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Effect of pacing and mexiletine on dispersion of repolarisation and arrhythmias in ΔKPQ SCN5A (long QT3) mice

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Abstract

Objective: It has been suggested that both pacing and treatment with mexiletine may reduce torsade de pointes (TdP) arrhythmias in patients with long QT syndrome 3 (LQT3), but it is not fully understood how these interventions could prevent TdP. We therefore studied the effects of pacing and mexiletine in mice with a heterozygous knock-in ΔKPQ SCN5A$^{\Delta/+}$ deletion (SCN5A-Tg), a murine LQT3 model. **Methods:** Three right and left ventricular monophasic action potentials (MAPs) were simultaneously recorded in Langendorff-perfused hearts of SCN5A-Tg and wild type (WT) littermates. AV block was induced, and pacing was performed at baseline and during mexiletine infusion (4 μg/ml). MAP recordings were analysed for action potential duration (APD), APD dispersion, and early afterdepolarisations (EADs) and related to spontaneous arrhythmias. **Results:** After inducing AV block, SCN5A-Tg hearts were bradycardic [SCN5A-Tg 532±60 vs. WT 284±48 ms cycle length (CL, mean±S.E.M., $P<0.05$(*))]. EADs occurred in 16/18, and polymorphic ventricular tachycardia (pVT) in 11/18 SCN5A-Tg but not in 19 WT. SCN5A-Tg had longer APD than WT hearts*. At CL of 200 ms and longer, APD dispersion was higher in SCN5A-Tg [dispersion (APD70): 12±3 ms vs. 5±2 ms at CL=200 ms*], and increased to 35±4 ms* directly prior to pVT episodes. Sudden rate accelerations initially increased APD dispersion due to EADs and APD alternans in SCN5A-Tg, but pacing then reduced APD dispersion. Pacing suppressed (n=9/9) and prevented (n=49/50) pVT. Mexiletine shortened APD at long CL*, and suppressed pVT (n=4/5*), but did not prevent pVT during normal rhythm. **Conclusions:** Bradycardia, increased dispersion of APD and EADs provoke ventricular ectopy and pVT in SCN5A-Tg hearts. Ventricular pacing reduces APD dispersion, suppresses EADs and prevents pVT in SCN5A-Tg hearts. These effects provide a pathophysiological rationale for pacing in LQT3.

Keywords: Antiarrhythmic agents; Arrhythmia (mechanisms); Bradycardia; Long QT syndrome; Membrane potential; Repolarization

1. Introduction

Mutations in the cardiac sodium channel gene SCN5A can cause long QT syndrome 3 (LQT3) [1–3], an inherited cardiac disorder that prolongs the ventricular action potential and causes sudden death from cardiac arrhythmias, specifically torsade de pointes (TdP). The disease is relatively rare but aggressive and can affect patients at an

Abbreviations: APD, action potential duration; AV, atrioventricular; CL, cycle length; EAD, early afterdepolarisations; ERP, effective refractory period; LQT3, Long QT syndrome 3; LV, left ventricle, left ventricular; MAP, monophasic action potential; pVT, polymorphic ventricular tachycardia; RV, right ventricle, right ventricular; SCN5A-Tg, mice with heterozygous knock-in ΔKPQ SCN5$^{\Delta/+}$ deletion; TdP, torsade de pointes

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early age, leading even to sudden infant death syndrome [4–6]. Cardiac events are less frequent but more often lethal than in other forms of long QT syndrome [7]. Therefore, clinical experience with the treatment of LQT3 patients is scarce and therapeutic recommendations concerning drug administration and implantation of pacemaker or defibrillator devices are discussed controversially.

In LQT3, TdP is known to occur preferably at rest [8]. LQT3 patients may suffer from resting bradycardia in addition to prolonged repolarisation [9,10], and some LQT patients, particularly children, develop functional atrioventricular nodal block (AV nodal block) immediately before the onset of TdP [11–13]. Prevention of bradycardia has therefore been suggested as a gene-specific therapy to prevent TdP in LQT3 [14].

To study the effect of bradycardia and therapeutic interventions on early afterdepolarisations (EADs), ventricular ectopy, and TdP-like ventricular tachyarrhythmias in LQT3, we studied intact hearts from heterozygous transgenic mice with a knock-in deletion $SCN5A^{\Delta/+}$ (SCN5A-Tg) lacking the same KPQ residues (Lys-1505, Pro-1506, Gln-1507 between domains III and IV in the sodium channel α-subunit) as LQT3 patients [15–19]. We measured action potential durations (APD), effective refractory period (ERP), dispersion of APD, occurrence of EADs and spontaneous tachyarrhythmias in intact hearts after AV nodal block and during ventricular pacing with and without administration of the sodium channel blocking drug mexiletine.

