Chapter 4

Atrial and ventricular tissue electrophysiology

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Atrial and ventricular heart tissue is composed of contractile cardiac muscle cells (cardiomyocytes), blood vessels, autonomic nerves, and connective tissues (Fig. 4.1). Several mechanisms have been hypothesized to underline sex differences in cardiac electrophysiology, including differences in cardiac ion channel expression levels and function; differences in autonomic tone and its alteration induced by sex hormones; and differences in three-dimensional architecture including heart size and cell-to-cell coupling. Clinically observable differences may stem from alterations in one or more of these factors. Sex differences in ion channel expression and hormonal effects are covered in other chapters of this book. Here, we offer a brief introduction to the structure and electrophysiology of atrial and ventricular tissue and then discuss some examples of sex-related differences reported in the literature. Specifically, sex-based differences related to the autonomic nervous system, sinoatrial node function, excitation–contraction coupling, action potential duration (APD), extracellular matrix structure, and cardiac tissue remodeling will be discussed.

Heart size

Heart sizes are similar in girls and boys [1]. After puberty, absolute heart mass is greater in men than in women by about 15%–30% [2].

Autonomic nervous system

The autonomic nervous system governs the heart output with two opposite branches of the system working collaboratively: stimulation of the sympathetic branch of the system is responsible for increase in heart rate and cardiomyocyte contractility, while parasympathetic stimulation leads to decrease heart rate and cell contractility. Autonomic nervous system plays important role in many arrhythmias, both in the atria and in the ventricles [3–5].

Sympathetic nerves innervate atrial and ventricular myocardium. Largest populations of cardiac ganglia are located near the sinoatrial and atrioventricular nodes of the heart with smaller collections of ganglia found on the superior left atrial surface, the internal septum, and the atrial appendage–atrial junctions (Fig. 4.2) [6]. The average numbers of ganglia in human hearts of either sex was estimated at 458 $\pm$ 43 in atrial tissues and 88 $\pm$ 7 ganglia in ventricular tissues with the estimated total number of intrinsic ganglionic neurons of 14,000 in a heart with no sex differences reported [7]. In animal studies, sex differences in cardiac ganglia were found in mice using neurochemical analysis [8]. In particular, neurotransmitter levels for norepinephrine (NE) and acetylcholine (ACh) were assessed and found generally similar between the sexes, except for the right atrium, where females had significantly higher levels of ACh. Furthermore, the levels of the main NE metabolite, dihydroxyphenylglycol in the right atrium, were also higher in females, suggesting higher NE turnover.

Governed by the autonomic nervous system, the basal heart rate in women is higher than in men [9,10]. This disparity is still observed in young girls versus boys, though to a smaller extend, suggesting that hormones alone do not explain the differences in heart rate. Furthermore, in these studies, the basal heart rate was different in men and women even after autonomic system was blocked with beta blockers or parasympathetic system blockers, suggesting that some of the sex differences in heart rate are in fact "intrinsic" and not related to differences in autonomic tone or hormones. In addition to these

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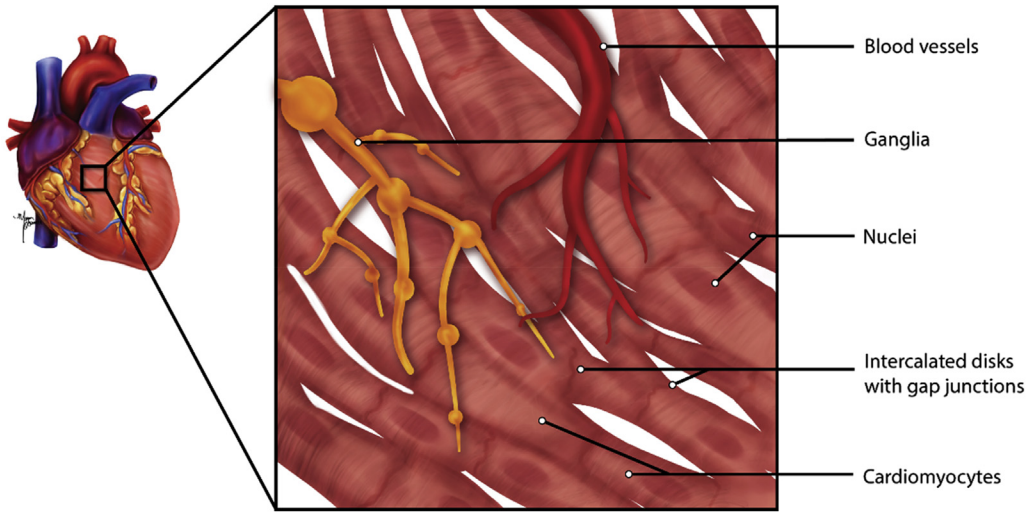

