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ORIGINAL CONTRIBUTION

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Gender-related differences in ventricular myocyte repolarization in the guinea pig

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Abstract It is well established that gender-differences exist in cardiac electrophysiology and these are thought to contribute to the increased risk of women, compared to men, for the potentially lethal ventricular arrhythmia, *torsades de pointes*. Data from animal models with abbreviated estrus cycles suggest that androgens may play a protective role in males. However, the role of female sex hormones in gender-differences in cardiac electrophysiology is less clear. This report describes gender differences in ventricular electrophysiology, investigated using the guinea pig heart. Ionic currents and action potentials were compared between ventricular myocytes isolated from male guinea pig hearts and those from females on the day of estrus (day 0) and 4 days post-estrus (day 4). The density of inward rectifier K^+ current (I_{K1}) at –120 mV was significantly greater in male myocytes than in female myocytes either at day 0 or day 4. The peak L-type Ca^{2+} current (I_{Ca}) at +10 mV was also significantly larger in male myocytes than in day 0 and day 4 female myocytes. Moreover, I_{Ca} differed significantly between day 0 and day 4 female myocytes, strongly suggesting that I_{Ca} density varies around the estrus cycle. Delayed rectifier (I_K) tail currents were significantly different between male and female day 4 myocytes. Action potential duration (at 90 % repolarization; APD_{90}) was significantly shorter in male myocytes than in female myocytes at day 0, but not at day 4, broadly consistent with the combined differences in I_K and I_{Ca} between the three groups. Taken together, our data are consistent with the contribution of multiple factors, rather than a single hormone, to gender differences in ventricular repolarization. Since female guinea pigs possess a conventional estrus cycle, our data suggest that this species may be well suited to elucidating the modulatory influence of ovarian steroids on ventricular repolarization and arrhythmic risk. Our findings suggest that further work examining the basis to gender differences in ventricular repolarization in the guinea pig is warranted.

Key words APD_{90} – delayed rectifier K^+ current – gender – inward rectifier K^+ current – L-type Ca^{2+} current – QT interval – sex – tail current – ventricular repolarization – whole-cell patch-clamp

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Introduction

It is well-established that women have higher resting heart rates than men, but that the rate-corrected QT interval (QTc) is longer in women (by 2 – 6%) [3, 35]. In addition to a longer QTc interval, women show a steeper rate-adaptation in QT interval than do men [19, 46]. There are also differences between men and women in the rising and descending slopes of the T wave, suggestive of possible gender differences in dispersion of repolarization [51]. These differences are important factors in the higher susceptibility of women than men to the potentially lethal ventricular tachyarrhythmia *torsades de pointes (TdP)*, associated with both familial and acquired long QT syndromes [9, 28, 35, 49]. The basis for the gender difference in risk of *TdP* is unclear but, since the difference in the QT interval becomes evident from puberty and gradually diminishes following menopause, it is likely to reflect the influence of sex hormones on ventricular repolarization [37]. Other electrocardiographic gender differences relate to the 'J' (or 'Osborn') wave, which is a late delta/secondary R wave thought to arise from transmural heterogeneity of early outward repolarizing current [2, 50]. The shorter JT interval of virilized women compared with normal women and the longer JT interval of orchiectomized men compared with normal men argue in particular for a role for testosterone in somehow shortening ventricular repolarization [4].

The role played by the ovarian steroids, estradiol and progesterone, in the gender differences in pro-arrhythmia is less clear, presumably due at least in part to variations in plasma hormone levels around the menstrual cycle and the use of hormone-based contraceptive therapies by women [35]. For example, women taking oral contraceptives are at increased risk of ventricular ectopy, suggesting a pro-arrhythmic action of either estrogen or progesterone [41]. Although hormone replacement therapy (HRT) has no effect on the QT interval, dispersion of the QT interval may be increased by HRT in post-menopausal women [1, 22]. Moreover, although the QT interval does not appear to change around the menstrual cycle in pre-menopausal women, the degree of drug-induced QT prolongation does vary with the menstrual cycle, being lower during the luteal phase than during menses and the peri-ovulatory period [40]. Thus, the change in QT interval in women has been found to relate inversely to circulating levels of progesterone, or the progesterone-to-estradiol ratio, and not to testosterone or estradiol individually [18, 35, 40].

Animal models are an important tool for the investigation of gender-differences in ventricular repolarization and risk of arrhythmia [35]. The role of testosterone in males is strongly supported by data from the rabbit [16, 25, 26, 35, 36], a species that also shows gender-linked differences in repolarization and susceptibility to drug-induced arrhythmia events [25, 36]. In rabbits, testosterone has been shown to influence both ventricular repolarization and the density of two key potassium currents in males: I_{Kr} (the rapid delayed rectifier K^+ current) and the outward component of I_{K1} (the inward rectifier K^+ current) [25, 26]. Data from studies using the rabbit are suggestive that estradiol may modulate ion channels, in particular the L-type Ca^{2+} current (I_{Ca}), and play a role in increasing pro-arrhythmic risk in rabbits [13, 34]. However, a recent study of ovariectomized and orchiectomized rabbits [36] provides evidence that the risk of excessive prolongation of repolarization in females may also involve sex-linked factors other than testosterone or estradiol.

