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INVITED REVIEW

Sex differences in mechanisms of cardiac excitation–contraction coupling

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Abstract The incidence and expression of cardiovascular diseases differs between the sexes. This is not surprising, as cardiac physiology differs between men and women. Clinical and basic science investigations have shown important sex differences in cardiac structure and function. The pervasiveness of sex differences suggests that such differences must be fundamental, likely operating at a cellular level. Indeed, studies have shown that isolated ventricular myocytes from female animals have smaller and slower contractions and underlying calcium transients compared to males. Recent evidence suggests that this arises from sex differences in components of the cardiac excitation–contraction coupling pathway, the sequence of events linking myocyte depolarization to calcium release from the sarcoplasmic reticulum and subsequent contraction. The concept that sex hormones may regulate intracellular calcium at the level of the cardiomyocyte is important, as levels of these hormones decline in both men and women as the incidence of cardiovascular disease rises. This review focuses on the impact of sex on cardiac contraction, in particular at the cellular level, and highlights specific components of the excitation–contraction coupling pathway that differ between the sexes. Understanding sex hormone regulation of calcium homeostasis in the heart may reveal new avenues for therapeutic strategies to treat cardiac dysfunction and cardiovascular diseases.

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Introduction

Clinical studies have identified striking differences between men and women in the incidence and expression of many different cardiovascular diseases. This should not be surprising; there are also sex differences in the normal structure and physiology of the heart. Although information is limited, there is evidence that myocardial electrical and contractile function differ between the sexes. Studies that have included both males and females have distinguished important differences in cardiac contractile function, even at the level of the individual cardiomyocyte. Additionally, cardiomyocytes possess receptors for major sex steroid hormones, and these hormones are thought to regulate myocardial function. This has led to considerable interest in identifying the cellular mechanisms responsible for sex differences in myocardial function. Indeed, recent studies have identified intriguing differences at several different levels of the excitation–contraction (EC) coupling pathway, which is responsible for the contraction of individual cardiomyocytes.

This review highlights our knowledge of the impact of sex on cardiac contraction and examines specific components of the EC coupling pathway that differ between males and females. Acute and chronic effects of sex hormones on components of this pathway are also considered. Current controversies are discussed, and important questions are raised for future research. Further understanding of the cellular mechanisms responsible for sex differences in myocardial function can begin to reveal why varying pathologies arise in males and females. This could lead to a better appreciation of the role of sex hormones in the regulation of cardiac function and to the development of more appropriate sex-specific therapies for cardiovascular diseases.

Sex differences in cardiac function

Important differences have been described in the incidence and susceptibility to cardiovascular disease in men and women [30, 84, 90]. For example, women typically experience coronary artery disease and myocardial infarction about 10 years later than men [87]. Hypertrophic and dilated cardiomyopathies both occur more frequently in men than in women, whereas Tako-Tsubo cardiomyopathy is seen almost exclusively in women [87]. Men also tend to develop heart failure with impaired systolic function as they age, while older women have a higher incidence of heart failure with preserved ejection fraction [87].

These differences in cardiac pathophysiology may originate, at least in part, from sex differences in the structure and function of the heart. In humans, echocardiographic studies have revealed that left ventricular (LV) mass is significantly smaller in women than in men [31, 32]. Echocardiography has also shown that females have reduced LV end-diastolic dimension [11, 111]. In addition, women have higher resting heart rates and longer corrected QT intervals than men [52, 125]. Radiographic studies using ventriculography have demonstrated that women possess a higher ejection fraction at rest than men [11]. Even so, men respond to exercise with a greater increase in ejection fraction than do women [52, 76]. This decrease in the ability of the female heart to respond to increased demand has been shown to be independent of sex differences in myocardial regulation by the sinus node or by autonomic tone [15]. This raises the question of whether individual cardiomyocytes from female hearts may have limited ability to augment contractile function, in particular in response to stimuli that increase demand.

Studies in animal models have provided additional evidence that cardiac contractile function differs between the sexes. In a working heart model, female rats have a smaller ejection fraction and exhibit less fractional shortening in comparison to age-matched males [95]. Studies in cardiac muscle strips from rats found similar results, in that responses were smaller and slower in females in comparison to males, particularly in conditions of higher demand such as rapid pacing rates [27, 65, 83]. By contrast, in vivo studies with Doppler echocardiography have reported no sex differences in functional parameters such as ejection fraction and fractional shortening in CD1 mice [104]. However, these in vivo results could be affected by the use of anaesthetics, which can alter cardiac function by decreasing heart rate and may abolish any sex differences that are present. Furthermore, indices such as ejection fraction and fractional shortening depend on LV loading status, which may differ between males and females. This may also mask sex differences in cardiac function.

Most experimental studies in hearts and cardiac muscle preparations have revealed lower myocardial contractile function in females. Sex differences in cardiac contractile function are likely to arise, at least in part, through differences in the ability of individual cardiomyocytes to contract. As cardiac contractions result from a transient rise in intracellular Ca^{2+} levels in cardiomyocytes [4, 35], they may reflect underlying sex differences in intracellular Ca^{2+} handling at the cellular level. The sequence of events that link cardiac excitation to Ca^{2+} release from the sarcoplasmic reticulum (SR) and contraction is known as EC coupling. This process will be briefly reviewed.

Cardiac excitation–contraction coupling

In cardiomyocytes, Ca^{2+} levels are tightly regulated via the EC coupling pathway. This pathway converts an electrical stimulus from the sinoatrial node into a mechanical contraction, causing the heart to eject blood. In ventricular myocytes, EC coupling is initiated when the action potential propagated along the cell membrane causes cellular depolarization, which activates voltage-sensitive L-type Ca^{2+} channels and results in an inward Ca^{2+} current [4]. L-type Ca^{2+} channels located within invaginations in the membrane are closely associated with Ca^{2+} release channels located on the SR [97]. These SR Ca^{2+} release channels are also known as ryanodine receptors (RyRs). Ca^{2+} influx triggers SR Ca^{2+} release by binding to RyRs, resulting in their opening and the subsequent release of SR Ca^{2+}, which gives rise to Ca^{2+} transients [37, 38].

The amount of Ca^{2+} released from the SR is much larger than the trigger Ca^{2+} that enters the cell, a phenomenon known as Ca^{2+}-induced Ca^{2+} release. The degree of amplification of Ca^{2+} influx to the resulting amount of Ca^{2+} released from the SR can be quantified by measuring a parameter known as EC coupling "gain". Experimentally, gain is calculated as the ratio of the amount of SR Ca^{2+} released (Ca^{2+} transient) per unit of Ca^{2+} current [6]. Factors such as elevated SR Ca^{2+} load, a decrease in temperature or ß-adrenergic stimulation have been shown to increase the gain of cardiac EC coupling [42, 100, 112]. By contrast, H-89, which inhibits protein kinase A (PKA), reduces the gain of EC coupling [81]. Therefore, modulation of EC coupling gain can potentially augment or inhibit SR Ca^{2+} release and contraction independently of changes in Ca^{2+} influx.

The rise in intracellular Ca^{2+} concentration caused by the Ca^{2+} transients allows Ca^{2+} to bind to the myofilaments, specifically troponin C, ultimately resulting in contraction of the myocyte [4, 35]. Relaxation occurs when the majority of Ca^{2+} is sequestered into the SR via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), while a much smaller amount of Ca^{2+} is removed from the cell predominantly by the Na^{+}/Ca^{2+} exchanger (NCX) [89]. Ca^{2+} reuptake in the SR is regulated by phospholamban (PLB), which inhibits SERCA. It is this tightly regulated sequence of events that

mediates the synchronous contraction of cardiomyocytes, and thus must be considered when evaluating cardiac contractile function in males and females.

SR Ca^{2+} is released in the form of discrete, subcellular Ca^{2+} release units called Ca^{2+} sparks. Ca^{2+} sparks are thought to result from the activation of a cluster of 6 to 20 RyR channels complexed with an L-type Ca^{2+} channel [20, 62], and many of these release units will fuse to form Ca^{2+} transients [49]. Spontaneous Ca^{2+} sparks can also occur in quiescent myocytes in the absence of L-type Ca^{2+} channel openings [20, 41]. The frequency and amplitude of spontaneous Ca^{2+} sparks is increased with increasing SR Ca^{2+} load [94]. In fact, spontaneous Ca^{2+} sparks are thought to represent a leak pathway to limit SR Ca^{2+} content under conditions of SR Ca^{2+} overload [94]. Changes in the properties of individual Ca^{2+} sparks, such as their size and frequency, can affect Ca^{2+} transient amplitudes, as well as SR Ca^{2+} content. Comparison of these unitary Ca^{2+} release events, between males and females for example, can reveal unique Ca^{2+} handling characteristics of individual cardiomyocytes.