FIGURE 4.1 Schematic of cardiac tissue. Cardiomyocytes connect through gap junctions, forming cardiac tissue, innervated by sympathetic ganglia and permeated by capillary blood vessels. *Illustration by Megan O'Connell and Andrea Kim.*

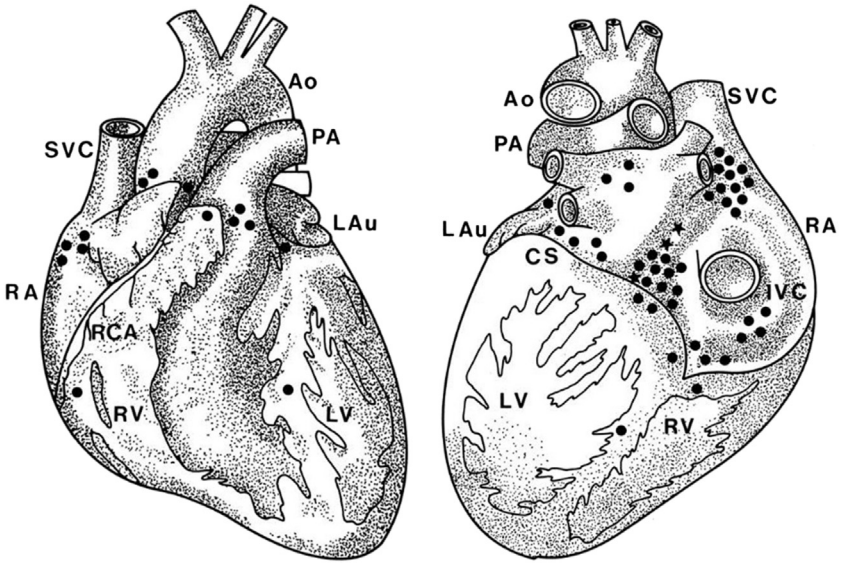

FIGURE 4.2 Anterior (left) and posterior (right) view of adult human heart showing topography of the cardiac ganglia. Ganglia are concentrated primarily at base of aorta (Ao) and pulmonary artery (PA). Para-sinoatrial (SA) nodal ganglia are concentrated primarily lateral to the right pulmonary veins. The para-atrioventricular (AV) nodal ganglia are on the epicardial surface superior to the coronary sulcus (CS) and within the interatrial septum. *Reproduced with permission from Singh S, Johnson PI, Lee RE, Orfei E, Lonchyna VA, Sullivan HJ, et al. Topography of cardiac ganglia in the adult human heart. J Thorac Cardiovasc Surg 1996;112(4):943–53.*

differences in the heart rate, the variability of the heart rate (measured by RR interval variability) is also different in males and females [11].

It has also been reported that male cardiomyocytes isolated from adult rats have a higher β-adrenergic receptor density than female cells and thus have an enhanced response to β-adrenergic stimulation [12]. Similar disparities were observed in isolated adult rabbit hearts, where isoproterenol response was significantly lower in female hearts, especially at the higher beating rate [13]. These authors further report that the reduced β-adrenergic responsiveness of female hearts was also associated with reduced arrhythmogenicity.

Sinoatrial and atrioventricular nodes

Autonomic nerve fibers extend through heart tissue with the right vagus nerve primarily innervating the sinoatrial (SA) node and the left vagus nerve innervating the atrioventricular (AV) node. Autorhythmic nodal cells are rhythmically

discharging governing the electrical signal propagation leading to heart contraction while SA node depolarization rate is constantly being affected by innervations from both the sympathetic and parasympathetic nervous system. Women have greater SA node automaticity, with a shorter sinus cycle length [14]. Sinus node recovery time and atrial refractory period are significantly longer in males compared to age-matched females (Fig. 4.3) [15]. These differences in clinical electrophysiology might be underlined by sex-related differences in the density of metabolically active cells in sinoatrial node. At least one study has reported by measuring phosphorus content in the isolated sinoatrial node tissues derived from Japanese monkeys that the density of the active cells in SA node is significantly higher in females than in males [16].

