Developmental changes of cardiac repolarization in rabbits: Implications for the role of sex hormones

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Abstract

Objectives: Firstly, to compare gender-dependent differences of cardiac repolarization in both adult and young rabbits. Secondly, to analyze the effect of gonadectomy on these gender differences in cardiac repolarization. **Methods:** We evaluated potential gender differences in cardiac repolarization with both microelectrode and ECG recordings. QT_{end}, JT_{end}, and $T_{peak\text{-}end}$ intervals and action potential durations at 30%, 50% and 90% of full repolarization were used to assess ventricular repolarization in adult (normal and gonadectomized) and young rabbits of both sexes. **Results:** Adult rabbits exhibited clear gender-related differences in repolarization evidenced by significantly longer JT_{end} and $T_{peak\text{-}end}$ intervals and significantly longer APD30, APD50 and APD90 in females. These gender-related differences in repolarization were absent in young rabbits and were abolished by gonadectomy. **Conclusions:** Developmental changes of repolarization are present in rabbits. These changes are in agreement with those reported in humans and may further support the role played by sex hormones in the modulation of cardiac repolarization.

Keywords: ECG; Gender; Hormones; Membrane potential; Repolarization

1. Introduction

There is an ever-increasing appreciation that cardiac repolarization is highly influenced by gender and gonadal steroids. Sex hormones have been reported to influence action potential duration (APD) in guinea pig, rat and rabbit isolated myocytes [1–4]. However, results are in some way contradictory. Some have shown a decrease in APDs [1,3] while others showed prolongation of repolarization [2,4] after the exposure to different concentrations of 17β-estradiol (between 1 and 100 μM/l) which are several orders of magnitude greater than the physiological level and, therefore, may be without significance in vivo.

Previous research has validated the use of a rabbit model that manifests gender-related differences in repolarization having characteristics similar to those in humans [5–10]. However, experimental data concerning sex differences in electrophysiological properties were derived largely from oophorectomized female adult rabbits long-term treated with gonadal steroids as surrogates for sex-based effects.

In this regard, it has been shown that endocardial APD of oophorectomized female adult rabbits long-term treated with 17β-estradiol was longer than that in oophorectomized female rabbits treated with 5α-dihydrotestosterone [8]. More recently, it has been also shown that gender-dependent differences were present in both control and gonadectomized rabbits, with control and oophorectomized female rabbits exhibiting longer APDs than control and

orchiectomized male rabbits mainly at 30% of repolarization [10].

On the other hand, a developmental component of these sex-related differences in repolarization has been shown in humans [11,12]. Whereas neonates and children before age 10 of both sexes show no difference in QT interval, adult women (from puberty to adulthood) have longer QT interval as compared with adult men. Moreover, it has been proposed that the gender-related differences in repolarization after puberty reflects abbreviation of QT interval in males rather than prolongation in females [13].

In order to gain insight into the mechanisms that underlie the gender-dependent differences in cardiac repolarization two important questions remain to be addressed: firstly, if the known gender-related differences in APDs are present before puberty and secondly, if they can be abolished by gonadectomy. Therefore, in the present study we analyzed the developmental change in cardiac repolarization of both male and female rabbits and compared these data with those obtained after gonadectomy.

2. Methods

2.1. General

A total of 44 New Zealand white rabbits (22 males and 22 females) divided into two groups, 24 adults at an age of 6 months and 20 young rabbits at an age of 30 days when the experiments were carried out, were included in the study. These groups were selected taking into account the evolution of the plasma gonadal hormone levels from birth to 6 months in both male and female New Zealand rabbits [14–16]. Ten of the 24 adult rabbits underwent gonadectomy under sterile conditions at least 2 weeks before they were included in the study. Therefore, the study was carried out in five oophorectomized female rabbits (OVX), five orchiectomized male rabbits (ORCH), seven adult male (AM), seven adult female (AF), 10 young male (YM) and 10 young female rabbits (YF). The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication ≠85-23, revised 1996).

