Gender determines the acute actions of genistein on intracellular calcium regulation in the guinea-pig heart

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Abstract

Objectives: The soy-isoflavone, genistein, appears to be cardioprotective partly through direct actions on the heart, although the relative benefits between men and women are not fully known. The purpose of the present study was to determine whether gender influences the acute electrophysiological actions of genistein at the level of isolated cardiac myocytes and to elucidate the mechanisms involved.

Methods: Left ventricular myocytes, isolated from weight-matched male and female guinea-pigs and rats, were field stimulated at a rate of 1 Hz in a superfusion chamber (37 °C). The effects of acute application of genistein on cell shortening and the Ca²⁺ transients were measured. Electrophysiological recordings were performed using single electrode voltage-clamp.

Results: Genistein increased cell contraction and the Ca²⁺ transients in a concentration-dependent manner in myocytes from male guinea-pigs [by 54 ± 11% and 22 ± 4%, respectively (mean ± S.E.M., p < 0.001, n = 18) at 40 μM], while having no significant corresponding effect in those from females. In contrast, genistein increased both parameters in myocytes obtained from male and female rats. The changes in guinea-pigs occurred despite inhibition of the L-type Ca²⁺ current in both sexes (n = 23, p < 0.001). In order to explain these observations, we measured sarcoplasmic reticulum (SR) Ca²⁺ contents by integrating the Na+/Ca²⁺ exchanger currents (I_{NCX}) following rapid caffeine application. Genistein increased I_{NCX} integrals by 27% in males (n = 12, p < 0.01) and 20% in females (n = 14, p < 0.01). The increased SR Ca²⁺ load in males, but not females, could be related to an impaired ability of the Na+/Ca²⁺ exchanger to extrude Ca²⁺.

Conclusions: We have demonstrated novel, gender-related differences in the acute cardiac actions of genistein, which can be attributed to actions at distinct points of the intracellular Ca²⁺ cycle. Our results suggest that genistein may afford greater cardioprotection in females than in males.

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Keywords: Calcium (cellular); Ca-channel; e-c coupling; Na/Ca-exchanger; Gender

1. Introduction

The observation that pre-menopausal women have a lower incidence of cardiovascular disease compared with men and post-menopausal women has fuelled speculation that estrogens and related compounds may be cardioprotective [1,2]. The phytoestrogen, genistein, is a major isoflavonoid component of soy and is thought to exert beneficial actions on the cardiovascular system through direct and indirect mechanisms [3]. The therapeutic advantages of phytoestrogens over mammalian estrogens are that they do not appear to have adverse effects on the female reproductive system [4] and can also be taken by men without inducing feminizing effects [5]. Whether genistein is equally beneficial to the cardiovascular systems of males and females is still a matter of debate. The long-term actions of genistein may be of greater benefit in females [4,6,7], while its acute vasorelaxing effects appear to be independent of gender [8]. It is becoming increasingly apparent that genistein also exerts direct actions on the heart, which may play an important role in cardioprotection [9,10], although the relative benefits of these actions between males and females are largely unknown.

Electrophysiological differences between males and females have been demonstrated across several species at the level of isolated cardiac myocytes. These include differences in action potential duration (APD), L-type Ca²⁺ currents (I_{Ca,L}) and Ca²⁺ transient amplitudes [11–13]. In addition, differences in the relative contribution of various Ca²⁺ influx and efflux systems during systole and diastole...
Fig. 1. Acute actions of genistein on cell shortening and Ca\(^{2+}\) transient parameters in male and female guinea-pig ventricular myocytes. (A) Bar graphs showing genistein effects at increasing concentrations. Results are expressed as a percentage change from control values in normal Tyrode (NT). The number of cells tested at each concentration is shown in parentheses. (B) Sample traces of cell shortening and the indo-1 ratio during steady-state field stimulation before and 2 min after the application of 40 μM genistein (Gen). (C) Bar graphs showing effects of 40 μM genistein on the times-to-peak cell shortening/Ca\(^{2+}\) transient (TTP, left) and times-to-half twitch relaxation/50% Ca\(^{2+}\) transient decay (R\(_{50}\), right). (D) Representative traces of genistein action on myocyte twitch kinetics. Amplitudes have been normalized to the same value to facilitate comparison. (*p<0.05, **p<0.01, ***p<0.001, ns = nonsignificant compared with control values in NT).
are known to exist between species. For example, sarcolemmal Ca\(^{2+}\) influx has been estimated to be 30% of SR Ca\(^{2+}\) content in guinea-pig ventricular myocytes compared with 3.5% in rats [14], and SR Ca\(^{2+}\) uptake via SERCA2a has been shown to play a relatively greater role than the Na\(^+\)/Ca\(^{2+}\) exchanger in the removal of cytoplasmic Ca\(^{2+}\) during diastole in rats compared with guinea-pigs [15,16].