The relevance to humans of the consequences of estradiol or testosterone administration to ovariectomized rabbits is questionable in two respects [16]. First, the normal female rabbit is an induced ovulator with very low circulating levels of estradiol and testosterone that are little affected by ovariectomy [35, 36]. Second, the circulating levels of estradiol and testosterone achieved by sub-cutaneous implantation of slow release pellets in ovariectomized rabbits are not physiological [34]. In contrast, laboratory guinea pigs are polyestrus all year around, have a conventional estrus cycle of approximately 16 days and circulating levels of estradiol that are reduced by ovariectomy [33, 45]. Although guinea pig myocytes lack the transient outward K^+ current (I_{to1}) that participates in early repolarization, they possess the principal ion currents contributing to the plateau and repolarization phases of the action potential [27, 52]. For these reasons we have recently suggested that the guinea pig may provide a valuable model to investigate the influence of the ovarian steroids on ventricular repolarization and arrhythmogenesis [16]. Currently, there is a profound lack of information concerning gender-related differences in ventricular repolarization in the guinea pig. The aim of this study, therefore, was to investigate and characterize gender-related differences in three ion currents (I_{Ca}, I_{K1} and I_K) well established to contribute to the plateau and repolarization phases of the guinea pig ventricular action potential.

Methods

Animals

Adult male and female Duncan-Hartley guinea pigs (400 – 500 g) (B&K Universal, UK) were housed under controlled lighting (lights on 05:00 – 19:00) and were provided with feed pellets and water *ad libitum*. Estrus cycles of female animals were recorded by daily examination of the vulva and vaginal membrane. Reddening of the vulva in combination with opening of the vaginal membrane is a well-established indication of the day of estrus [32, 45]

and was therefore used to establish day 0 of the female cycle. It has been suggested that the ratio of progesterone to estradiol is important in the risk of ventricular arrhythmias in women [18, 35, 40]. For this reason, in this study female guinea pigs at day 0 (estrus) and day 4 of the estrus cycle were used, since day 0 corresponds to the peak of plasma estradiol levels and day 4 corresponds to the peak in the plasma progesterone-to-estradiol ratio [45].

Myocyte isolation

Male and female guinea pigs (400 – 500 g) were killed by a UK Home Office approved 'Schedule 1' method and ventricular myocytes were isolated as described previously [53]. Once isolated, cells were stored at 4 °C in a high-K^+, low-Cl^- solution (Kraft-Brühe, KB solution) containing (in mmol/L): L-glutamate 100; KCl 30; Na-pyruvate 5; taurine 20; creatine 5; succinic acid 5; Na_2ATP 2; β-OH butyrate 5; glucose 20; $MgCl_2$ 5; EGTA 1; HEPES 10 (pH adjusted to 7.2 with KOH). Cells were stored in this solution for at least 1 h prior to use. Only cells that showed a clear rod-shaped and striated appearance and that did not exhibit spontaneous contractions were chosen for recording.

Electrophysiological recordings

Isolated myocytes were plated into a perfusion chamber on the stage of an inverted microscope (Olympus CK-40, Olympus UK Ltd, London, UK) and superfused with a Tyrode's solution containing (in mM) at 35 °C: NaCl 134, KCl 4, $MgCl_2$ 1.2, $CaCl_2$ 1, HEPES 10, D-glucose 10, pH 7.4 (NaOH). Patch-pipettes were pulled from borosilicate glass to a tip resistance of 2.4 MΩ – 3.0 MΩ when filled with the internal solution. Since previous reports using the rabbit have demonstrated a role for K^+ and Ca^{2+} currents in gender-related differences in cardiac ion currents [25, 26, 34], a K^+-rich internal solution was used, containing (in mM): $KMeSO_4$ 130, KCl 10, EGTA 10, HEPES 10, $CaCl_2$ 1, $MgCl_2$ 2, MgATP 1, Na_2GTP 0.4, pH 7.2 (KOH). The free Ca^{2+} concentration of this solution was estimated to be 25 nM. The low Na^+ concentration and the presence of EGTA in this solution were chosen to minimize the contribution of $[Ca^{2+}]_i$-dependent currents, such as the Na^+/Ca^{2+} exchange current [12, 20, 24]. Tip potentials were offset electronically prior to giga-seal formation. Whole-cell currents and membrane potentials were passed through a 5 kHz lowpass Bessel filter and recorded at 10 kHz to the hard disk of an Apple Macintosh G3 computer using an Axoclamp 200B patch-clamp amplifier (Axon Instruments Inc, USA) and Pulse software (HEKA Elektronik GmbH, Germany). For voltage-clamp recordings, currents were elicited from a holding potential of –40 mV to inactivate voltage-gated Na^+ currents and T-type Ca^{2+} currents [30]. Currents were normalized to cell capacitance as a measure of cell size. For action potential recordings, no holding current was applied and 1 ms supra-threshold current pulses (typically 100 pA) were applied every 5 s. This corresponded to the inter-pulse interval of the voltage-clamp experiments, and would be expected to facilitate recovery from inactivation of currents on repeated stimulation. Action potential duration at 90% repolarization (APD_{90}) was measured as the time from the peak of the action potential to the time at which the action potential amplitude had decreased by 90%. For each cell, APD_{90} values were obtained for 10 consecutive action potentials during steady-state stimulation and the mean of these was then determined. Analysis of voltage-clamp and action potential data was performed using IgorPro 3.1 (Wavemetrics Inc., USA). Data were obtained from at least 10 myocytes, isolated from at least two hearts from each group (male, female day 0, female day 4).

