Sex and strain differences in adult mouse cardiac repolarization: importance of androgens

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Abstract

Objective: Gender differences in mouse cardiac repolarization have been reported to be due to the stimulatory action of androgens on the ultrarapid delayed rectifier K+ current (I_{Kur}) and its underlying Kv1.5 channel. To confirm the regulation of ventricular repolarization by androgens, the present study compared two strains of mice (CD-1 and C57BL/6) that present different androgen levels.

Methods and results: Measurement of testosterone levels in different strains of mice (CD-1, C57BL/6, C3H and FVB) revealed that male C57BL/6 mice had very low levels of testosterone, whereas males of the other strains displayed normal testosterone levels. Furthermore, whole-cell voltage clamp recordings in isolated ventricular myocytes showed that the current density of I_{Kur} in male C57BL/6 mice was similar to that in female mice but smaller with respect to male CD-1 mice. Androgen replacement in male C57BL/6 mice as well as in castrated male CD-1 mice shortened ventricular repolarization, increased I_{Kur} current density, and increased expression of Kv1.5 channels.

Conclusion: Strain and gender differences observed in mouse cardiac repolarization can be explained by different androgen levels. As a consequence, androgens are major regulatory factors in cardiac repolarization and special attention should be paid to the hormonal status of the animal when studying hormonal regulation of cardiac repolarization.

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1. Introduction

In earlier studies, we demonstrated that CD-1 mice exhibited sex-specific differences in cardiac repolarization and that male mice subjected to castration presented a cardiac phenotype resembling that of female mice [6,25]. Indeed, both females and castrated male mice displayed longer action potential duration (APD) and QTc intervals compared to males. This prolonged repolarization was associated with a specific decrease in the density of the ultrarapid delayed rectifier K+ current (I_{Kur}) and of its underlying K+ channel isoform, Kv1.5. These results strongly suggested that gender difference in repolarization might be due to the action of androgens.

Previous studies have reported different blood testosterone levels in different strains of mice [1,23]. In these reports, the CD-1 mouse, the strain used in our studies and one of the most commonly used strain, exhibits vigorous reproductive activity and aggressive behaviours associated with physiological levels of testosterone. In contrast, the C57BL strains were considered as being chronically androgen deficient as indicated by subnormal levels of serum testosterone [1]. Since the C57BL strain could be viewed as a naturally occurring mouse model with low androgen levels, we took advantage of this particularity to further confirm the role of male sex hormones in the regulation of ventricular repolarization.
In the present study, we postulated that since male C57BL/6 mice have low level of androgen, they should exhibit similar ventricular repolarization than their female counterparts and delayed repolarization compared to the male CD-1 mice. In addition, we also tested the hypothesis that androgen replacement in male C57BL/6 mice as well as in orchiectomized male CD-1 mice would shorten ventricular repolarization, as a result of a higher expression of \( I_{Kur} \) and Kv1.5. Findings obtained in this study confirm these hypotheses and provide strong evidence that male sex hormones regulate cardiac repolarization.

2. Methods

2.1. Animals

Animal handling followed the Canadian Council Animal Care guidelines and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Adult CD-1 (\( n=59 \)), C57BL/6 (\( n=35 \)), C3H (\( n=18 \)), FVB (\( n=18 \)) mice of both sexes were used.

2.2. Dihydrotestosterone (DHT) replacement

Mice were treated with subcutaneous DHT pellet (7.5 mg/pellet, 90-day release) (Innovative Research of America, FL, USA). This DHT dosage regimen has been selected to evoke physiological concentrations (see Results).

2.3. Measurements of androgen levels

Testosterone and DHT levels were assessed by radioimmunoassay (Diagnostic Systems Laboratories, TX, USA) and ELISA (American Laboratory Products, NH, USA) following the manufacturer’s instructions.

2.4. Myocytes isolation

Animals were heparinized, anaesthetized by inhalation of isoflurane and then sacrificed by cervical dislocation. Single myocytes were then isolated from the right ventricular free wall using the protocol we described previously [6,25].