The aim of the present study was to determine whether genistein exerts different actions in male and female cardiac myocytes and to elucidate the mechanisms involved. We demonstrate clear gender-related differences in the effects of genistein on cell shortening and Ca\(^{2+}\) transient amplitude in guinea-pigs, but not in rats, and further show that the findings in guinea-pigs can be related to gender differences at distinct points during cardiac excitation–contraction coupling and diastolic Ca\(^{2+}\) removal. Since the regulation of intracellular Ca\(^{2+}\) in the guinea-pigs is closer to that seen in humans compared with rats [17], our findings may be of clinical relevance.

2. Methods

2.1. Animals

Sexually mature, weight-matched male and female Dunkin Hartley guinea-pigs (450–550 g) and Sprague–Dawley rats (225–250 g) were used in the studies and housed in single-sex cages. Female animals were chosen at random when required in an attempt to control for the varying stages of their estrus cycles. All investigations conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Cell isolation

Single left ventricular myocytes were freshly prepared by enzymatic dissociation as previously described [18]. Cells were stored in Dulbecco’s modified Eagle’s medium at room temperature and used within 8 h of isolation.

2.3. Measurements of cell shortening and Ca\(^{2+}\) transients

Cells were loaded with 10 \(\mu\)M of the acetoxymethyl ester form of the Ca\(^{2+}\)-sensitive fluorescent dye, indo-1 (Molecular Probes) for 25 min at room temperature. All experiments were subsequently performed at 37 °C. Myocytes were placed on the coverslip base of a bath chamber, on the stage of an inverted microscope (Nikon). Cells were superfused with normal Tyrode, NT (containing in mM: NaCl 140, KCl 6, CaCl\(_2\) 2, MgCl\(_2\) 1, glucose 10, HEPES 10, pH adjusted to 7.4 using NaOH) and electrically field stimulated at 1 Hz by means of a pair of platinum electrodes positioned on either side of the chamber. A single rod-shaped myocyte with clear striations and good contractions during field stimulation was chosen and shortening followed at one end with a video edge-detector system. Light at 365 nm was used to excite the fluorescent dye in the cells and the indo-1 ratio obtained from the 405/485 nm fluorescence emission ratio. Cell shortening and indo-1 ratios were recorded once a steady-state was attained (usually after 2 min) and expressed as a percentage of baseline values in NT taken from the same cell. This allowed direct comparison of the indo-1 ratios measured in the presence and absence of genistein to be made and avoided the need for calibration into absolute Ca\(^{2+}\) concentrations, which can be associated with errors from dye compartmentalization. Genistein has previously been shown to have no effect on the optical parameters of indo-1 [9].

2.4. Electrophysiology

Electrophysiological recordings were made with an Axoclamp-2B amplifier controlled by pClamp8 software (Axon
Instruments). Myocytes were impaled with high resistance (20–35 MΩ) borosilicate glass microelectrodes filled with a solution of 2 M KCl, 5 mM HEPES and 100 mM EGTA at pH 7.2. APDs at 50% and 90% repolarization (APD<sub>50</sub> and APD<sub>90</sub>, respectively) were recorded in current-clamp mode. Voltage-clamp experiments were performed using discontinuous single electrode voltage-clamp (5–6 kHz) and the peak cadmium-sensitive I<sub>Ca,L</sub> recorded in NT and then in the presence of 40 μM genistein. Myocytes were held at −40 mV and test pulses (200 ms duration) were imposed from −45 to +50 mV to elicit a Ca<sup>2+</sup> current over a range of potentials. Steady-state activation parameters of I<sub>Ca,L</sub> were obtained from the relationship between membrane conductance and the imposed potential. Steady-state inactivation parameters were analyzed with double-pulse protocols. Conditioning pulses (200 ms) ranging from −55 to +50 mV were imposed from a holding potential of −50 mV. Five milliseconds after the end of each conditioning pulse, a test pulse to +5 mV (200 ms) was applied to elicit the Ca<sup>2+</sup> current.