2.2. Experimental protocol

The animals were anesthetized with sodium thiopenthal (20 mg/kg) or ketamine (15 mg/kg) i.v. for the obtention of ECG recordings. The assessment of ECG variables characterizing cardiac repolarization on the body surface was carried out in a precordial lead exhibiting the T wave of greatest amplitude. This lead was localized on the 4th left intercostal space at 2 cm from the esternal border. The ECG variables of repolarization include: the JT interval, measured from the J point to the end of the T wave (JT_{end}), the $T_{peak\text{-}end}$ interval measured from the peak to the end of the T wave and the QT_{end} interval. $T_{peak\text{-}end}$ was included as a measurement that reflects transmural dispersion of repolarization. In order to clarify the relationship between these ECG variables and repolarization from a single endocardial cell during intracellular recordings it can be mentioned that the normal repolarization process of ventricular myocardial cells is not entirely synchronous; some cells recover early and some later, resulting in a normal heterogeneity among ventricular cells in the time to reach full repolarization. Under normal conditions, the shortest AP occurs in the epicardium and the longest in the M region, and the endocardial AP is intermediate. Following endocardial stimulation the epicardium is the last to depolarize but the first to repolarize providing for a T wave displaying the same polarity of the QRS. Therefore, the start of the T wave is caused by the more rapid decline of the plateau of the epicardial AP, creating a voltage gradient between epicardium and the M cells. The gradient increases as the epicardial AP continues to repolarize, reaching a maximum with full repolarization of the epicardium; this point marks the peak of the T wave. Finally, full repolarization of the M cells marks the end of the T wave [17]. On the other hand, heart rate plays a major role among the many sources of variation in repolarization intervals and correction formulas can be applied to normalize them for heart rate. Numerous empirical formulas have been proposed to correct the QT interval for the heart rate at which the interval was measured. Correction formulas were, in general, derived from regression analyses of QT versus RR interval plots using different equations; however, none of these formulas is entirely accurate. Nevertheless, in the present study all the electrocardiographic intervals measuring cardiac repolarization are expressed as absolute values in ms or corrected for heart rate. The correction for heart rate was done with a linear regression model that referred repolarization interval durations to an RR interval of 1000 ms [18]. The procedure applied for all the repolarization intervals was the same and it is exemplified below for QT_{end} interval:

$$QT_{end}c = QT_{end}\,(\text{ms}) + \text{slope of QT/RR relationship} \times (1000 - \text{RR}\,(\text{ms})).$$

The ECG signals were amplified by an ECG amplifier (Gould ECG amplifier) and digitized at 12 bit resolution with a sampling interval of 1 kHz (Lab-PC A/D acquisition board from National Instruments). Simultaneously, the ECG signals were stored on a computer hard disk with custom-made software for posterior analysis. Immediately, the animals were killed by cervical dislocation and the hearts were quickly removed and immersed in Tyrode's solution equilibrated at 37 °C with 100% O_2. The solution contained (mM/l) NaCl 136.5, KCl 2.7, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.33, HEPES 5 and dextrose 5.5. Endocardial tissues from the left ventricle were dissected from males and females and placed together with their endocardial

surface facing up in a 10-ml tissue bath perfused with Tyrode's solution (37 °C, pH 7.4) at a rate of 400 ml/h. The bath was connected to ground with an Ag/AgCl wire. Preparations were impaled with 3 M KCl-filled glass capillary microelectrodes with tip resistances from 10 to 15 MΩ coupled by an Ag/AgCl junction to an amplifier with high-input impedance and input capacity neutralization (WPI KS-700).

Transmembrane AP were displayed on a storage oscilloscope, digitized at a sampling rate of 10 kHz (Lab-PC A/D acquisition board from National Instruments) and simultaneously stored in a computer hard disk with custom-made software for posterior analysis. APs from at least two different sites were obtained in each animal, only those with a resting membrane potential (RP) more negative than −75 mV and a maximum rate of depolarization (V_{max}) greater than 90 V/s were considered for the analysis. A stabilization period of 3 h was permitted during constant stimulation at a cycle length (CL) of 1000 ms. Frequency-dependent changes in the AP characteristics were assessed during drives at CL of 5000, 1000, 500 and 300 ms. Stimulation was accomplished using rectangular pulses of 2 ms in width and twice diastolic threshold. Pulses were delivered with a programmable stimulator and stimulus isolation units through two thin bipolar silver wire teflon-coated electrodes. Acquisition of action potential for analysis was achieved only after steady state at each CL was reached (usually between 3 and 5 min after changing the pacing rate). Resting periods of 5 min at a CL of 1000 ms were interposed between tests.

