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Ca^{2+}-activated Cl^- current is antiarrhythmic by reducing both spatial and temporal heterogeneity of cardiac repolarization

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ABSTRACT

The role of Ca^{2+}-activated Cl^- current ($I_{Cl(Ca)}$) in cardiac arrhythmias is still controversial. It can generate delayed afterdepolarizations in Ca^{2+}-overloaded cells while in other studies incidence of early afterdepolarization (EAD) was reduced by $I_{Cl(Ca)}$. Therefore our goal was to examine the role of $I_{Cl(Ca)}$ in spatial and temporal heterogeneity of cardiac repolarization and EAD formation.

Experiments were performed on isolated canine cardiomyocytes originating from various regions of the left ventricle; *subepicardial*, *midmyocardial* and *subendocardial* cells, as well as *apical* and *basal* cells of the midmyocardium. $I_{Cl(Ca)}$ was blocked by 0.5 mmol/L 9-anthracene carboxylic acid (9-AC). Action potential (AP) changes were tested with sharp microelectrode recording. Whole-cell 9-AC-sensitive current was measured with either square pulse voltage-clamp or AP voltage-clamp (APVC). Protein expression of TMEM16A and Bestrophin-3, ion channel proteins mediating $I_{Cl(Ca)}$, was detected by Western blot.

9-AC reduced phase-1 repolarization in every tested cell. 9-AC also increased AP duration in a reverse rate-dependent manner in all cell types except for subepicardial cells. Neither $I_{Cl(Ca)}$ density recorded with square pulses nor the normalized expressions of TMEM16A and Bestrophin-3 proteins differed significantly among the examined groups of cells. The early outward component of $I_{Cl(Ca)}$ was significantly larger in subepicardial than in subendocardial cells in APVC setting. Applying a typical subepicardial AP as a command pulse resulted in a significantly larger early outward component in both subepicardial and subendocardial cells, compared to experiments when a typical subendocardial AP was applied.

Inhibiting $I_{Cl(Ca)}$ by 9-AC generated EADs at low stimulation rates and their incidence increased upon beta-adrenergic stimulation. 9-AC increased the short-term variability of repolarization also.

We suggest a protective role for $I_{Cl(Ca)}$ against risk of arrhythmias by reducing spatial and temporal heterogeneity of cardiac repolarization and EAD formation.

Abbreviations: 4-AP, 4-aminopyridine; 9-AC, 9-anthracene carboxylic acid; AP, action potential; APD, action potential duration; APD_{90}, action potential duration at 90% of repolarization; APVC, action potential voltage-clamp; $Ca_v1.2$, pore forming subunit of L-type Ca^{2+} channel; $[Ca^{2+}]_{cleft}$, Ca^{2+} concentration in the dyadic cleft; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration in bulk cytoplasm; CL, cycle length; DAD, delayed afterdepolarization; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; EAD, early afterdepolarization; ENDO, subendocardial cell; EPI, subepicardial cell; $I_{9\text{-}AC}$, 9-anthracene carboxylic acid-sensitive current; $I_{Ca,L}$, L-type Ca^{2+} current; $I_{Cl(Ca)}$, Ca^{2+}-activated Cl^- current; I_{K1}, inward rectifier K^+ current; I_{Kr}, rapid component of delayed rectifier K^+ current; I_{Ks}, slow component of delayed rectifier K^+ current; I_{NCX}, Na^+/Ca^{2+} exchange current; I_{SK}, small conductance Ca^{2+}-activated K^+ current; I_{to1}, transient outward K^+ current; ISO, isoproterenol; LQT2, type 2 long QT syndrome; LTCC, L-type Ca^{2+} channel; MID, midmyocardial cell; RMP, resting membrane potential; SR, sarcoplasmic reticulum; SV, short-term variability of repolarization; TdP, torsades de pointes ventricular tachycardia; V_{max}, maximal rate of depolarization.