Sarcoplasmic reticulum (SR) Ca<sup>2+</sup> loads were measured using rapid application of caffeine and integrating the resulting inward Na<sup>+</sup>/Ca<sup>2+</sup> exchanger currents (I<sub>NCX</sub>) [19]. Myocytes were voltage-clamped at −80 mV and subjected to a train of 10 pre-pulses (1 Hz) to +30 mV. Caffeine (10 mM) was then rapidly applied to the superfusing solution for 6 s to produce a sudden, sustained release of Ca<sup>2+</sup> from the SR and elicit the I<sub>NCX</sub>. Na<sup>+</sup>/Ca<sup>2+</sup> exchanger function was assessed by measuring the time constants (taus) of repolarizing tail currents following membrane depolarization [20]. Myocytes were voltage-clamped at −60 mV and subjected to a test potential to 0 mV (200 ms) in control solution. This was repeated after 2 min in the presence of genistein and finally with 0Na<sup>+</sup>/0Ca<sup>2+</sup> solution (containing in mM: LiCl 140, MgCl<sub>2</sub> 1, glucose 10, HEPES 10, EGTA 0.75, pH adjusted to 7.4 using KOH) in order to inactivate the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. The final trace in 0Na<sup>+</sup>/0Ca<sup>2+</sup> solution was subsequently subtracted from the other two traces to give traces in which the tail currents during repolarization predominantly reflected Na<sup>+</sup>/Ca<sup>2+</sup> exchanger function. Monoequiline expression curves were fitted to the declining phase of the tail currents to produce time constants, taud, which were taken as a measure of Na<sup>+</sup>/Ca<sup>2+</sup> exchanger activity.

2.5. Drugs

Genistein was purchased from Sigma-Aldrich and dissolved in DMSO to make a stock solution of 100 mM. The maximum final concentration of DMSO carrier used, 0.05%, had no significant effect on cell shortening or the Ca<sup>2+</sup> transients (n = 7).

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. and analyzed using multivariate ANOVA and Bonferroni post-test (PRISM 4 software) unless otherwise stated. A value of p < 0.05 was considered significant.

![Fig. 3. Gender differences in effects of genistein on the action potential duration (APD). (A) Representative action potential profiles during steady-state stimulation in normal Tyrode (NT) and in the presence of 40 μM genistein (Gen). (B) Bar graphs showing effects of genistein on the times-to-50% and 90% repolarisation (APD<sub>50</sub> and APD<sub>90</sub>, respectively) in males and females. (**p < 0.001).](image-url)
3. Results

Left ventricular myocytes obtained from male and female guinea-pigs were of comparable sizes as demonstrated by similar cell capacitances [224 ± 12 pF in males (n = 25) and 243 ± 12 pF in females (n = 27)] and resting cell lengths [109 ± 3 μm (n = 22) and 102 ± 4 μm (n = 22), respectively]; p = nonsignificant using unpaired t-test for both capacitance and cell lengths.

3.1. Effects of genistein on cell shortening and Ca\textsuperscript{2+} transient parameters in guinea-pig ventricular myocytes

Genistein markedly increased cell shortening and the Δ indo-1 ratio in myocytes obtained from male guinea-pigs in a concentration-dependent manner (Fig. 1). In the presence of 40 μM genistein, these parameters were increased by 54 ± 11% (n = 18, p < 0.001 compared with control using one way ANOVA and Bonferroni post-test).
and 22 ± 4% ($p<0.001$), respectively. Expressed as a percentage of cell length, 40 μM genistein increased mean cell shortening from 4.1 ± 0.4% to 6.3 ± 0.7%. These changes generally occurred over the course of 2 min. In contrast, 40 μM genistein had no significant effect on cell shortening or Ca$^{2+}$ transient amplitude in female myocytes. Cell shortening was decreased in the presence of 1 μM genistein in females ($p<0.05$ using paired $t$-test), although this failed to show significance when considered with the other concentrations using one-way ANOVA.

