

Effects of Species-Dependent Differences in Action Potential Shape in Setting β -Adrenergic-Stimulation Induced Current

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Abstract

In canine (D) and human, but not guinea pig (GP), ventricular myocytes, a spike-and-dome profile (SaD), supported by I_{to} , characterizes ventricular repolarization. β -adrenergic stimulation (by isoprenaline, ISO) shortens action potential (AP) duration (APD) in D (and human) myocytes, but prolongs it in GP ones.

Aim: The aim of this work is to clarify whether SaD is the main factor determining the direction of APD response to β -adrenergic stimulation.

Methods: AP-clamp with D epicardial, D endocardial, and GP waveforms was applied at different diastolic intervals (DI) to measure ISO-induced current (I_{iso}) in GP myocytes. Dynamic Clamp was used to test the effect of I_{to} introduction, and of the resulting SaD, on ISO modulation of GP repolarization.

Results: In AP-clamp at DI 1750 ms, I_{iso} was more inward with both D and GP waveforms. In Dynamic-Clamp, SaD introduction failed to change the direction of ISO-induced APD changes in GP myocytes.

Conclusions: SaD profile alone may not account for differences between D and GP in terms of APD response to β -adrenergic-stimulation. Further differences of AP profile and/or diverse contributions of Ca^{2+} and K^+ currents between the two species may be involved.

1. Introduction

In cardiac myocytes, the action potential (AP) repolarization profile is species-dependent, reflecting differences in the underlying ionic currents. In human and canine (D) myocytes, but not guinea-pig (GP) ones, a spike-and-dome (SaD) profile characterizes initial repolarization. SaD consists of fast initial repolarization (supported by I_{to}), followed by a secondary depolarization, caused by the sum of I_{to} decay (inactivation) and ensuing I_{CaL} (re-activation). I_{to} expression and SaD prevail in sub-epicardial myocytes

and, mainly because of slow I_{to} recovery, SaD magnitude is rate-dependent. The β -adrenergic agonist isoproterenol (ISO) elicits opposite effects on AP duration (APD) depending on cell type and pacing rate [1]. In canine epicardial (DEPI) myocytes, with large SaD, ISO shortens APD; this is associated with marked elevation of the whole plateau phase. In canine endocardial (DENDO) myocytes, with small SaD, ISO leaves APD almost unchanged, with the dome only mildly elevated [1]. In GP myocytes, ISO prolongs APD with only minor effects on the early plateau level [2]. ISO effects on APD are larger at slow pacing rate, thus matching SaD rate-dependency. AP contour results from a feed-back interplay between current and membrane potential, the effect of current modulation may well depend on the initial AP contour. Therefore, the correlation between SaD magnitude and APD response to ISO suggests that the two aspects might be causally related [1]. However, this hypothesis has never been directly tested. The aim of this work is to clarify whether the AP contour, in particular the presence of the SaD, may account for APD variations under β -adrenergic stimulation. If this was the case, conditions or interventions affecting SaD magnitude would be expected to deeply affect APD response to sympathetic activation, a conclusion of practical relevance.

2. Methods

This investigation conforms to the Guide to the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and to the guidelines for Animal Care endorsed by the Universities of Milan and Debrecen.

Adult Dunkin-Hartley GP were anesthetized by 100 mg/kg sodium thiopental and euthanised by cervical dislocation. Ventricular myocytes were isolated by using a retrograde coronary perfusion method previously published [2], with minor modifications.

Adult beagle dogs were anesthetized with intramuscular

injection of 10 mg/kg ketamine HCl + 1 mg/kg xylazine HCl. Ventricular myocytes were dissociated by using the segment perfusion technique [1].

2.1. Membrane potential and current measurements

Measurements were performed by the patch-clamp technique in the whole-cell configuration at 36.5 ± 0.5 °C. The pipette solution contained (mM): 100 K⁺ Aspartate, 45 KCl, 0.4 CaCl₂, 3 MgCl₂, 5 HEPES, 1 EGTA, 0.4 GTP-Na⁺, 5 ATP-K⁺, and 5 creatine phosphate Na⁺, adjusted to pH 7.2 with KOH. Extracellular solution was composed (mM) by 144 NaCl, 5.6 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 5 HEPES, 11 Glucose. pH was adjusted to 7.4 with NaOH. ISO was dissolved in this solution to obtain 10 nM concentration. Membrane capacitance (126.4 ± 19 pF) and series resistance were measured in every cell.

2.2. Current-clamp experiments

GP, DEPI and DENDO myocytes were paced at cycle length (CL) of 2000 ms. Action potentials were recorded (I-clamp mode; I=0) before and during steady-state exposure to ISO (10 nM). APD was measured at 90% repolarization.

Average AP waveforms were obtained from at least 7 cells for each group after normalizing each record time-scale to APD measured at 90% repolarization.

