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Cardiac action potential duration and calcium regulation in males and females

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ABSTRACT

Adult women have longer QT intervals compared with men of a similar age, indicating differences in the speed of repolarisation of the ventricles. We investigate the influences of gender on ventricular electrophysiology and intracellular Ca^{2+} regulation of the guinea pig heart. Comparing sexually mature animals, females exhibited a significantly longer APD. Peak L-type Ca^{2+} current (I_{CaL}) was larger in females and when this current was inhibited with nifedipine the gender differences in APD were removed. APD differences also disappeared when the SR was depleted of Ca^{2+}. Inactivation of I_{CaL} during a clamp step is faster in females but slower during an action potential and SR Ca^{2+} content is larger. We suggest that gender differences in APD result from variation in the kinetics of I_{CaL} stemming from alterations to Ca^{2+} release.

Introduction

Despite the anatomical similarity of the heart in men and women, gender differences in cardiac physiology and pathophysiology are increasingly recognised. The action potential duration (APD) in female guinea-pigs is longer than that in males [1,2] and gender differences in some underlying ionic currents may be influenced by the stage of oestrus [1]. There also appear to be gender differences in cardiac contraction and cellular Ca^{2+} regulation. Schaible and Scheuer [3] found that the hearts of male rats had greater ejection fractions and fractional shortening than those from their female counterparts. At the cellular level, the Ca^{2+} transient is smaller [4,5] and the rate of cellular relaxation slower [4] in ventricular myocytes from female rats compared with males.

The subcellular mechanisms underlying these gender differences in cardiac contractile function are not understood. In this study we measure action potential shape and duration and correlate this with the L-type Ca^{2+} current (I_{CaL}), a main current that underlies the action potential plateau. Since the length of the action potential influences the amount of Ca^{2+} stored in the sarcoplasmic reticulum (SR) and therefore the amount of Ca^{2+} available for contraction we also investigated how SR content varies with gender.

Methods

Animals. We used male and female Dunkin Hartley guinea-pigs since their ECG [6], the ionic currents shaping the ventricular action potentials and excitation–contraction coupling in this species are similar to humans [7,8]. Peak plasma testosterone in guinea pigs is achieved at 7 weeks of age equivalent to 530 g in weight so we used males over this weight and designated them as being sexually mature [9]. Females were designated mature if their weight was greater than 330 g, this being the average weight at first oestrus [10]. Mature female animals were chosen at random stages of their oestrus cycle. The investigation conforms to 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).

Myocyte isolation. Left ventricular myocytes were isolated using a modification of a previous method [11]. Myocytes were stored in Dulbecco's modified Eagle's medium solution at room temperature and used within 8 h.

Experimental solutions. Cells were dropped onto laminin-coated glass coverslips that formed the floor of a low volume chamber on the stage of an inverted microscope. They were superfused with normal Tyrode (NT) at 37 °C which had the following composition (mM): NaCl 140, KCl 6, glucose 10, HEPES 10, $MgCl_2$ 1 $CaCl_2$ 2, pH 7.4. Nifedipine was prepared as a 20 mM stock solution dissolved in ethanol, protected from light and added to NT as required. Chromanol 293B was prepared as a 30 mM stock solution dissolved in DMSO. The final concentrations of ethanol (0.0001% [vol/vol]) and DMSO (0.001% [vol/vol]) had no significant effects on membrane currents. Caffeine was added as a solid to the NT solution.

Electrophysiology. Electrophysiological parameters were measured using an Axoclamp-2B system and pCLAMP software (Axon Instruments). To preserve the intracellular milieu, cells were impaled with sharp (30–50 MΩ) micropipettes filled with a solu-

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tion containing 2 M KCl, 5 mM HEPES, pH 7.2. Current amplitudes were normalised to cell capacitance. Action potentials were elicited with 10 ms suprathreshold current pulses (typically 1.5 nA) applied at a rate of 1 Hz. Action potentials were measured in NT and in the presence of 2 μM nifedipine after steady state had been achieved (between 30 and 70 stimuli). Action potential duration measurements are expressed as the time to 50 (APD_{50}) and 90% (APD_{90}) repolarisation. Voltage-clamp experiments were performed using the Axoclamp in discontinuous single electrode voltage clamp mode. Gain (0.3–0.8 nA/mV) and switching rate (4–6 kHz) were advanced to maximum values allowing complete settling of current without overshoot. L-type Ca^{2+} current (I_{CaL}) was measured by holding at −40 mV and applying 200 ms long steps in 10 mV increments from −40 to +50 mV at 0.1 Hz. Each test step was preceded by a short SR loading protocol of five steps (each of 200 ms duration) from −80 to 30 mV applied at 0.5 Hz. I_{CaL} was measured as nifedipine-sensitive current. The inactivation phases of I_{CaL} were best fitted by double exponential decays.