Analysis of the cell shortening and Ca$^{2+}$ transient kinetics revealed further gender differences. Genistein (40 μM) significantly decreased the time-to-peak (TTP) shortening in males, while having no significant effect in females (Fig. 1C). There was a significant effect of gender on TTP shortening ($p<0.05$) from testing with multivariate ANOVA. Similarly, genistein decreased the TTP Ca$^{2+}$

Table 1

<table>
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<tr>
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<td>Activation $V_{50}$ (mV)</td>
<td>$-10.7±1.1$</td>
<td>$-10.4±1.0$</td>
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<td>$-13.1±0.9$</td>
<td>$-12.4±1.2$</td>
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<td>$K$ (mV)</td>
<td>$6.6±0.5$</td>
<td>$6.9±0.36$</td>
<td>24</td>
<td>$5.7±0.2$</td>
<td>$6.0±0.5$</td>
<td>27</td>
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<tr>
<td>Inactivation $V_{50}$ (mV)</td>
<td>$-20.5±2.0$</td>
<td>$-21.5±2.1$</td>
<td>24</td>
<td>$-21.2±1.1$</td>
<td>$-29.9±3.1^{***}$</td>
<td>15</td>
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<tr>
<td>$K$ (mV)</td>
<td>$-3.6±1.1$</td>
<td>$-4.1±1.2$</td>
<td>24</td>
<td>$-4.2±0.5$</td>
<td>$-5.7±1.2$</td>
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$V_{50}$ is the membrane potential at which half the channels are activated or inactivated and $K$ is the slope factor ($n$ = number of cells; $^{***}p<0.001$ using multivariate ANOVA).

![Diagram](image-url)
transient in males, but not females, although gender interaction just failed to show significance \((p=0.054)\). Genistein significantly shortened the time-half relaxation from peak shortening \((R_{50})\) and time-to-50% decay of the \(\text{Ca}^{2+}\) transient in both male and female myocytes, with no significant gender interaction.

### 3.2. Genistein actions in male and female rat ventricular myocytes

In view of known species differences in intracellular \(\text{Ca}^{2+}\) handling, we investigated whether our above findings of gender differences in genistein action were also apparent in rat ventricular myocytes. We found that 40 \(\mu\text{M}\) genistein significantly increased cell shortening and the \(\text{Di}

# 3.3. Effects of genistein on action potential duration

Genistein did not significantly affect \(\text{APD}_{50}\) or \(\text{APD}_{90}\) in male myocytes \((n=22)\). In contrast, genistein shortened both \(\text{APD}_{50}\) and \(\text{APD}_{90}\) in female myocytes \((n=29, p<0.001\) for both times compared with equivalent times in \(\text{NT}\)) (Fig. 3). The gender-related effect of genistein on \(\text{APD}\) was highly significant (gender interaction \(p<0.01\) for both \(\text{APD}_{50}\) and \(\text{APD}_{90}\)).

### 3.4. Genistein effects on the \(\text{Ca}^{2+}\) current

Genistein markedly inhibited peak \(I_{\text{Ca,L}}\) in both male and female myocytes over the range of potentials tested without altering the shape of the current–voltage relationship (Fig. 4). There was no significant difference in the degree of genistein-induced inhibition of absolute \(I_{\text{Ca,L}}\) (pA/pF) between males and females. However, the percentage decrease in \(I_{\text{Ca,L}}\) from control values was greater in females compared with males—genistein decreased peak \(I_{\text{Ca,L}}\) by \(47 \pm 4\%\) in male myocytes \((n=23)\) and \(59 \pm 3\%\) in female myocytes \((n=27)\) \((p<0.05\) using unpaired \(t\)-test). Inhibition of \(I_{\text{Ca,L}}\) occurred very rapidly within several seconds in both sexes and was reversible upon washout in \(\text{NT}\).