Excitation–contraction coupling

The electrical stimuli from the sinoatrial node is translated into cardiomyocyte contraction through excitation–contraction coupling governed by Ca^{2+} influx through the voltage-sensitive L-type Ca^{2+} channels and subsequent release of sarcoplasmic reticulum Ca^{2+} storage through the ryanodine receptor. Ventricular cardiomyocytes are rich in special transverse tubules (T-tubules) that represent extensions of the cell membrane (sarcolemma) that penetrate into the center of the cardiomyocyte, helping to conduct impulses from the cell membrane down into the sarcoplasmic reticulum. There are fewer T-tubules in atrial cardiomyocytes, and almost no T-tubules in nodal cells or in Purkinje fibers. Massive release of Ca^{2+} from sarcoplasmic reticulum allows Ca ions to bind to the myofilaments, specifically to troponin C, resulting in contraction of cardiomyocytes. Sex differences in mechanisms of cardiac excitation–contraction coupling have been reported in animal models (see Ref. [17] for review). In isolated rat ventricular cardiomyocytes, despite similar Ca^{2+} current density, Ca^{2+} transients were smaller in females than in males. Furthermore, the excitation–contraction coupling gain (the ratio of Ca^{2+} release/inward Ca^{2+} current) was also significantly lower in females than in males [18]. But in another study in guinea pig heart pea L-type Ca^{2+} current was larger in females suggesting that sex differences in APD result from variation in the kinetics of L-type Ca^{2+} current stemming from alterations to Ca^{2+} release (Fig. 4.4) [19]. Cyclic adenosine monophosphate (cAMP)/PKA is an important signaling pathway in excitation–contraction coupling. It has been shown that estrogen suppresses sarcoplasmic reticulum Ca^{2+} release and that this is regulated, at least in part, by the cAMP/PKA pathway, improving our understanding of female-specific cardiomyocyte mechanisms [20]. Blocking Ca^{2+} release from the sarcoplasmic reticulum has larger impact on isoproterenol-induced changes in female compared with male cardiomyocytes [21].

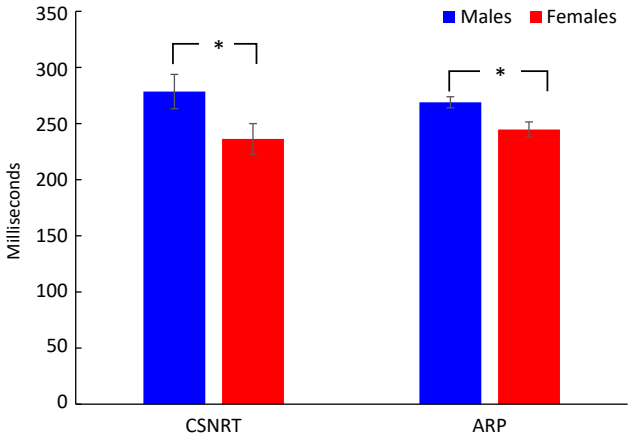

FIGURE 4.3 Sex differences in corrected sinus node recovery time (CSNRT) and atrial refractory period (ARP), error bars represent ± SEM, $*P < .05$. *Figure generated based on data previously published Sanjeev S, Karpawich PP. Developmental changes in sinus node function in growing children: an updated analysis. Pediatr Cardiol 2005;26(5):585–588.*

Cardiac action potential

Cardiac action potential is a change in cardiomyocyte membrane potential due to inward and outward currents of ions across the membrane. The sex-specific variations in ion channels expression are believed to lead to different atrial and ventricular action potential profiles between males and females (Fig. 4.2) and to ultimately translate to differences in electrocardiographic parameters recorded from male and female patients in clinic (see Ref. [22] for review). Specifically, in adult human, female hearts have reduced expression of potassium repolarizing channels [23], L-type calcium current is higher in female base (but not apex) cardiomyocytes [24], and sodium current is higher in female epicardial and endocardial cells (but not in midmyocardial cells). [25], leading to differences in morphology and duration of cardiomyocyte action potential and ultimately underlying longer QT intervals, and shorter QRS, PR intervals, and P-wave duration observed in female electrocardiogram (Fig. 4.5) [26,27].

