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Optical mapping of ventricular arrhythmias in LQTS mice with SCN5A mutation N1325S

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Abstract

Transgenic expression of *SCN5A* mutation N1325S creates a mouse model for type-3 long QT syndrome (LQT3), TG-NS/LQT3. Optical mapping is a high temporal resolution fluorescence mapping system that records 256 action potentials simultaneously in a Langendorff-perfused heart. Here for the first-time, we provide a spatial view of VT in a genetic LQT3 model using optical mapping. Spontaneous VT was detected in TG-NS/LQT3 hearts, but not in littermate control hearts. VT was initiated primarily by activation of a new firing focus as well as functional conduction block of new activation waves. New firing was initiated at many different locations in the heart, suggesting that “increased automaticity” is a key mechanism for initiation of VT. The sustained VT was maintained by a re-entry mechanism. Nifedipine, an L-type calcium channel blocker, decreased the frequency of VT, indicating the involvement of abnormalities of the calcium homeostasis in the genesis of VT in TG-NS/LQT3 mice.

Keywords

transgenic mouse model; long QT syndrome (LQTS); LQT3; ventricular arrhythmia; sudden death; cardiac sodium channel gene *SCN5A*; mutation; optical mapping; reentry arrhythmia; increased automaticity

Long QT syndrome (LQTS) is a cardiac disorder characterized by prolonged QT interval on the surface electrocardiogram (ECG), syncope, and sudden cardiac death due to ventricular tachyarrhythmias (VT) [1]. In 1995, Wang et al. reported that mutations in the cardiac sodium channel gene *SCN5A* caused type-3 LQTS, LQT3 [2,3]. However, to date the detailed pathogenic mechanisms for initiation and maintenance of VT by *SCN5A* mutations remain largely unexplored.

The N1325S mutation in *SCN5A* was one of the first series of mutations identified for LQT3 [2]. In 2004, we generated a transgenic mouse model for LQT3 (TG-NS/LQT3) with cardiac selective over-expression of a mutant human *SCN5A* gene with mutation N1325S [4]. As LQT3 mutations in *SCN5A* are gain-of-function mutations, transgenic overexpression of these

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mutations in the mouse heart is expected to result in clinical features associated with LQTS. Indeed, the TG-NS/LQT3 mice exhibited the clinical features of LQT3, including the prolonged QT interval and development of spontaneous polymorphic VT, syncope, and sudden cardiac death [4]. In this study, we used an optical mapping system to directly visualize the initiation and maintenance of polymorphic VT in the TG-NS/LQT3 mouse model. We demonstrated that Langendorff-perfused TG-NS/LQT3 murine hearts developed a high frequency of spontaneous VT without any pacing stimulation, but no VT was detected in wild-type hearts. Further, a calcium channel blocker, nifedipine, reduces the frequency of VT, suggesting that abnormalities of calcium homeostasis play a role in development of VT. We show that multifoci firing initiates VT, which validates the role of automaticity in the genesis of VT, and reentry maintains VT in TG-NS/LQT3 mice. Our results provide the first spatial view of VT in the genetic mouse model for LQT3.

Materials and methods

Generation of transgenic mouse model and genotyping

TG-NS/LQT3 transgenic mice were created by selective cardiac expression of mutant human *SCN5A* with LQTS-causing mutation N1325S using the mouse myosin heavy chain- α promoter as described previously [4]. Two lines of mice were generated, L3 with 1–2 copies of transgene and L12 with 10 copies of transgene [4]. The mice were genotyped as described previously [4]. This study was approved by the Cleveland Clinic Animal Research Committee.

Langendorff perfusion of the mouse heart

The mouse was heparinized with 100 U of heparin before its sacrifice. The heart was quickly excised, cannulated, placed into a perfusion chamber, and immobilized mechanically by slightly pushing it against the chamber wall. No electrical-contraction de-couplers were used to avoid side effects of such agents [5]. The perfusate was oxygenated (95% O₂, 5% CO₂) modified Tyrode's solution containing (in mM): NaCl 128.2, CaCl₂ 1.3, KCl 4.7, MgCl₂ 1.05, NaH₂PO₄ 1.19, NaHCO₃ 25, and glucose 11. Temperature and pH were continuously maintained at $36 \pm 0.5^\circ\text{C}$ and 7.30 ± 0.05 , respectively. The perfusion pressure was maintained to a range of 55–80 mmHg by adjusting the flow rate (1.5–2 ml/min).