Statistical analysis

Data are reported as the mean ± standard error of the mean (S.E.M). Current-voltage relations and the voltage-dependent activation of tail currents were analyzed by two-way ANOVA. All other data were compared using one-way ANOVA followed by Student-Newman-Keuls multiple comparisons *post-hoc* test. $P < 0.05$ was accepted as being significant.

Results

Gender-related differences in current-voltage relations

Ionic currents were elicited from a holding potential of –40 mV by 200 ms pulses to potentials between –120 mV and +60 mV. Figure 1A shows representative, normalized current traces in myocytes from male, female day 0 and female day 4 guinea pig hearts. There was no significant difference in the holding current level (male: 1.99 ± 0.18 pA/pF, n = 12; female day 0: 1.92 ± 0.11 pA/pF, n = 11; female day 4: 1.92 ± 0.14 pA/pF, n = 10; one-way ANOVA). Data from individual cells within each group were pooled and the mean current at each voltage calculated. The mean current-voltage (I-V) relations between –120 mV and –40 mV are shown in Fig. 1C. In all three groups, large inward currents were recorded from potentials negative to –80 mV, corresponding to the inward rectifier K^+ current (I_{K1}) [21, 27, 42, 43]. Voltage commands over the range between –70 mV and –40mV

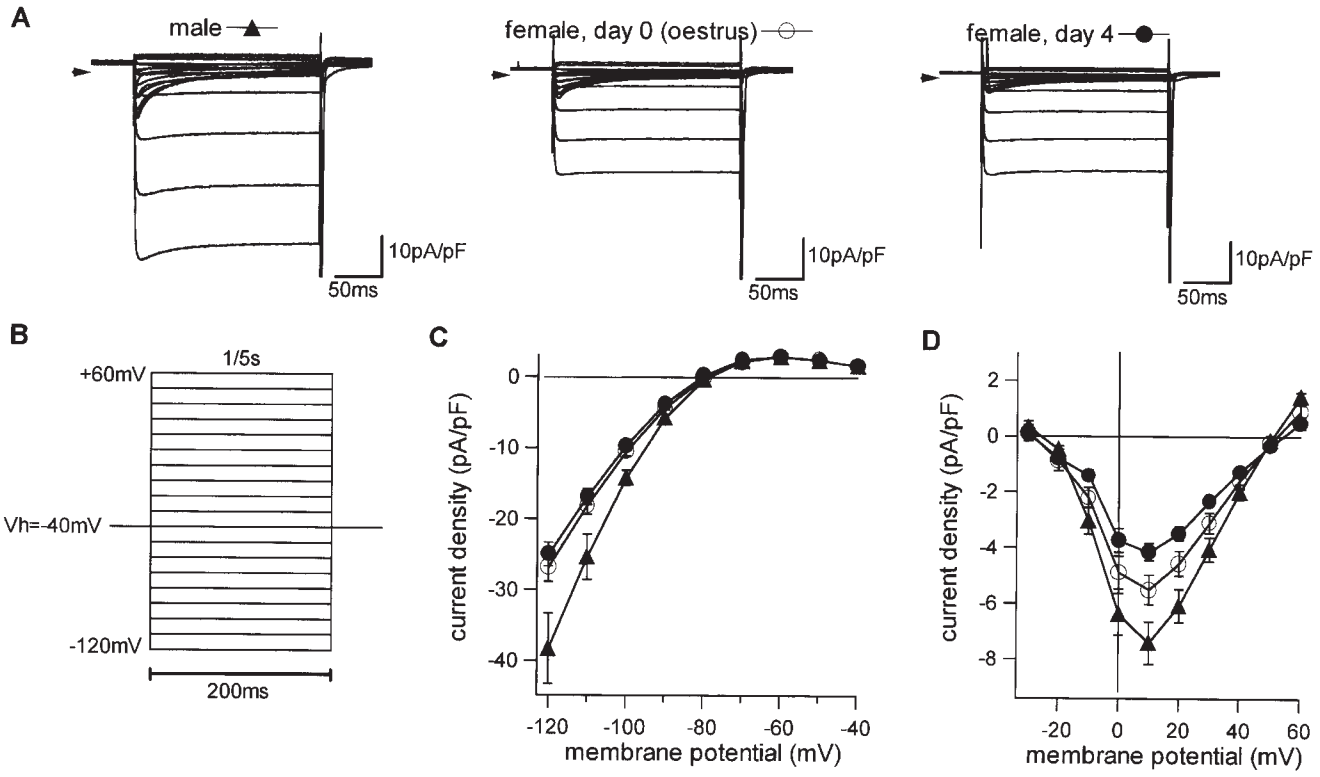


Fig. 1 Gender-related differences in current-voltage relations. **A** Representative families of current traces from ventricular myocytes isolated from male, female day 0 (estrus) and female day 4 guinea pigs elicited using the voltage-pulse protocol shown in B. Arrows indicate zero current level. **B** Voltage-pulse protocol. 200 ms pulses were applied 1/5 s from a holding potential of –40 mV to potentials varying from –120 mV to +60 mV in 10 mV increments. **C** Mean peak minimum current-voltage relations measured relative to the zero current level near the start of voltage-pulses from –120 mV to –40 mV, inclusive. Filled triangles, males (n = 12); open circles, females at day 0 (estrus; n = 11); closed circles, females day 4 (n = 10). Vertical bars show S.E.M. **D** Mean peak minimum current-voltage relations measured relative to the zero current level near the start of voltage-pulses from –30 mV to +60 mV, inclusive. Note difference from C in scale of y-axis. Filled triangles, males (n = 12); open circles, females at day 0 (estrus; n = 11); closed circles, females day 4 (n = 10). Vertical bars show S.E.M.