One important pathway in the regulation of EC coupling in cardiomyocytes is the cyclic adenosine monophosphate (cAMP)/PKA pathway. Upon activation of the β-adrenergic receptor, adenylyl cyclase will increase the conversion of ATP into cAMP, which will activate PKA. PKA phosphorylates various components of the EC coupling pathway and causes an increase in inotropy and lusitropy [5]. L-type Ca^{2+} channel phosphorylation will increase the size of the Ca^{2+} current [59], thus causing a larger Ca^{2+}-induced Ca^{2+} release from the SR [5]. Phosphorylation of PLB alleviates its inhibition on SERCA activity and increases SR Ca^{2+} uptake, resulting in a faster Ca^{2+} transient decay [67]. Phosphorylation of troponin I causes faster relaxation by promoting dissociation of Ca^{2+} from the myofilaments [127]. A second protein kinase implicated in the regulation of cardiac contractile function is the Ca^{2+}-calmodulin-dependent kinase II (CaMKII). CaMKII also phosphorylates various components of the EC coupling pathway to cause an increase in lusitropy and inotropy, though to a lesser extent than PKA [5, 17, 126]. As PKA and CaMKII increase contraction size by increasing the size of the Ca^{2+} transients, these pathways are important to consider when examining contractile function in individual myocytes. Sex differences in either of these kinases could contribute to differences in SR Ca^{2+} release and contraction in cardiomyocytes from males and females.

Contractile function in isolated ventricular myocytes from males and females

Over the past decade, a number of studies have investigated whether sex differences in the ability of individual cardiomyocytes to contract contribute to the differences in cardiac contractile function reported in studies of intact heart and isolated cardiac muscle preparations. Some studies in field stimulated rat ventricular myocytes have reported either no difference in peak contraction between males and females [113] or an increase in contraction in cells from females [96], although this latter study found that sex differences were abolished when external Ca^{2+} levels were increased. However, as shown in Table 1, these studies were performed at room temperature, rather than at 37 °C, and myocytes were paced at stimulation frequencies between 0.5 and 0.8 Hz, which is far below the physiological heart rate in rats [98]. Other studies have used physiological or close to physiological stimulation frequencies (e.g. 1 to 4 Hz) to investigate sex differences in cardiac contractile function at the cellular level. Figure 1a depicts representative examples of contractions, Ca^{2+} transients and Ca^{2+} currents recorded simultaneously in voltage clamp experiments by Farrell et al. [40]. Mean data from this study shows that peak contractions are significantly smaller in ventricular myocytes from female rat hearts in comparison to cells from males (Fig. 1b). Additional field stimulation and voltage clamp experiments have revealed that cells from young adult female rats have smaller and slower contractions in comparison to age-matched males [29, 40, 55]. Whether this applies to all species is not clear, as one study performed in mice reported no sex difference in myocyte contraction size [46]. Studies examining contraction and relaxation of isolated ventricular myocytes from male and female rats are summarized in Table 1. Taken together, most studies show that sex differences in cardiac contraction are present at the cellular level, in particular if myocytes are paced at physiological rates. As cardiomyocyte contraction is proportional to pacing frequency in cells from rodents [68], it is perhaps not surprising that sex differences in cardiac contractile function are more apparent at rapid pacing rates. Further, as discussed earlier, studies in intact hearts, papillary muscles and ventricular trabeculae found that sex differences were more prominent at rapid stimulation rates. These results support the conclusion that sex differences in cardiac contractility are due, at least in part, to differences in the ability of individual cardiomyocytes to contract.

Sex differences in the contraction size of individual cardiomyocytes could arise from differences in the contractile machinery in cells from males and females. Therefore, the abundance and properties of myofilament proteins have been compared in male and female hearts. Female rat ventricles have been reported to possess higher levels of both α- and β-myosin heavy chain (MHC) and of sarcomeric actin mRNA in comparison to males [92]. However, there is no sex difference in the ratio of α- to β-MHC [92]. Even so, the possibility remains that there are sex differences in the rate of translation or in post-translational modifications of MHC proteins, and this could be evaluated with newer experimental approaches.

Table 1 Contractile function in isolated ventricular myocytes from female rats in relation to responses in cells from males

Functional change	Model	Frequency (Hz)	[Ca^{2+}] (mM)	Temperature (°C)	References
↓ Shortening ↔ Relaxation	Rats	2	1	37	[40]
↓ Shortening ↔ Relaxation	Rats	2, 4	1	37	[55]
↓ Shortening Slower relaxation	Rats	1	0.5–2	25	[29]
↑ Shortening ↔ Relaxation	Rats	0.5	1.5	Room temperature	[96]
↔ Shortening Slower relaxation	Rats	0.5	3.5	Room temperature	[96]
↔ Shortening	Rats	0.8	1	–	[113]

To determine whether there were functional differences in myofilaments from male and female hearts, Petre et al. [83] compared the Ca^{2+} sensitivity of myofilaments in skinned ventricular trabeculae from cats of both sexes. They compared maximal force in response to increasing concentrations of Ca^{2+} and found no sex difference in either the maximal force generated or in the concentration of Ca^{2+} required to produce 50 % of maximal force (EC_{50} values). Similarly, Schwertz et al. [96] found that the maximal developed force did not differ in skinned muscle fibres from male and female rats. However, myofibrillar ATPase activity was higher in females at any given Ca^{2+} concentration, which suggests that contractile proteins in female hearts may have greater Ca^{2+} sensitivity when compared to males [96]. Therefore, it is possible that the contractile machinery within female rat hearts is actually more sensitive to Ca^{2+}, and thus responds to lower intracellular Ca^{2+} concentrations than males. Certainly, there is no evidence that reduced myofilament responsiveness to Ca^{2+} explains the smaller contractions characteristic of myocytes from female hearts. Further research is required to confirm this finding, and to determine whether this is true in other animal models, as well as in humans.

Sex differences in Ca^{2+} transients in isolated ventricular myocytes

The amplitude of cardiac contraction depends not only on the myofilaments but also on the magnitude of the rise in intracellular Ca^{2+} [5]. Thus, the smaller contractions characteristic of cardiomyocytes from female animals may arise from sex differences in the size of the underlying Ca^{2+} transients. Figure 1a (top panel) depicts representative Ca^{2+} transients recorded in voltage clamp experiments from myocytes from young adult male and female rats [40]. As shown in this figure and in the mean data in Fig. 1c, peak Ca^{2+} transients are larger in cardiomyocytes from male hearts in comparison to female hearts. In fact, many studies using either field stimulation or voltage clamp techniques have determined that ventricular myocytes from female rats have smaller Ca^{2+} transient amplitudes in comparison to cells from males [29, 40, 65, 114]. By contrast, other investigations have reported no difference in Ca^{2+} transient amplitudes between the sexes in both mouse and rat models [46, 55, 124]. Differences in pacing frequency, temperature and/or species are known to affect intracellular Ca^{2+} handling [33, 86, 100], and variations in these parameters between studies may help explain these divergent results. Additional factors such as diastolic Ca^{2+} levels may also be important, as discussed below.

There is growing experimental evidence that contractions are substantially smaller in ventricular myocytes from females than males, in particular when cells are paced at rapid rates. However, the corresponding changes in peak Ca^{2+} transients are less dramatic, and some studies even report no sex difference in Ca^{2+} transient amplitudes [46, 55, 124]. It is important to note that the magnitude of cardiac contractions depends not only on the peak Ca^{2+} transients but also on the level of resting (diastolic) Ca^{2+} in the cell [4, 99]. For the same amount of activating Ca^{2+}, higher levels of diastolic Ca^{2+} result in larger contractions [4, 99]. Interestingly, diastolic Ca^{2+} levels are significantly lower in cardiomyocytes from female rats when compared to male rats [40, 55], although no sex differences are seen in murine myocytes [46]. Additional experiments that explore sex differences in cardiomyocytes exposed to varying diastolic Ca^{2+} levels could determine if lower diastolic Ca^{2+} levels are contributing to smaller contractions in females.