2.5. Electrophysiological recordings

The whole-cell voltage and current recording methods, data acquisition, voltage-clamp protocols and analysis methods have been described previously [5]. Pipettes had resistances in the range 1.5–4 MΩ when filled with the following solution (mM): 110 K\(^+\)-aspartate, 20 KCl, 8 NaCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 10 BAPTA, 4 K\(_2\)ATP and 10 HEPES (pH 7.2 with KOH). Voltage-clamp currents were low-pass filtered at 1 kHz with a four-pole Bessel analog filter, digitized at 4–10 kHz. All experiments were carried out at room temperature (20–22 °C). To measure \( I_{Kur} \), we applied 100 µM 4-aminopyridine (4-AP, which blocks \( I_{Kur} \)) [5,9,18,25] in combination with an inactivating prepulse (which blocks \( I_{so} \)) and recorded the 4-AP-resistant K\(^+\) current, or \( I_{ss} \). We then measured \( I_{Kur} \) by subtracting currents recorded in the absence or presence of 4-AP (\( I_{Kss} - I_{ss} \)). The description and validation of these pharmacological and biophysical methods has been recently published [5].

2.6. Western blot analysis

Protocols used for protein isolation and Western blots analysis were identical to those previously reported [6]. Proteins were prepared from mouse ventricles (3 pooled hearts/sample) homogenized in Tris-EDTA (TE) buffer (pH: 7.4) containing protease inhibitors (Leupeptin, Aprotinin, Benzanidime, Phenylmethyl sulfonyl fluoride [PMSF], and Na\(_3\)VO\(_4\)). The homogenate was centrifuged at 10,000×g. The supernatant was ultracentrifuged three times at 200,000×g for 20 min. The pellet was resuspended in TE buffer containing the protease inhibitors and 0.6 M KCl to dissolve contractile proteins. The pellet corresponds to the sarcosomal-enriched proteins. Proteins were separated by electrophoresis, transferred to nitrocellulose membranes. Membranes were then blotted with anti-Kv1.5 (1:500; Upstate, Lake Placid, NY, USA). Ponceau S was used to confirm uniformity of the protein loading and transfer. Bands were quantified by densitometry using Multi-Analyst program (Bio-Rad, CA, USA).

2.7. ECG recordings

Mice were anaesthetized with pentobarbital (65 mg/kg, ip) and placed on heating pads to maintain their body temperature at 37 °C. Platinum electrodes were positioned and lead I surface ECG were acquired using the Biopac System MP100 at a rate of 2 kHz. The signal was amplified, filtered at 100 Hz (low pass) and 60 Hz (notch filter). Recordings were analyzed using AcqKnowledge 3.7 program by two observed in a blinded fashion. QT intervals were corrected for the heart rate (QTc) using the correction formula for mice QTc=QT/(RR/100)\(^{1/2} \) [19] where RR corresponds to the time between two consecutive R waves.

2.8. Statistical analysis

Values are presented as mean±S.E.M. and \( n \) refers to the number of different cells. An unpaired Student’s \( t \)-test or one-way analysis of variance (ANOVA) with a Tukey post test were used when appropriate. For the comparison of androgen levels, the nonparametric tests Kruskal–Wallis with a Dunns post test, or Mann–Whitney were used.
P-values smaller than 0.05 were considered statistically significant.

3. Results

Fig. 1 shows testosterone and DHT levels in male and female CD-1, C57BL/6, C3H and FVB mice. Females of all strains had similar low levels of both androgens. The male CD-1, C3H and FVB mice had normal levels of testosterone within the physiological range observed in men, which varied from 3 to 11 ng/ml [4]. In contrast, the male C57BL/6 mice had low plasma testosterone concentrations. Moreover, the male C57BL/6 and C3H mice had low levels of DHT while the male of the CD-1 and FVB strains had normal DHT levels.

We used two strains of mice (CD-1 and C57BL/6) that present different androgen levels to further assess the association between androgen level and cardiac repolarization. We compared electrophysiological properties of C57BL/6 and CD-1 mice of both sexes. More precisely, we focused on $I_{Kur}$ based on the fact that the relatively prolonged ventricular repolarization of female and orchiectomized male CD-1 compared to normal male CD-1 mice is associated with a specific decrease in this K⁺ current [6,25]. Fig. 2 presents typical examples as well as mean data for the current density of $I_{Kur}$ recorded in ventricular myocytes isolated from male and female CD-1 and C57BL/6 mice. $I_{Kur}$ was measured as the 4-AP-sensitive (100 μM) current elicited by 500 ms voltage step ranging from −110 to +50 mV from a holding potential of −80 mV (for more details, see Ref. [5]). The current density of $I_{Kur}$ at +30 mV was similar for male and female C57BL/6 mice (25.6±2.6 and 24.5±3.7 pA/pF, respectively). However, in male CD-1 mice, $I_{Kur}$ density of 37.5±3.3 pA/pF was significantly higher than that in male C57BL/6 mice (25.6±2.6 pA/pF, p<0.05). Consistent with previous results [25], $I_{Kur}$ density in male CD-1 mice was significantly higher than that