elicited comparatively small outward currents, also largely representing I_{K1} [21]. The mean I-V relations from –30 mV to +60 mV are shown in Fig. 1D. Pulses from –20 mV to +40 mV elicited inward currents, representing predominantly L-type Ca^{2+} current (I_{Ca}) [17, 23, 27]. The I-V relations between –120 mV and +60 mV of the three groups were found to be significantly different by two-way ANOVA ($P < 0.01$), indicating the existence of gender-related differences in the whole-cell ion currents in guinea pig ventricular myocytes. The greatest differences between the three groups appeared to be at –120 mV and +10 mV, potentials at which the contribution of, respectively, I_{K1} and I_{Ca} to the peak inward currents predominates [17, 21, 23, 27, 42, 43]. Further comparison between these two currents in the three groups was therefore made.

Gender-related differences in I_{K1}

Marked differences between male and female myocytes in I_{K1} density were found. I_{K1} was measured as the steady-state current at the end of 200 ms pulses to –120 mV. Example current traces are shown in the left-hand panel of Fig. 2A and the mean current densities are shown in the right-hand panel. Male myocytes showed the greatest I_{K1} density with female day 0 and day 4 myocytes having noticeably lower current densities. The I_{K1} density among the three groups was significantly different ($P < 0.05$, one-way ANOVA) and *post hoc* analysis showed that I_{K1} in myocytes from female day 0 and female day 4 hearts were significantly different ($P < 0.05$) from male myocytes. However, there was no difference between female day 0 and day 4 myocytes. In contrast to the data at –120 mV, there was no difference between the three groups of myocytes in the outward current density at –60 mV (see Fig. 1C), suggesting little gender-related difference in the density of outward I_{K1} under these conditions.