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1. Introduction

In cardiac arrhythmias the regular electrical activity of the heart is altered. Some of these arrhythmias can be life-threatening tachyarrhythmias like torsades de pointes ventricular tachycardia (TdP) and ventricular fibrillation (VF). These tachyarrhythmias can result in sudden cardiac death which is the major cause of mortality in Europe and in the USA [1,2].

Cardiac action potentials (APs) characteristically differ between regions of the working myocardium. Inhomogeneity of repolarization is a peculiarity of even healthy myocardium and includes transmural dispersion of repolarization [3], the apico-basal gradient in repolarization [4] as well as different repolarization times in left versus right ventricular muscle [5]. This regional heterogeneity contributes to development of ECG waves [6] whereas the increase of this heterogeneity is considered to be arrhythmogenic by possible reentry formation [7,8].

QT interval prolongation is considered as a risk factor of cardiac arrhythmias as it promotes the development of the highly arrhythmogenic early afterdepolarizations (EADs) and TdP [9]. Short-term variability of repolarization has a higher predictive value than the extent of QT interval prolongation [10]. This temporal heterogeneity of repolarization can be measured on isolated cells and monitored as the variability of ventricular AP duration (APD) [11,12].

Abnormalities of intracellular Ca^{2+} homeostasis (Ca^{2+} overload and spontaneous Ca^{2+} release from the sarcoplasmic reticulum (SR)) may also influence the onset of TdP [13]. Spontaneous SR Ca^{2+} release activates the Ca^{2+} removal mechanisms of the cell generating a transient inward current leading to membrane depolarization (delayed afterdepolarization, DAD) and triggered activity. The source of the transient inward current can be the Na^+/Ca^{2+} exchange current (I_{NCX}) operating in forward mode or the activation of Ca^{2+}-activated Cl^- current ($I_{Cl(Ca)}$) [14,15].

$I_{Cl(Ca)}$ is present in mammalian myocardium where it contributes to early repolarization [16] and to DAD formation at least in Ca^{2+}-overloaded cells [15,17]. On the contrary, incidence of both EADs and DADs evoked by various stimulations was increased in the presence of 9-anthracene carboxylic acid (9-AC), an inhibitor of $I_{Cl(Ca)}$ in canine ventricular cells [18] suggesting an antiarrhythmic role for $I_{Cl(Ca)}$. Similarly, $I_{Cl(Ca)}$ may play an important role in the prevention of arrhythmias in acidosis in rabbit ventricular myocytes [19]. This however was not the case in an ovine EAD model where 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), another inhibitor of $I_{Cl(Ca)}$, barely had any action [20]. These conflicting results need further clarification to determine the pro-, or antiarrhythmic role of $I_{Cl(Ca)}$.

The molecular identity of $I_{Cl(Ca)}$ is still controversial but TMEM16A (also known as Anoctamin1 or Ano1) [21–23] and Bestrophins are the most likely candidates, the latter ones at least as the Ca^{2+}-sensitive channel subunits [24]. More and more information is available on the physiological functions of TMEM16 in smooth muscle [25], neuronal and other tissues [26]. Moreover, TMEM16A is expressed in murine ventricle and it was confirmed to be responsible for $I_{Cl(Ca)}$ in ventricular myocytes [27]. Previously we have confirmed the expression of TMEM16A and Bestrophin-3 on both canine and human isolated left ventricular cardiomyocytes [28]. It was also shown that these two proteins co-localize with each other and $Ca_v1.2$ suggesting a direct control of $I_{Cl(Ca)}$ by Ca^{2+} entry through L-type Ca^{2+} channels (LTCCs) in canine ventricular myocytes [28]. Although a previous publication found no evidence for the existence of $I_{Cl(Ca)}$ in healthy human ventricular myocytes [29] but this was not confirmed later. Only two other publications used human cells but in both studies the examined cells were obtained from patients with end-stage heart failure [20,30] and only extrapolated to healthy human tissue from the fact that heart failure does not change the density of $I_{Cl(Ca)}$ in sheep ventricle [20].