It is well established that Ca^{2+} transients in isolated cardiomyocytes from female rats have slower decay rates when compared to males [29, 65, 114]. A reduction in the rate of Ca^{2+} transient decay suggests that the rate of Ca^{2+} reuptake into the SR must be slower in myocytes from females. To investigate this possibility, expression levels of the major SR Ca^{2+} ATPase cardiac isoform, SERCA2a, have been compared in ventricles from male and female rats. No differences in either SERCA2a protein or mRNA levels in the ventricles of male and female rats have been

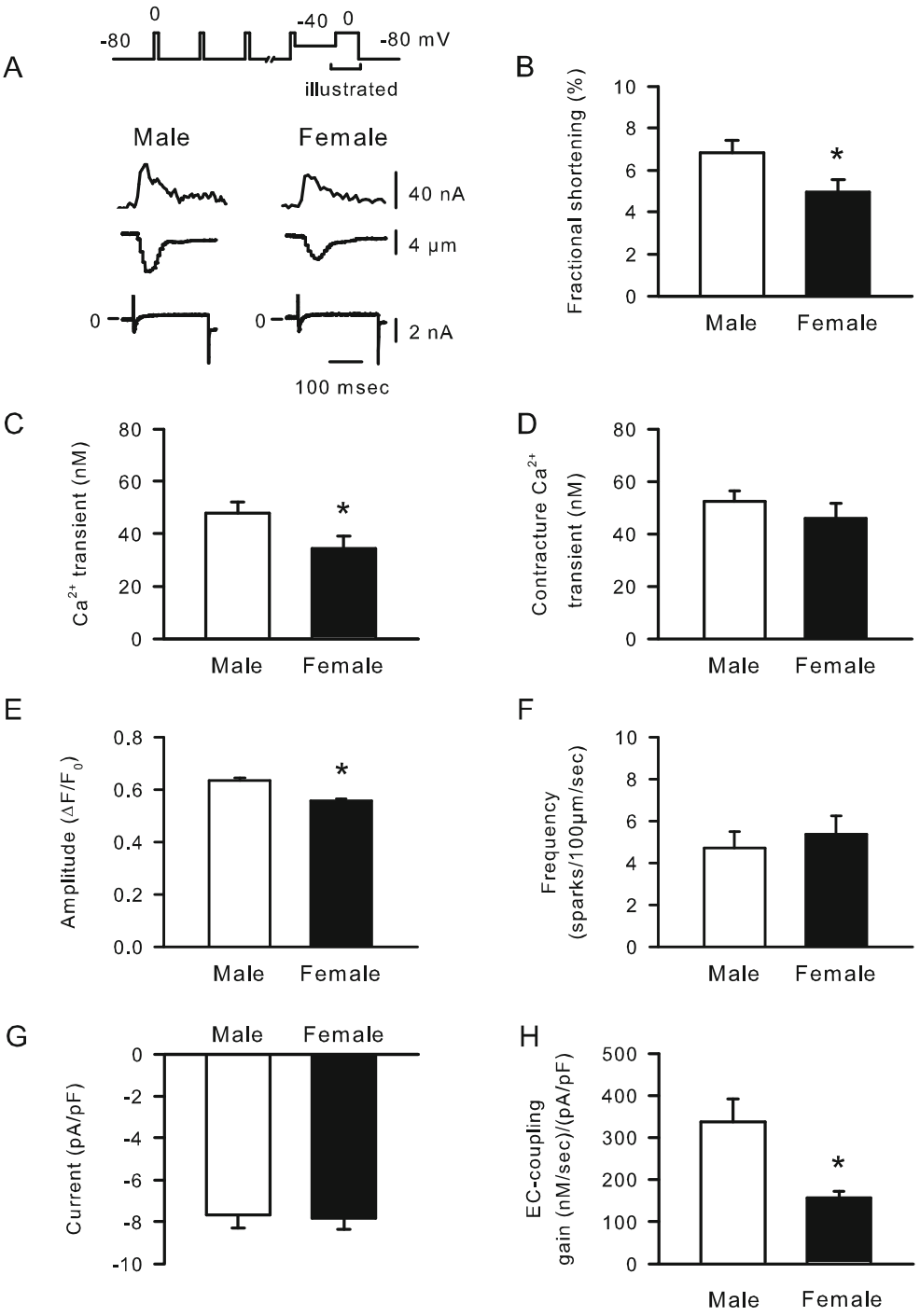

Fig. 1 Mechanisms of cardiac EC coupling differ between the sexes. **a** Sample recordings of Ca^{2+} transients (*top*), contractions (*middle*) and Ca^{2+} current (*bottom*) in ventricular myocytes from 3-month-old male and female rats. Responses were simultaneously activated by a voltage clamp test step from −40 to 0 mV. **b**, **c** Contractions and Ca^{2+} transients were significantly smaller in myocytes from females when compared to males. **d** SR Ca^{2+} content, evaluated by the rapid application of 10 mM caffeine, was similar in the two groups. **e**, **f** Ca^{2+} spark frequency did not differ between the sexes, but Ca^{2+} spark amplitudes were smaller in cells from females when compared to males. **g** Peak Ca^{2+} currents were similar in myocytes from males and females. **h** The gain of EC coupling (SR Ca^{2+} release per unit Ca^{2+} current) was much lower in cells from females compared to males. Reprinted from Farrell et al. [40] with permission

observed [19, 23, 106]. PLB, the intrinsic regulator of SERCA, was also evaluated in hearts from male and female rats. Similar levels of PLB protein and mRNA expression were found in the ventricles of both males and females [19, 23, 106]. Therefore, differences in the rate of decay of the Ca^{2+} transients are not due to alterations in SERCA2a or PLB protein levels. Future work should determine whether post-translational modifications that alter the activity of SERCA2a or PLB differ between the sexes.

One area that requires further investigation is differences in the cAMP/PKA pathway in cardiomyocytes from males and females. Activation of this pathway plays an important role in increasing SR Ca^{2+} release and contraction size. Thus, in theory, sex differences in cAMP levels, PKA activity or other components of this pathway could contribute to the observed sex differences in components of cardiac EC coupling. For example, phosphorylation of PLB via PKA is known to accelerate relaxation of ventricular myocytes [67].

Reduced levels of phosphorylated PLB could diminish SERCA2a activity and account for the slower rates of Ca^{2+} transient decay characteristic of ventricular myocytes from female hearts in comparison to males. Similarly, a decrease in PLB phosphorylation by CaMKII could account for the slower rate of decay of Ca^{2+} transients in females. Further investigation of these pathways in cardiomyocytes from male and female animals may be helpful in understanding the cellular basis for sex differences in cardiac contractile function.

SR Ca^{2+} handling in cardiomyocytes from males and females

The magnitude of cardiac contractions and underlying Ca^{2+} transients depends upon SR Ca^{2+} content [6]. If cells from female hearts have lower SR Ca^{2+} content than males, then this could explain why less Ca^{2+} is released upon depolarization. Therefore, several studies have evaluated the possibility that the amount of SR Ca^{2+} available for release differs between the sexes. These studies have measured SR Ca^{2+} content by rapidly applying 10–20 mM caffeine to cardiomyocytes. Figure 1d shows mean results obtained by Farrell et al. [40]. Mean peak caffeine-induced Ca^{2+} transients were similar in cardiomyocytes from male and female rats. Other studies in rats have shown that SR Ca^{2+} stores are similar in cardiomyocytes from males and females [19, 29, 40, 55, 124]. By contrast, one study in guinea pig ventricular myocytes showed that SR Ca^{2+} content was, in fact, higher in myocytes from females when compared to cells from age-matched males [73]. Therefore, there is considerable evidence that SR Ca^{2+} content is either similar in cardiomyocytes from males and females or is actually higher in myocytes from female animals, depending upon the species examined. These findings demonstrate that lower SR Ca^{2+} content is unlikely to be responsible for smaller Ca^{2+} transients that are observed in myocytes from female animals.