Direct measurements of human cardiomyocyte action potentials are possible from nontransplantable or end-stage failing hearts, but these samples are available in very limited quantities. There thus exists a need for an alternative cardiovascular physiology model. Sex-related findings on action potential parameters in primary cardiomyocytes isolated from human and animal hearts as well as human cardiomyocytes, derived from stem cells, and experiment-based computer simulations are reviewed in this section.

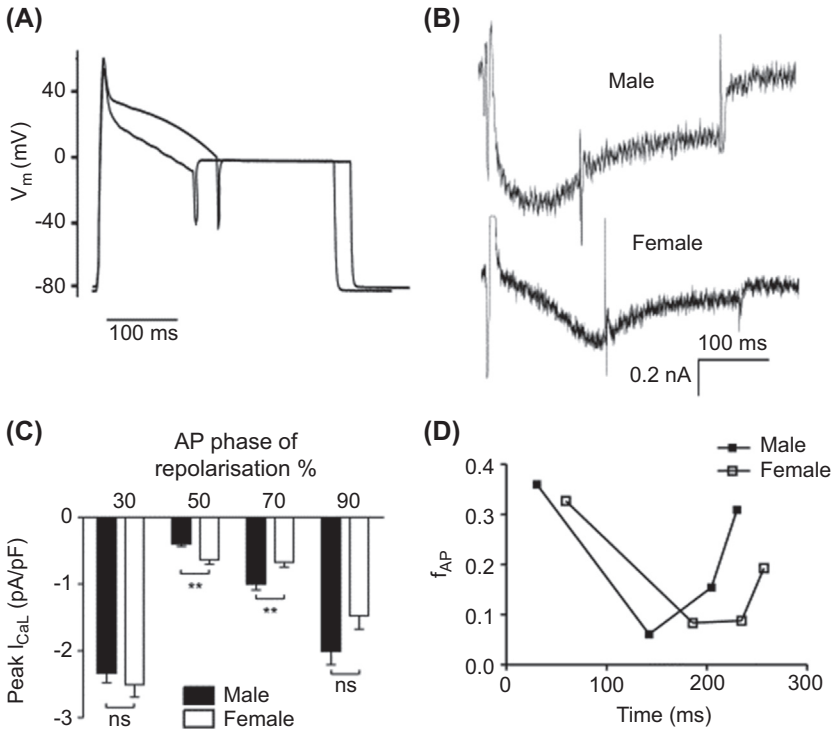

FIGURE 4.4 Gender differences in L-type calcium channel (I_{CaL}) availability during action potential clamp. (A) Shows typical stimulus files and (B) representative currents elicited from these protocols. (C) Illustrates pooled data ICaL at 30%, 50%, 70%, and 90% repolarization. (N = 12−16; $*P < .05$, $**P < .01$). (D) Illustrates the differences in availability (fAP) of ICaL during male and female action potentials. *Reproduced with permission from Mason SA, MacLeod KT. Cardiac action potential duration and calcium regulation in males and females. Biochem Biophys Res Commun 2009;388(3):565−70.*