![Fig. 1](image-url)

Fig. 1. Comparison of androgen levels between four different mouse strains. (A) Mean serum testosterone levels measured in 2- to 3-month-old CD-1, C57BL/6, C3H and FVB mice of both sexes. (B) Mean serum DHT levels measured in 2- to 3-month-old CD-1, C57BL/6, C3H and FVB mice of both sexes. Numbers on the top of each bar represents number of mice studied (*p<0.03 vs. male CD-1 mice).

![Fig. 2](image-url)

Fig. 2. Comparison of $I_{Kur}$ density between CD-1 and C57BL/6 mice of both sexes. (A) Superimposed current records corresponding to $I_{Kur}$, or the 4-AP-sensitive K⁺ current, in male and female CD-1 and C57BL/6 mouse ventricular myocytes. (B) Mean $I$–$V$ relationships for $I_{Kur}$ obtained in male CD-1 (n=18), female CD-1 (n=14), male C57BL/6 (n=49) and female C57BL/6 (n=21) mice (*p<0.05 vs. male C57BL/6; †p<0.05 vs. female CD-1).
obtained in female CD-1 mice (at +30 mV, 24.2 ± 3.9 pA/pF, p < 0.05). In addition, we also compared the activation and inactivation kinetics of \( I_{Kur} \) between male C57BL/6 and CD-1 mice and found no strain difference for these parameters (data not shown).

Androgen regulation of cardiac repolarization was examined using chronic DHT treatment. DHT was favored over testosterone as the androgenic compound because it cannot be converted into estrogens, as opposed to testosterone, hence avoiding a potential confounding factor. Castrated male mice were treated with a subcutaneous implant of DHT. Fig. 3A and B presents DHT and testosterone levels in three groups of male CD-1: intact, ORC, and DHT-treated ORC mice. The bar graphs show that castration leads to a complete loss of both male sex hormones levels and that DHT treatment restores physiological concentrations of

![Graph](image-url)

**Fig. 3.** Comparison of male sex hormones between three groups of male CD-1 mice: intact, castrated and DHT-treated castrated males. (A) Mean serum testosterone levels in male CD-1 (\( n=9 \)), ORC (\( n=3 \)) and ORC+DHT (\( n=3 \)) mice (\( *p < 0.05 \) vs. intact males). (B) Mean serum DHT levels in male CD-1 (\( n=10 \)), ORC (\( n=5 \)) and ORC+DHT (\( n=5 \)) mice.

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**Fig. 4.** Effect of castration and DHT treatment on the density of ventricular \( I_{Kur} \) and Kv1.5 in male CD-1 mice. (A) Typical examples of \( I_{Kur} \) in 4- to 5-month-old intact, ORC and ORC+DHT male CD-1 mouse ventricular myocytes. (B) Mean peak current density measured at +30 mV for \( I_{Kur} \) obtained in male CD-1 (\( n=38 \)), ORC (\( n=58 \)) and ORC+DHT (\( n=24 \)) myocytes (\( *p < 0.05 \) vs. intact and DHT-treated males). (C) Sarcolemmal-enriched proteins from ventricles of intact males (lanes 1–2), ORC (lanes 3–4) and ORC+DHT (lanes 5–6) CD-1 mice subjected to immunoblotting (100 μg protein/lane). For these experiments, two samples per group were studied but for each of these samples, proteins were extracted from three different mice. Thus results presented in this figure represent a total of six control mice, six ORC mice and six DHT-treated ORC mice. Membranes were probed with anti-Kv1.5 (75 kDa). The lower panel represents the Ponceau S staining of the membrane used to analyze Kv1.5. (D) Bar graphs showing mean values (± standard deviation) of the relative abundance of Kv1.5 protein expression determined by densitometry. Relative abundance was calculated with value for intact male CD-1 mice as a reference of 100%. The numbers in parenthesis represent the individual values.
DHT but not those of testosterone. These results are consistent with the fact that DHT cannot be converted into testosterone. Fig. 4A presents $I_{Kur}$ (or the 4-AP-sensitive current) recorded in ventricular myocytes isolated from intact, ORC, and DHT-treated ORC male mice. As illustrated in Fig. 4B, the density of $I_{Kur}$ was smaller in ORC mice compared to both intact male and DHT-treated animals. Fig. 4C shows the results of a Western Blot analysis performed on sarcolemmal-enriched proteins isolated from ventricles of each group. Kv1.5, the K$^+$ channel underlying $I_{Kur}$, was present in all groups but displayed higher expression in both intact and DHT-treated male mice. The bands depicted on the lower panel correspond to proteins of the same membrane used to study Kv1.5. The proteins, coloured with Ponceau S, were equally loaded ruling out the possibility that unequal loading could account for the observed difference in Kv1.5 protein expression. We measured Kv1.5 density for each of the three groups studied by densitometry. These results are presented in Fig. 4D.

