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Original article

I_{Ks} restricts excessive beat-to-beat variability of repolarization during beta-adrenergic receptor stimulation

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ABSTRACT

In vivo studies have suggested that increased beat-to-beat variability of ventricular repolarization duration (BVR) is a better predictor of drug-induced torsades de pointes than repolarization prolongation alone. Cellular BVR and its dynamics before proarrhythmic events are poorly understood. We investigated differential responses of BVR in single myocytes during I_{Ks} blockade versus I_{Kr} blockade and late-I_{Na} augmentation, under the influence of β-adrenergic receptor stimulation. Transmembrane action potentials were recorded from isolated canine left-ventricular midmyocytes at various pacing rates. I_{Ks} was blocked by HMR1556, I_{Kr} by dofetilide. Late I_{Na} was augmented by sea anemone toxin-II. Isoproterenol was added for β-adrenergic receptor stimulation. BAPTA-AM buffered intracellular Ca^{2+}. SEA0400 partially inhibited the Na^{+}–Ca^{2+} exchanger. BVR was quantified as variability of action-potential duration at 90% repolarization: $\Sigma(|APD90;\ i+1\ \text{minus}\ APD90;\ i|)/[nbeats\times\sqrt{2}]$ for 30 consecutive action potentials. Baseline BVR was significantly increased by I_{Kr} blockade and late-I_{Na} augmentation, especially at slow pacing rates. β-adrenergic stimulation restabilized these BVR changes. In contrast, I_{Ks} blockade caused very little change in repolarization when compared to baseline conditions, but predisposed the myocyte to increased BVR during β-adrenergic stimulation, especially at fast rates. BAPTA-AM and SEA0400 reduced this excessive BVR and eliminated early afterdepolarizations. In conclusion, β-adrenergic receptor stimulation exaggerates BVR during I_{Ks} blockade, indicating a BVR-stabilizing role of β-adrenergic-sensitive I_{Ks}. Loss of I_{Ks} plus overriding of Ca^{2+}-dependent membrane currents, including inward Na^{+}–Ca^{2+} exchange current, conspire to proarrhythmic BVR under these conditions.

1. Introduction

Beat-to-beat variability of ventricular repolarization duration (BVR) occurs as an apparently random alteration of the repolarization duration (measured from transmembrane or monophasic action potentials (APs), local activation-recovery intervals or QT intervals) in consecutive heart beats at stable rates. Proarrhythmic conditions caused by I_{Kr} blockade [1–3], I_{Kr}-plus-I_{Ks} blockade [3] and late-I_{Na} augmentation [4] are characterized by significant increases of BVR. When increased, BVR is a better predictor of drug-induced torsades de pointes (TdP) than repolarization prolongation alone, at least in canine and rabbit models [1–3], and in selected human patients [5].

In the case of selective I_{Ks} inhibition *in vivo*, BVR remains unchanged despite mild QT prolongation [3,6]. However, upon the addition of intense β-adrenergic receptor stimulation (βAS) pronounced repolarization instability occurs, as evident from amplified BVR, and this precedes the triggering of early afterdepolarizations (EADs), ventricular extrasystoles and TdP [6]. Various antiarrhythmic interventions (e.g., intravenous KCl administration, $I_{K,ATP}$ activation, steady-state ventricular pacing) that prevent TdP are characterized by stabilization of BVR when often the QT interval is still prolonged [7]. Collectively, these data indicate that a prominent rise of BVR heralds torsadogenic instability of the heart, whereas its decline is an expression of restabilization of electrical activity.

Cellular studies have revealed that the mechanisms underlying BVR reside, at least partly, in the cardiac myocyte. Under baseline conditions at fixed-rate pacing, isolated ventricular myocytes express temporal (i.e., beat-to-beat) variability of the action-potential duration (APD) [1,8]. A direct relationship exists between rate-dependent APD and BVR, even during random changes in pacing rate [9]. Pharmacological interventions to influence ion channels that operate during the AP plateau can markedly alter baseline BVR. I_{Kr} blockade [1,8,10] not only increases the cellular APD, but also BVR, while promoting the generation of EADs. Similar effects were noted for late-I_{Na} augmentation with sea

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anemone toxin-II (ATXII) [11]. Conversely, the blockade of late-I_{Na} with tetrodotoxin [8] or ranolazine [11,12] largely suppresses these proarrhythmic repolarization changes. Finally, intracellular Ca^{2+} chelation with EGTA reduces BVR in myocytes [8].

In contrast, little is known about the contribution of the potassium current I_{Ks} to BVR stability in cardiac myocytes. I_{Ks} function is prominent during βAS when it promotes AP shortening by increased protein-kinase-A-dependent activation, and during I_{Kr} block when it limits AP prolongation by time-dependent activation [10,13,14], thus providing critical "repolarization reserve" when other repolarizing currents are impaired [15,16]. BVR exaggerates significantly when I_{Ks} is inhibited after AP preprolongation with the I_{Kr} blocker almokalant [10].

In this study we investigated the properties of BVR in single canine left-ventricular (LV) myocytes during superfusion with standard-buffer solution containing physiological concentrations of ions (baseline) and the differential responses of BVR during selective I_{Ks} blockade versus I_{Kr} blockade and late-I_{Na} augmentation, in the absence or presence of βAS. Our findings indicate a protective role of I_{Ks} in preventing excessive BVR and EAD generation during βAS, and a mechanistic contribution of inward Na^+–Ca^{2+} exchange to these proarrhythmic sequelae.

2. Materials and methods

This investigation 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). Animal handling was in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU).