2. Methods

2.1. Isolated heart setup

Studies were carried out according to institutional guidelines and approved by the institutional animal care and use committee. The study adhered to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH publication No. 85-23, revised 1996). The experimental setup has been described before [20]. In brief, transgenic mice expressing the mutant ΔKPQ SCN5A gene causing LQT3 in patients (SCN5A-Tg) [19] and their wildtype (WT) littermates were anaesthetised using urethane (2 g/kg) and a digital six-lead ECG was recorded for 10 s (CORINA, GE Hellige Marquette Medical Systems, Freiburg, Germany, software custom-adapted for murine ECG). The heart was excised via a median thoracotomy and immediately immersed in warm and oxygenated buffer. The aorta was cannulated and retrogradely perfused with 37 °C Krebs–Henseleit buffer containing (in mmol/l): NaCl 118; $NaHCO_3$ 24.88; KH_2PO 41.18; glucose 5.55; Na-pyruvate 2; $MgSO_4$ 0.83; $CaCl_2$ 1.8; KCl 4.7. The buffer was equilibrated with a 95% O_2–5% CO_2 gas mixture. The heart was mounted on a vertical Langendorff apparatus (Hugo Sachs, March-Hugstetten, Germany) ensuring a perfusion pressure at 100±5 mmHg and a coronary flow of 4±1 ml/min.

A 2.0 French octapolar mouse electrophysiologic catheter with 0.5 mm electrodes spaced at 0.5 mm (CIB'ER MOUSE, NuMED, Hopkinton, NY, USA) was inserted into the right atrium and right ventricle (RV) and twisted toward the RV septum for pacing and recording of intracardiac electrograms. ECG recordings were obtained from Ag–AgCl electrodes immersed in superfused sponges flanking the isolated heart. Signals were amplified and filtered by an ECG amplifier at a bandwidth of 0.1 to 300 Hz (Hugo Sachs). Three monophasic action potentials (MAPs) were continuously and simultaneously recorded from the right and left ventricular (LV) epicardium. Stable MAP recordings were obtained by miniaturised MAP catheters mounted on custom-made spring-loaded electrode holders ensuring perpendicular application of the electrode tip and constant electrode contact pressure [20]. Recording positions were at an equal distance from the ventricular base. MAPs were stable for more than 30 min and reproducible for more than 2 h of recording. MAP recordings were preamplified using a DC-coupled preamplifier (Model 2000, EP Technologies, San Jose, CA, USA). All preamplified signals were digitised and stored using a custom-made semi-automatic software [21] adapted for analysis of murine repolarisation [22,20].

2.2. Protocols

After an equilibration period of 10 min to verify stability of the preparation, signal quality, and intrinsic rhythm, the AV node was ablated by applying gentle pressure at the septal portion of the anulus fibrosus by a pair of surgical tweezers. AV nodal block was confirmed by discordant atrial and ventricular activity in the ECG. The spontaneous ventricular rhythm was observed for 10 min, followed by RV constant pacing via the octapolar catheter at physiological rates [100–200 ms cycle length (CL)] and bradycardic rates (300–600 ms CL). When pacing was stopped, pauses were observed [23,20]. When spontaneous arrhythmias occurred, we performed ventricular pacing (100–600 ms pacing CL) or added mexiletine (4 μg/ml perfusate) to suppress arrhythmias, followed by a wash-out period to confirm re-occurrence of arrhythmias. Furthermore, sudden rate accelerations were performed by ventricular pacing in steps from 600 to 200 ms pacing CL and from 400 and 300 to 100 ms pacing CL. Pacing was continued until steady state was reached. The ERP was determined by programmed stimulation using trains of eight S1 stimuli and 2 ms decrements of the extra stimulus, S2. All ERP measurements were repeated to ensure reproducibility. The protocols were performed at baseline and with mexiletine at a concentration of 4 μg/ml.

2.3. MAP measurements

Data were manually reviewed using an adapted version of a previously published MAP analysis program [21]. APD at 50, 70, and 90% repolarisation was analysed manually and via the MAP analysis program [21]. MAP signal quality criteria included the following specific features: stable baseline and MAP morphology, fast upstroke without inflection or negative spikes, amplitude >1 mV, and rapid first phase of repolarisation. Due to the typical murine MAP wave shape of early repolarisation, 0% repolarisation was measured at the peak of the MAP upstroke and 100% repolarisation was measured during electrical diastole [24,22,20]. Dispersion of repolarisation was calculated as the difference between the maximal and the minimal APD of the three simultaneously recorded MAPs. Activation time was measured as the delay between stimulus artefact in the endocardial pacing catheter and the upstroke of the MAP. Mean activation time was calculated as the mean interval of the three epicardial MAPs. The ERP/APD ratio was calculated as the quotient of the effective refractory period as measured during RV endocardial pacing, and APD90 of the RV MAP [25]. Post repolarisation refractoriness was calculated as ERP minus APD90 [26].