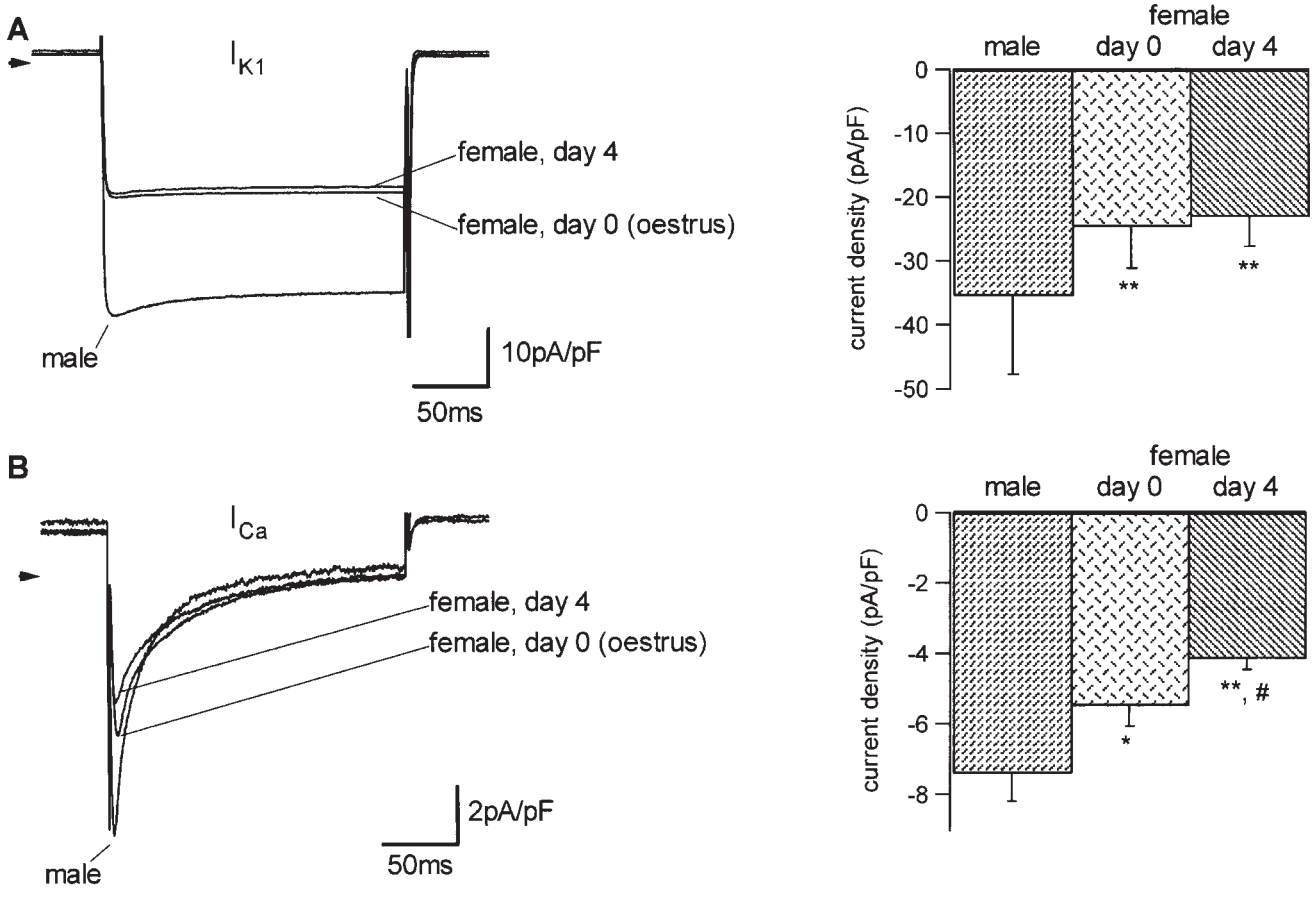
Gender-related differences in I_{Ca}

Striking differences in I_{Ca} density were found between the three groups of myocytes. I_{Ca} was measured as the peak inward current on depolarization to +10mV. Representative current traces are shown in the left-hand panel of Fig. 2B and the mean current densities are shown in the right-hand panel. The greatest mean I_{Ca} density was in male myocytes and the lowest in female day 4 myocytes, with female day 0 myocytes being intermediate (Fig. 2B). The current densities were significantly different among the three groups ($P < 0.05$, one-way ANOVA). *Post hoc* analysis showed that I_{Ca} density in both female day 0 ($P < 0.05$) and day 4 ($P < 0.01$) was significantly different from the current density in male myocytes. In addition, the density of I_{Ca} in female day 4 was significantly different from that in female day 0 ($P < 0.05$), strongly suggesting that the Ca^{2+} current varies around the estrus cycle.

Gender-related differences in I_K tails

The delayed rectifier K^+ current (I_K) plays an important role in phase 3 repolarization of the ventricular action potential [15, 27, 39, 44] and has been reported to be larger in male than in female rabbit ventricular myocytes [25]. Therefore, in addition to investigating I_{K1} and I_{Ca} it was important to determine any gender-related differences in guinea pig I_K. To achieve this, the tail currents elicited on repolarization to –40 mV from depolarizing pulses (voltage protocol as shown in Fig. 1B) were measured as the difference between the peak of the tail current and the holding current [14, 15]. In myocytes from all three groups, tail currents were activated voltage-dependently following pulses positive to –40 mV (Fig. 3A and B). The voltage-dependence of tail current activation is shown in Fig. 3B, tail currents activating with a half-

Fig. 2 Gender-related differences in I_{K1} and I_{Ca}. **A** Left-hand panel shows representative inward current traces at –120 mV, representing largely I_{K1}, from ventricular myocytes isolated from male, female day 0 (estrus) and female day 4 guinea pigs. Arrows indicate zero current level. Right-hand panel shows mean current-densities from male (n = 12), female day 0 (n = 11) and female day 4 (n = 10) myocytes. Vertical bars show S.E.M. **B** Left-hand panel shows representative inward current traces at +10mV, representing largely I_{Ca}, from ventricular myocytes isolated from male, female day 0 (estrus) and female day 4 guinea pigs. Arrows indicate zero current level. Right-hand panel shows mean current-densities from male (n = 12), female day 0 (n = 11) and female day 4 (n = 10) myocytes. Vertical bars show S.E.M. *P < 0.05 compared to male; **P < 0.01 compared to male; #P < 0.05 compared to female day 0 in a Student-Newman-Keuls *post-hoc* test