2.1. Cell-isolation procedure

Twenty-three adult female beagle dogs weighing 12.8 ± 0.3 kg (range: 10.5–15.0 kg; 9–31 months of age) were used for the myocyte isolations. Anesthesia was induced with 45 mg/kg pentobarbital. Once full anesthesia was reached, the chest was opened via a left thoracotomy and the heart was excised and placed in an O_2-gassed Ca^{2+}-free standard buffer solution at approximately 4 °C. The cell-isolation procedure was the same as previously described [17]. Briefly, the left anterior descending coronary artery was cannulated and perfused. After ~20 min of collagenase perfusion and subsequent washout of the enzyme, the epicardial surface layer was removed from the LV wedge until a depth of ≥3 mm was reached. Softened tissue samples were collected from the midmyocardial layer underneath while contamination with the endocardium was avoided. Samples were gently agitated, filtered and washed. LV midmyocytes were stored at room temperature in standard buffer solution and only quiescent rod-shaped cells with clear cross-striations were used for the experiments.

2.2. Cellular recordings

Transmembrane APs were recorded at 37 °C bath temperature using high-resistance (30–60 MΩ) glass microelectrodes filled with 3 M KCl. Intracellular pacing was done at various cycle lengths (CLs) and only cells that showed a spike-and-dome AP morphology were accepted for the experiments. Myocyte contractions were recorded with a video edge motion detector (Crescent Electronics, Sandy, UT, USA).

2.3. Solutions and agents

The standard buffer solution used for the experiments was composed of (in mM): NaCl 145, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 11 and HEPES 10, pH 7.4 with NaOH at 37 °C. HMR1556 ((3R,4S)-(+)-*N*-[3-hydroxy-2,2-dimethyl-6-(4,4,4-trifluorobutoxy)chroman-4-yl]-*N*-methylmethanesulfonamide) was used to block I_{Ks}. At 500 nM it blocks the current completely [10], while exerting minimal, if any, effects on I_{Kr} and other ion currents [6,18,19]. I_{Kr} was selectively blocked by 1 μM dofetilide (Apin Chemicals, Abingdon, United Kingdom). Late I_{Na} was augmented by 20 nM ATXII (Sigma-Aldrich, Zwijndrecht, The Netherlands). Intracellular Ca^{2+} was buffered with 5 μM BAPTA-AM (Invitrogen, Breda, The Netherlands). The Na^+–Ca^{2+} exchanger was inhibited by 300 nM or 1 μM SEA0400. At these concentrations SEA0400 has little effect on I_{CaL}, the cytosolic Ca^{2+} transient or contraction of canine ventricular myocytes, whereas it blocks Na^+–Ca^{2+} exchange by 28-80% (reverse mode > forward mode) depending on cytosolic Ca^{2+} concentration [20,21]. HMR1556, dofetilide, ATXII, BAPTA-AM and SEA0400 were initially dissolved in dimethyl sulfoxide and then diluted so that the concentration of the solvent was <0.1% in the superfusate, a concentration that has no measurable effects on AP or ionic currents. βAS was applied as 100 nM isoproterenol. This agent was originally dissolved in distilled water containing 30 μM ascorbic acid and then stored in the dark at 4 °C until use.

2.4. Data analysis and statistics

BVR was quantified as variability of APD at 90% repolarization (APD90): Σ(|APD90; i + 1 minus APD90; i|) / [nbeats × √2] for 30 (or a minimum of 10) consecutive APs [1]. In addition, we calculated the coefficient of variability (CV) of APD90 as the percentage of standard deviation/mean APD90 [8].

Quantitative data are expressed as mean ± SEM. Intergroup comparisons were made with Student's *t* test for unpaired and paired data groups, after testing for the normality of distribution. Differences were considered statistically significant if $P<0.05$.

3. Results

3.1. Baseline characteristics of BVR in canine LV myocytes

Figure 1A shows a histogram of 300 consecutive APD90s in a representative contracting myocyte during pacing at a CL of 1000 ms. APD90 was normally distributed about a mean of 276 ms with a SEM of 0.5 ms. Similar results were obtained from 13 cells. A correlation coefficient of 0.90 was calculated between the histogram and a normal Gaussian fit. This indicated that APD90 variability within the canine myocyte occurs randomly, which is consistent with previous data from guinea pig [8]. In 33 cells, pacing rate was varied between CLs of 350 and 4000 ms. APD90 increased on slowing of the pacing rate, as expected [22]. Single representative and pooled APD90 data are shown in Fig. 1B.

BVR averaged 5.9 ± 0.5 ms at CL 1000 ms (Fig. 1B). Significant increases were calculated at both the fastest and slowest pacing rates; at CL 350 ms BVR was 10.3 ± 2.3 ms and at CL 4000 ms 11.3 ± 1.3 ms (both $P<0.05$ vs. CL 1000 ms). Similar patterns were found for CV. At CL 1000 ms CV averaged 2.6 ± 0.2%, increasing to 4.7 ± 0.8% and 4.4 ± 0.5% at the same extreme rates (both $P<0.05$ vs. CL 1000 ms; Fig. 1B). Thus, at (very) fast pacing, rate-dependent adaptation of APD90 (further decrease) was clearly discordant with that of both BVR and CV (increases). Beat-to-beat alternans of both APD90 and contraction amplitude explained this discordance, as demonstrated in Fig. 1C, left panel. APD90 alternans occurred as a very ordered process (interchanging between two values), whereas BVR was apparent as a more random APD90 behavior, generally at slower rates (Fig. 1C, right panel; same cell). This is further exemplified in the Poincaré plots of Fig. 1D. These data suggest distinct mechanisms for BVR and APD90 alternans.

Both BVR and CV expressed similar patterns of rate-dependent repolarization variability. As BVR incorporates the beat-to-beat