DJ, Ji X, Boluyt MO, Cheng H, Lakatta EG, Koch WJ (1998) Age-associated reductions in cardiac $ß_1$ - and $ß_2$ -adrenoceptor responses without changes in inhibitory G proteins or receptor kinases. J Clin Invest 101:1273–1282
75. Xu A, Hawkins C, Narayanan N (1993) Phosphorylation and activation of the Ca^{2+}-ATPase of cardiac sarcoplasmic reticulum by Ca^{2+}/calmodulin-dependent protein kinase. J Biol Chem 268:8394–8397
76. Xu A, Narayanan N (1998) Effects of aging on sarcoplasmic reticulum Ca^{2+}-cycling proteins and their phosphorylation in rat myocardium. Am J Physiol 275:H2087–H2094
77. Yin FC, Spurgeon HA, Weisfeldt ML, Lakatta EG (1980) Mechanical properties of myocardium from hypertrophied rat hearts. A comparison between hypertrophy induced by senescence and by aortic banding. Circ Res 46:292–300

78. Younes A, Boluyt MO, O'Neill L, Meredith AL, Crow MT, Lakatta EG (1995) Age-associated increase in rat ventricular ANP gene expression correlates with cardiac hypertrophy. Am J Physiol 38:H1003–H1008
79. Zhang SJ, Zhou YY, Xiao RP et al (2000) Age-associated reduction in recovery of the equilibrium state of myocyte length during reduced interstimulus intervals at higher stimulation rates. Biophys J 78:227A (Abstract)
80. Zhu X, Altschafl BA, Hajjar RJ, Valdivia HH, Schmidt U (2005) Altered Ca^{2+} sparks and gating properties of ryanodine receptors in aging cardiomyocytes. Cell Calcium 37:583–591