We further investigated whether the gender difference in percentage inhibition of \(I_{\text{Ca,L}}\) by genistein could be attributed to fundamental differences in voltage dependence of \(\text{Ca}^{2+}\) channel activation or inactivation. The relationships between membrane potentials and \(\text{L}\)-type \(\text{Ca}^{2+}\) channel conductance were fitted with the Boltzmann function:

\[
G = G_{\min} + \frac{(G_{\max} - G_{\min})}{1 + \exp\left(\frac{V - V_{50}}{K}\right)}
\]

where \(G\) is the conductance at membrane potential \(V\), \(G_{\min}\) and \(G_{\max}\) are the minimum and maximum conductances, respectively, and \(K\) is the slope factor. This allowed the membrane potential, \(V_{50}\), when half the channels are activated \((d_{\text{on}}\) variable) or inactivated \((f_{\text{off}}\) variable) to be determined in the presence and absence of genistein. Table 1 summarizes the effects of 40 \(\mu\text{M}\) genistein on \(V_{50}\) and \(K\) of channel activation and inactivation in male and female myocytes. Genistein did not significantly alter these parameters in male myocytes \((n=24)\). Interestingly however, genistein decreased \(V_{50}\) of inactivation to a more negative potential in female myocytes \((n=15, p<0.001\) compared with control; gender interaction \(p<0.01\)), producing a leftward shift in the inactivation curve (Fig. 5).

### 3.5. SR \(\text{Ca}^{2+}\) loads and \(\text{Na}^{+}/\text{Ca}^{2+}\) exchanger function

We next investigated the effect of genistein on SR \(\text{Ca}^{2+}\) content in order to explain our finding of increased/unchanged \(\text{Ca}^{2+}\) transient amplitudes in the presence of genis-
tein despite a simultaneous decrease in $I_{\text{Ca,L}}$. Fig. 6A shows representative traces of $I_{\text{NCX}}$ integrals, which are greater in the presence of genistein compared with control solution in both males and females. Genistein increased the SR Ca\(^{2+}\) content by 27% in males (from 0.23 $\pm$ 0.02 to 0.29 $\pm$ 0.03 pC/pF, $n = 12$, $p < 0.01$) and 20% in females (from 0.30 $\pm$ 0.03 to 0.36 $\pm$ 0.03 pC/pF, $n = 14$, $p < 0.01$) (Fig. 6B). There was no significant difference in the degree of SR Ca\(^{2+}\) increase between males and females from multivariate ANOVA. Control experiments using two consecutive caffeine releases in NT (rather than a second release in the presence of genistein) revealed no significant change in SR Ca\(^{2+}\) load between the caffeine releases (results not shown).

To determine whether the increased SR Ca\(^{2+}\) loads were secondary to an impaired ability of the Na\(^+/\)Ca\(^{2+}\) exchanger to extrude Ca\(^{2+}\), we investigated the effects of genistein on the time constants of tail current relaxation, tau. Single exponentials could be fitted to the relaxation phase of the tail currents, as shown in the sample traces in Fig. 7A. Genistein significantly prolonged taus in male myocytes from 36 $\pm$ 3 to 63 $\pm$ 7 ms ($p < 0.05$, $n = 17$), but had no significant effect in female myocytes (taus measured 96 $\pm$ 28 ms in NT and 86 $\pm$ 20 ms in genistein) (Fig. 7B). There was a significant effect of gender on the actions of genistein on tau ($p < 0.05$).

4. Discussion

Our study has revealed for the first time fundamental gender differences in the acute actions of the widely consumed isoflavone genistein in guinea-pig ventricular myocytes. These differences are present at distinct points of the intracellular Ca\(^{2+}\) cycle (summarized in Fig. 8) and result in an overall increase in contraction of male myocytes, but little or no change in females. Our findings of
stimulatory actions of genistein on cell shortening and Ca\(^{2+}\) transient amplitude in myocytes from both male and female rats highlights the species dependence of these effects and provides important clues as to the mechanisms involved.