To quantify the time course of frequency adaptation of APD after sudden rate accelerations, APD was analysed at 50, 70 and 90% repolarisation in each of the first 100 beats after sudden rate accelerations.

2.4. Evaluation of arrhythmias

All recordings were scrutinised online and offline for EADs, triggered activity, bigemini, triplets, quadruplets and spontaneous monomorphic and polymorphic ventricular tachycardia (pVT). The arrhythmia CL was measured offline on a beat per beat basis. Arrhythmias were classified as monomorphic, polymorphic, and with or without fractionated action potentials using MAP and ECG criteria. For the purpose of this study, we analysed arrhythmias during spontaneous rhythm [23,20].

2.5. Statistical analysis

Statistical analysis was carried out using a JMP software package (Version 3, SAS Institute, USA). Paired and unpaired *t*-tests, univariate analysis of variance (ANOVA) analyses and ANOVA analyses for multiple comparisons and repetitive measurements were used for comparison of continuous parameters at different CLs in WT and SCN5A-Tg at baseline and with mexiletine. Fisher's exact test was used to compare arrhythmia occurrence rates. Values are reported as mean±S.E.M. throughout the text. Differences were considered significant at a two-tailed alpha level of $P<0.05$ and are marked by an asterisk (*) throughout the text.

3. Results

3.1. Bradycardia, EADs and arrhythmias

AV nodal block rendered SCN5A-Tg hearts extremely bradycardic compared to WT [spontaneous ventricular CL 532±60 ms ($n=18$ SCN5A-Tg) vs. 284±48 ms ($n=19$ WT), $P<0.05$, Fig. 1]. A difference between spontaneous CL during sinus rhythm was neither detected in vivo (SCN5A-Tg 133±4 ms vs. WT 125±5, $n=12$ per group, $P>0.2$) nor ex vivo before induction of AV nodal block (SCN5A-Tg 127±6 ms vs. WT 130±7 ms, $P>0.2$, $n=5$ per group). After inducing AV nodal block, EADs occurred in 16, and repetitive ventricular ectopy and pVT in 11 of 18 SCN5A-Tg hearts with AV nodal block, but not in 19 WT hearts (Figs. 1 and 2, $P<0.05$). EADs occurred in all three recording sites, but more often in the RV (13/16 hearts) compared to LV free wall (4/16 hearts) and LV septum (3/16 hearts) as well as compared to LV free wall and septum together ($P<0.05$). EADs and triggered ectopy only developed after AV nodal block, and preferably at long intrinsic CLs (>600 ms): episodes of pVT were preceded by a pause of more than 1000 ms in 10/11 hearts with pVT. Mean CL during the first half of the arrhythmia was 137±7 ms ($n=11$ hearts) and shorter than the atrial CLs at the time of arrhythmias (Fig. 2B). During pVT, MAPs were fractionated and the QRS axis in the ECG was changing (Fig. 2B). Prolongation of CL and a lesser degree of fractionation of MAPs and ECG preceded spontaneous termination of the arrhythmia (Fig. 2A and B). Episodes of pVT lasted 7.0±0.6 s (range from 3.8 to 9.4 s, $n=11$ hearts). Usually, pVT occurred in repetitive episodes separated by pauses of more than 1000 ms.

3.2. Action potential prolongation and bradycardia-dependent increased dispersion

Steady-state APD was prolonged in SCN5A-Tg hearts during fix frequent pacing at pacing CLs from 100 to 400 ms (Fig. 1). During steady-state pacing, mean MAP duration was not significantly different between recording sites, although dispersion was present in individual hearts. Only at long pacing cycle lengths of 200 ms and more, dispersion of APD was increased in SCN5A-Tg hearts (Fig. 1). Directly prior to the pause preceding pVT, APD (APD70: 117±5 ms vs. 51±1 ms, $P<0.05$, all values measured in the five beats directly prior to pVT episodes versus five consecutive beats during spontaneous rhythm without pVT) and APD dispersion (dispersion of APD70: 35±4 ms vs. 13±1 ms, $P<0.05$) were further increased in SCN5A-Tg hearts during spontaneous rhythm when com-