Inward rectifying K^+ current (I_{K1}) was measured as Ba^{2+} (1 mM) sensitive current using a voltage ramp (1.6 mV/s) from −100 to +20 mV. Delayed rectifier currents were measured by holding at −50 mV and applying 3 s steps in 10 mV increments from −40 to +50 mV at 0.2 Hz. I_K was separated into chromanol-sensitive (I_{Ks}) and insensitive (I_{Kr}) currents using 30 μM chromanol-293B to selectively block I_{Ks}. In action potential clamp experiments the action potentials used as commands were recorded from male and female cells best representing the average mature male and female action potential profiles. To maintain consistent cellular Ca^{2+} loading, a drive train consisting of 10 complete average male or female action potentials preceded the final action potential which was interrupted at 30%, 50%, 70% and 90% repolarisation and clamped to −40 mV for 2 ms followed by a step to 0 mV for 200 ms at which point it was clamped back to the command resting membrane potential.

SR load measurements. SR Ca^{2+} load was measured by integration of the caffeine-induced inward Na^+/Ca^{2+} exchanger current (I_{NCX}). Prior to initial caffeine exposure, myocytes were subjected to a loading protocol consisting of a train of current pulses applied at 0.5 Hz eliciting 20 action potentials. After the action potential train, myocytes were voltage clamped to their resting membrane potential and 20 mM caffeine in NT rapidly applied. To assess differences in SR Ca^{2+} reloading, myocytes were subjected to caffeine re-exposure following a shorter loading protocol consisting of a train of four action potentials.

Statistical analysis. All data are expressed as mean ± SEM and *N* is the number of cells. When appropriate, statistical analyses were made using the one or two tailed unpaired Student's *t*-test or an ANOVA with the Bonferroni post-test. A *P*-value <0.05 was taken as statistically significant.

Results

Action potential duration

We measured APD in cells isolated from mature males and females and confirmed that females have a longer APD (Fig. 1) both at APD_{50} (males = 142 ± 5 ms, $N = 81$; females = 185 ± 5 ms, $N = 156$, $P < 0.001$) and APD_{90} (males = 221 ± 5 ms, $N = 81$; females = 255 ± 5 ms, $N = 156$, $P < 0.001$). Cell capacitances were not significantly different ($P > 0.05$) between males (220 ± 8 pF, $N = 74$) and females (207 ± 6 pF, $N = 119$) and there was no dependency of APD on cell size. The peak amplitudes of the action potentials were not different (males = 61 ± 2 mV, female = 65 ± 2 mV; $P > 0.05$).

Action potentials measured in nifedipine

Action potential shortening was observed following application of 2 μM nifedipine (Fig. 1B) in both males and females, but shortening was greater in females such that the previously identified gender differences disappeared at APD_{50} (Fig. 1C) and reversed at APD_{90} (Fig. 1D).

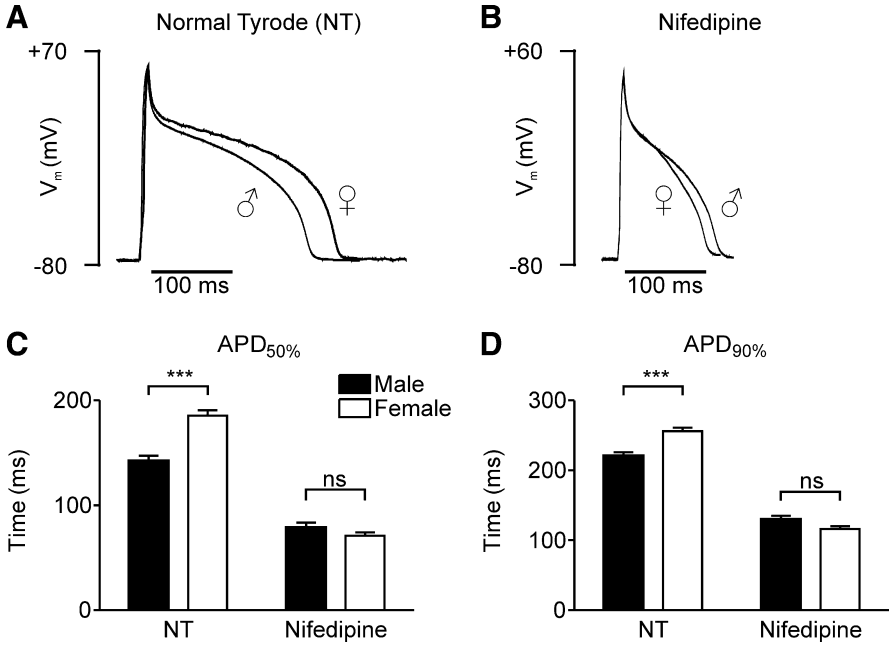

Fig. 1. Differences in action potential duration in male and female cardiac myocytes. (A) Shows example traces under normal conditions. (B) Shows the effect of application of nifedipine. (C and D) Show mean data of the duration at 50% and 90% repolarisation, respectively.