Resting membrane potential (RP), amplitude of the APs (APA), maximum rate of depolarization during phase 0 (V_{max}) and duration of the APs at 30% (APD30), 50% (APD50) and 90% (APD90) of full depolarization were determined as illustrated in Fig. 1.

2.3. Data analysis

Data are reported as mean±S.E.M., statistical analysis was performed using analysis of variance (ANOVA) and Bonferroni's or Dunnett's test when appropriate. A value of $P<0.05$ was considered significant.

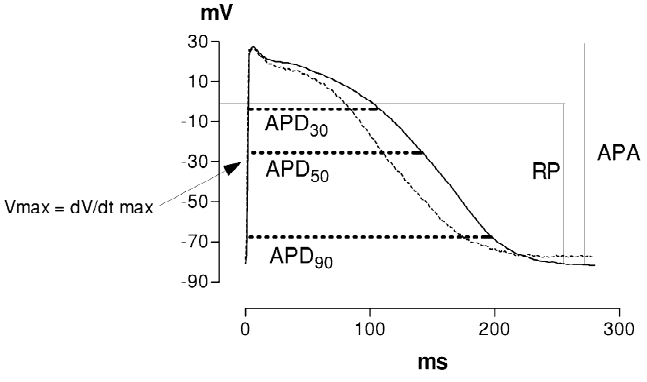

Fig. 1. Representative action potential recordings from AM, AF at a CL of 500 ms illustrating action potential parameters. Solid lines represent female APs while dotted lines represent male APs.

3. Results

3.1. ECG analysis of cardiac repolarization

Electrocardiographic data were obtained to determine whether there is a gender difference in the duration of total repolarization, through the measurement of the baseline QT_{end} and JT_{end} intervals. Mean±S.E.M. data are summarized in Fig. 2. It can be seen that when raw data of ECG recordings were analyzed, AF showed a slightly longer JT_{end} interval than AM. In contrast, gonadectomized rabbits exhibited a slightly longer JT_{end} in ORCH as compared with OVX, while it was similar between YF and YM. Because heart rate was different among the three groups of rabbits (mean±S.E.M. values of RR intervals were 277±17 ms and 281±15 ms for YF and YM, 369±24 ms and 359±29 ms for AF and AM, 304±19 ms and 380±30 ms for OVX and ORCH, respectively), corrected ECG data for heart rate were also analyzed. Clear gender differences can be seen, reflected by a significantly shorter JT_{end}c interval in AM as compared with AF. On the other hand, gonadectomized rabbits exhibited an inverse pattern with a JT_{end}c being significantly longer in ORCH as compared with OVX. When multiple comparisons were made it becomes evident that AM exhibited a shorter repolarization duration than the other groups since AM showed the shortest JT_{end}c as compared with AF, YF, YM, OVX and ORCH. Finally, transmural dispersion of repolarization, reflected by the duration of the $T_{peak\text{-}end}$ interval [17], was significantly shorter in AM as compared with AF. These data are illustrated in Fig. 3.

3.2. Gender differences in action potentials

AP recordings were made from isolated endocardial ventricular strips obtained from the left ventricle of both male and female rabbits. All preparations were driven at CLs between 300 and 5000 ms. No significant differences were found in RP, APA and V_{max} among males and females for the three groups of animals studied. Mean±S.E.M. values are shown in Table 1.

APD was highly dependent on CL in both males and females of young and gonadectomized rabbits. It was maximal at a CL of 1000 ms and significantly decreased at both longer and shorter CLs at any of the three levels of repolarization duration analyzed. On the other hand, adult rabbits exhibited a different behaviour of the CL/APD relationship between males and females. Both AF and AM exhibited APD30, APD50 and APD90 that were maximal at a CL of 1000 ms and significantly decreased at longer