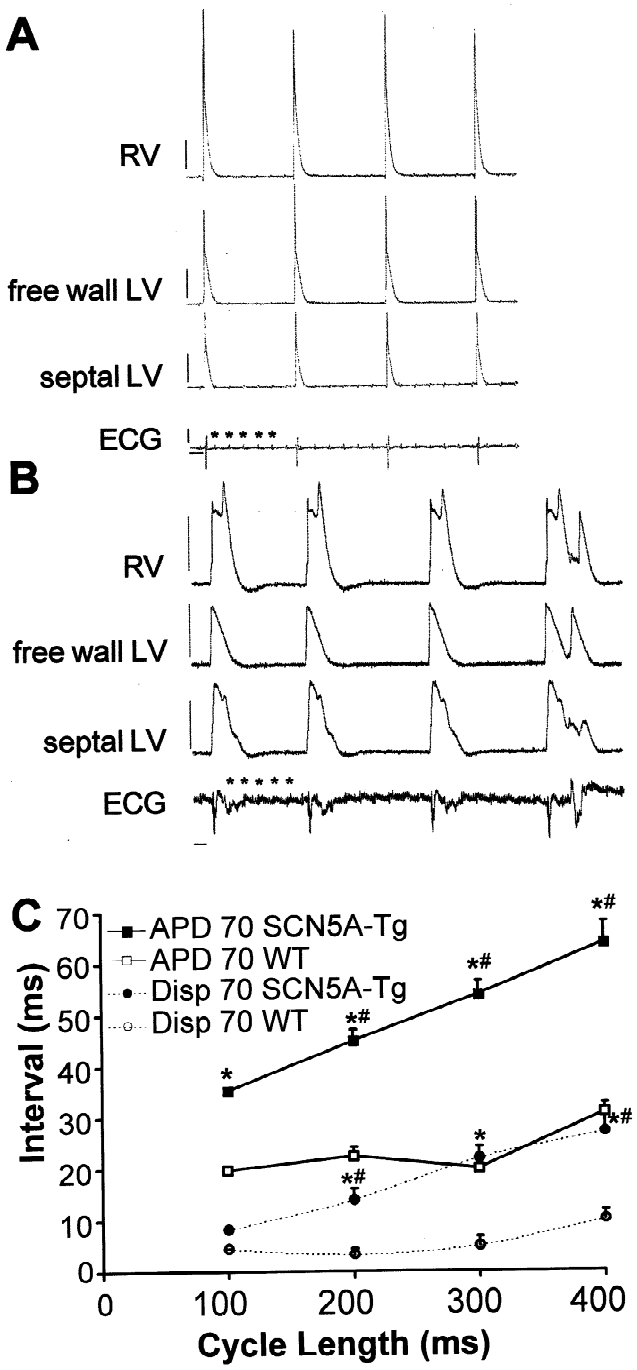

Fig. 1. Prolonged action potentials, increased dispersion, and EADs. Simultaneous recording of epicardial MAPs from the right ventricle (RV), left ventricular (LV) free wall and septal LV, and lead I of the volume conducted ECG in spontaneously beating hearts after AV block in WT (A) and SCN5A-Tg hearts (B). Asterisks (*) indicate five consecutive P waves in the tissue bath ECG in (A) and (B). Calibration bars indicate 1 mV and 100 ms. Early afterdepolarisations (EADs) are recorded in the RV and septal LV, but not on the LV free wall. After the fourth beat, a premature beat is elicited, probably initiated by an EAD in the RV. The premature beat is conducted to the LV free wall and appears to turn back to the RV (re-entry). (C) Mean action potential duration (APD) and APD dispersion of the three MAPs at 70% repolarisation WT ($n=17$) and SCN5A-Tg ($n=21$) hearts at different pacing cycle lengths. Asterisks (*) indicate significant differences between genotypes, # indicate significant differences to a cycle length of 100 ms. APD50 and APD90 showed similar differences.

pared to values during spontaneous rhythm without pVT. Ventricular cycle length was also increased directly prior to pVT episodes (654±86 ms prior to TdP vs. 466±33 ms in SCN5A-Tg hearts without TdP, $P=0.04$).

3.3. Effect of pacing

RV pacing completely suppressed EADs and pVT in nine of nine hearts when it was begun during a period of repetitive pVT. Pacing at CLs of 100–200 ms CL was more potent than pacing at longer CLs from 300 to 600 ms. Ventricular pacing at constant CLs also prevented pVT episodes when pacing was begun before pVT occurrence (no pVT episodes during constant pacing at 100–200 ms CL in 49/50 SCN5A-Tg hearts). Pacing was so effective in preventing arrhythmia episodes that we avoided pauses in the pacing protocols in order to complete them without occurrence of pVT.

During the first beats after sudden rate accelerations, EADs and regional APD alternans developed in 9/18 SCN5A-Tg hearts (Fig. 3A–C), and temporarily resulted in enhanced dispersion of repolarisation (Fig. 3D). However, APD and APD dispersion decreased progressively during sustained rapid pacing and reached a steady state below the values measured for the long CL (Fig. 3).