Optical mapping of the mouse heart

The fluorescence was excited by a DC-powered light source at 520 ± 45 nm. The emission was collected above 610 nm by a 16×16 element square matrix of photodiodes, which is coupled to a computerized data conditioning and acquisition system. Data was filtered at 1 kHz and sampled at a rate of 1,894 frames per second, yielding temporal resolution of 528 μsec . The field of view was 7×7 mm in all experiments.

Recording of optical action potentials

Fifteen minutes after cannulation, the heart was stained with a voltage-sensitive dye di-4-ANEPPS (10 μM , Molecular Probes) for 5 to 10 min. Action potentials on anterior surface were recorded for additional 30 min. The electrocardiogram was recorded immediately after the heart was cannulated until the perfusion was completed. To determine the frequency of VT occurred in isolated transgenic hearts, 23 transgenic mice from TG-NS/LQT3 L3, 15 from TG-NS/LQT3 L12, and 43 wild type control mice were analyzed. The transgenic and wild type littermate control mice were sex- and age-matched.

In pharmacological studies, mexiletine hydrochloride (15 μM) or nifedipine (1 μM) was constantly injected in the perfusion solution prior to cannulation to maintain the final concentration based on the perfusion rate of 1.5 ml/min. Eight to sixteen TG-NS/LQT3 L12

mice were used in each drug-treated group. The drugs were applied in the same protocol: 30 min treatment, 60 min washout and then additional 30 min treatment.

The software programs used for signal analysis in this study were previously described [6]. These programs automatically calculate maps of activation, repolarization and action potential duration (APD) from 256 optical recording windows.

Statistical analysis

All values are expressed as mean $\pm$ SEM. The paired t-test was used to evaluate the drug effects on ECG parameters before and after treatments. $P < 0.05$ was set for significance.

Results

High frequency of VT in Langendorff-perfused TG-NS/LQT3 L12 hearts and suppression of VT by mexiletine and nifedipine

We recorded ECGs from Langendorff perfused murine hearts, which allowed easy detection of VT. No E-C decouplers nor voltage sensitive dye, di-4-ANEPPS, were applied to avoid any side effects of such reagents. A high frequency of VT was detected in TG-NS/LQT3 hearts. The frequency of polymorphic VT developed spontaneously in transgenic hearts was 15.8% (3 out of 22) in TG-NS/LQT3 L3 and 73.3% (11 out of 15) in TG-NS/LQT3 L12 mice (Fig. 1A). The polymorphic VT in each transgenic heart appeared as multiple episodes (from 4 to 10 runs or above) lasting from a few beats to minutes and self-terminated in the tested period (40 minutes from cannulating). No episode of VT was detected in the littermate control hearts (Fig. 1A, NTG).

A high frequency of VT developed in TG-NS/LQT3 L12 mouse hearts provided a novel system to determine the effects of various pharmacological agents on VT and VF in this arrhythmia model. We tested two drugs, mexiletine (late persistent sodium current blocker) [7] and nifedipine (L-Ca²⁺ channel blocker) on the frequency of spontaneous VT in TG-NS/LQT3 L12 mice. Nifedipine was included in this study to evaluate the contribution of intracellular Ca²⁺ handling to the occurrence of polymorphic VT. Mexiletine at a concentration of 15 μ M dramatically reduced the frequency of polymorphic VT from 73.3% to 12.5% (Fig. 1A). Interestingly, nifedipine (1 μ M) was also able to reduce the frequency of polymorphic VT from 73.3% to 31.3% (Fig. 1A). The effects of mexiletine and nifedipine on mouse ECGs are summarized in Fig. 1B. Mexiletine slowed the heart rate, reduced the QT interval, and prolonged the PR interval. Nifedipine did not change these parameters significantly.

Ventricular extrasystoles precedes initiation of VT and reentry maintains VT

We recorded ventricular action potentials at 256 different windows after the heart was stained with a voltage-sensitive dye di-4-ANEPPS (10 μ M, Molecular Probes). In total, we were able to capture 19 images of action potential recording showing the transition from sinus rhythm to VT. Among these, 13 episodes showed activation of new firing foci at clearly different locations while the other 6 episodes exhibited only a subtle activation pattern shift, but suddenly accelerated firing (i.e. firing at the same or very close locations). These results suggest that new activation firing can occur at any location in the heart.