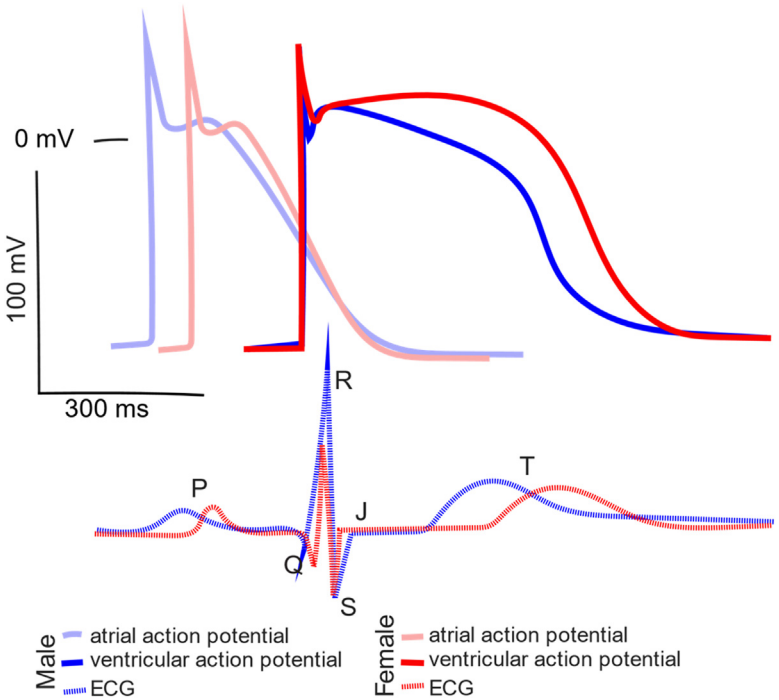

FIGURE 4.5 Sex-specific atrial and ventricular action potentials. *Figure courtesy of Anna Gams, Department of Biomedical Engineering, Efimov Lab, George Washington University, redrawn from Zipes DP, Jalife J, Stevenson WG. Cardiac electrophysiology: from cell to bedside 2018; Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on Atrial cardiomyopathies: definition, characterisation, and clinical implication. J Arrhythm 2016;32(4):247−278.*

Cardiomyocytes isolated from human hearts

Action potential recordings in ex vivo human heart preparations are scarce, and even when APD is recorded in both, cardiomyocytes isolated from male and female hearts, the reported data often contain averaged parameters, but no sex-specific data [30,31]. In Ref. [32], authors isolated midmyocardial left ventricular cardiomyocytes from explanted hearts of five male and five female patients undergoing cardiac transplantation and recorded action potentials using whole-cell patch-clamp technique. The results were consistent with the hypothesis that the long-observed longer QT interval duration in female versus male humans is underlined by longer cardiomyocyte APD. Female cardiomyocytes had significantly longer APD at each of the repolarization stages, including APD at 90% repolarization (APD_{90}) of 866 ± 60 ms in female cardiomyocytes versus 672 ± 43 ms in male cardiomyocytes at 2-s cycle length ($P < .05$) and 375 ± 29 ms (female) versus 271 ± 36 ms (male) at 20% repolarization (APD_{20}). There were no statistically significant differences observed in action potential properties beyond APD in this study, including cell capacitance, resting membrane potential, maximal action potential amplitude, maximal depolarization velocity, or maximal repolarization velocity.

Recorded ex vivo atrial action potentials from female and male patients in atrial fibrillation did not exhibit significant differences in shape, except that action potentials from women had slower upstroke velocity [33]. 652 action potentials were recorded from right atrial appendage preparations from 520 male and female patients.

Animal-isolated cardiomyocytes and tissues

Use of adult cardiomyocytes isolated from the heart tissue of rats and other animals has been a golden standard for in vitro studies of cardiac electrophysiology since 1970s [34]. While APD_{90} is commonly regarded to be longer in females than in males in earlier studies, such supporting data were typically from studies in isolated cardiomyocytes at room temperature with slow (nonphysiologic) pacing rates; a closer review of the literature shows that studies are divided on whether APD or the QT interval in females is longer or similar to corresponding values in males. Studies conducted in intact tissue preparations at physiologic temperatures and pacing rates were more likely to show similar APDs in males and females (see Ref. [17] for review).

APD was measured in many animal models using various experimental samples and approaches. APD_{20}, APD_{50}, and APD_{90} in mice ventricle cardiomyocytes was significantly longer in females compared with male mice [35,36] and attributed to lower expression of potassium channels and smaller repolarizing currents in female cells. Furthermore, in orchiectomized mice, APD_{20}, APD_{50}, and APD_{90} as well as QTc intervals were all significantly longer than in control male mice, suggesting a role of androgen on repolarization. APD_{90} was reported to be 11% longer in female versus male rabbit cardiomyocytes paced at 1 Hz (672.7 ± 3.7 vs. 604.9 ± 3.6 ms, $n = 26$ (24 hearts), 29 (24 hearts), $P < .01$ for female vs. male) [21], while these sex-specific differences in rabbit action potentials were absent in young rabbits and abolished by gonadectomy [37]. APD of female cardiomyocytes was longer than that of male cells paced at the same slow rate in canine heart [38]. An animal study that took in account estrus cycle found that APD_{90} was longer in female guinea pig ventricular cardiomyocytes at as compared to that of a male on day 0 of the cycle, but at day 4 the duration was equal in both male and female cells, supporting role of hormones in sex-specific cardiac electrophysiology [39].