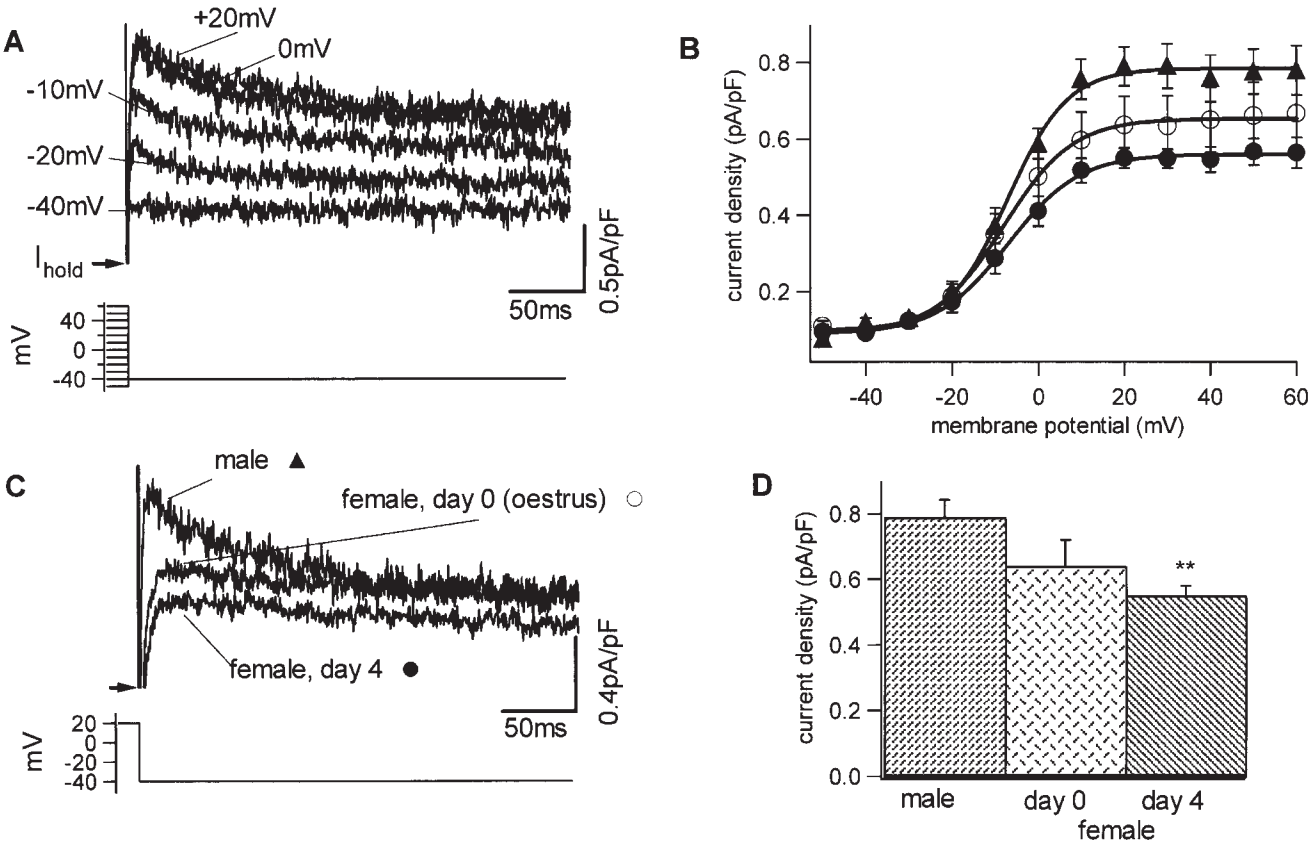


Fig. 3 Gender-related differences in delayed rectifier tail currents. **A** Family of tail currents on repolarization to –40 mV from pulse potentials from –40 mV to +60 mV demonstrating the voltage-dependent activation of the tail currents. Arrow indicates holding current level. Lower panel shows segment of the pulse protocol corresponding to the tail currents. Traces obtained from a male cell. **B** Voltage-dependent activation of tail currents in myocytes from male (filled triangles, n = 12), female day 0 (open circles, n = 11) and female day 4 (filled circles, n = 10). Data are mean ± S.E.M. Solid lines represent fits to a Boltzmann equation of the form: $I_{tail} = I_{max}/(1+\exp((V_{0.5}-V_m)/V_s))+c$, where I_{max} is the maximum tail current magnitude, $V_{0.5}$ is the voltage of half-maximal activation, V_m is the pulse potential, V_s is a slope factor and c is a constant. Fitted values for male, female day 0 (estrus) and female day 4 respectively were as follows: I_{max}, 0.685 pA/pF, 0.561 pA/pF & 0.470 pA/pF; $V_{0.5}$, –7.2 mV, –8.0 mV & –7.1 mV; V_s, 7.0 mV, 8.0 mV & 8.3 mV; c, 0.100 pA/pF, 0.093 pA/pF & 0.091 pA/pF. **C** Representative current traces on repolarization to –40 mV from a pulse potential of +20 mV from ventricular myocytes isolated from male, female day 0 (estrus) and female day 4 guinea pigs. Holding currents have been subtracted. **D** Mean tail current-densities from male (n = 12), female day 0 (n = 11) and female day 4 (n = 10) myocytes. Vertical bars show S.E.M. **P < 0.05 compared to male in a Student-Newman-Keuls *post-hoc* test

maximal voltage of approximately –7 mV and being maximally activated following potentials of +20 mV and positive. In this regard, it is important to note that whilst guinea pig ventricular myocytes exhibit both I_{Kr} and I_{Ks} sub-types [14, 44], the fact that I_K tail magnitude did not increase following commands to potentials greater than +20 mV suggests that, with our voltage protocols, I_{Kr} is likely to have predominated. Normalized I_K tails from the three groups were compared by two-way ANOVA of the voltage-dependent activation of the tail currents (Fig. 3B); this revealed that the three groups differed significantly ($P < 0.01$). However, there were no significant differences in the half-maximal voltages of activation of the three groups (Fig. 3B). To examine further the differences in I_K density between the three groups, the tail current density after depolarization to +20 mV was measured and compared. Example traces from which the holding currents have been subtracted are shown in Fig. 3C. Figure 3D shows the mean current densities of the three groups, which were significantly different (one-way ANOVA, $P < 0.02$). As found for the I_{Ca} inward currents (Fig. 2B), male myocytes showed the largest mean tail current density and female day 4 myocytes the lowest, with the mean current density in female day 0 myocytes being intermediate to the two. *Post hoc* analysis revealed that the tail currents of female day 4 myocytes were significantly different from male myocytes ($P < 0.05$). However, the differences between female day 0 and male myocytes and between female day 0 and day 4 myocytes were not significant.