2.3. Action-potential clamp

Average AP waveforms from the different myocyte types (GP, DEPI and DENDO) were applied in V-clamp mode, at the relevant CLs, to GP myocytes. To avoid any influence of diastolic interval (DI), the latter was kept constant, i.e. made independent of APD changes, by suitably adjusting CLs. At steady-state stimulation, ISO (10 nM) was applied and ISO-induced current (I_{iso}) was evaluated by subtraction (I in ISO - I in control) [3].

2.4. Dynamic clamp

GP myocytes do not express I_{to} . A modelled I_{to} , suitable to reproduce canine SaD, was injected in GP myocytes by the dynamic-clamp technique [4]. In brief: an I_{to} numerical model was derived from Heijman's one [5], by considering only the I_{to1} component and adjusting parameters to reproduce in GP myocytes the SaD profile peculiar of DEPI myocytes at a CL of 2000 ms. Electrical activity was recorded from the GP myocyte at CL 2000 ms, fed to the I_{to} model; the modeled current was injected in the myocyte in a virtually real-time loop. The response of my-

ocyte AP to ISO could thus be recorded in the absence and presence of the SaD profile respectively.

3. Results

3.1. Current-clamp experiments: ISO effects on APD

This set of experiments tests the effect of ISO on spontaneously generated AP profiles. Under this setting, reproducing the physiological condition, the feed-back between membrane current and potential is intact; thus both primary and secondary effects contribute to set the final AP contour (closed-loop condition). At CL 2000 ms, the effect of ISO on APD differed among GP, DEPI and DENDO cardiomyocytes. In GP myocytes ISO prolonged APD by $21 \pm 2.5\%$ ($p < 0.05$). In DEPI myocytes ISO shortened APD by $15.4 \pm 3.3\%$ ($p < 0.05$). In DENDO myocytes, ISO did not affect APD significantly ($+1.58 \pm 4.8\%$; NS).

3.2. AP-clamp experiments: ISO effects on membrane current

This set of experiments tests the effect of AP profile on the primary ISO-induced modification of membrane current, expressed as I_{iso} . As the AP profile is clamped, it cannot feed-back on current to determine its secondary changes (open-loop condition). Repetition CL was adapted to keep DI constant at 1750 ms at the value observed in control at CL of 2000 ms. At this preliminary stage, AP-clamp data are available for GP myocytes only.

At DI 1750 ms I_{iso} profile markedly differed between native (GP) or canine waveforms. For all waveforms I_{iso} had an early inward ($-I_{iso}$) and a late outward ($+I_{iso}$) component. Under the GP waveform I_{iso} occurred through most of repolarization, and peaked at the transition between phases 2 and 3. $+I_{iso}$ was observed only during terminal phase 3. Under canine AP waveforms, I_{iso} appeared as a transient just after the AP upstroke, and it was significantly larger for the DENDO than for the DEPI waveform (mean $-I_{iso}$ 5.68 ± 0.67 vs 3.73 ± 0.53 pA/pF; $p < 0.05$). $+I_{iso}$ appeared close to phase 2-3 transition (i.e. when $-I_{iso}$ was maximal under the GP waveform) and was comparable between DEPI and DENDO waveforms (mean $+I_{iso}$ 0.49 ± 0.07 vs 0.45 ± 0.12 pA/pF, NS).

3.3. Dynamic-clamp experiments: impact of SaD profile on APD response to ISO

In this set of experiments, albeit membrane current is changed by modelled current injection, the myocyte AP is recorded in I-clamp mode. Therefore this is again a closed-loop condition. In control conditions, introduction of the

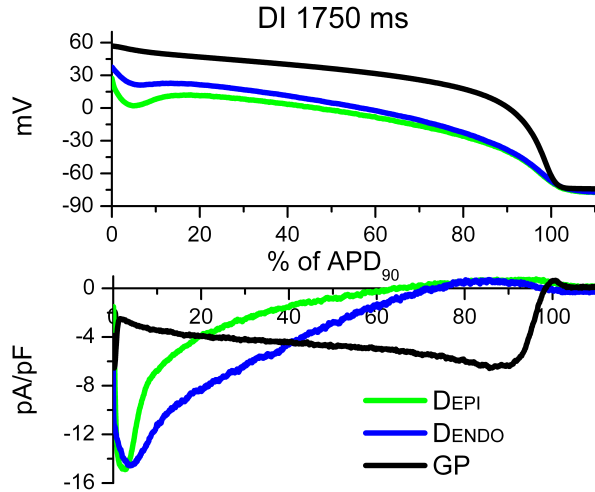


Figure 1: Sample traces of I_{iso} recorded under GP (black), DENDO (blue) and DEPI (green) AP waveforms at DI 1750.

DEPI-like SaD profile shortened GP APD by $5.7 \pm 3.4\%$, a result consistent with the APD prolonging effect of I_{to} blockade in canine myocytes [6]. Thus far, only preliminary data are available for the impact of SaD profile on ISO-induced APD modulation in GP myocytes at a CL of 2000 ms. According to these data, ISO prolonged GP APD similarly whether or not the SaD profile was introduced ($+20.7 \pm 1.8\%$ vs $+21.4 \pm 2.5\%$; NS).