3.4. Effect of mexiletine

During steady state pacing, mexiletine (4 μg/ml) shortened APD in SCN5A-Tg hearts at long pacing CLs (400 and 600 ms, Fig. 4A). At short pacing CLs, in contrast, APD was not significantly altered (Fig. 4A). Mexiletine thereby decreased the difference between the longest and the shortest steady-state APD from 53 to 26 ms at 70% repolarisation (Fig. 4A, $P<0.05$). Mexiletine furthermore decreased the occurrence of APD alternans and EADs during sudden rate accelerations. Transient prolongation of APD was attenuated by mexiletine: during the first 50 beats after a sudden rate acceleration from 600 to 200 ms CL, APD at 50, 70 and 90% repolarisation was shorter in RV and LV MAPs with mexiletine when compared to baseline (e.g., APD70 mexiletine vs. baseline: RV MAP 36±0.3 vs. 52.9±0.4, LV free wall 54.8±0.2 vs. 65.1±0.3 ms, LV septum 41.7±0.2 vs. 60.2±0.4 ms, all $P<0.05$). During the following 50 beats (beat 51–100 after the rate acceleration), APD further shortened both at baseline and with mexiletine (APD70 baseline: RV MAP 44.2±0.6*, LV free wall 46.5±1.0, LV septum 45.2±0.7* ms; APD70 mexiletine: 39.7±0.3, 46.3±0.9, 38.8±0.5 ms, *: all $P<$ 0.05, Fig. 4).

Mexiletine prolonged the ERP at twice diastolic pacing threshold from 55±6 to 91±11 ms at a pacing CL of 200 ms ($P<0.05$), resulting in an increase of the ERP/APD ratio from 0.77±0.06 to 1.14±0.14 ($P<0.05$) and in post repolarisation refractoriness of 7±11 which was not present at baseline (SCN5A-Tg at baseline: −19±6 ms,

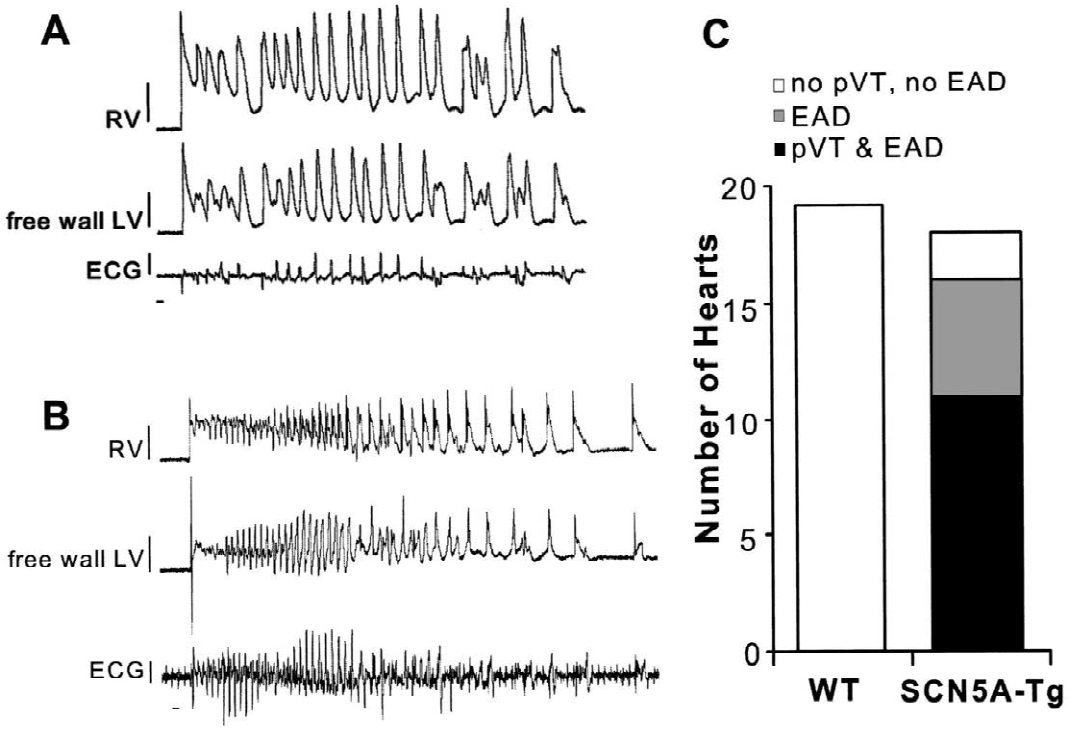

Fig. 2. Repetitive ventricular ectopy and polymorphic ventricular tachycardias (pVTs). (A, B) MAPs from right (RV) and left (LV) free wall and the tissue bath ECG during early afterdepolarisations (EAD) and triggered repetitive ventricular ectopy (A), and during EAD-triggered pVT (B) in SCN5A-Tg hearts. Arrhythmias occurred during spontaneous rhythm after long pauses (>1000 ms). Calibration bars indicate 1 mV and 100 ms. In B, atrial cycle length (visible in the ECG during pauses) is longer than pVT cycle length (117±2 ms versus 124±0 ms, $P<0.05$) and pVT has an undulating QRS axis in the ECG. (C) Occurrence of pVTs in WT and SCN5A-Tg. Shown is the number of hearts without EAD (white), with EADs only (grey), and with pVT (black) for each genotype. $P<0.05$ for WT versus SCN5A-Tg.