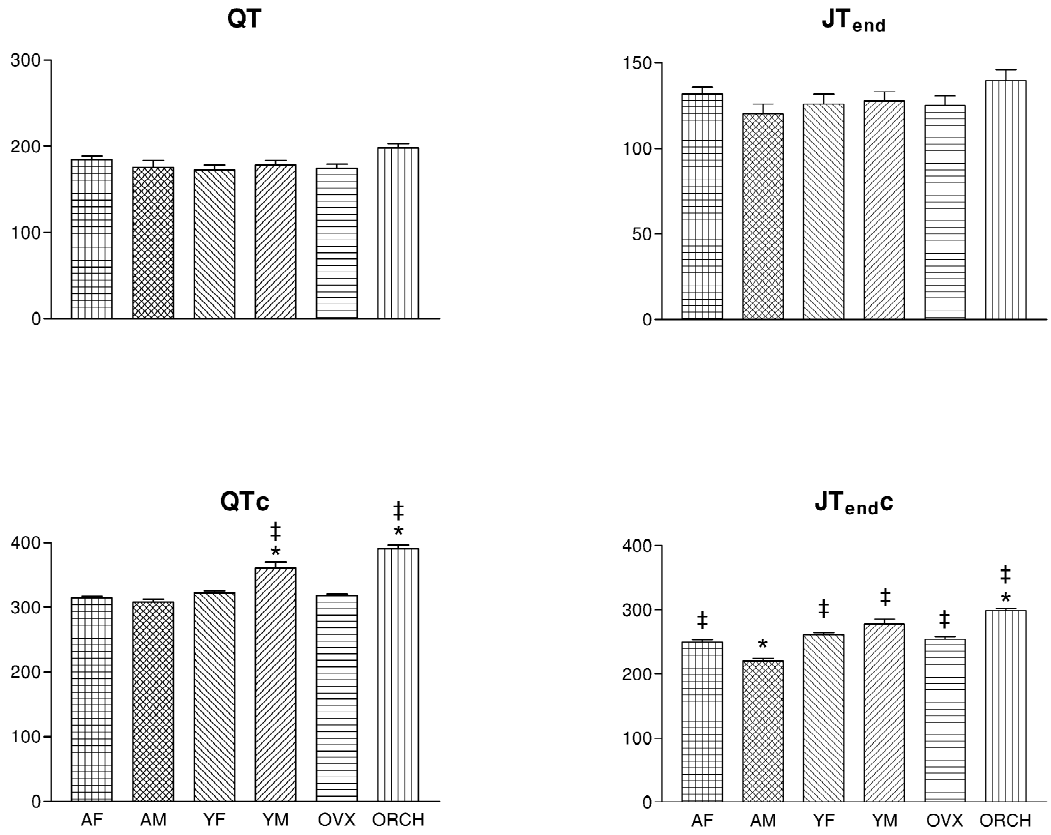

Fig. 2. Mean±S.E.M. values of raw (upper panel) and corrected (lower panel) QT_{end} (left) and JT_{end} (right) intervals. *$P<0.05$ against females; ‡$P<0.05$ against AM.

CLs. In contrast, at shorter CLs, while APD30, APD50 and APD90 significantly shortened between 1000 ms and 300 ms CLs in AF, APDs remained nearly constant as the CLs were decreased from 1000 to 300 ms in AM. These results are summarized in Fig. 4.

There were also clear differences among groups in APDs as a function of gender, as illustrated in Fig. 4. AM exhibited significantly shorter APDs at 30%, 50% and 90% of full repolarization as compared with AF. In contrast, this gender-related difference in the duration of repolarization was not observed between YF and YM or between OVX and ORCH at any of the three levels of repolarization duration analyzed.

4. Discussion

The major findings of our studies are: (1) young rabbits do not exhibit the clear gender-dependent differences in the duration of cardiac repolarization that are present in adult rabbits and (2) these gender-related differences in cardiac repolarization are abolished by gonadectomy.

At the ECG level we found a significantly shorter $JT_{end}c$ interval in AM as compared with AF. In the gonadectomized group, in contrast this pattern was inverted with AM exhibiting the longest $JT_{end}c$. On the other hand, transmural dispersion of repolarization was found to be shorter in males as reflected by a significantly shorter $T_{peak\text{-}end}$ in both AM and YM when compared with AF and YF, respectively.

Our ECG data from adult animals are in agreement with those reported by Liu et al. [6]. These authors showed that the QT interval of female rabbits was significantly longer than that of male rabbits at CLs of 2.3 s but not at CLs of 0.4 s. In our data, the gender differences in repolarization at the ECG level were evident only with the corrected values for heart rate. The presence of a longer $T_{peak\text{-}end}$ interval in AF is consistent with the increase in transmural dispersion of repolarization found by Pham et al. in the same gender [10] and with the increased risk of torsades de pointes found in female [7,9].