Altogether these results strongly suggest that treatment with DHT in ORC male CD-1 mice shortens repolarization by up-regulating Kv1.5 and increasing $I_{Kur}$ density.

We then examined the possibility that DHT replacement would also accelerate ventricular repolarization in male C57BL/6 mice by a similar effect on $I_{Kur}$ and Kv1.5. Because male C57BL/6 mice have low androgen levels, the presence of the androgen receptor in their cardiac tissues was not definitive. Thus, using Western Blot analysis, we first verified the presence of these receptors in ventricles of male C57BL/6 mice (data not shown). Given that these mice had androgen receptors in their ventricle and should

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**Fig. 5.** DHT levels in male C57BL/6 mice treated or not with DHT. Mean serum DHT levels in 2- to 3-month-old male C57BL/6 ($n=4$) and in male C57BL/6 mice treated with a DHT pellet ($n=5$), $p=0.008$.

**Fig. 6.** Effect of DHT treatment on the density of ventricular $I_{Kur}$ and Kv1.5 in male C57BL/6 mice. (A) Typical examples of $I_{Kur}$ recorded from ventricular myocytes isolated from male C57BL/6 and male C57BL/6+DHT mice. (B) Mean $I–V$ relationships for $I_{Kur}$ obtained in male C57BL/6 ($n=32$) and male C57BL/6+DHT ($n=49$) mice ($p<0.05$). (C) Sarcolemmal-enriched proteins from ventricles of male C57BL/6 (lanes 1–3) and C57BL/6+DHT (lanes 4–6) mice were subjected to immunoblotting (3 pooled ventricles/lane; 100 µg protein/lane). Membranes were probed with anti-Kv1.5 (75 kDa). The lower panel represents Ponceau S staining of the same membrane used to study Kv1.5. (D) Bar graphs comparing relative abundance of Kv1.5 protein expression determined by densitometry. Relative abundance was calculated with value for intact male C57BL/6 mice as a reference of 100% ($p<0.01$).
therefore be able to respond to an androgen treatment, we implanted DHT pellets in male C57BL/6 mice. The bar graphs presented in Fig. 5 show that physiological concentrations of DHT were reestablished by DHT replacement in male C57BL/6 mice. We then measured the current density of $I_{Kur}$ in ventricular myocytes of these mice. The superimposed current traces presented in Fig. 6A correspond to $I_{Kur}$ recorded in male C57BL/6 mice treated or not with DHT. Fig. 6B shows that mean current density of $I_{Kur}$ was significantly larger in DHT-treated animals (e.g., at +30 mV, male C57BL/6: 25.6 ± 2.6, +DHT: 40.6 ± 3.7 pA/pF, p < 0.001). In addition, $I_{Kur}$ density was significantly different between male C57BL/6 mice treated with DHT and female C57BL/6 mice. In fact, the density of $I_{Kur}$ in DHT-treated male C57BL/6 mice very much resembles that measured in male CD-1 mice (at +50 mV: 55 ± 5, 53 ± 5 pA/pF, respectively). To verify whether this increase in $I_{Kur}$ was due to a corresponding increase in the expression of Kv1.5 in ventricles of DHT-treated mice, Western Blot analysis was carried out in ventricular tissues isolated from C57BL/6 mice treated or not with DHT. Fig. 6C shows that the expression level of Kv1.5 in DHT-treated mice is much higher compared with intact male C57BL/6 mice. Fig. 6D shows that when we quantify Kv1.5 density for each of the two groups studied, we found that the increase in Kv1.5 in DHT-treated male C57BL/6 was statistically significant (p = 0.01). Similar to the findings obtained with the DHT-treated ORC CD-1 mice, these results also indicate that