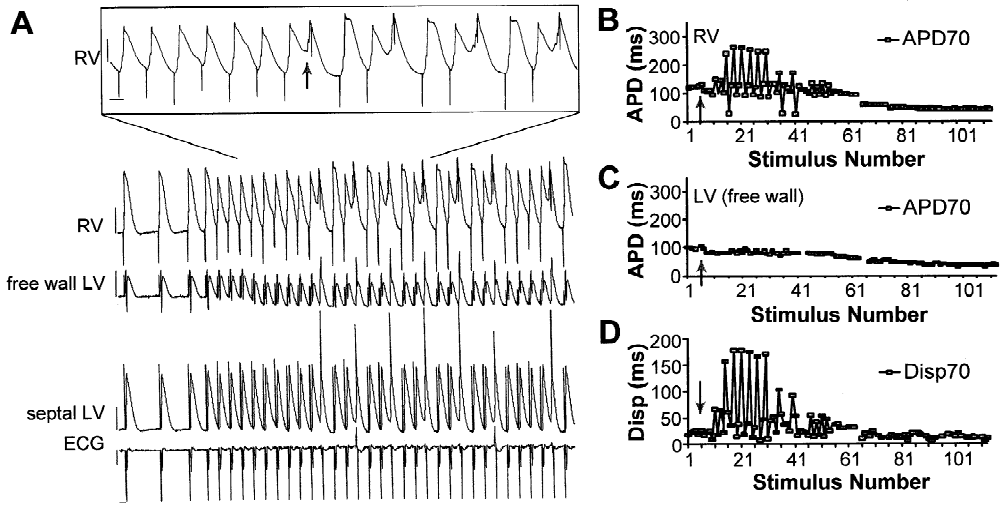

Fig. 3. Dynamic dispersion of repolarisation in a SCN5A-Tg heart. (A) Right ventricular (RV), left ventricular (LV) free wall and septal LV MAPs and tissue bath ECG during a sudden rate acceleration (600–200 ms pacing cycle length). Action potentials in RV and LV lengthen and EADs (arrow in the inset) appear in the RV MAP, activating earlier than the pacing stimulus as seen in the ECG and in the inset. Inset: Enlargement of the RV MAP recording. Calibration bars indicate 100 ms and 1 mV. (B, C) APD at 70 repolarisation (APD70) versus stimulus number measured in (A) in the RV (B) and the LV free wall (C) MAP. Rate acceleration (arrow) induces a transient increase in APD and regional APD alternans. APD shortens progressively during pacing and reaches steady state after around 100 paced beats. Similar observations were made at APD50. (D) APD dispersion of the three MAPs at 70% repolarisation (Disp70) in (A). Rate acceleration (arrow) induces a transient increase in APD dispersion. After a transient increase, dispersion decreases during continued pacing.