Results from recording of a series of continuous optical action potentials from log files 268 to 289 are shown in Fig. 2. The traces in Fig. 2A were about 30 seconds upstream of that in Fig. 2B. In Fig. 2A, repetitive extrasystoles were observed and indicated by boxes. Two extrasystolic beats b and d were mapped to compare with their pre-extrasystolic (sinus) beats a and c. Isochronal activation maps showed that the firing direction in b and d was suddenly changed to an opposite direction from a and c (also see Supplementary Video 1 and 2). Thirty

seconds after extrasystoles b and d, the initiation of an episode of VT was detected (Fig. 2B). These results suggest that the VT episodes are preceded by repeated occurrence of multiple ventricular extrasystoles, which are accompanied by firing from a new focus. Interestingly, sustained VT was clearly maintained by a re-entry pattern as shown in Fig. 2B (also see Supplementary Video 3).

The three transition beats before the transition from sinus rhythm to VT as well as three beats after VT were optically mapped (Fig. 3I-II). Three isochronal activation maps in row I represented the activation patterns from 3 continuous sinus beats before VT. Starting with the first beat of VT, activation started from a totally different location and moved to a different direction. These results suggest that the initiation of VT is accompanied with ectopic firing from different locations. Furthermore, the firing from the ectopic foci conducted at a slowed conduction rate (16, 20, and 22 ms) compared to non-extrasystolic beats (5, 10, and 11 ms) (Fig. 3, rows I and II).

We also mapped the action potentials when the VT terminated (see **rows III and IV**, Fig. 3). At the second beat after VT, two wave-fronts of activation collided (middle panel of IV). Row IV shows that after VT, the firing has not returned to sinus yet, but the conduction became accelerated (from 22–28 ms in row III to 5–7 ms in row IV). In total, we captured 18 episodes with the transition from VT back to sinus rhythm. Among these episodes, 15 shifted foci back to the original firing sites from ectopic foci, whereas the other three presented subtle shift in firing foci and were terminated by suddenly slowed firing.

Effect of mexiletine on activation of action potentials in TG-NS/LQT3 L12 hearts

In the optical mapping study, perfusion of mexiletine prevented development of VT. After washout of mexiletine, VT re-developed (see ECG in Fig. 4). The direction of activation firing was different after washout (beat 1, 2–4) from in the presence of mexiletine. Interestingly, initiation of a VT episode was started with reentry (Fig. 4, beat 9) and maintained by reentry thereafter (Fig. 4, Beat 11–19). On the activation map, it was apparent that the firing rate and conduction velocity were normalized in the presence of 15 μ M mexiletine (the first map, Fig. 4, 12 ms). In the two ectopic beats and VT episode after the mexiletine washout, conduction was propagated at a slower rate, 35 ms and 40 ms, respectively.

Discussion

Our studies on epicardial surface ventricular action potentials from the perfused hearts using the optical mapping system showed that polymorphic VT was mainly preceded by ventricular extrasystoles and maintained by a reentrant mechanism in this model. Some episodes of VT were initiated by automatically accelerated heart activation firing from the seemingly same focus or a nearby focus. Furthermore, extrasystoles and re-entry were associated with slow conduction of activation wave-fronts. These results provide the first spatial view of VT in a genetic mouse model for LQT3.

Detection of multiple activation foci that can initiate VT in TG-NS/LQT3 hearts is consistent with the automaticity theory of arrhythmogenesis. Non-suppressed automaticity of ventricular cells will cause abnormal initiation of firing, resulting in ectopic beats and VT. Increased automaticity in TG-NS/LQT3 hearts may be related to our previous finding that TG-NS/LQT3 myocytes presented abnormal APD restitution with increasing rate [4]. APD in these myocytes prolonged dramatically with increasing pacing rate. At a cycle length of 300 ms or above, myocytes failed to maintain pacing and EADs developed during the recording. But, the mechanism underlying abnormal APD restitution remains unknown.