Human cardiomyocytes derived from stem cells

Relatively recently a new model of human cardiac electrophysiology became available to researchers. Discovered in 2007 [40], induced pluripotent stem cells (iPSCs) can be differentiated in multiple organ−specific cell types, including cardiomyocytes of various lineages, including ventricular-, atrial-, and nodal-like cardiomyocytes. Blood samples or skin biopsy samples for iPSC-derived cardiomyocytes production can be collected from adult consented subjects of either sex. The resulting sex-specific iPSC-derived cardiomyocytes are expected to carry genetic information of the donor, including sex-related differences as well as variations related to possible disease status (normal vs. genetic cardiovascular disease carriers). These cells have been proposed for disease modeling as well as an in vitro model for predicting drug-induced ventricular arrhythmias, Torsade de Pointes [41,42], as well as an integrated subject-specific model for prediction of an individual clinical response [43,44].

A few iPSC-based studies have been done so far that studied specifically the effects of donor's sex on the electrophysiological characteristics of resulting iPSC-derived cardiomyocytes. In one study, three male and three female commercially available iPSC-derived cardiomyocyte lines were used to measure baseline electrophysiological characteristics and drug-induced effects [45]. The authors matched male and female lines based on the baseline spontaneous beating rate and report female Fridericia rate-corrected [46], repolarization duration to be longer as compared to male, in two out of three pairs.

In our own unpublished experiments, we have recorded baseline characteristics of subject-specific iPSC-derived cardiomyocytes obtained from a larger sample of male and female young normal healthy subjects (7 women and 10 men) using voltage-sensitive dye optical

recordings of cellular APD at 37°C and compared these data to the clinical electrocardiographic recordings of QT interval in these subjects. While clinical Fridericia rate-corrected QTc duration was longer in female subjects (410 ± 14 ms in females vs. 388 ± 11 ms in males), the average cellular Fridericia rate-corrected ADPc was shorter in female subject–specific iPSC-derived cardiomyocytes (278 ± 31 ms in females vs. 304 ± 27 ms in males) (Fig. 4.6).

Donor-specific iPSC-derived cardiomyocyte technology is an exciting and promising new model in cardiac electrophysiology, but it needs thorough validation and verification to further develop iPSC-based assays and understand this approach limitations. Further development of the reprogramming and differentiation protocols is also needed to address known morphological and functional immaturity of iPSC cardiomyocytes as well as high variability in the characteristics of the cells developed in different laboratories.

In silico modeling

Mathematical models of human ventricular cardiomyocytes with sex-specific ion channel currents available from the experimental data predict longer APD in female cells, steeper APD–heart rate relationship, and greater susceptibility to early afterdepolarizations. Male cells, on the other side, are predicted to have more prominent phase-1 repolarization [47]. Mathematical models of male and female ventricular tissue that take into account differences in ion channel expression and hormone effects suggested that female susceptibility to alternans stems from longer action potentials and further confirms that increased incidence of ventricular arrhythmias (like Torsade de Pointes) has its roots in the sex-based differences in ventricular electrophysiology [48].

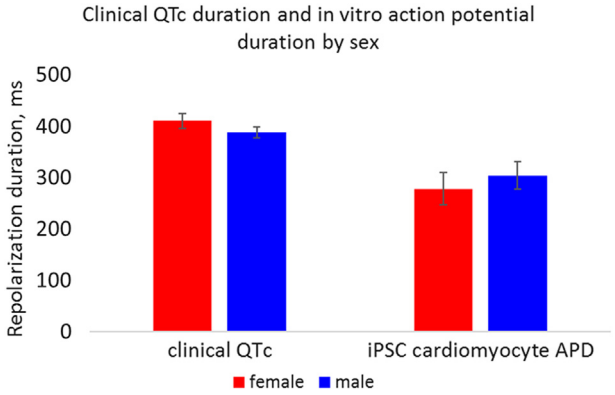

FIGURE 4.6 Fridericia rate-corrected QT interval duration in normal, healthy, young male and female subjects ($N_{female} = 7$, $N_{male} = 10$) compared to Fridericia rate-corrected action potential duration at 90% repolarization measured in subject-specific induced pluripotent stem cell–derived cardiomyocytes using voltage-sensitive optical recordings. Error bars represent ±1 SD.