We have also shown that APDs differed significantly at 30%, 50% and 90% of full repolarization for CLs longer than 300 ms in adult animals, being shorter in males as compared with females. In contrast, these differences were not present in young rabbits.

Our data are consistent with those reported by Hara et al. who showed that APDs were significantly different at CLs greater than 500 ms, with APs being longer in oophorectomized rabbits treated with estradiol as compared with those treated with dihydrotestosterone at either 30% and 90% of full repolarization. Moreover, APD90 of

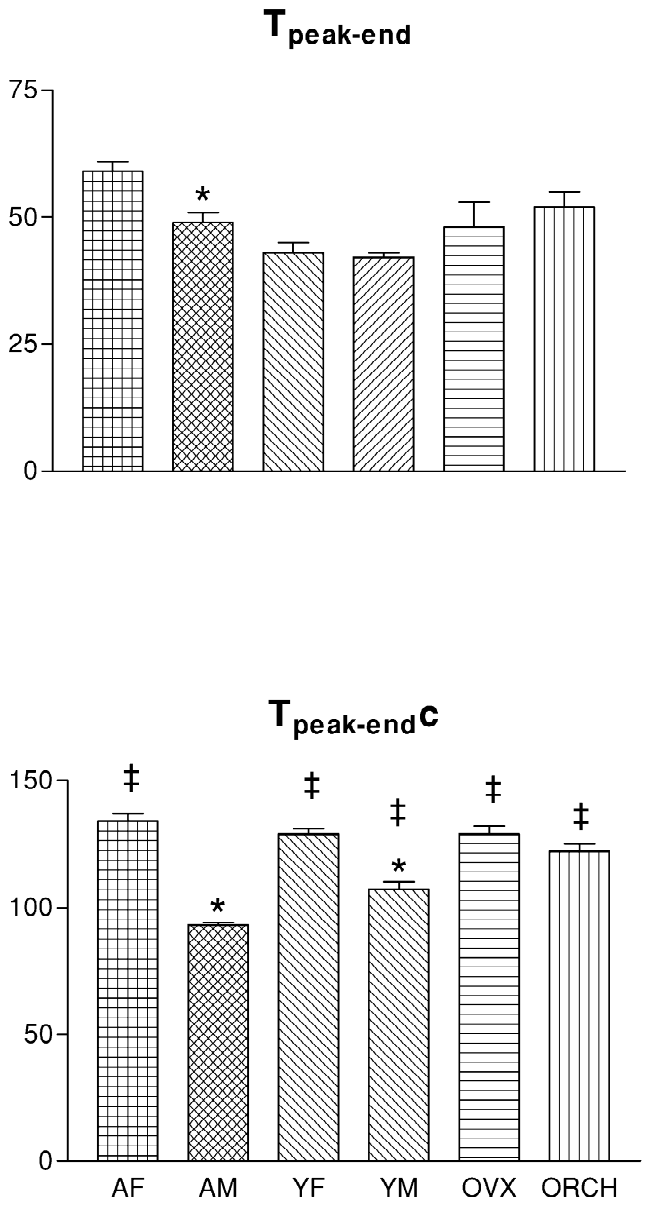

Fig. 3. Mean±S.E.M. values of raw (upper panel) and corrected (lower panel) values of $T_{peak\text{-}end}$ interval. *$P<0.05$ against females; ‡$P<0.05$ against AM.

the estradiol group tended to become longer than that of the oophorectomized group treated with placebo as CL increased [8]. Also, our data showed that both young and gonadectomized rabbits exhibited a similar biphasic APD/CL relationship. APDs were maximal at CLs of 1000 ms and decreased for shorter and longer CLs. The decrease in APD observed at long CLs has been attributed to the kinetics of the recovery from inactivation of the transient outward potassium current (I_{to}) and the decrease in APDs observed at short CLs has been considered dependent on a rate-dependent decrease in Ca current [19,20].

We have also shown that the sex-dependent differences seen in adult rabbits at 30%, 50% and 90% of full repolarization were abolished by gonadectomy. These results differed from those reported by Pham et al. [10], who showed the persistence of sex-related differences in APD30 after gonadectomy and argue against extragonadal factors underlying gender differences in repolarization.