To determine whether differences in the abundance of the major SR Ca^{2+} release channel (RyR2) could account for differences in SR Ca^{2+} release between the sexes, several studies have measured RyR2 expression levels in rat ventricles. Interestingly, RyR2 protein and mRNA levels are significantly higher in females when compared to males [23, 124]. Therefore, a reduction in the level of RyR2 protein does not account for the smaller Ca^{2+} transient amplitudes in cardiomyocytes from females. Alternatively, sex differences in Ca^{2+} transients could be explained by alterations in the magnitude and/or duration of individual SR Ca^{2+} release units (Ca^{2+} sparks) from RyR2 channels. To investigate this possibility, Farrell et al. [40] compared spontaneous Ca^{2+} sparks in quiescent myocytes from male and female rats. Figure 1e shows that mean Ca^{2+} spark amplitudes were significantly smaller in cells from females when compared to males [40]. However, this study also found that there were no differences between the sexes when spark frequency (Fig. 1f) or spark width (not shown) was examined. Females also exhibited lower SR Ca^{2+} spark durations (time-to-peak and decay time) when compared to males [40]. By contrast, the only other study to examine Ca^{2+} sparks in males and females contradicted these results, and reported an increase in both Ca^{2+} spark amplitude and duration in myocytes from female rats [124]. However, this latter study was performed at room temperature, while Farrell et al. [40] examined Ca^{2+} sparks at physiological temperature. Previous work has shown that Ca^{2+} sparks recorded at room temperature are larger, have longer durations and occur more frequently when compared to sparks recorded at physiological temperature [41]. It is possible that, in the study by Yaras et al. [124], cooling may have masked sex differences that would be present at physiological temperature in vivo. This is important to consider because many contemporary studies of EC coupling are still conducted at room temperature, which represents a condition of profound hypothermia that influences many aspects of cardiac EC coupling [100].

If Ca^{2+} sparks are indeed smaller and shorter in myocytes from females, this offers valuable insight into the subcellular basis for sex differences in SR Ca^{2+} release and contraction. Smaller Ca^{2+} sparks would be expected to summate and give rise to smaller Ca^{2+} transients in cardiomyocytes from females. As the amount of Ca^{2+} released during a spark is regulated by the intrinsic gating of the RyR2 [16], sex differences in the opening of individual RyR2 channels could be responsible for smaller Ca^{2+} transients in males. Thus, an important area for future research is determining whether there are sex differences in the gating of RyR2 channels in the SR. Alternatively, as RyR2 levels are actually higher in females [23, 124], it is possible that there are post-translational modifications in males that could contribute to increased SR Ca^{2+} release by increasing channel opening. Phosphorylation by PKA or CaMKII has been shown to increase the Ca^{2+}-dependent activation and opening of RyRs [5, 126]. As such, reduced SR Ca^{2+} release in myocytes from female hearts could be a result of a lower level of basal PKA or CaMKII activation. Even in the absence of β-adrenergic receptor stimulation, lower basal PKA or CaMKII activity could cause less RyR2 phosphorylation and suppress SR Ca^{2+} release. Future research into the cAMP/PKA and CaMKII pathways in male and female hearts could be helpful in understanding the cellular basis for sex differences in EC coupling.

Myocytes from male animals respond to isoproterenol, a non-selective β-adrenergic receptor agonist, with a larger increase in contractions, Ca^{2+} transient amplitudes and Ca^{2+} current in comparison to females [29, 113]. Isoproterenol also causes a larger increase in diastolic Ca^{2+} and SR Ca^{2+} content in cardiomyocytes from males [19, 29]. These

effects may be explained by the observation that isoproterenol increases cAMP levels substantially more in cardiomyocytes from males than females [113]. This would cause a greater activation of PKA in male hearts than in females, and thus higher levels of phosphorylation of relevant targets such as L-type Ca^{2+} channels, RyR2 and PLB. Further studies are required to fully understand the mechanisms that underlie sex differences in responses to β-adrenergic receptor activation and to determine whether these mechanisms contribute to sex differences in EC coupling even in the absence of β-adrenergic stimulation.

Electrophysiological studies of EC coupling in ventricular myocytes from males and females

It has been well documented that the size of the Ca^{2+} transients in ventricular myocytes is directly proportional to the magnitude of the L-type Ca^{2+} current [6]. To determine whether smaller Ca^{2+} transients in females could be explained by a decrease in Ca^{2+} current density, Ca^{2+} currents have been compared in myocytes from males and females. Representative Ca^{2+} current recordings from voltage clamp experiments in ventricular myocytes from male and female rats are shown in the lower panel of Fig. 1a [40]. Mean data clearly show that there is no sex difference in Ca^{2+} current density (Fig. 1g). Indeed, most investigations utilizing ventricular myocytes from rats, mice or guinea pigs have reported no sex difference in Ca^{2+} current density [9, 40, 46, 55, 65, 124]. In addition, a few studies in various animal models have reported that Ca^{2+} current density is actually higher in myocytes from females in comparison to males [73, 113, 123]. Taken together, there is considerable evidence that a reduction in Ca^{2+} current density does not explain the smaller Ca^{2+} transients and contractions observed in cardiomyocytes from female animals.

Only one study has reported that Ca^{2+} current density is lower in myocytes from female guinea pigs [56]. However, this study was designed to determine whether Ca^{2+} current density fluctuated with the oestrous cycle and therefore utilized female guinea pigs that were cycling regularly. Interestingly, they found that Ca^{2+} current differed between the sexes only on day 4 of the oestrous cycle, which corresponds to the peak in plasma progesterone-to-estradiol levels [56]. However, the influence of the oestrous cycle may not be a factor in most experimental studies, as female rodents housed in groups typically do not cycle regularly [1, 74, 119] unless they are induced to cycle by exposure to the pheromones present in male urine [118]. Still, the oestrous stage may be an important variable to consider in studies of female animals if they are exhibiting regular oestrous cycles.

A number of investigations have used molecular approaches to determine whether L-type Ca^{2+} channel expression differs in male and female myocardium. The levels of mRNA for $Ca_V1.2$, a subunit of the L-type Ca^{2+} channel, have been evaluated in hearts from males and females. There is little agreement on whether $Ca_V1.2$ mRNA levels differ between the sexes, as levels have been shown to increase, decrease or not change at all in ventricles from females when compared to males [23, 102, 106], reflecting inherent limitations in such an approach. A more direct approach is to evaluate the levels of $Ca_V1.2$ protein expression in hearts from males and females. These studies showed that female animals have significantly higher levels of $Ca_V1.2$ protein in comparison to males [23, 102]. Similarly, Vizgirda et al. [113] used a radioligand binding approach and reported an increase in the density of dihydropyridine receptors (L-type Ca^{2+} channels) in the ventricles of female rats. If females do have higher levels of $Ca_V1.2$, it is interesting that most studies have found that Ca^{2+} current density does not differ between the sexes. It is possible that there are sex differences in the activity of individual L-type Ca^{2+} channels. For example, if myocytes from male animals had a higher basal level of PKA activation, then this would result in more Ca^{2+} channel phosphorylation that could compensate for the decrease in Ca^{2+} channel expression. Future research should focus on this possibility, as well as other post-translational modifications that may give rise to sex differences in EC coupling.

When cardiomyocytes are regularly paced to reach steady state, Ca^{2+} efflux from the cytosol must match the Ca^{2+} entering the cytosol upon depolarization [7]. The primary mechanism responsible for Ca^{2+} efflux is the NCX, so studies have compared the abundance and activity of the NCX in hearts from males and females. One study found no difference in the NCX current in ventricular myocytes isolated from male and female pig hearts [117]. Conversely, studies in the rabbit model have found an increase in NCX current in myocytes from the base of female ventricles when compared to males [18]. In addition, the expression of NCX1 protein, the major cardiac NCX isoform, is increased in female rabbit hearts [18]. These results are supported by two other studies that showed an increase in NCX protein and mRNA levels in myocytes from female rats in comparison to males [23, 106]. Taken together, these results suggest that both the level of NCX expression and its activity are higher in females than in males, at least in the rat model. Higher NCX activity could contribute to the lower diastolic Ca^{2+} concentrations and smaller Ca^{2+} transients observed in myocytes from female rats.

Most of the evidence available to date indicates that Ca^{2+} current density is similar in cardiomyocytes from males and females, but contractions and Ca^{2+} transients are smaller in females. This suggests that the "gain" of EC coupling, which is the amount of SR Ca^{2+} released per unit Ca^{2+} current, may be lower in cardiomyocytes from females. To

directly explore this idea, Farrell et al. [40] measured the gain of EC coupling in voltage clamp experiments where Ca^{2+} current density and Ca^{2+} transients were measured simultaneously. They found that gain was substantially lower in female rat ventricular myocytes when compared to age-matched males. Mean data from this study are shown in Fig. 1h. These data show that less SR Ca^{2+} is released in response to a given trigger Ca^{2+} stimulus in myocytes from females than in males. As previously suggested, future research should examine the possibility that males possess increased phosphorylation levels as a result of higher PKA or CaMKII activity. This could potentially explain the larger SR Ca^{2+} release in response to the same amount of trigger Ca^{2+}. Alternatively, it has been suggested that sex steroid hormones are responsible for the decrease in EC coupling gain in female myocytes in comparison to males [71]. Further understanding of the cardiomyocyte pathways activated by the major sex steroid hormones is required.

