

Transmural conduction time in an early repolarization syndrome model

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Abstract. In the pathological aspect of J wave syndrome, delayed depolarization is defined as the difference in local conduction velocity of the ventricular myocardium. If polymorphic ventricular tachycardia is induced without local conduction velocity heterogeneity, this contradicts the delayed depolarization theory. In the present study, the transmural conduction time at was evaluated at several transmural locations in a canine early repolarization model. The transmural pseudo-electrocardiogram and endocardial/epicardial action potentials were recorded from coronary-perfused canine left ventricular wedge preparations (n=18). The I_{to} agonist NS5806 (9-10 μM), Ca^{2+} channel blocker verapamil (2 μM) and acetylcholine (ACh) (2 μM) were used to pharmacologically mimic early repolarization syndrome genotypes. The transmural conduction times were measured at five fixed epicardial unipolar electrodes before and after the perfusion of provocative agents. The transmural conduction time was defined as the time from endocardial stimulation to the maximal negative deflection (dV/dt) of the endocardial electrogram at the unipolar electrode. Polymorphic ventricular tachycardia developed in 14/18 preparations. In the transmembrane action potentials, there was no definite delayed phase 0 upstroke in any induced polymorphic ventricular tachycardia preparations. In all preparations, the transmural conduction time increased significantly after perfusing the I_{to} agonist NS5806, verapamil and Ach; however, the increase was only 2.6 ± 0.4 msec, and dispersion of the transmural conduction time did not exhibit significant heterogeneity (7.16 ± 0.93 vs. 7.76 ± 1.21 msec; $P=0.240$). In the early repolarization model, polymorphic ventricular tachycardia was induced without

any regional conduction velocity heterogeneity. This finding suggests that local depolarization heterogeneity would not be a major contributor to the generation of ventricular arrhythmia in the early repolarization syndrome wedge preparation model.

Introduction

Early repolarization patterns are characterized by J point elevation, distinct J waves combined with or without ST-segment elevation or slurring in the terminal portion of the QRS wave in inferolateral electrocardiogram (ECG) leads. An early repolarization pattern on ECGs has traditionally been considered to be benign (1,2); however, according to previous experimental studies, a more pathological prognosis has been suggested (3,4), and subsequently confirmed in clinical studies reported by Haïssaguerre *et al* (5) and Nam *et al* (6,7).

The early repolarization hypothesis could be explained in terms of ionic and cellular mechanisms. The ionic and cellular mechanisms associated with generation of an early repolarization pattern are similar to those responsible for J wave manifestation and ST-segment elevation in Brugada syndrome (BrS) (4). A net outward shift in the balance of the current active in the early phases of the epicardial action potential (AP), which is secondary to reduction of I_{Ca} or I_{Na} or augmentation of I_{K-ATP} , has been demonstrated to underlie the early repolarization ECG pattern and substrate responsible for the development of life-threatening arrhythmias associated with early repolarization syndrome (ERS) (8-12).

The delayed depolarization hypothesis could be expressed as follows. An *SCN5A* loss-of-function mutation leads to reduced sodium current, which may in turn decrease the V_{max} of the membrane AP and thus reduce membrane excitability. This may result in conduction delays. The conduction delay between the right ventricular apex (RVa) and right ventricular outflow tract (RVot) generates the BrS coved-type ECG.

However, debates on the mechanism underlying J wave syndrome including BrS and ERS have been ongoing for decades (13). Theoretically, both the two mechanisms-delayed depolarization theory and early repolarization theory are feasible. Recently, Haïssaguerre *et al* (14) defined ERS with/without a conduction delay. In other words, they described

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both ERS with delayed depolarization and ERS with early repolarization.

The delayed depolarization theory may be explained by regional conduction velocity heterogeneity in the ventricular myocardium (15). Thus, if polymorphic ventricular tachycardia (pVT) develops without any regional conduction velocity heterogeneity, it would be less compatible with the delayed depolarization. The aim of the present study was to identify whether pVT could develop without regional conduction velocity heterogeneity. In the present study, the transmural conduction time was evaluated at several transmural locations using a canine ERS wedge preparation model.