Action potential dispersion

APD dispersion across heart wall is widely considered as potentially arrhythmogenic. Transmural gradient of repolarization time and APD was estimated from T-wave morphology in electrocardiograms of 5376 healthy men and women [49]. Both epi- and endo-cardiac APD was shorter in men than in women, by 18 and 14 ms, respectively. The estimated crossmural RT and APD gradients were the same in both sexes, but the duration of phase 2 plateau of the epicardial action potential was 19 ms longer in women.

Study using isolated cardiomyocytes from epi-, endo-, and mid-myocardium of canine hearts of both sexes found no differences in epi- and endo-APD, but the action potential of the cells derived from midmyocardium was significantly longer in female samples (e.g., at 1 Hz, female vs. male: 288 ± 21 vs. 237 ± 8 ms; $P < .05$), resulting in greater transmural APD heterogeneity in females [38]. Sex differences in transmural APD dispersion was concluded to be underlined by the differences in ionic currents in female and male hearts. Consistent with clinical and isolated cardiomyocyte studies, in silico modeling has also predicted larger transmural APD heterogeneity in females [47].

Much less is known about sex differences in APD gradients between the right ventricle and left ventricle, or between apex and base. Even when data seem to exist for animals of both sexes (see i.e., [50,51]), the sex-specific gradient data are not reported. There were no statistically significant differences in male and female ventricular action potentials (APD_{90}) or calcium transient duration (CaD_{80}) measured in either the left ventricle base (left) or apex (right) of adult rabbit hearts (Fig. 4.7) [13].

Contractility

Force–frequency relationship is an important intrinsic regulatory mechanism of cardiac contractility. In normal myocardium, the heart contractility force increases with the increase in heart rate [52]. The positive force–frequency relationship is maintained in absence of sarcoplasmic reticulum function in rabbit, but not in rat myocardium [53]. Positive force–frequency relationship is observed in both male and female cat isolated trabeculae, but at higher pacing frequencies (1.5 and 2.0 Hz), force produced by male trabeculae is significantly higher than that produced by trabeculae from females with a significant sex differences in the overall force–frequency response (30% higher in males) [54]. Consistent with these findings, isoprenaline, a beta-adrenergic agonist, induces significantly higher calcium transient increase in