The data reviewed above indicate that sex differences in EC coupling are likely due to alterations in the regulation of SR Ca^{2+} release, rather than differences in Ca^{2+} influx, at least when cells are activated by identical square pulses in voltage clamp experiments. However, SR Ca^{2+} release and cardiac contraction in vivo are triggered by an action potential, not a square voltage clamp waveform. Investigations in various animal models have compared action potential characteristics between the sexes. Studies in cardiomyocytes from C57BL/6 mice, rats and guinea pigs have found that action potential durations at 50 % and 90 % repolarization (APD_{50} and APD_{90}) are similar in males and females [9, 10, 40, 65]. However, this finding has not been observed in all studies. APDs are prolonged in CD-1 female mice when compared to males [109]. APD is also prolonged in ventricular myocytes from the midmyocardium of female dogs [123]. There is evidence that ventricular myocytes from female guinea pigs have longer APDs than males [73], although another study found longer APDs in females only when the animals were in the "oestrus" stage, where estradiol levels had peaked [56]. Interestingly, prolongation of the APD in female mice is also most readily apparent when mice are in the "oestrus" stage [93]. Differences in the species used, source of the ventricular myocytes used and/or the oestrous stage may account for these divergent results. Nonetheless, these studies show that APD is either similar in cardiomyocytes from males and females or that it is prolonged in females. As prolongation of the action potential increases Ca^{2+} influx [25], this may help compensate for the reduction in SR Ca^{2+} release in female myocytes in vivo. Additional experiments to explore the relationship between APD and myocardial Ca^{2+} handling in myocytes from males and females are needed. Important functional differences in cardiac EC coupling mechanisms in myocytes from female rodents in comparison to males are summarized in Fig. 2.

The impact of sex steroid hormones on cardiac EC coupling mechanisms

The risk of cardiovascular disease is lower in pre-menopausal women in comparison to age-matched men; this difference disappears as early as 5 years post-menopause [8, 53, 54]. As menopause is marked by a decrease in the production of ovarian hormones, it is thought that female sex hormones are cardioprotective, and their decline contributes to the increased risk of cardiovascular disease during the aging process [69]. Indeed, there is some evidence that hormone replacement therapy reduces the risk of cardiovascular disease in post-menopausal women, although this is controversial [78]. It is less often appreciated that men also experience an age-dependent decline in the levels of sex hormones during andropause, at the same time as their risk of cardiovascular disease rises [30, 61]. Testosterone supplementation, which is currently used to treat various conditions linked to low androgen states, is being explored for the treatment of cardiovascular diseases [61]. These observations have led to considerable interest in the mechanisms by which sex steroid hormones may modify cardiovascular function.

The knowledge that individual cardiomyocytes possess receptors for all three major sex steroid hormones (oestrogen, progesterone and testosterone) has led to the idea that effects of sex hormones on the myocytes themselves may be important in understanding their role in cardiovascular disease [30, 79]. Sex hormones are thought to act via specific receptors to elicit both non-genomic (acute) and genomic (chronic) effects on cardiac contractile function [30, 79]. As such, it is possible that sex hormones play an important role in mediating the sex differences in EC coupling observed in cardiomyocytes from male and female animals. The following discussion will first consider acute and chronic effects of female sex hormones on cardiac function. This will be followed by consideration of the impact of male sex hormones on the heart.

Acute effects of female sex hormones on EC coupling

As neonatal and adult cardiomyocytes possess oestrogen receptors [47, 91], there has been considerable interest in the acute (non-genomic) effects of oestrogen on cardiomyocyte function. Therefore, a number of studies have explored the effects of acute administration of oestrogen on components of the EC coupling pathway. It is well established that acute application of supra-physiological concentrations (e.g. 0.1 to 100 μM) of the major female sex steroid hormone, 17β-estradiol, to ventricular myocytes causes a marked reduction in the magnitude of the Ca^{2+} current in myocytes from mice, rats, guinea pigs, rabbits and even humans [3, 57, 77, 105, 110]. Meyer et al. [77] showed that 10 μM 17β-estradiol elicited a similar inhibitory action on the Ca^{2+} current

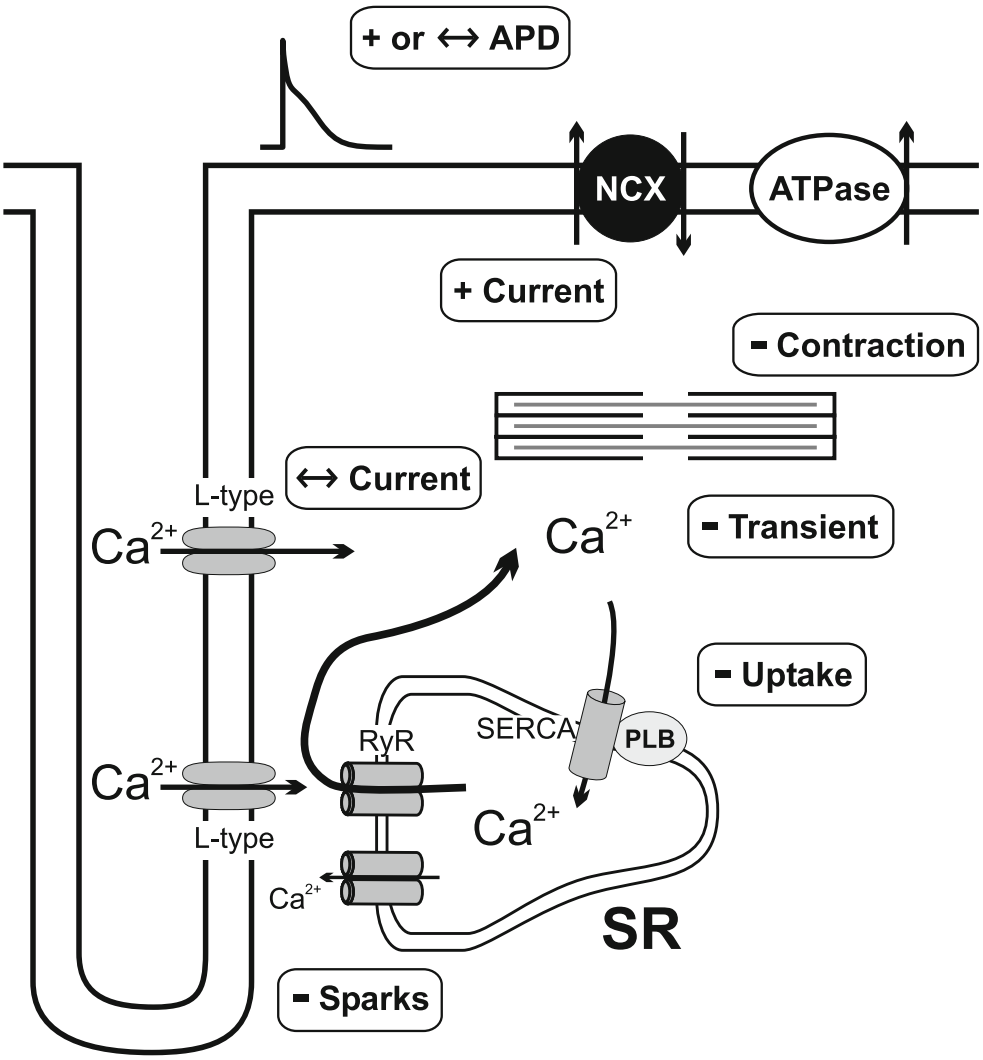

Fig. 2 Differences in the major components of cardiac excitation–contraction (EC) coupling in ventricular myocytes from female animals in comparison to males. L-type Ca^{2+} current density does not differ between males and females, although NCX activity is higher in myocytes from females. However, Ca^{2+} transient amplitudes and SR Ca^{2+} spark amplitudes are decreased in females in comparison to males. Therefore, females have lower EC coupling gain (Ca^{2+} transient per unit Ca^{2+} current). Female myocytes have smaller contractions in comparison to males. Contractions are also slower to relax in female myocytes, which is likely a result of reduced SR Ca^{2+} uptake via SERCA. Additionally, APD is either unchanged or prolonged in myocytes from females in comparison to cells from males. These sex differences in EC coupling are thought to contribute to reduced contractile function in myocytes from females in comparison to males. *APD* action potential duration, *NCX* Na^{+}–Ca^{2+} exchanger, *PLB* phospholamban, *RyR* ryanodine receptor, *SERCA* sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase, *SR* sarcoplasmic reticulum