Gender-related differences in action potential duration

Broadly speaking, for the three currents investigated (I_{K1}, I_{Ca} and I_K) it was found that male myocytes had the largest mean current densities and female day 4 myocytes the lowest. Since, unlike inward I_{K1}, I_{Ca} and I_K contribute to the plateau and repolarization phases of the action potential; it is not clear how the combination of these differences in current densities might affect ventricular repolarization. To investigate further the contribution of gender-related differences in these ion currents to ventricular repolarization, action potential recordings were made from the male, female day 0 and female day 4 myocytes immediately following completion of the voltage clamp recordings described in Figs. 1–3. Representative examples of signal-averaged action potentials from the three groups are shown in Fig. 4A. Mean APD_{90} values are compared in Fig. 4B. The longest action potentials were recorded from the female day 0 (estrus) cells and the shortest action potentials were recorded from the male cells. Female day 4 myocytes had mean APD_{90} values intermediate between the two other groups. The differences in APD_{90} between the three groups were significantly different ($P < 0.05$, one-way ANOVA), demonstrating that under these recording conditions guinea pig ventricular myocytes show gender-related differences in repolarization. *Post hoc* analysis revealed that the APD_{90} of male and female day 0 myocytes were significantly different ($P < 0.05$), but that female day 4 myocytes were not significantly different from either of the other two groups. The differences in APD_{90} between male and female day 0 myocytes is consistent with the gender-related differences in ventricular repolarization reported for the rabbit and human [3, 25, 35].

Discussion

This study represents the first report of gender differences in cardiac electrophysiology in the guinea pig. Moreover, to the best of our knowledge, the demonstration of differences in I_{Ca} in female myocytes isolated at day 0 and day 4 of the estrus cycle (Fig. 2A) represents the first report of changes in a cardiac ion current post-estrus, regardless of species. The guinea pig is a species commonly used for cardiac electrophysiology, since the ventricular myocytes have an action potential with a high plateau and possess most of the ion currents thought to be present in the human [27, 52]. Moreover, the frequency dependence of the action potential and of Class III drug action make the guinea pig a suitable species for the study of drug-induced QT prolongation and pro-arrhythmia [11, 38, 48]. Thus, the present data are consistent with a recent suggestion that the guinea pig may be of value in the study of gender differences in cardiac electrophysiology and drug-induced pro-arrhythmia and in particular for the investigation of the influence of sex hormones on ventricular repolarization in the female [16]. Several aspects of the data merit discussion.

Gender-related differences in I_{K1}

As previously reported in rabbit ventricular myocytes, I_{K1} density was greater in male than female myocytes (Fig. 2A) [25]. The gender differences in I_{K1} density in the rabbit have been suggested to reflect the influence of testosterone; our results showing no differences in I_{K1} density between female guinea pig myocytes isolated on day 0 and day 4 of the estrus cycle are consistent with this proposal [26]. However, in contrast to the rabbit, the gender differences in the present study were in inward but not outward I_{K1} [25]. Nevertheless, the data from the two species are similar in the respect that gender differences

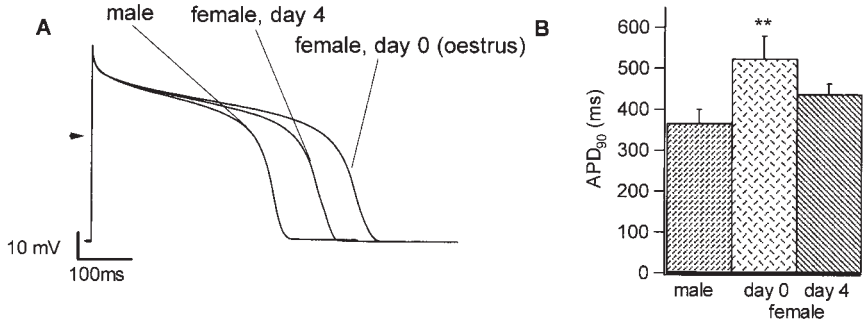


Fig. 4 Gender-related differences in action potentials. **A** Traces show representative time-averaged action potentials recorded from left-ventricular myocytes isolated from male, female day 0 (estrus) and female day 4 guinea pigs. Traces represent the average of ten consecutive action potentials. **B** Mean APD_{90} from male (n = 12), female day 0 (n = 11) and female day 4 (n = 10) myocytes. Action potentials were recorded from the same cells as reported in Fig. 1. **$P < 0.05$ compared to male in a Student-Newman-Keuls *post-hoc* test

in I_{K1} may not occur across the entire voltage range, observations that cannot simply be explained by differing levels of expression of I_{K1} channel subunits [16, 26].

