Apamin induces early afterdepolarizations and torsades de pointes ventricular arrhythmia from failing rabbit ventricles exhibiting secondary rises in intracellular calcium

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BACKGROUND A secondary rise of intracellular Ca^{2+} (Ca_i) and an upregulation of apamin-sensitive K^+ current (I_{KAS}) are characteristic findings of failing ventricular myocytes. We hypothesize that apamin, a specific I_{KAS} blocker, may induce torsades de pointes (TdP) ventricular arrhythmia from failing ventricles exhibiting secondary rises of Ca_i.

OBJECTIVE To test the hypothesis that small conductance Ca^{2+} activated I_{KAS} maintains repolarization reserve and prevents ventricular arrhythmia in a rabbit model of heart failure (HF).

METHODS We performed Langendorff perfusion and optical mapping studies in 7 hearts with pacing-induced HF and in 5 normal control rabbit hearts. Atrioventricular block was created by cryoablation to allow pacing at slow rates.

RESULTS The left ventricular ejection fraction reduced from 69.1% [95% confidence interval 62.3%–76.0%] before pacing to 30.4% [26.8%–34.0%] ($N = 7$; $P < .001$) after pacing. The corrected QT interval in failing ventricles was 337 [313–360] ms at baseline and 410 [381–439] ms after applying 100 nmol/L of apamin ($P = .01$). Apamin induced early afterdepolarizations (EADs) in 6 ventricles, premature ventricular beats (PVBs) in 7 ventricles, and polymorphic ventricular tachycardia consistent with TdP in 4 ventricles. The earliest activation site of EADs and PVBs always occurred at the site with long action potential duration and large amplitude of the secondary rises of Ca_i. Apamin induced secondary rises of Ca_i in 1 nonfailing ventricle, but no EAD or TdP were observed.

CONCLUSIONS In HF ventricles, apamin induces EADs, PVBs, and TdP from areas with secondary rises of Ca_i. I_{KAS} is important in maintaining repolarization reserve and preventing TdP in HF ventricles.

KEYWORDS Action potential duration; Apamin; Optical mapping; Potassium channels; Torsades de pointes

ABBREVIATIONS **APD** = action potential duration; **AV** = atrioventricular; **Ca_i** = intracellular Ca^{2+}; **EAD** = early afterdepolarization; **HF** = heart failure; **$I_{Ca,L}$** = L-type Ca^{2+} current; **I_{KAS}** = apamin-sensitive K^+ current; **LV** = left ventricular; **PCL** = pacing cycle length; **PVB** = premature ventricular beat; **QTc** = corrected QT; **SK** = small conductance Ca^{2+} activated K^+; **TdP** = torsades de pointes; **V_m** = membrane potential

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Introduction

Ventricular arrhythmia is a major cause of death in patients with heart failure (HF).[1] Multiple randomized clinical trials[2–4] conducted in patients with HF documented increased ventricular arrhythmias or mortality in patients randomized to the drug treatment arm, suggesting that HF predisposes patients to drug-induced arrhythmia. A recent study confirmed that there is enhanced sensitivity to drug-induced QT interval lengthening in patients with HF owing to left ventricular (LV) systolic dysfunction.[5] The mechanisms by which HF increases the risk of drug-induced arrhythmia and reduces drug safety remain poorly understood. Previous studies showed that HF is associated with the downregulation of multiple K^+ currents[6,7] but the upregulation of apamin-sensitive K^+ current (I_{KAS}) conducted through the small conductance Ca^{2+} activated K^+ (SK) channels.[8] Drugs that block these K^+ currents may reduce repolarization reserve, prolong action potential duration (APD), and increase

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propensity of ventricular arrhythmia. The upregulation of I_{KAS} in failing ventricles was recently reproduced by Bonilla et al[9] in a canine model of HF. In addition to I_{KAS} upregulation, failing ventricular myocytes are known to develop a slow secondary rise of intracellular Ca^{2+} (Ca_i) that prolongs the Ca_i transient duration.[10] The prolonged availability of Ca^{2+} may activate the I_{KAS} to counterbalance the downregulation of other K^+ currents, thereby maintain repolarization reserve and prevent ventricular arrhythmias in HF. If this hypothesis is correct, then blocking the I_{KAS} by apamin should reduce the repolarization reserve and promote ventricular arrhythmias in failing but not normal ventricles. This hypothesis has important implications in drug safety in HF because inadvertent blocking of I_{KAS} by food or drugs may increase the incidence of ventricular arrhythmia and sudden cardiac death. The purpose of the present study was to perform optical mapping studies in failing rabbit ventricles to test the hypotheses that apamin, a specific I_{KAS} blocker, induces early afterdepolarizations (EADs) and torsades de pointes (TdP) ventricular arrhythmias from areas with secondary rises of Ca_i in failing ventricles.