in cells from humans, guinea pigs and rats, regardless of whether the cells were from males or females. Acute application of high concentrations (10 to 30 μM) of 17β-estradiol also reduces the amplitudes of Ca^{2+} transients and contractions in isolated ventricular myocytes [57, 110]. Interestingly, stimulation of oestrogen receptor α with PPT (4,49,499-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl) trisphenol; 100 nM) has been shown to decrease myofilament Ca^{2+} sensitivity [64]. There is also evidence that oestrogen can acutely modify electrophysiological properties of the heart. Langendorff-perfused female rabbit hearts [21] showed that 17β-estradiol (1 to 30 μM) prolonged APD and increased the incidence of arrhythmias induced by the Class III antiarrhythmic drug, sotalol. These studies provide evidence that acute application of supra-physiological concentrations of 17β-estradiol can affect components of the EC coupling pathway. However, serum concentrations of 17β-estradiol range from 70 to 1,300 pM depending upon the menstrual cycle in premenopausal women [30]. Serum estradiol levels are reported to be even lower in rodent models and are very difficult to accurately and consistently measure [51, 75, 93, 121]. Even so, all studies of the acute effects of oestrogen on cardiomyocytes to date have utilized very high concentrations of 17β-estradiol. It would be interesting to investigate acute effects of picomolar concentrations of 17β-estradiol in vitro.

The other major female sex hormone is progesterone, and previous studies have shown that progesterone receptors are also present on cardiomyocytes [45]. There is some evidence that the acute application of progesterone can affect various components of the EC coupling pathway. One study found that acute treatment with very high concentrations of progesterone (1 to 3 μM) increased APD in Langendorff-perfused female rabbit hearts [21]. By contrast, lower concentrations of progesterone (e.g. 100 nM) have been shown to reduce APD in cardiomyocytes isolated from female guinea pigs [80]. This effect was found to be due mainly to a progesterone-mediated increase in the magnitude of the

slow delayed rectifier K^+ current (I_{Ks}), which is an important contributor to cellular repolarization [80]. Progesterone (100 nM) also attenuates the increase in L-type Ca^{2+} current that occurs in response to β-adrenergic stimulation with isoproterenol [80]. Together, these studies suggest that relatively high concentrations of progesterone (0.1 to 3 μM) can affect myocardial function at the cellular level. However, these studies have all utilized relatively high concentrations of the hormone; circulating levels of progesterone range from 1 to 64 nM in premenopausal women [30] and between 3 and 55 nM in rodents [60, 121]. Whether lower concentrations of progesterone affect the contractile function of cardiomyocytes in vitro should be further examined.

The impact of ovariectomy on EC coupling

Inferences about the effects of chronic exposure to gonadally derived oestrogen on cardiac function have been drawn from investigations where animals have been subjected to bilateral ovariectomy (OVX), in the absence and/or presence of oestrogen replacement. Bilateral OVX causes a dramatic reduction in circulating levels of the primary female sex hormone, 17β-estradiol [22, 34, 58, 88, 122]. Several studies have compared contractile function in ventricular myocytes isolated from sham-operated and OVX rodents, with conflicting results. Two studies found an increase in contraction size in cells from OVX rats in comparison to sham controls after 3–6 weeks of steroid withdrawal [28, 122]. By contrast, contractions were reported to be smaller in myocytes isolated from rats following 9–10 weeks of steroid withdrawal [14, 88]. Results from these studies are summarized in Table 2. Interestingly, in all cases, these effects of OVX on cardiac contractile function were reversed when 17β-estradiol was replaced [14, 28, 88, 122]. It is not clear why differing results were obtained in these studies, but it is possible that the time frame of steroid withdrawal is important in the observed effects on cardiac contraction at the cellular level. Additional investigation of the impact of varying time periods of OVX may be revealing.

A number of studies also have investigated whether OVX affects SR Ca^{2+} release at the level of the cardiomyocyte. Figure 3a shows mean results obtained by Fares et al. [39] from voltage clamp experiments performed on isolated cardiomyocytes from sham-operated and OVX mice. These data show that OVX causes a marked increase in mean peak Ca^{2+} transients in myocytes from OVX animals when compared to sham controls. Most other investigations in the rat model have also shown that peak Ca^{2+} transient amplitudes are increased by OVX when compared to sham-operated controls, and this effect is reversed by 17β-estradiol replacement [28, 39, 63, 70]. Together, these studies have investigated periods of hormone withdrawal as short as 3 weeks and as long as 26 weeks (Table 2), suggesting that the period of hormone restriction may not affect the observed results [28, 39, 63, 70]. In contrast to these findings, two studies have reported that peak Ca^{2+} transients are either unchanged or reduced following OVX in the rat model [14, 88]. However, these latter studies used slow pacing rates (e.g. 0.5 Hz) and buffers that contained only 0.5 to 1 mM Ca^{2+}, factors that have been shown to reduce SR Ca^{2+} loading and eliminate sex differences in cardiac EC coupling [83]. These factors may have reduced the amount of SR Ca^{2+} available for release in OVX myocytes.

Insights into the effects of chronic oestrogen exposure on cardiac contractile function have also been drawn from comparisons of myofilament Ca^{2+} sensitivity in cardiac tissues from sham-operated and OVX rats. There is good agreement that myofilament Ca^{2+} sensitivity is increased in OVX hearts after 10 weeks of steroid withdrawal when

Table 2 Components of cardiac EC coupling in ventricular myocytes from OVX animals in relation to responses in sham-operated controls

Parameter	Change	Model	Steroid withdrawal (weeks)	References
Contraction	↑ Shortening	Rats	3	[28]
			6	[122]
	↓ Shortening	Rats	9	[88]
			10	[14]
Ca^{2+} transient	↑ Amplitude	Rats	3	[28]
			6	[63]
			9	[70]
		Mice	26	[39]
	↔ Amplitude	Rats	9	[88]
	↓ Amplitude	Rats	10	[14]
Myofilaments	↑ Ca^{2+} sensitivity	Rats	10	[14]
		Rats	10, 14	[115]
SR Ca^{2+} sparks	↑ Frequency, amplitude	Mice	26	[39]
SR Ca^{2+} content	↑ Stores	Rats	6	[63]
		Mice	26	[39]
Ca^{2+} current	↔ Density	Mice	26	[39]
Diastolic Ca^{2+}	↔ Levels	Rats	3	[28]
			6	[63]
			10	[14]
	↑ Levels	Rats	9	[70]
			9	[88]
		Mice	26	[39]
NCX current	↑ Density	Rats	6	[63]