![Image of graphs showing current density and Western Blot analysis results]

Fig. 7. Effect of DHT treatment on APD recorded in male C57BL/6 mice. (A) Typical examples of action potential recorded in ventricular myocytes isolated from male C57BL/6 and male C57BL/6+DHT mice. Action potentials were evoked by injection of brief (2–5 ms) stimulus currents (0.4–0.7 nA) at rates of 1 to 4 Hz. For the examples shown in this figure, resting membrane potential were −79 mV for the male C57BL/6 and −76 mV for C57BL/6+DHT; for the corresponding groups, the mean resting potential was −75 ± 1 and −74 ± 1 mV, respectively; p=0.6. (B) Bar graph showing the mean APD (± S.E.M.) at 20%, 50%, and 90% repolarization in male C57BL/6 (n=41) and male C57BL/6+DHT (n=39) mouse myocytes (*p<0.05). APD$_{20}$, male C57BL/6: 5 ± 0.9 ms; +DHT: 3 ± 0.2 ms; p=0.008; APD$_{50}$, male C57BL/6: 10 ± 2 ms; +DHT: 6 ± 0.5 ms; p=0.01; APD$_{90}$, male C57BL/6: 32 ± 3 ms; +DHT: 23 ± 2 ms; p=0.02.

![Image of graphs showing QT intervals]

Fig. 8. Effect of DHT treatment on QT intervals of male C57BL/6 mice. Examples of lead I surface ECG obtained from 2- to 3-month-old male C57BL/6 mice treated or not with DHT. Lower panel: table comparing mean QT, QTc and heart rate (HR) in male C57BL/6 and male C57BL/6+DHT mice (*p<0.05). n represents the number of mice studied.
treatment with DHT significantly affects $I_{Kur}$ and Kv1.5 in C57BL/6 mice. Ventricular action potentials (AP) were then recorded in both groups. Fig. 7A depicts examples of action potential recordings obtained in C57BL/6 mice treated or not with DHT. In line with the larger density of $I_{Kur}$, the action potential were significantly shorter in the DHT-treated group compared with the intact males, as shown in Fig. 7B. Fig. 8 presents examples of lead I surface ECG recordings obtained in one control and one DHT-treated male C57BL/6 mice. We were particularly interested in the measurement of the QT interval, which reflects ventricular repolarization. As shown by the examples, the QT interval was shorter in male C57BL/6 mice treated with DHT compared to control male C57BL/6 mice. The lower panel of Fig. 8 presents mean data for QT, QTc and heart rate and shows that QT and QTc intervals were significantly shorter in the DHT-treated group compared to the controls.

4. Discussion

4.1. Summary of main findings

The major findings of this study are: (1) male C57BL/6 mice display similar ventricular repolarization as their female counterparts but show delayed repolarization with respect to male CD-1 mice, which have androgen levels of about six to seven times higher than C57BL/6 levels, and (2) androgen replacement in male C57BL/6 mice as well as in orchietomized male CD-1 mice shortened ventricular repolarization, associated with higher expression of Kv1.5 and larger $I_{Kur}$ current density. These results are consistent with an action of androgens on protein expression, implying an effect of androgens either on transcription, translation, trafficking, or a combination of all of these factors. These results provide convincing evidence that male sex hormones regulate cardiac repolarization in mice.

4.2. Choice of the mouse strains

To assess the association between androgen levels and cardiac repolarization, two strains of mice were selected. CD-1 mice were selected because of (1) normal levels of both male sex hormones, (2) being mentioned by others as a good strain for endocrinological or reproductive studies involving physiological levels of androgens [1] and, (3) our use in previous studies on gender differences and the effects of androgen deficiency on cardiac repolarization. C57BL/6 mice were chosen because of their low plasma testosterone levels. However, when we also checked for the DHT level we found that not only the C57BL/6/6 males had low level of DHT but also the males of the C3H line. The explanation for the low DHT level in this line of mice was unknown. We decided to measure DHT levels in 4- to 5-month-old C3H mice and found that the DHT level was more than six-fold higher in the older C3H mice (data not shown). It is possible that the enzyme responsible for the conversion of the testosterone into DHT, the 5α-reductase, is more abundant or effective in older C3H mice. We repeated this experiment for the C57BL/6 mice and found that DHT levels remained low even in the 4- to 5-month-old mice (data not shown). Therefore, since the concentrations of both androgens were found to be low in the male C57BL/6 mice (2- to 3- and 4- to 5-month-old animals), this strain represented a better model to study the effect of low levels of androgen on ventricular repolarization.