We have previously shown that the ECG pattern of cardiac repolarization differed between men and women and that these differences attenuated as the age of the subjects increased [21]. We have also shown that the ECG pattern of repolarization of women exhibiting a virilization syndrome, due to polycystic ovarian disease or hyperplasia of the adrenal cortex, was significantly different from that of castrated men. However, the differences were the opposite to those seen in the normal population [22]. These findings further suggest the importance of testosterone in accounting for the sex differences in myocardial repolarization. However, APDs from ORCH were not significantly different from those of AM, as it would be expected considering the hypothesis of testosterone-dependent modulation of repolarization. Nevertheless, since ORCH exhibited APDs that were not different from those of AF and considering that APDs from AF were significantly longer than those of AM, it can be concluded that APDs from ORCH were intermediate between AF and AM. It is possible that significant differences between ORCH and AM would be achieved with a greater number of animals included or if the assessment of APD was carried out after a longer time from gonadectomy.

Modulation of APD occurs largely as a consequence of a fine balance among depolarizing and repolarizing currents that are responsible for the generation of the AP. A growing body of experimental evidence, suggesting possible effects of gonadal steroids on K^+ and Ca^{2+} currents, has been reported in recent years. In this regard, Liu et al. demonstrated smaller IK1 and IKr densities in female than in male rabbit ventricle [6]. Also, Dricci et al. demonstrated that chronic treatment with gonadal steroids downregulates potassium channel expression in the rabbit heart. Both mRNA levels of HK2 and IsK were markedly reduced in OVX long-term-treated with estradiol and dihydrotestosterone [5].On the other hand, direct evidence from patch-clamp studies confirmed that testosterone opened K^+ channels in single coronary myocytes or in vascular smooth muscle of the rat aorta [23,24].

The shortening of APD30 and APD50 in AM implies that androgen (specifically testosterone) rather than estrogen contributes to the gender-dependent differences seen in adult rabbits. Therefore it can be postulated that the gender-related differences in cardiac repolarization seen in adult rabbits would be a consequence of a testosterone-dependent difference in the expression and current densities of potassium currents.

The identification of receptors for gonadal hormones in the heart provided a rationale to support the hypothesis of direct hormonal modulation of repolarization. In this regard, androgen receptors have been identified both in atria and ventricles [25] whereas estrogen receptors appear

Table 1
Characteristics of action potentials

	RP (mV)				APA (mV)				V_{max} (V/s)			
	300	500	1000	5000	300	500	1000	5000	300	500	1000	5000
AF	−84	−82	−83	−83	108	110	112	109	187	185	193	202
	±0.9	±1.3	±1.1	±1.4	±2.4	±2.4	±1.6	±1.9	±19.6	±20.5	±19.0	±13.5
AM	−78	−79	−80	−82	103	106	107	107	161	168	172	173
	±1.1	±1.3	±1.7	±1.8	±2.0	±1.4	±1.6	±1.7	±14.6	±14.7	±15.6	±14.2
YF	−83	−84	−84	−84	109	109	111	110	232	238	247	238
	±1.0	±0.9	±0.8	±0.8	±1.3	±2.1	±1.0	±0.7	±25.6	±25.1	±25.8	±23.1
YM	−81	−83	−84	−83	106	109	110	108	214	220	219	218
	±0.9	±0.7	±0.6	±0.8	±0.9	±1.1	±1.1	±1.0	±14.5	±15.1	±15.1	±15.3
OVX	−84	−86	−87	−87	109	112	111	110	218	227	236	219
	±1.9	±1.8	±1.6	1.9	±2.2	±1.8	±1.7	±1.5	±21.4	±18.4	±18.7	±17.5
ORCH	−84	−85	−87	−86	108	109	109	106	167	170	178	173
	±1.1	±1.3	±1.6	±1.6	±1.2	±0.9	±1.0	±1.5	±10.2	±8.1	±9.1	±11.3

to be largely confined to atrial myocytes [26]. The present data may further contribute to support the hypothesis of a genomic mechanism for the modulation of repolarization by gonadal steroids. Therefore and because we acknowledge that an important limitation of the present study would be the lack of testosterone and estrogen plasma level determinations, in another group of 18 rabbits with similar characteristics of those where electrophysiological measurements of repolarization were assessed (less than 30 days from birth for young rabbits, $n=6$; more than 6 months from birth for adult rabbits, $n=6$ and at least 3 weeks from surgery for gonadectomized rabbits, $n=6$), the serum levels of both estradiol and testosterone plasma levels were analyzed. Mean±S.E.M. values of testosterone were 0.25±0.16, 6.6±1.3, 0.04±0.03, 0.38±0.16, 1.47±0.61 and 0.85±0.10 ng/ml for AF, AM, YF, YM, OVX and ORCH, respectively. AM plasma levels of testosterone were significantly different from those of YF,