The goal of our study was to assess the possible role of $I_{Cl(Ca)}$ in spatial and temporal heterogeneity of cardiac repolarization using two techniques which are probably the closest to physiological conditions.

First, conventional sharp microelectrode recordings were performed to assess the role of $I_{Cl(Ca)}$ on AP of cells isolated from various regions of the left ventricular myocardium (subepicardial, midmyocardial and subendocardial layers and the apical and basal parts of the midmyocardial layer). Second, action potential voltage-clamp (APVC) technique was used to record $I_{Cl(Ca)}$ profiles as 9-AC-sensitive current ($I_{9\text{-}AC}$) under experimental conditions designed to closely mimic physiological conditions (with preserved intracellular Ca^{2+} homeostasis). The normalized expression of TMEM16A and Bestrophin-3 proteins were calculated from Western blot images performed on isolated myocytes obtained from the previously mentioned regions.

Our results indicate that $I_{Cl(Ca)}$ reduces both regional (transmural and apico-basal) and temporal (short-term variability of APD) heterogeneity of repolarization suggesting an antiarrhythmic role for the current. Neither $I_{Cl(Ca)}$ density recorded with square pulses nor the normalized expressions of TMEM16A and Bestrophin-3 proteins differed significantly among the examined cells of various origin. $I_{Cl(Ca)}$ densities measured with APVC in subepicardial cells were larger than that in subendocardial ones probably due to the larger Ca^{2+} entry through LTCCs during subepicardial APs.

2. Methods

A detailed description of the applied methods including electrophysiology protocols, composition of solutions, molecular biological reagents, etc. is provided in the Online Supplement.

All animal handling and laboratory procedures conform 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), and to our Institutional Animal Care and Use Committee approved protocols (license no. 10/2011/DEMÁB). Chemicals and reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) if not specified otherwise. All experiments except for molecular biological techniques were performed at 37 °C and pH = 7.4.

Experiments were performed in enzymatically isolated myocytes obtained from various regions of the canine left ventricle. Following digestion, thin slices were peeled off from both epicardial and endocardial surfaces of the left ventricle containing subepicardial (EPI) and subendocardial (ENDO) cells, respectively. Midmyocardial (MID) cells were obtained from the middle portion of the left ventricular free wall. In separate experiments, cells were collected from the apical and basal parts of the midmyocardial layer of the left ventricular wall.

APs were measured with sharp microelectrode recording during steady-state stimulation. Ionic currents were recorded using conventional whole-cell voltage clamp and APVC techniques. In the latter case the cell's own action potential was used as a command signal to record current profiles. $I_{Cl(Ca)}$ was dissected using 0.5 mmol/L 9-AC and presented as $I_{9\text{-}AC}$ (Supplementary Fig. 1). 9-AC is suitable to study $I_{Cl(Ca)}$ as it evoked maximal effect on AP in 0.5 mmol/L concentration and did not alter L-type Ca^{2+} current ($I_{Ca,L}$), I_{Kr}, the slow component of delayed rectifier K^+ current (I_{Ks}), and the inward rectifier K^+ current (I_{K1}) [31]. Moreover, neither the transient outward K^+ current (I_{to1}) nor the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was influenced by 0.5 mmol/L 9-AC (Supplementary Fig. 2). Furthermore, 9-AC-sensitive current recorded in rabbit myocytes was completely different from apamin-sensitive current (Supplementary Fig. 3) but identical to the CaCCinh-A01-sensitive current (Supplementary Fig. 4) suggesting the lack of 9-AC action on small conductance Ca^{2+}-activated K^+ current (I_{SK}) channels and the suitability of 9-AC for the study of $I_{Cl(Ca)}$.

Short-term variability of repolarization (SV) was evaluated from a series of 50 consecutive APs evoked by 1 Hz steady-state stimulation and presented in Poincaré diagrams to visualize drug-induced changes in SV as previously [12]. The overall probability of differences between consecutive APD_{90} values in each cell was averaged and plotted (Fig. 6E, F) to illustrate any changes in SV.