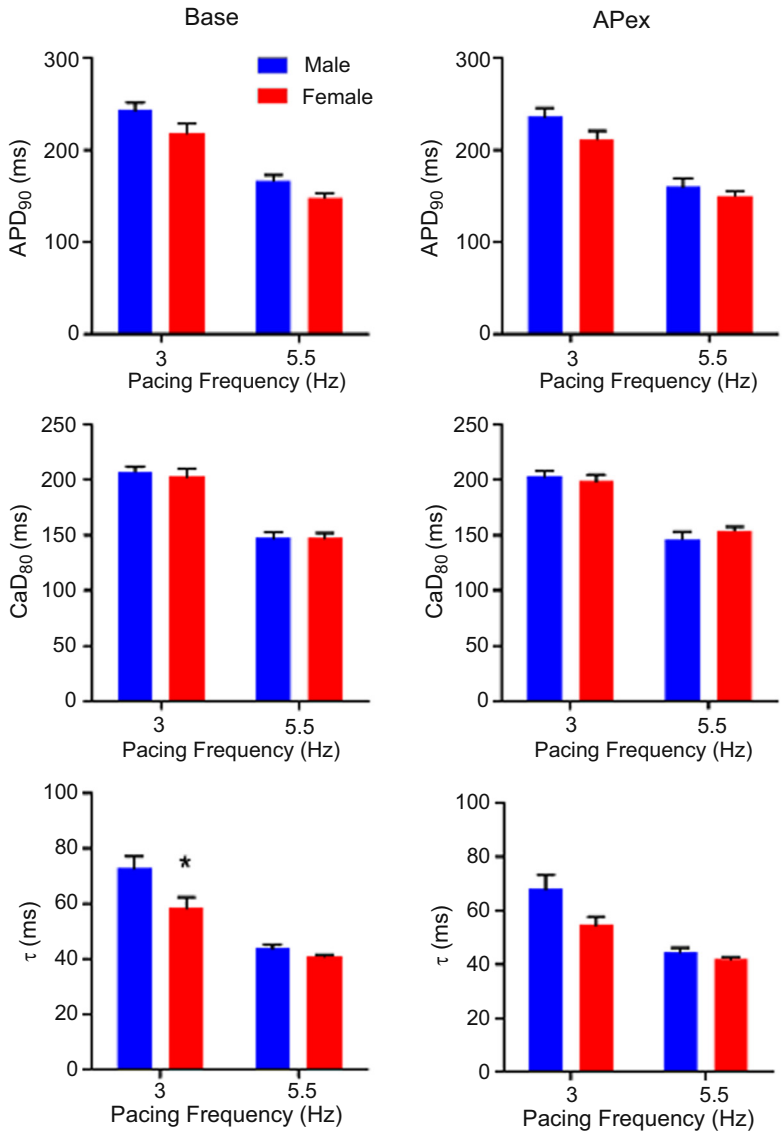

FIGURE 4.7 Sex differences in steady-state ventricular action potentials (APD_{90}) or calcium transient duration (CaD_{80}) measured in either the left ventricle base (left) or apex (right) were statistically insignificant in isolated rabbit hearts paced at 3 and 5.5 Hz. The time constant of calcium recovery was significantly lower at the base of hearts isolated from female rabbits and paced at 3 Hz but was not different at faster pacing rate (5.5 Hz). *Reproduced from open source publication Hoeker GS, Hood AR, Katra RP, Poelzing S, Pogwizd SM. Sex differences in beta-adrenergic responsiveness of action potentials and intracellular calcium handling in isolated rabbit hearts. PLoS One 2014;9(10):e111411.*

male rat isolated cardiomyocytes as compared with female cardiomyocytes [55].

Gap junctions

Cardiomyocytes attach to one another with specialized cell junctions called intercalated discs, forming strong mechanical links between cardiomyocytes and responsible for the tissue integrity. The electrical currents are transmitted from cell to cell through gap junctions. One role of gap junctions is to permit the passage, from cell to cell, of currents that are essential for action potential propagation through cardiac tissue. Gap junctions facilitate ion transfer between cells producing intracellular electrical coupling and allowing for the cells to contract in synchrony. Gap junctions are formed by membrane proteins known as connexins (Cx), with connexin 43 (Cx43) being predominant in the ventricular tissue. The abnormalities in Cx43 lead to conduction defects

and contractile dysfunction. It has been shown that Cx43 mRNA and protein expression were higher in female than male adult rat cardiomyocytes [56,57], thus favoring cardioprotection in female cardiomyocytes.

Extracellular matrix structure

Cardiomyocytes comprise 75% of the heart tissue volume, but only about one-third of the total number of cells in adult heart with the rest being a complex structure of connective tissue [58]. Extracellular matrix is composed of collagen, elastin bundles, and interconnected basement membranes. This 3D structure orients cardiomyocytes and mechanically couples them to blood vessels and nerve fibers. mRNA levels for collagen type I and cytoskeletal actin levels are significantly higher in the female heart as compared with the age-matched male heart in rat [59]. In humans, collagen type I, III, and IV are higher in adult males, while with aging (after 50), these proteins were higher in females [60]. This dimorphism is likely to contribute to the sex differences in heart remodeling.

Remodeling

Physiological and pathological conditions (exercise, pregnancy, heart abnormalities that cause heart to work harder, hypertension, etc.) may lead to heart tissue remodeling. Heart responds to myocardial wall stress by adding new sarcomeres in parallel to existing sarcomeres, increasing heart wall thickness (concentric hypertrophy). New sarcomeres can be added in series to existing sarcomeres (eccentric hypertrophy) characterized by dilation of the left ventricle without changes of the chamber wall thickness, observed, i.e., after endurance training. There is some evidence that with age the decrease in the total number of cardiomyocytes due to apoptosis is more prevalent in males compared to females, consistent with age-related decrease in left ventricle mass in men, but not women [58,61]. Worse diastolic and longitudinal function with advanced age or elevated load appears in both sexes, but a significant increase of torsion is more pronounced in women [62]. Ventricular cardiomyocyte numbers decline with age and this may be more prominent in men than in women; ventricular cardiomyocyte volume increases with age and this may be more pronounced in men than in women [63].

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