4.1. Gender differences in the effects of genistein on the Ca\(^{2+}\) current

Although genistein inhibited \(I_{\text{Ca,L}}\) in cardiac myocytes from both male and female guinea-pigs, we found a greater percentage inhibition in females. This may be related to a lower basal \(I_{\text{Ca,L}}\) in females compared with males. Estrogen appears to normally down-regulate the expression of L-type Ca\(^{2+}\) channels since \(I_{\text{Ca,L}}\) density has been reported to be increased in estrogen receptor deficient mice [21]. It is therefore conceivable that there is a greater expression and subsequent redundancy of cardiac L-type Ca\(^{2+}\) channels in male guinea-pigs (which have lower circulating 17\(\beta\)-estradiol levels) with the result that genistein-induced block of \(I_{\text{Ca,L}}\) is less pronounced in males. This would be consistent with our finding of a greater control peak \(I_{\text{Ca,L}}\) in males. In addition, genistein appears to exert an additional inhibitory action on \(I_{\text{Ca,L}}\) in females by shifting Ca\(^{2+}\) channel inactivation to more negative potentials.

4.2. Genistein effects on SR Ca\(^{2+}\) content and Na\(^{+}\)/Ca\(^{2+}\) exchanger function

We and others have previously reported that the endogenous mammalian estrogen, 17\(\beta\)-estradiol, inhibits \(I_{\text{Ca,L}}\) in both guinea-pigs and rats [22,23], which produces the expected decrease in APD, Ca\(^{2+}\) transient amplitude and cell contraction [22]. Our intriguing findings of increased or unaltered contraction, in myocytes from both species, despite inhibition of \(I_{\text{Ca,L}}\), imply that genistein must exert additional actions. Furthermore, the findings of genistein-induced gender differences in guinea-pigs, but not rats, suggest that these additional actions are likely to be related to effects on Ca\(^{2+}\) handling at the SR and Na\(^{+}\)/Ca\(^{2+}\) exchanger, which are markedly different between the two species [14,15].

We found that genistein increases SR Ca\(^{2+}\) content in guinea-pig ventricular myocytes from both sexes. In males, this appears to be secondary to impaired Na\(^{+}\)/Ca\(^{2+}\) exchanger function, as evidenced by prolonged tail current taur in the presence of genistein. Consequently, removal of cytoplasmic Ca\(^{2+}\) during diastole via SERCA2a becomes relatively more important, resulting in an increased SR Ca\(^{2+}\) load and Ca\(^{2+}\) transient amplitude. In contrast, genistein did not significantly alter Na\(^{+}\)/Ca\(^{2+}\) exchanger function in females, suggesting that a different mechanism is responsible for increasing SR Ca\(^{2+}\) content in females. One possibility may be that genistein impairs SR Ca\(^{2+}\) release in female myocytes through interactions with the SR Ca\(^{2+}\) release channel, the ryanodine receptor (RyR), leading to a build up of Ca\(^{2+}\) within the SR. This would be consistent with our observations that genistein did not alter Ca\(^{2+}\) transient amplitudes or TTP shortening/Ca\(^{2+}\) transient in females, despite increasing SR Ca\(^{2+}\) content. A greater SR Ca\(^{2+}\) load increases the open probability of the RyR [24] and would therefore be expected to accelerate
TTP shortening/Ca\(^2+\) transient, as was the case in males. Ovariection in rats has been demonstrated to produce a decrease in the \(K_\Delta\) of the RyR, which can be reversed by estrogen replacement [25]. This suggests that estrogen may modulate a change in the properties of the RyR and adds plausibility to our hypothesis that genistein may also modulate RyR function in a similar way.

4.3. Differential effects of genistein on the action potential

Our finding of gender differences in the effects of genistein on APD is highly suggestive of additional actions on other ionic currents, in particular \(K^+\) currents. The lack of overall effect of genistein on APD in myocytes from male guinea-pigs can be explained if the isoflavone exerts dual inhibitory actions on Ca\(^2+\) and \(K^+\) currents. Such individual actions would shorten and prolong APD, respectively, with the net result being that of no change. In contrast, genistein may exert a relatively greater effect on Ca\(^2+\) over \(K^+\) current inhibition in females and thereby shorten APD.