OVX ovariectomy, *NCX* Na^+–Ca^{2+} exchanger, *SR* sarcoplasmic reticulum

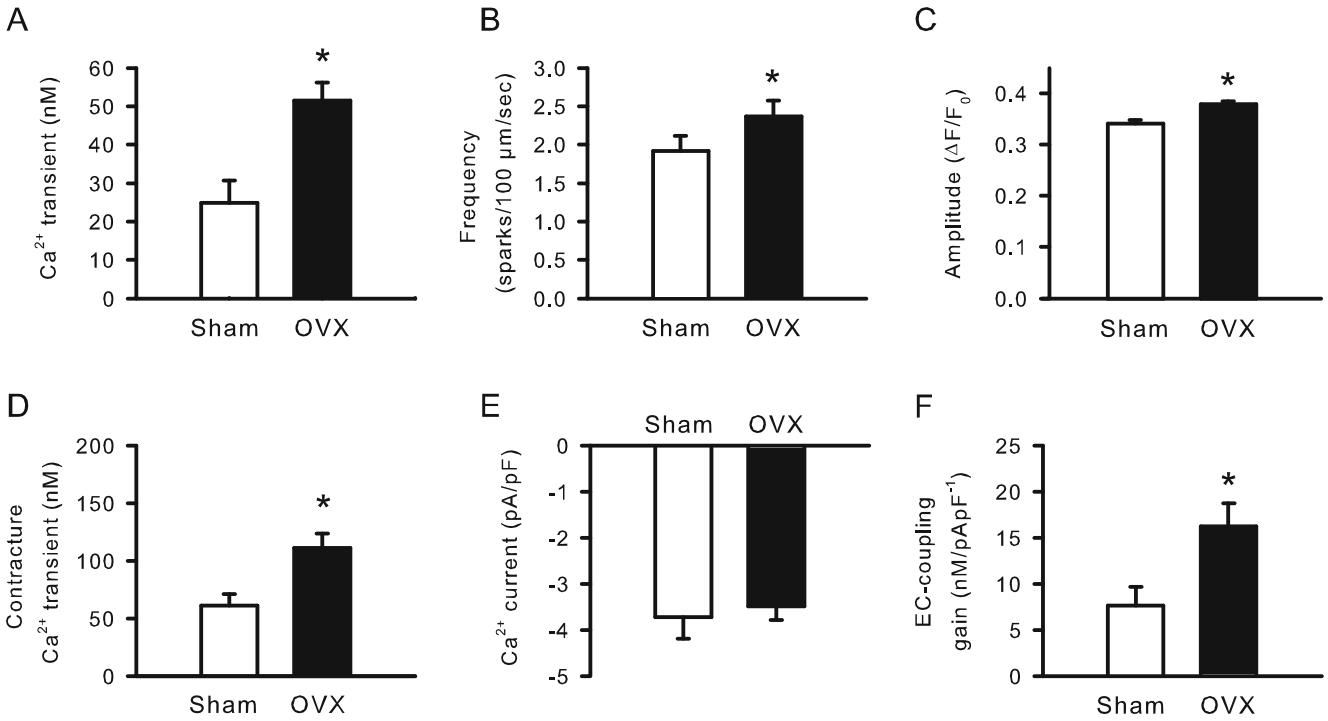

Fig. 3 Ovariectomy modifies components of EC coupling to enhance SR Ca^{2+} release in isolated ventricular myocytes. EC coupling mechanisms were compared in ventricular myocytes isolated from 8-month-old female mice that had either a bilateral OVX or sham operation at 1 month of age. Ca^{2+} transients and Ca^{2+} currents were activated by a voltage clamp step from −40 to 0 mV. **a** Ca^{2+} transient amplitudes were significantly larger in myocytes from OVX mice when compared to sham-operated controls. **b**, **c** Spontaneous Ca^{2+} sparks were larger and occurred more frequently in OVX myocytes when compared to sham controls. **d** OVX increased SR Ca^{2+} content, as measured via rapid application of 10 mM caffeine. **e** Ca^{2+} current densities were similar in myocytes from OVX and sham-operated animals. **f** OVX increased the gain of EC coupling. Reprinted from Fares et al. [39] with permission

compared to sham-operated controls [14, 115, 116]. Furthermore, treatment with oestrogen replacement reversed these changes in myofilament Ca^{2+} sensitivity [116]. Together with the majority of studies that show that OVX increases SR Ca^{2+} release, this sensitization of the myofilaments to Ca^{2+} would be expected to augment cardiac contractile function. However, whether OVX increases contraction at the level of the cardiomyocyte remains controversial and is an interesting area for additional exploration.

The finding that OVX causes an increase in Ca^{2+} transient amplitudes suggests that oestrogen may suppress SR Ca^{2+} release. To further examine this possibility, Fares et al. [39] compared the occurrence and properties of individual Ca^{2+} units (spontaneous Ca^{2+} sparks) in ventricular myocytes from sham-operated and OVX mice. Although spark duration was unaffected by OVX, they found that spontaneous Ca^{2+} sparks were more frequent and larger in amplitude in myocytes from OVX mice in comparison to cells from sham controls (Fig. 3b, c and Table 2). This finding demonstrates that unitary SR Ca^{2+} release is enhanced by chronic oestrogen withdrawal and suggests that oestrogen may reduce Ca^{2+} transient amplitudes by suppressing Ca^{2+} release at the level of the individual SR Ca^{2+} release unit. Additional experiments to determine whether oestrogen replacement could reverse these changes would provide further support for this idea.

One factor that may modify Ca^{2+} release in OVX myocytes is SR Ca^{2+} content. Thus, SR Ca^{2+} content has been compared in cardiomyocytes isolated from sham-operated and OVX rodents. Fares et al. [39] used the rapid application of 10 mM caffeine to measure SR Ca^{2+} load. They showed that OVX increased SR Ca^{2+} content in murine cardiomyocytes, as shown in the mean data illustrated in Fig. 3d. Indeed, there is good agreement that cardiomyocytes from OVX mice and rats have increased SR Ca^{2+} stores in comparison to sham-operated controls [39, 63]. These data indicate that OVX increases the amount of Ca^{2+} available in the SR, which would be expected to contribute to the larger Ca^{2+} transients characteristic of OVX myocytes. This increase in SR Ca^{2+} content may explain the increased frequency of Ca^{2+} sparks in OVX cells. Spontaneous Ca^{2+} sparks are thought to be a pathway to limit SR Ca^{2+} overload [94], so the increased spark frequency in OVX cells likely occurs in response to the increase in SR Ca^{2+} content.

Elevated SR Ca^{2+} content can also lead to spontaneous release of a larger amount of SR Ca^{2+} and trigger delayed afterdepolarizations, leading to cardiac arrhythmias [103]. Interestingly, OVX has been shown to promote the

spontaneous release of SR Ca^{2+} [39]. This may explain previous observations that OVX increases arrhythmias in myocardial ischemia and during adrenergic stimulation [24, 107] and could, in part, explain the increase in risk for arrhythmias in post-menopausal women [82]. Taken together, these results suggest that oestrogen suppresses SR Ca^{2+} release, limits SR Ca^{2+} content and inhibits the spontaneous release of SR Ca^{2+}. This may contribute to the cardioprotective effects of oestrogen observed in both clinical and laboratory studies.

Since the magnitude of SR Ca^{2+} release is directly proportional to the amount of Ca^{2+} entering the cell upon depolarization [6], differences in Ca^{2+} current between OVX and sham controls could account for the variability in Ca^{2+} transient amplitudes. Previous studies have shown that $Ca_V1.2$ protein expression is higher in hearts from OVX rats in comparison to sham controls [22]. However, Fares et al. [39] directly measured L-type Ca^{2+} current in ventricular myocytes from sham-operated and OVX mice and found that peak Ca^{2+} currents were similar in the two groups, as shown in Fig. 3e. Mean data from this study also showed that Ca^{2+} current amplitudes were similar in sham and OVX cells across a range of voltages [39]. This indicates that the increased Ca^{2+} transients observed in OVX cardiomyocytes are not a result of increased Ca^{2+} influx. In fact, the same study reported that OVX increased the gain of EC coupling in comparison to sham controls, as shown in Fig. 3f [39]. Overall, these studies suggest that female sex steroid hormones, such as oestrogen, reduce SR Ca^{2+} release by modifying the gain of EC coupling, not by suppressing Ca^{2+} influx.

Whether OVX affects resting Ca^{2+} levels in isolated cardiomyocytes has been investigated in a number of studies, as summarized in Table 2. When diastolic Ca^{2+} levels were quantified 3 to 6 weeks after OVX, diastolic Ca^{2+} levels were similar in sham and OVX myocytes [28, 63]. One study has reported that diastolic Ca^{2+} levels were unchanged even 10 weeks after OVX [14], although most studies that looked at longer times after OVX (e.g. 9 to 26 weeks) found that diastolic Ca^{2+} levels were higher in cardiomyocytes from OVX animals in comparison to sham controls [39, 70, 88]. These findings suggest that prolonged periods of oestrogen withdrawal may increase resting Ca^{2+} levels in ventricular myocytes. This could promote impaired relaxation and diastolic dysfunction in older female hearts that are chronically exposed to lower ovarian hormone levels, and further studies of this are warranted.

Elevated diastolic Ca^{2+} suggests that Ca^{2+} extrusion mechanisms may be compromised by OVX. Thus, several studies have evaluated whether OVX modifies Ca^{2+} handling by affecting Ca^{2+} sequestration and/or removal mechanisms. There is evidence that the levels of SERCA2a protein are unchanged after OVX [22, 63, 88], although two studies have found that SERCA2a levels decline following longer periods of hormone withdrawal (e.g. 10 weeks [12, 13]). However, whether this impairs SR Ca^{2+} sequestration has not been established. While total PLB and phospho-Ser^{16} PLB are not affected by OVX, phospho-Thr^{17} PLB has been shown to decrease with OVX [12, 13], which could actually enhance SR Ca^{2+} uptake. Additional studies to clarify this issue would be of interest. There is no evidence that NCX protein levels are increased by OVX [22, 63], although functional studies have reported an increase in NCX activity in cardiomyocytes isolated from rats 6 weeks after OVX [63]. This would be expected to promote Ca^{2+} extrusion and reduce resting Ca^{2+} levels, at least in the short term. Whether this persists with longer periods of ovarian hormone withdrawal has not yet been determined.