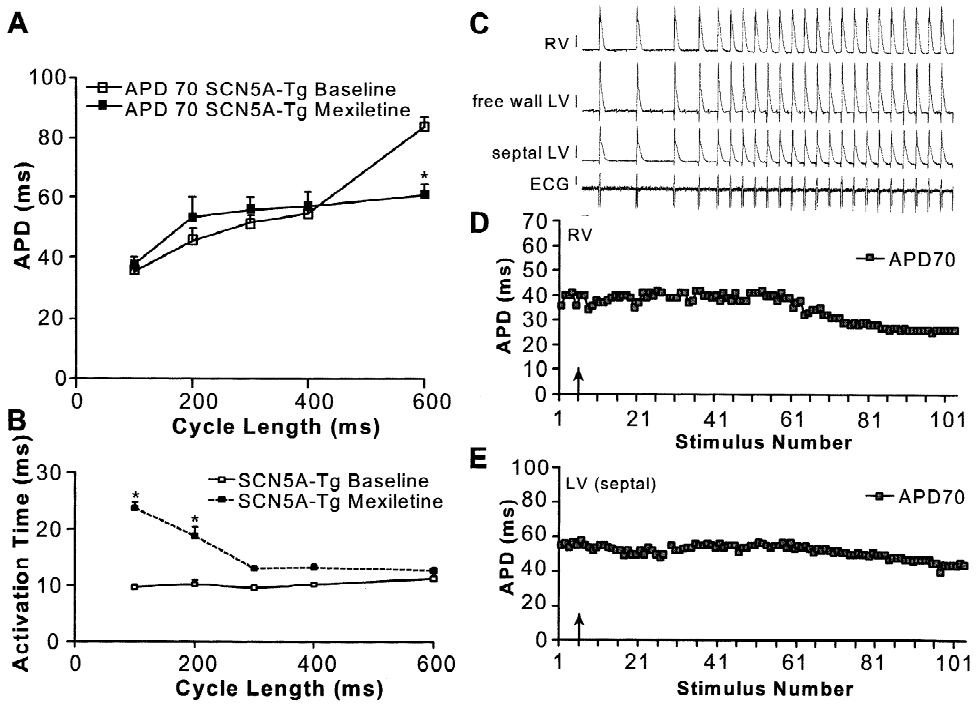

Fig. 4. Effect of mexiletine in SCN5A-Tg hearts. (A) Mean action potential duration (APD) at 70% repolarisation (APD70) of the three MAPs during steady state pacing at cycle lengths from 100 to 600 ms at baseline and with mexiletine ($n=17$ hearts per group). Results were similar for APD50 and APD90. Mexiletine reduced APD at a cycle length of 600 ms. (B) Mean activation time of the three MAPs during steady state pacing at CLs from 100 to 600 ms. Mexiletine prolonged activation times at short CLs (100 and 200 ms, $P<0.05$). (C) RV and LV MAPs and a tissue bath ECG during a sudden rate acceleration (600–200 ms) with mexiletine. Calibration bars indicate 100 ms and 1 mV. (D, E) APD70 measured in the RV (D) and the septal LV (E) versus stimulus number as measured in the upper example.

$n=7$, $P<0.05$). Values for WT showed the same trend but did not reach significance (PRR: WT at baseline -7.4 ± 8.1, WT with mexiletine -1.6 ± 7.6). Activation times were increased by mexiletine in WT (data not shown) and SCN5A-Tg hearts, especially at short pacing CLs (Fig. 4B). Furthermore, mexiletine aggravated the intrinsic bradycardia in SCN5A-Tg hearts and increased the pacing threshold from 1.3 ± 0.1 to 2.0 ± 0.3 mA ($P<0.05$).

When given during periods of pVT, mexiletine suppressed arrhythmias in 4/5 SCN5A-Tg hearts. When given before the onset of pVT, mexiletine did not completely prevent episodes of pVT: in 5/10 SCN5A-Tg hearts in whom mexiletine was added to the perfusate to assess APDs, refractory periods and activation times, spontaneous pVT occurred despite the presence of mexiletine in the perfusate.

4. Discussion

4.1. Main findings

In this ΔKPQ SCN5A knock-in mouse heart model of LQT3, pVT were preceded by slow intrinsic ventricular rhythm, prolonged APD, increased dispersion of APD, and EADs. Transgenic hearts exhibited intrinsic bradycardia after AV nodal block. Fix frequent ventricular pacing at physiological rates prevented bradycardia, reduced APD and APD dispersion, and completely suppressed EADs and ventricular arrhythmias. Sudden rate accelerations resulted in a transient increase in APD dispersion. Mexiletine partially suppressed arrhythmias, possibly by preventing APD prolongation at long CLs and by producing post repolarisation refractoriness, but aggravated intrinsic bradycardia. These data suggest a pathophysiological rationale for pacing in LQT3.

4.2. Altered SCN5A-Tg whole-heart electrophysiology

Concordant with microelectrode studies, APD was prolonged in intact hearts from SCN5A-Tg mice [19]. This effect was more pronounced at long pacing CLs, probably due to the increased late inward sodium current in the mutant SCN5A channel [27]. SCN5A-Tg hearts exhibited a markedly slowed intrinsic ventricular rhythm after AV nodal block. This may be due to a reduced early sodium influx or to an increased late sodium influx that could suppress spontaneous activation in ventricular pacemaker cells [28]. When bradycardia was present, dispersion of APD was markedly increased in SCN5A-Tg hearts. This novel finding may be due to regional heterogeneity of the depolarising current (I_{Na}). Increased APD dispersion has been identified as a proarrhythmic factor in another

transgenic model of LQT [29], and similar proarrhythmic effects of bradycardia have been reported in a model of drug induced LQT3 [30]. Our data demonstrate that an increased dispersion of APD contributes to spontaneous arrhythmias in this transgenic model of LQT3, and that intrinsic bradycardia aggravates these proarrhythmic effects.

4.3. Spontaneous pVT

Spontaneous polymorphic ventricular tachycardias occurred in more than half of SCN5A-Tg hearts after AV block, but not in WT hearts. Some characteristics of pVT are comparable to TdP in LQT3: arrhythmia episodes occurred after long pauses and were preceded by EAD and enhanced dispersion of APD. Arrhythmia episodes were of polymorphic nature with undulating QRS axes and occurred repetitively. Although a detailed mapping of the initiating sequence of pVT episodes was beyond the scope of our study, these characteristics suggest that pVT in this model were initiated by EADs and maintained in a substrate of functional re-entry based on increased dispersion of APD [20]. Comparable with our data, extreme action potential prolongation in association with EADs was seen in microelectrode recordings from the isolated RV in the same model at long CLs [19]. Here we show that heterogeneous action potential prolongation increases dispersion of APD at long CLs in the intact SCN5A-Tg heart, thereby increasing dispersion of refractoriness and enabling functional re-entry.