We are unaware of any evidence demonstrating differential gender effects of genistein on \(K^+\) currents, although there is independent evidence that (1) genistein affects \(K^+\) currents and (2) intrinsic \(K^+\) channel expression is gender-related. In relation to the first point, several investigators have reported that genistein inhibits a number of \(K^+\) currents in guinea-pig ventricular myocytes, including \(I_{K1}\), \(I_{Ks}\) and \(I_{K,ATP}\) [26–28]. Evidence for the second point comes from studies performed on several different species. Lui et al. [29] studied the three major repolarizing \(K^+\) currents in the rabbit heart and found \(I_{Kr}\) density to be 20% lower in females compared with males. Trepanier-Boulay et al. [12] reported that the longer APD present in myocytes obtained from female mice can largely be attributed to a lower expression of Kv1.5 and subsequently a decrease in its corresponding \(K^+\) current, \(I_{Kur}\). This finding cannot be extrapolated to guinea-pigs, however, since \(I_{Kur}\) is not present in this species.

4.4. Interactions via the estrogen receptor

Genistein has been demonstrated to interact with nuclear estrogen receptors (ER) [30] and may therefore potentially exert acute effects via putative membrane-associated ERs. Consequently, it is possible that our findings of gender differences in guinea-pigs may therefore be related to differences in ER expression between males and females [31]. Against this, however, is our previous finding of acute ER-independent actions of genistein in ventricular myocytes obtained from male guinea-pigs [9]. On the other hand, it is possible that gender differences in the expression of key Ca\(^2+\) regulatory proteins, which appear to be altered by sex hormones [32,33], may be relevant. This remains to be determined from future studies.

4.5. Clinical considerations

If we consider suppression of myocardial contractility to be an advantageous action in patients with cardiovascular disease (similar to the acute effects of \(\beta\)-blockers and Ca\(^2+\) channel antagonists), then our findings suggest that low concentrations of genistein (1 \(\mu\)M) may be beneficial to females while higher concentrations (40 \(\mu\)M) may possibly be detrimental to males. This assumes that results from experiments performed on guinea-pigs can be applied to humans. While many aspects of cardiac excitation–contraction coupling are common to guinea-pig and human myocytes, further experiments on human cells and/or in vivo studies in human subjects are required before firm clinical conclusions can be made.

The use of phytoestrogens as an alternative to traditional hormone replacement therapy (HRT) is becoming increasingly popular [3], especially in light of recent trials showing that HRT may in fact increase the risk of coronary heart disease among healthy post-menopausal women [34]. Our findings that phytoestrogens possess distinct cardiovascular actions to mammalian estrogens suggest that clinical trial data on HRT cannot be generalized to other estrogenic treatments, which may prove to be beneficial. Again, further human studies are required to determine whether phytoestrogens do indeed decrease cardiovascular risk in post-menopausal women.

4.6. Study limitations

It is impossible to use male and female animals that are both weight- and aged-matched. We chose to use weight-matched guinea-pigs in order to obtain cardiac myocytes of comparable sizes, although this necessarily meant that the male animals were younger than their female counterparts. Several reports have suggested that age may affect certain aspects of cardiac excitation–contraction coupling [11,35], raising the possibility that some of the differences reported here resulted from age- rather than gender-related factors. However, all our experiments with genistein were paired within the same cell (and therefore same sex), so that the parameters measured were assessed before and after the application of genistein. In addition, the use of multivariate ANOVA to compare overall effects between males and females allowed for baseline gender differences to be taken into account.

We chose to use a genistein concentration of 40 \(\mu\)M in most of our experiments in order to accentuate any gender-related differences, even though this is greater than the plasma concentration found in humans after consumption of a soy-rich drink (reported to reach levels of up to 2 \(\mu\)M [8]). Nonetheless, our findings may be relevant since isoflavones are extensively distributed throughout the body following absorption and so the concentration found at the tissue level may be even greater due to local accumulation [36].
4.7. Conclusion

In summary, the overall cardiac actions of genistein in guinea-pigs appear to be gender-dependent. The mechanisms responsible can be related to specific actions at distinct points of the intracellular Ca\(^{2+}\) cycle. Our findings support the notion that genistein may play a greater role in cardioprotection in females than in males, although further studies with human cells and/or clinical trials are required.

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