Gender-related differences in I_{Ca}

The finding of significant differences between male and female cardiac myocytes in I_{Ca} density is in marked contrast with the results of Rosen and colleagues from rabbit ventricular myocytes [34]. In the present study, male myocytes had the greatest mean I_{Ca} density, female myocytes at day 4 of the estrus cycle, the smallest and female day 0 myocytes had an intermediate I_{Ca} density significantly different from either of the other two groups (Fig. 2B). Thus, our data are consistent with the contribution of multiple factors to the gender differences in I_{Ca} density. Nevertheless, our data from female myocytes showing a greater I_{Ca} density on the day of estrus, when plasma estradiol levels can be expected to be highest [45], are consistent with the suggestion that circulating estradiol can modulate I_{Ca} density in female rabbits [34]. Acute administration of high concentrations (3 μM – 30 μM) of 17 β-estradiol to guinea pig atrial and ventricular myocytes has been reported to reduce L-type Ca^{2+} currents [31, 47]. However, the concentrations of estradiol used in those studies [31, 47] are approximately one thousand-fold higher than the actual range of circulating estradiol concentrations during the normal estrus cycle of guinea pigs (1 nM – 10 nM) [33, 45]. Thus, the physiological relevance of the previously reported inhibitory action of high estradiol concentrations on guinea pig cardiac I_{Ca} remains unclear, whilst our results are consistent with the suggestion that in the female the presence of plasma estradiol can up-regulate cardiac L-type Ca^{2+} currents [34]. Moreover, although the mechanism for the gender-related differences in ion currents remains unclear, their observation in isolated myocytes in the absence of externally applied hormones in this study is consistent with a mechanism independent of acute effects of steroid hormones on the cell membrane *per se*.

Gender-related differences in I_K tails

The finding of the largest I_K tails in male myocytes (Fig. 3) is consistent with the previous report of gender-related differences in I_K in rabbits, with I_{Kr} having a lower baseline density in myocytes from females [25] than in those from males. It has been suggested that the larger I_K currents of male compared with female rabbit myocytes reflect the influence of testosterone on I_{Kr} density in male rabbits [25, 26]. However, the observation in the present study that the differences between female and male myocytes in tail currents were significant at day 4 of the estrus cycle, but not at day 0, suggests that additional factors to testosterone may modulate I_K density in females. As previously demonstrated by Drolet and colleagues [7, 8], the use of short duration depolarizing pulses (200 ms in the present study) will have resulted in the relatively selective activation of I_{Kr}. However, it is important to note that whilst the data in Fig. 3A, B are consistent with the suggestion that I_{Kr} dominated our I_K tail measurements, we cannot exclude the possibility that the gender-related differences in I_K tails shown in Fig. 3 may reflect heterogeneous effects of gonadal steroids on both I_{Kr} and I_{Ks} components. For example, both estradiol and testosterone have been reported to reduce cardiac expression of the K^+ channel subunit gene, KCNE1 (also known as IsK or minK), which contributes to cardiac I_{Ks}, in ovariectomized rabbits [6]. On the other hand, expression in the rat uterus of KCNE1 has been suggested to depend on estrogen [10]. Taking these factors collectively, it is clear that in future investigation of gender-related differences in I_K, individual consideration of each of I_{Kr} and I_{Ks} will be warranted.

Gender-related differences in APD_{90}

The observation that the difference between male and female myocytes in APD_{90} was significant at day 0 of the estrus cycle, but not day 4, demonstrates that male-female differences in ventricular repolarization vary with the estrus cycle of female guinea pigs. Outward I_{K1} density varied little between the three groups, suggesting that differences in action potential duration are unlikely to have been mediated primarily by changes to I_{K1} under our conditions. In contrast, both I_{Ca} and I_K are active over plateau voltages and are widely recognized to play important roles in setting action potential duration. The differences between groups in I_{Ca} cannot alone account for the observed differences to APD_{90}, as I_{Ca} density was greatest in male myocytes, which had the shortest APD_{90}. However, the difference in I_K tail density between male and females myocytes was significant at Day 4, which would offset to a degree the difference in I_{Ca}. Nevertheless, the difference in APD_{90} between male and female myocytes was significant at Day 0, and not at Day 4, consistent with a key role for I_{Ca} in the gender differences in APD_{90}. Taken together, our data on I_{Ca}, I_K and APD_{90} are consistent with the contribution of (a) multiple factors, rather than of a single hormone and (b) changes to more than one ion channel type to gender differences in ventricular repolarization.

Strengths and limitations of the present study

In order to complete both current and action potential recordings from the same cells before the onset of significant current run-down, the current components were

not separated in the present study, either pharmacologically or by ionic substitution. Thus, although the peak inward current measured at +10 mV will have represented predominantly L-type Ca^{2+} current, the presence of contaminating outward K^+ currents (mostly I_K) may have caused an underestimation of peak I_{Ca} amplitude. Since both I_K and I_{Ca} appear to be influenced by the estrus cycle, further studies in which the current components are separated are required to confirm and define in more detail the gender-differences revealed in the present study. It may also be of value to investigate additional ionic currents/channels to those that have formed the focus of this initial study.

The major strength of the present study is that it clearly demonstrates gender-related differences in cardiac ion currents and repolarization in isolated guinea pig ventricular myocytes. As such it provides 'proof of concept' that, since female guinea pigs possess a conventional estrus cycle analogous to the situation in humans, this species offers a valuable additional model to the rabbit for the examination of gender-related differences in ventricular repolarization. The guinea pig may be of particular value for the investigation of the role of the sex hormones in modulation of cardiac electrophysiology in the female. Further work to study the gender differences in ventricular repolarization using the guinea pig is now warranted. It has been suggested that there are gender differences in susceptibility to atrial fibrillation, and the guinea pig may also be of value to investigate gender-related differences in atrial ion currents and ion channel remodeling [5, 29].

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