4.3. Relation to previous studies

Findings presented here along with data reported by other groups demonstrate that the presence of androgens is associated with faster repolarization [6,10,17,20]. Furthermore, this notion is supported by many clinical studies documenting that ventricular repolarization can be affected under various situations in which there is a chronic change in male sex hormones including normal development, pathological conditions, castration, and steroid treatment [3,10,15,21,26].

Since our initial observation showing gender-related difference in murine repolarization [25], other laboratories have also examined repolarization in male and female mouse ventricles [7,29]. Wu and Anderson [29] documented longer action potential at 90% of repolarization in female compared to male ventricular myocytes. Moreover, they reported that in females $I_{to}$ was smaller and $I_{sus}$ larger. These results differ somewhat from our findings. Indeed, under our experimental conditions, the only current that was smaller in female CD-1 mice was $I_{Kur}$ and as for the C57BL/6 mice, all the $K^+$ currents were similar for both genders. Many reasons could explain this apparent discrepancy between these studies. First, Wu and Anderson used a different strain of mice (C57BL/6J males crossed with 129SVE females). The animals were studied at 10–12 months of age as opposed to our studies where young adults (2–3 months) were used. They isolated left ventricular myocytes without specification of the origin of the cells (e.g., endocardial, epicardial or a mixed sample of both types of cells). Finally, the methods of separation of the $K^+$ currents were different. For example, $I_{Kslow}$ (composed of $I_{Kur}$ and $I_{sa}$) or what they designated as $I_{sus}$ was measured as the residual current recorded at the end of the depolarizing pulse (450 ms), during which $I_{Kur}$ significantly inactivates.

Very recently, another study examining gender difference in mouse ventricle was published by Brunet et al. [7]. In line with our results, they reported that $I_{Kur}$ (or $I_{Kslow,1}$) was smaller in CD-1 females. However, as opposed to our findings, they were unable to detect sex difference in the expression levels of Kv1.5. This inconsistency could possibly be due to different experimental conditions. The present paper, however, provides strong evidence for the up-regulation of Kv1.5 by androgens. Indeed, both castrated CD-1 and normal C57BL/6 displayed higher expression of
Kv1.5 when they were treated with DHT. This is consistent with the well-known genomic action of androgens [16]. Of note, 14 out of 15 consecutive nucleotides of the androgen response element (ARE) are present in the 5′-flanking region of the mouse Kv1.5 coding region [18]. Therefore, our observation showing that male CD-1 mice have high levels of androgens compared to females and present higher Kv1.5 density than females is in accordance with these observations.

Drici et al. reported sex differences in the occurrence of polymorphic ventricular arrhythmias in mice but did not observe any significant sex differences in electrophysiological parameters measured by optical mapping. They postulated that differences in the expression of cardiac ionic channels can account for these sex-dependent polymorphic ventricular arrhythmias, yet they did not observe any significant difference in the mRNA expression of different K⁺ channels including Kv1.5 [8]. This absence of difference may be explained by the use of Northern blots which may not be sensitive enough to detect a sex difference in Kv1.5 expression.

### 4.4. Male sex hormones concentrations

Others investigators have measured testosterone levels in male C57BL/6 mice [1,12]. Even if the male mice studied belonged to the same strain (C57BL/6) and were of similar age (2- to 3-month-old) than those studied in the present paper, there was clearly some inter-study variability between these reports. It is likely that this disparity can be attributed to differences in the experimental conditions or the techniques used (ELISA or RIA). Thus, it seems difficult to compare results obtained in different studies with the same strain and therefore, it is crucial for strain comparison to compare them under the same experimental conditions as it was the case in the present study.