We studied the late sodium current blocker, mexiletine, and the L-type Ca^{2+} channel blocker, nifedipine for their effects on VT. Both mexiletine and nifedipine were shown to reduce the frequency of polymorphic VT in TG-NS/LQT3 hearts. The finding that nifedipine greatly reduced the frequency of VT implicates a novel mechanism, i.e. abnormalities of calcium handling, for the pathogenesis of VT in TG-NS/LQT3 mice. Our recent observations that verapamil was effective in decreasing the incidence of TG-NS/LQT3 action potential alternans and in eliminating the poor APD restitution with an increasing stimulation rate provide additional support that an abnormality in Ca^{2+} handling is a contributing factor for development of VT in the LQT3 mice [8]. Given the net excess of Na^{+} ions due to the late sodium current generated by mutation N1325S [4], it is likely that the state of intracellular Ca^{2+} ions could be affected due to the activation of the reverse mode of the sodium-calcium exchanger (Ca^{2+} influx and Na^{+} extrusion). Further studies will test this hypothesis. Moreover, it is important to test whether other *SCN5A* mutations will act by similar mechanisms as N1325S. Future studies should intend to replicate the findings from the mouse in LQT3 patients, if heart tissues become available from patients. The studies of molecular mechanisms of VT in LQTS patients are severely impeded by the lack of patient tissues, thus studies in genetic mouse models are critical in exploring novel leads. If confirmed in the humans, our finding may have clinical implications in the treatment of VT associated with LQT3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Yong S, Tian X, Wang Q. LQT4 gene: the missing ankyrin. *Molecular Interventions* 2003;3:131–136. [PubMed: 14993420]
2. Wang Q, Shen J, Li Z, Timothy K, Vincent GM, Priori SG, Schwartz PJ, Keating MT. Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia. *Hum Mol Genet* 1995;4:1603–1607. [PubMed: 8541846]
3. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. *SCN5A* mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995;80:805–811. [PubMed: 7889574]
4. Tian XL, Yong SL, Wan X, Wu L, Chung MK, Tchou PJ, Rosenbaum DS, Van Wagoner DR, Kirsch GE, Wang Q. Mechanisms by which *SCN5A* mutation N(1325)S causes cardiac arrhythmias and sudden death in vivo. *Cardiovasc Res* 2004;61:256–267. [PubMed: 14736542]
5. Baker LC, Wolk R, Choi BR, Watkins S, Plan P, Shah A, Salama G. Effects of mechanical uncouplers, diacetyl monoxime, and cytochalasin-D on the electrophysiology of perfused mouse hearts. *Am J Physiol Heart Circ Physiol* 2004;287:H1771–H1779. [PubMed: 15191898]
6. Cheng Y, Mowrey KA, Nikolski V, Tchou PJ, Efimov IR. Mechanisms of shock-induced arrhythmogenesis during acute global ischemia. *Am J Physiol Heart Circ Physiol* 2002;282:H2141–H2151. [PubMed: 12003822]
7. Dumaine R, Wang Q, Keating MT, Hartmann HA, Schwartz PJ, Brown AM, Kirsch GE. Multiple mechanisms of Na^{+} channel-linked long-QT syndrome. *Circ Res* 1996;78:916–924. [PubMed: 8620612]
8. Yong SL, Ni Y, Zhang T, Tester DJ, Ackerman MJ, Wang QK. Characterization of the cardiac sodium channel *SCN5A* mutation, N1325S, in single murine ventricular myocytes. *Biochem Biophys Res Commun.* in press

A

	No treatments	Mexiletine (15 μ M)	Nifedipine (1 μ M)
NTG	0/43 (0%)	N/A	N/A
LQT3 mice:			
TG-LQT3 L3	3/22 (15.8%)	N/A	N/A
TG-LQT3 L12	11/15 (73.3%)	1/8 (12.5%)	5/16 (31.3%)

B **Fig. 1.**

Suppression of VT by mexiletine and nifedipine. A: Frequency of VT before and after treatments in non-transgenic mice (NTG) and TG-NS/LQT3 L3 and TG-NS/LQT3 L12 mice modeling LQT3. B: Summary of ECG parameters affected by mexiletine and nifedipine. L12: TG-NS/LQT3 L12; L12-mex: TG-NS/LQT3 L12 treated with mexiletine; L12-nif: TG-NS/LQT3 L12 treated with nifedipine. Stars represent $p < 0.05$.

Fig. 2.

Isochronal maps for the initiation and maintenance of VT in TG-NS/LQT3 L12. A: An action potential recording 30s before VT (see B). Four extrasystoles were observed and boxed. Among these extrasystoles, the isochronal maps of b and d as well as sinus beats a and c were shown at the low panel. Note that the moving direction of the activation wave is from white traces to orange ones. B: A typical action potential trace recorded during VT (left) and its isochronal map (right). The arrow points to the propagation direction of wavefront during reentry.

Fig. 3.

Isochronal maps for the transition and termination of VT in TG-NS/LQT3 L12. Six maps in row I and II represent 6 continuous beats with transition from sinus firing (3 beats) to VT (3 beats), while the 6 maps from row III and IV represent 6 continuous beats with transition from VT (3 beats) to the slowed heart beats (3 beats). The middle is the recorded action potential. Note that the moving direction of the activation wave is from white traces to orange ones.

Fig. 4.

Reoccurrence of VT in TG-NS/LQT3 L12 when mexiletine was withdrawn. The action potential recording (middle) represents an episode of VT (beats 9–19) eight minutes after mexiletine was washed out. The first map is a sinus beat in the presence of mexiletine. The others are the representative maps from beats 1–19. Note that the moving direction of the activation wave is from white traces to orange ones.