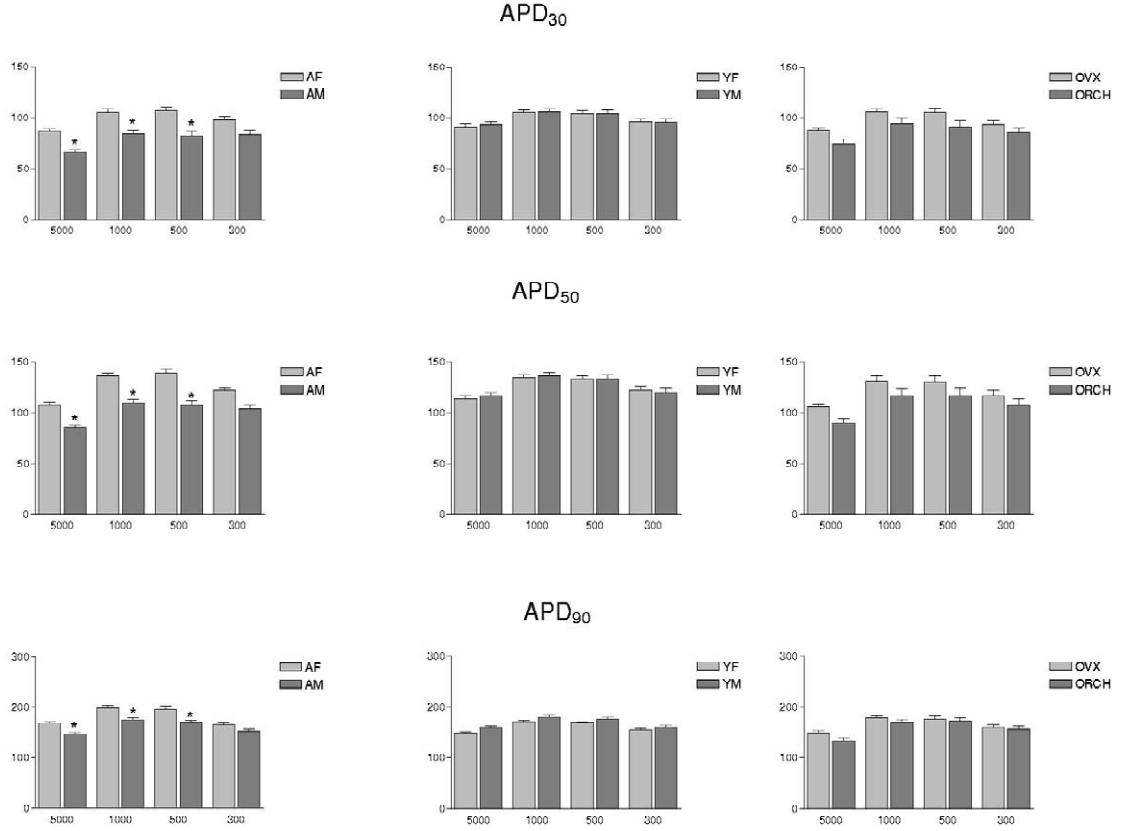

Fig. 4. Gender-dependent differences of APD30, APD50 and APD90 in both adult (left), young (middle) and gonadectomized (right) animals as a function of CL. Only adult rabbits exhibited significant gender-related differences of APD.

YM, OVX and ORCH. On the other hand, estradiol levels were not significantly different among the three groups of rabbits as it was previously reported [14–16]. The significant differences found in the testosterone plasma levels may further support the role that this hormone plays in the modulation of cardiac repolarization. The existence of very low levels of testosterone in young and gonadectomized male rabbits may justify the lack of significant sex-related differences in APDs in these groups of rabbits.

In summary, in the present study we provided experimental evidence demonstrating developmental changes in cardiac repolarization in rabbits that are similar to those described in humans and parallel with the plasma levels of testosterone as a function of age. Our results may help, further, to gain insight into the intricate mechanisms implicated in the effect of gender and sex hormones on ventricular repolarization.

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