Methods

Surgical preparation

Rapid pacing protocol was conducted to induce HF in 7 New Zealand white rabbits.[8,11] Five normal rabbits were also studied as controls. Echocardiography was performed before and after high-rate pacing. The hearts were harvested and Langendorff perfused with oxygenated 37°C Tyrode's solution that includes (in mmol/L) NaCl 125, KCl 4.5, $NaHCO_3$ 24, NaH_2PO_4 1.8, $CaCl_2$ 1.8, $MgCl_2$ 0.5, dextrose 5.5, and bovine serum albumin 100 mg/L with a pH of 7.40. Cryoablation of atrioventricular (AV) node was then performed to reduce ventricular rate. We performed simultaneous membrane potential (V_m) and Ca_i optical mapping according to methods published elsewhere.[12,13] More detailed descriptions are included in an Online Supplement.

Experimental protocol

Pseudoelectrocardiogram was monitored by using 2 electrodes placed at the left atrium and the right ventricle, respectively. A bipolar electrode was used to pace the right ventricle with an output at 2.5 times the diastolic pacing threshold. Dynamic pacing protocol[14] was performed and the optical signals were mapped at different pacing cycle lengths (PCLs). We started to acquire optical mapping signal after at least 30 paced beats at the same PCL. An S1/S2/S3 short-long-short pacing protocol (S1 30 beats with S1–S1 300 ms, a long S1–S2 of 1000 or 2000 ms, and an S2–S3 starting from 300 ms and gradually shortened to the ventricular effective refractory period) was used to simulate the electrocardiographic characteristics that initiate the TdP ventricular tachycardia in humans.[15] Apamin (100 nmol/L) was then added to the perfusate, and the protocol was repeated 30 minutes later. Nifedipine (2 μmol/L) was then added in 4 of the 7 failing rabbit hearts to determine whether it prevents the development of TdP. The same protocol was also conducted in 5 normal rabbit hearts for comparison.

Data analysis

APD_{80} was measured at the level of 80% repolarization of APD, and mean APD_{80} was calculated for all available ventricular pixels. A *secondary rise of* Ca_i is defined as the spontaneous increase in Ca_i at the downslope of the primary Ca_i released.[10] Continuous variables are expressed as mean [95% confident interval]. Paired Student *t* tests were used to compare continuous variables measured at baseline and during apamin infusion. Comparison of prevalence of EAD inducibility between baseline and during apamin infusion was performed by using paired McNemar test. A $P \leq .05$ was considered statistically significant.

Results

Induction of HF

All 7 rabbits developed significant symptoms and signs of HF, including tachypnea, poor appetite, cardiomegaly, and pleural effusion. The LV ejection fraction reduced from 69.1% [62.3%–76.0%] before pacing to 30.4% [26.8%–34.0%] ($P < .001$) after pacing. LV end-diastolic diameter increased from 12.3 [11.5–13.12] to 18.1 [16.6–20.0] mm ($P < .001$) and the LV end-systolic diameter from 7.9 [7.0–8.8] to 15.7 [14.3–17.2] mm ($P < .001$). The Langendorff perfused rabbit hearts had sinus rhythm with sinus cycle length 405 [341–469] ms and normal 1:1 AV conduction before AV node cryoablation. All rabbits developed complete AV block after 1–3 attempts of cryoablation, with mean ventricular escape cycle lengths of 1757 [1217–2297] ms. After the addition of apamin, the average spontaneous ventricular escape rate did not change significantly (1757 [1176–2338] ms; $P = .99$). The sinus (atrial) cycle length did not change significantly after cryoablation (457 [366–549] ms; $P = .33$) or after adding apamin (434 [323–545] ms; $P = .62$).

Effects of apamin on QT interval and ventricular arrhythmias in failing ventricles

Apamin significantly prolonged corrected QT (QTc) interval during spontaneous escape rhythm. The QTc interval was 337 [313–360] ms at baseline and 410 [381–439] ms after applying 100 nmol/L of apamin ($P = .01$). Figure 1A shows an example of QT prolongation after apamin administration. Figure 1B shows the effects of apamin on QTc intervals in all 7 hearts studied. In addition to prolonging QTc intervals, apamin led to the development of EADs in 6 ventricles. The ventricle without EAD had a mean APD of 208 ms (at a PCL of 500 ms), which was within the range of APDs (188–261 ms at a PCL of 500 ms) in ventricles with EADs. Premature ventricular beats (PVBs) were observed in 7 ventricles and polymorphic ventricular tachycardia consistent with TdP in 4 ventricles.