To elucidate the molecular mechanisms responsible for enhanced SR Ca^{2+} release following OVX, several studies have examined the contribution of phosphorylation of EC coupling components. Application of isoproterenol causes a greater increase in Ca^{2+} current, Ca^{2+} transients and PKA activity in myocytes from OVX rats compared to sham controls [58]. This may be due, at least in part, to the higher levels of β_1-adrenergic receptors observed in OVX cardiomyocytes [108, 122]. Even acute application of physiological concentrations of 17β-estradiol (0.1 to 1 nM) attenuates the increase in cAMP levels caused by isoproterenol application [66]. Although no basal differences in cAMP levels are observed, myocytes from OVX rats have increased basal PKA expression and activity [58, 63]. Interestingly, Kravtsov et al. [63] showed that pharmacological inhibition of basal PKA abolished the increase in SR Ca^{2+} release in myocytes from OVX rats. Furthermore, the basal increase in PKA activity was abolished when OVX rats were chronically treated with oestrogen replacement [58]. This suggests that oestrogen may attenuate PKA expression and/or activity in cardiomyocytes. There is also some evidence that implicates the CaMKII pathway in Ca^{2+} dysregulation in OVX hearts. Levels of CaMKIIδ and phosphorylated CaMKII protein are higher in ventricular tissue from OVX compared to sham controls [70]. This could augment SR Ca^{2+} release, as CaMKII-dependent phosphorylation of RyR2 is known to increase the amplitude and duration of Ca^{2+} sparks [50]. Together, these observations suggest that enhanced phosphorylation of various components of the EC coupling pathway may augment SR Ca^{2+} release and ultimately cause intracellular Ca^{2+} dysregulation following sex hormone withdrawal in females. Further studies that focus on sex hormone modulation of signalling pathways that regulate EC coupling could be highly informative.

Even though the ovaries are the primary source of oestrogens, gonadally derived steroid hormones are not the only source of systemic oestrogens in female animals. Other cell types including bone and adipocytes express the enzyme aromatase, which can convert testosterone to 17ß-

estradiol [101]. Aromatase is expressed in both neonatal tissues and cardiomyocytes [48, 85], as well as in the adult rodent heart [2]. This raises the interesting possibility that androgens can be locally converted to oestrogens in the myocardium [2]. Whether exposure of cardiomyocytes to sex steroid hormones can be regulated at the local tissue level is an important area for further investigation.

Acute and chronic effects of male sex hormones on EC coupling

Previous studies have shown that functional androgen receptors are present on cardiomyocytes [47, 72]. Therefore, some studies have investigated the effects of both acute and chronic application of testosterone on cardiomyocyte function. Er et al. [36] found that acute application of supra-physiological concentrations of dihydrotestosterone (100 nM) to ventricular myocytes from female rats caused a marked reduction in the magnitude of the Ca^{2+} current. Interestingly, these authors also showed that treatment with similar amounts of dihydrotestosterone for 24 h had the opposite effect and enhanced Ca^{2+} current [36]. This increase in Ca^{2+} current density was associated with an increase in expression of the α1C subunit of the Ca^{2+} channel [36]. Acute treatment with 100 nM dihydrotestosterone had no effect on Ca^{2+} spark frequency or amplitude, but 24 h treatment increased the frequency of SR Ca^{2+} sparks, without affecting their amplitude [36]. Interestingly, 24 h of exposure to 100 nM dihydrotestosterone had no effect on SR Ca^{2+} stores in cardiomyocytes [36, 44]. By contrast, 24 h of treatment with dihydrotestosterone caused an increase in both Ca^{2+} transient amplitudes and fractional shortening [36, 44]. These results suggest that both acute and chronic exposure to high concentrations of testosterone affect intracellular Ca^{2+} handling. While acute exposure may limit Ca^{2+} entry, chronic (up to 24 h) exposure increases Ca^{2+} influx and augments both SR Ca^{2+} release and contraction. However, physiological levels of testosterone are in the range of 10–35 nM in adult men [30] and fluctuate around a similar range in rodent models [75]. Additional studies that use more physiological concentrations of testosterone and longer exposure times could be informative.

Very few studies have evaluated the impact of long-term male gonadal steroid hormone withdrawal on cardiac contractile function. Golden et al. [43] used gonadectomised male rats to examine the effect of testosterone withdrawal on cardiac EC coupling. They showed that, 16 weeks after gonadectomy, isolated cardiomyocytes exhibited a significantly slower rate of shortening and relaxation in comparison to sham controls, although there was no difference in peak contraction [43]. When rats were subjected to sex hormone replacement, these differences between sham and gonadectomised animals were abolished [43]. Other studies have examined cardiac muscle strips and isolated myocytes from rats 2 weeks after gonadectomy and reported a decrease in both peak contraction and peak Ca^{2+} transient amplitudes, as well as slower decay of both responses when compared to controls [26, 120]. Importantly, these changes were reversed after testosterone replacement [26]. These findings indicate that removal of male gonadal steroid hormones inhibits SR Ca^{2+} release and suggest that testosterone may augment SR Ca^{2+} release. This agrees with the results of previous studies, described above, where testosterone was acutely applied to cardiomyocytes for 24 h. Future studies should investigate the specific components of EC coupling that are affected by testosterone and identify the intracellular signalling pathways that may be implicated in these effects.

Summary

The past decade has seen advances in our understanding of the impact of sex on cardiac contractile function, although much remains to be investigated. There is growing evidence that peak contractions are smaller and slower in intact hearts and cardiac muscle preparations from females when compared to males. This is due, in large part, to a decrease in the ability of individual cardiomyocytes to contract. Sex differences at several different locations in the EC coupling pathway have been implicated (Fig. 2). Most notably, new studies have shown that cardiomyocytes from female hearts exhibit a marked decrease in the gain of EC coupling, which translates to a decrease in SR Ca^{2+} release. This has been observed as lower peak Ca^{2+} transients and smaller individual SR Ca^{2+} sparks in myocytes from females, in comparison to males. Nonetheless, little is known about the specific signalling pathways implicated in these sex differences, and this limits the ability to translate these findings to new therapeutic strategies.

Although information is limited, sex differences in cardiac contractile function may be linked to effects of the major sex hormones on components of the EC coupling pathway. There is some evidence that acute application of high concentrations of 17β-estradiol, progesterone or testosterone can modify EC coupling mechanisms. However, whether more physiological concentrations of these hormones have similar effects is not yet clear. There is evidence from studies in ovariectomized rodents that chronic reduction in circulating 17β-estradiol modifies EC coupling mechanisms. Importantly, OVX causes a marked increase in the gain of EC coupling, resulting in larger peak Ca^{2+} transients and larger Ca^{2+} sparks. These results strongly suggest that oestrogen suppresses SR Ca^{2+} release and contributes importantly to the reduction in EC coupling gain present in cardiomyocytes from females. OVX also promotes cardiomyocyte Ca^{2+} dysregulation, including elevated SR Ca^{2+}

content and larger unitary Ca^{2+} release events. This SR Ca^{2+} overload promotes the spontaneous release of Ca^{2+} from the SR. This could increase susceptibility to a range of different cardiovascular diseases in low oestrogen states, such as in older, post-menopausal women. New research that explores the intracellular signalling pathways involved in these effects could lead to the identification of new targets for the treatment of cardiovascular diseases in older women.

There is a growing literature that suggests that oestrogen contributes to sex differences in myocardial contractile function, although this work is far from complete. Even less attention has been paid to the effects of testosterone on cardiac contraction, either at the intact heart level or at the cellular level. Intriguing evidence suggests that chronic reduction in circulating testosterone levels reduces the contractility of individual cardiomyocytes and suppresses Ca^{2+} transients. This raises the possibility that testosterone may increase intracellular Ca^{2+} levels through effects on EC coupling mechanisms, although this has not yet been investigated. If the loss of testosterone reduces intracellular Ca^{2+} levels in older men, this could suppress contractile function and contribute to systolic dysfunction in this population.

These findings that implicate sex hormone-regulated signalling pathways in the modulation of cardiac contractile function are potentially very important. If sex hormones such as oestrogen and testosterone are primary determinants of EC coupling gain, understanding the signalling pathways that regulate these actions can shed light on unique factors responsible for differences in cardiovascular disease expression in older men and women. This could lead to the identification of potential targets for the development of new agents to treat diseases such as cardiac contractile dysfunction and systolic heart failure in both men and women.

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