4.4. Effect of pacing

Pacing at physiological rates effectively suppressed and prevented pVT. This antiarrhythmic effect was associated with shorter APD, a decreased dispersion of APD, and with suppression of EADs. Pacing thereby both eliminated the trigger for spontaneous arrhythmias, afterdepolarisations and triggered activity, and reduced the substrate for functional re-entry by decreasing dispersion of repolarisation.

Late I_{Na} in the ΔKPQ SCN5A mutation shows rate-dependent slow inactivation as shown by whole-cell patch clamp recordings in transfected HEK293 cells [27]. Rate dependent reduction of the late inward sodium current may cause shortening of the action potential at short CLs, thereby also reducing APD dispersion (see above). Similar to our data in a transgenic intact heart model of LQT3, QT shortening in response to cardiac pacing has been reported in a drug-induced cellular model mimicking the SCN5A defect [31] and in patients [8]. LQT3 patients are expected to derive special benefit from anti-bradycardic pacing [32]. In a family with the 1795insD mutation of the SCN5A gene, pacemaker therapy has proven protective [28]. Our data demonstrate for the first time that pacing reduces dispersion of APD and prevents EADs and pVT in this transgenic model of LQT3.

SCN5A-Tg hearts showed marked intrinsic ventricular bradycardia after AV nodal block. During sleep, normal individuals may experience short episodes of sinus bradycardia or of 2:1 AV nodal block with junctional escape rhythms. These arrhythmias are usually asymptomatic. In LQT3 patients, in contrast, such short bradycardia episodes may be sufficient to provoke EADs and pVT. In addition to the deleterious effects of slow ventricular rates, sudden rate accelerations caused a transient increase in APD ([19], Fig. 3) and APD dispersion (Fig. 3). These observations may suggest that rate-smoothing algorithms could be beneficial in pacing therapy of LQT3 patients.

4.5. Effect of sodium channel block by mexiletine

By shortening APD at long CLs, mexiletine decreased the chance of spontaneous EADs occurring at long APDs and also reduced the transient APD prolongation after sudden rate accelerations. Similar effects of mexiletine on APD and APD dispersion have been reported in pharmacological models of LQT3 [31,30]. Shortening of the QT interval by mexiletine has also been reported in patients [8]. Our study reproduces these findings in a transgenic model of LQT3. With mexiletine, refractory periods were longer than APD, resulting in post repolarisation refractoriness, comparable to the effects of other sodium channel blockers [25,26] and the combined use of mexiletine and sotalol [33]. Post repolarisation refractoriness has been shown to have antiarrhythmic effects in experimental and clinical studies [25,26]. By preventing excitability during late repolarisation of the action potential, post repolarisation refractoriness may directly suppress spontaneous ventricular activity related to afterdepolarisations, thereby as well suppressing EADs and triggered arrhythmias. In contrast to pacing, however, mexiletine did not completely prevent pVT in our study, possibly because mexiletine aggravated bradycardia in SCN5A-Tg hearts.

In this intact heart study, we could not determine the molecular effect of mexiletine on the cellular level and on the gating properties of the mutated sodium channel. Lidocaine and mexiletine at low concentrations preferentially block late openings of the ΔKPQ sodium channel [34,35] without greatly modifying peak sodium current. This implies that pharmacological shortening of the action potential in patients might be possible without compromising conduction severely [36]. Lidocaine has been shown to augment closed-state-inactivation of the R1623Q mutated sodium channel [37]. Mexiletine could as well drive the ΔKPQ sodium channel from closed into the inactivated state, thereby attenuating late reopenings and bursts [38]. Furthermore, mexiletine rescued the expression defect in another SCN5A mutation, M1766L, in embryonic kidney cells [38].

5. Implications

Our data demonstrate that prevention of bradycardia by pacing suppressed pVT in this SCN5A knock-in mouse heart model of LQT3. Patients with this mutation, and potentially other patients with LQT3, may benefit from pacing at physiological rates using a rate-smoothing algorithm. Mexiletine caused both pro- and antiarrhythmic effects in this model, and although the antiarrhythmic effects prevailed, did not appear as safe as pacing for the prevention of pVT.

There are important differences in heart rate, repolarising currents, and size between murine and human hearts, and results from measurements in transgenic mice therefore need to be transferred to the clinical setting with caution [39]. Nonetheless, our findings in this transgenic LQT3 model correspond well to the limited observations in patients. The pathophysiological observations may provide a rationale for preventive pacing to avoid pauses and sudden rate accelerations during bradycardia in LQT3.

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