An important factor that has to be considered when measuring sex hormones is the age of the animal. Different groups have studied the effect of age on testosterone levels in mice. Krishnamurthy et al. [13] studied wild-type CSV129 male mice from postnatal day 7 to 70. Testosterone levels were already substantial at day 7, stayed stable until day 35, reached a peak at day 42, and returned to similar baseline mean testosterone levels at days 56 and 63 before reaching another peak at day 70. This study shows that for a given strain of mice, under similar experimental conditions, there are week-to-week fluctuations in testosterone levels, even during adulthood period. Jones et al. [12] studied two groups of male C57BL/6 mice with mean age of 74 and 151 days old. Mean testosterone levels were higher in the older group but there was no mention whether or not this difference was statistically significant. Finally, Shapiro et al. [23] showed that testosterone levels were similar between mature male CD1 mice (98 days) and old mice (515 days). In keeping with this last study, we found no difference in testosterone or DHT levels between 2- to 3-month-old and 4- to 5-month-old male CD-1 mice (data not shown). Similarly, the testosterone and DHT values of the C57BL/6 male mice were also comparable between the two age groups.

### 4.5. Effect of estrogen on cardiac repolarization

The present study focused on the regulation of \( I_{\text{Kur}} \) by male sex hormones based on the observation that the gender difference observed in this current was abolished following castration. However, female sex hormones have also been implicated in the regulation of K⁺ currents in a variety of tissues including uterus, smooth and cardiac muscles [2,24,28]. Most of the studies focusing on cardiac K⁺ channels show that acute administration of estrogen decreased K⁺ current density [2,27]. This inhibitory effect of estrogen has been reported for the transient outward (\( I_{\text{to}} \)) [2] and the slow delayed rectifier (\( I_{\text{ks}} \)) K⁺ currents [27]. Theoretically, this action of estrogen could contribute to explain why females have longer cardiac repolarization than males. However, the concentration of estrogen used to elicit such effect exceeded the physiological concentration range. Furthermore, results from our group and other laboratories demonstrated that mouse and rat \( I_{\text{to}} \) display no gender difference [7,14,25]. In addition to its fast action, long-term effects of estrogen on cardiac repolarization have also been studied. Saba et al. [22] studied the effect of ovariectomy and estrogen treatment on mouse ECG parameters. Neither ovariectomy nor estrogen administration affected QT interval. Another group reported prolongation of QT interval and APD in a transgenic mouse model lacking estrogen receptors (ERKO) [11]. The authors suggested that this prolongation was due to increased Ca²⁺ currents but no study was undertaken to verify the implication of K⁺ currents in this phenotype. In summary, estrogens are potential regulators of cardiac repolarization but more studies are required to delineate their functional roles.

### 4.6. Limitations of the study

This study provides important information about gender and strain differences in cardiac repolarization of murine models. However, the fact that mouse and human ventricles share some but not all components of repolarizing K⁺ currents may prevent direct extrapolation of these findings to the human situation. Indeed, although \( I_{\text{Kur}} \) is an important repolarizing K⁺ current in mouse ventricular myocytes, this current is prominent in atrial but not in ventricular myocytes of humans. Nevertheless, these studies are relevant for many reasons. First, similar to human, mouse displays gender differences in ventricular repolarization. Moreover, mouse cardiac K⁺ currents/channels can be regulated by variations in male sex steroid hormones levels and as mentioned earlier, growing clinical evidence also suggests that androgens regulate cardiac repolarization in humans. Thus, the
mouse could be viewed as a useful model to study the general mechanisms of sex-related differences in cardiac repolarization. Furthermore, access to transgenic mice that lack sex steroid hormones receptors provides a valuable tool to further study the actions of these hormones and receptors on cardiac repolarization. It is also possible that in humans the density of different repolarizing K+ currents such as IKr and IKs may be similarly modified by sex steroid hormones. On that matter, ongoing studies in our laboratory are aimed at exploring the cellular and molecular mechanisms underlying gender difference in human repolarization.

In conclusion, this study provides useful insight into how androgens influence basic cardiac electrophysiology. Indeed, our results strongly suggest that there is a clear association between androgen levels and fIKr/Kv1.5 density in adult mouse ventricular myocytes. Moreover, it appears that special attention should be paid to the hormonal status of the strain of mice (or of any other animal models) used when studying hormonal regulation of cardiac repolarization. For similar reasons, it would also be important to ascertain that the animals studied have reached sexual maturity.

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