4. Discussion

The data obtained can be summarised as follows: 1) in closed-loop conditions, ISO effects on APD are opposite between GP and DEPI myocytes; DENDO has an intermediate response; 2) in open-loop conditions, within a single species (GP) I_{iso} profile under GP, DEPI and DENDO waveforms substantially differs. The differences are concordant with the species-difference observed in close-loop conditions (i.e. the transition between inward and outward I_{iso} occurs earlier during the AP, as expected to shorten APD, when canine waveforms are applied); 3) introduction of the SaD profile is not adequate to switch GP APD response to ISO from prolongation to shortening.

4.1. Closed-loop (I-clamp) vs open-loop (AP-clamp) ISO effects

In GP myocytes, ISO changed plateau potentials only slightly, but markedly prolonged APD. This is consistent with $-I_{iso}$ being relatively small, but persisting almost to repolarization end. In canine myocytes, ISO-induced APD shortening correlates with the earlier switch between inward and outward I_{iso} , but occurs in spite of the prominent

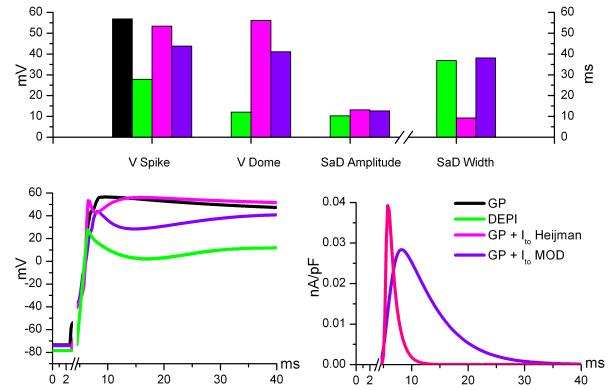


Figure 2: Comparison of SaD parameters in GP, DEPI, and GP + two different examples of I_{to} injection. To better shape GP AP towards DEPI one, we modified the Heijman I_{to} model to match some key parameters of the DEPI SaD. *V Spike*: voltage peak reached by the upstroke. *V Dome*: voltage peak reached by the dome. *SaD Amplitude*: electrical span of the SaD. *SaD Width*: temporal extension of the SaD.

inward I_{iso} observed during the plateau phase. Although with differences in current magnitude, the I_{iso} pattern, shown here when DEPI waveforms were applied to GP myocytes, is very similar to that previously shown by applying DEPI waveforms to the canine myocytes from which they were recorded [1]. Therefore, I_{iso} pattern (or time-course) seems to be dictated mostly by AP profile, rather than by species differences in the kinetics of repolarizing currents. On the other hand, I_{CaL} density is larger in GP myocytes [7] [8], thus accounting for the larger inward I_{iso} observed in GP (present data) as compared to dog [1]. Repetition of AP-clamp experiments in canine myocytes under conditions strictly uniform with those of the present study is currently ongoing to confirm this preliminary conclusion. The mismatch between the prominent $+I_{iso}$ occurring during the plateau of DEPI waveform (open-loop) and ISO-induced APD shortening (closed-loop) necessarily calls into play secondary changes occurring as a consequence of membrane potential feed-back on current. Large inward I_{iso} is consistent by the observed ISO-induced plateau elevation; we speculate that the latter may in turn enhance I_{Ks} activation, thereby leading to overall APD shortening. Specifically designed experiments are required to confirm this hypothesis.

4.2. Role of the SaD in determining ISO effects on APD

Although preliminary, the present results seem to negate a central role of SaD in dictating APD response to ISO.

This is apparently in contrast with [1] the recent observation that in midmyocardial myocytes I_{to} blockade (by 4-AP), and the consequent reduction of SaD magnitude, blunts ISO-induced APD shortening. While the role of 4-AP ancillary effects cannot be ruled out, the discrepancy deserves a more careful discussion. In the present experiments, SaD was introduced in the context of a GP action potential, featuring a plateau phase substantially positive as compared to that of DEPI myocytes. Moreover, whereas ISO globally elevated the plateau in DEPI myocytes, it only affected its repolarization velocity in GP myocytes. The impact of SaD may theoretically differ between the two contexts. Furthermore, as judged from the differences in I_{iso} profile between GP and canine waveforms (Figure 1), SaD, possibly in combination with the lower plateau, strongly enhances early inward current, but it accelerates its reversal, an effect indeed compatible with APD shortening. Therefore, from the present preliminary data we can at best conclude that SaD insertion per se is not adequate to shift APD response in GP myocytes. This, in fact, does not negate a role of SaD (and I_{to}) in ISO-induced APD shortening in canine myocytes. Experiment designed to assess the role plateau level in determining APD response to ISO are now ongoing. The previously reported contribution of SaD in setting I_{CaL} time-course [8] is confirmed by the present AP-clamp recordings (Figure 1) showing larger but more quickly reversing inward I_{iso} under canine AP waveforms.

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