Materials and methods

Wedge preparations and electrogram recordings. All experiments were conducted in accordance with the guidelines for the management and use of laboratory animals by the Institutional Animal Care and Use Committee. The study was approved by the Animal Care and Use Committee at Chonnam National University, Gwangju, Republic of Korea (approval number, CNU IACUC-H-2017-33). Detailed methods for the wedge isolation and electrogram recording from coronary-perfused canine left ventricle (LV) wedge preparations have previously been reported (16,17). The animals were supplied by Orient Bio, Inc. Briefly, adult mongrel dogs (age, ~1 year-old, weight, 20-35 kg; 9 males, 9 females) underwent anticoagulation with heparin (300 units/kg IV) and were anesthetized with pentobarbital (30-35 mg/kg IV). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution [cold (4°C) Tyrode's solution containing 12 mmol/l KCl]. Death of the animal was confirmed by personnel trained to recognize cessation of vital signs in euthanasia. The wedge preparations (2.2-2.4x2.0-2.2x1.8-1.9 cm³) were transmurally dissected from the inferolateral free wall of the LV. The second or third diagonal artery ostium was cannulated using a handmade small plastic cannula and fixed using a thread, while the cardioplegic solution [Tyrode's solution containing NaCl (129 mM), KCl (12 mM), NaH₂PO₄ (0.9 mM), NaHCO₃ (20 mM), CaCl₂ (1.8 mM), MgSO₄ (0.5 mM) and glucose (5.5 mM)] was delivered via the cannula. Non-perfused tissue was carefully cut using a razor blade. After being placed in a tissue bath, Tyrode's solution containing KCl (4 mM) was delivered using a roller pump (Cole Parmer Instrument Co., Ltd.) at a constant flow rate of 8-10 ml/min and warmed to 37±0.5°C. The temperature was maintained at 37±0.5°C throughout the experiment. The pH was 7.4.

The preparation was equilibrated in the tissue bath until electrically stabilized for ~1 h, while being stimulated at a cycle length of 1,000 msec using bipolar silver electrodes, insulated up to the tips. The preparations were stimulated endocardially. A transmural pseudo-ECG was obtained using two electrodes, consisting of AgCl half cells placed in the tissue bath. The tip of the two electrodes were placed along the same axis as the transmembrane AP recordings. The distance between endocardial or epicardial surface and the pseudo-ECG electrode was 1.0-1.5 cm; the epicardial electrode was connected to the positive input of the ECG amplifier.

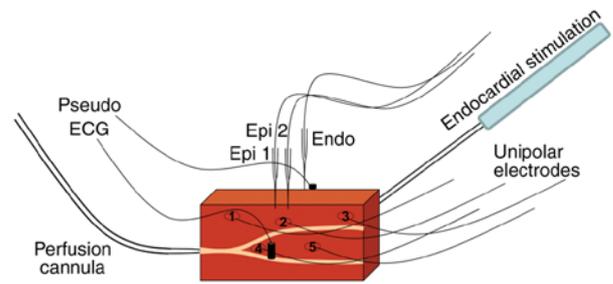


Figure 1. Schematic image of a left ventricular canine coronary-perfused wedge preparation indicating the recording electrodes used. Transmembrane action potentials were acquired simultaneously from two epicardial sites and one endocardial site using floating microelectrodes. A pseudo-ECG was acquired using two AgCl electrodes placed across the preparation. Five unipolar electrodes were evenly distributed on the epicardial surface. ECG, electrocardiogram; Endo, endocardial; Epi, epicardial.

The transmembrane APs were acquired simultaneously from two epicardial sites (distance from epicardial 1 to 2 was 5-10 mm) and one endocardial site using floating microelectrodes (DC resistance=10-20 MΩ) filled with 2.7 mol/l KCl; each microelectrode was connected to a high-input impedance amplifier. Five unipolar electrodes (1, 2, 3, 4 and 5) were evenly distributed on the epicardial surface (Fig. 1). Each individual microelectrode had its own reference electrode at the shortest possible distance. The virtual bipolar electrograms were derived as the difference of the adjacent two unipolar electrograms (1-2, 2-3, 1-4, 2-4, 2-5 and 3-5 pairs).

Measurement of the transmural conduction time. Spike2 for Windows version 7 (Cambridge Electronic Design Ltd.) was used to record and analyze ECGs, electrograms and APs. All electrograms and analyses were manually checked by three independent observers. The transmural conduction time was defined as the time from the endocardial pacing to the maximal negative deflection (dV/dt) of the signal at the unipolar electrode. The transmural conduction times were measured prior to the perfusion of provocative agents and immediately before pVT development at the five fixed epicardial unipolar electrodes and two fixed epicardial floating microelectrodes. The transmural conduction times at these seven fixed transmural locations were evaluated. Conduction time dispersion was defined as the difference between the longest and the shortest conduction time.

Arrhythmia induction. The ERS model setup and arrhythmia induction procedure were conducted according to the previously reported methods (3,9). To produce the pharmacologically similar ERS genotypes, the I_{to} agonist NS5806 (9-10 μM), calcium channel blocker verapamil (2 μM) and acetylcholine (ACh; 2 μM) were added to the perfusate. All preparations were endocardially stimulated at a cycle length of 1,000 msec. When ventricular arrhythmias occurred spontaneously after the perfusion of provocative agents, this was referred to as arrhythmia inducibility.

Statistical analysis. For continuous variables, a paired Student's t-test was used to analyze differences between

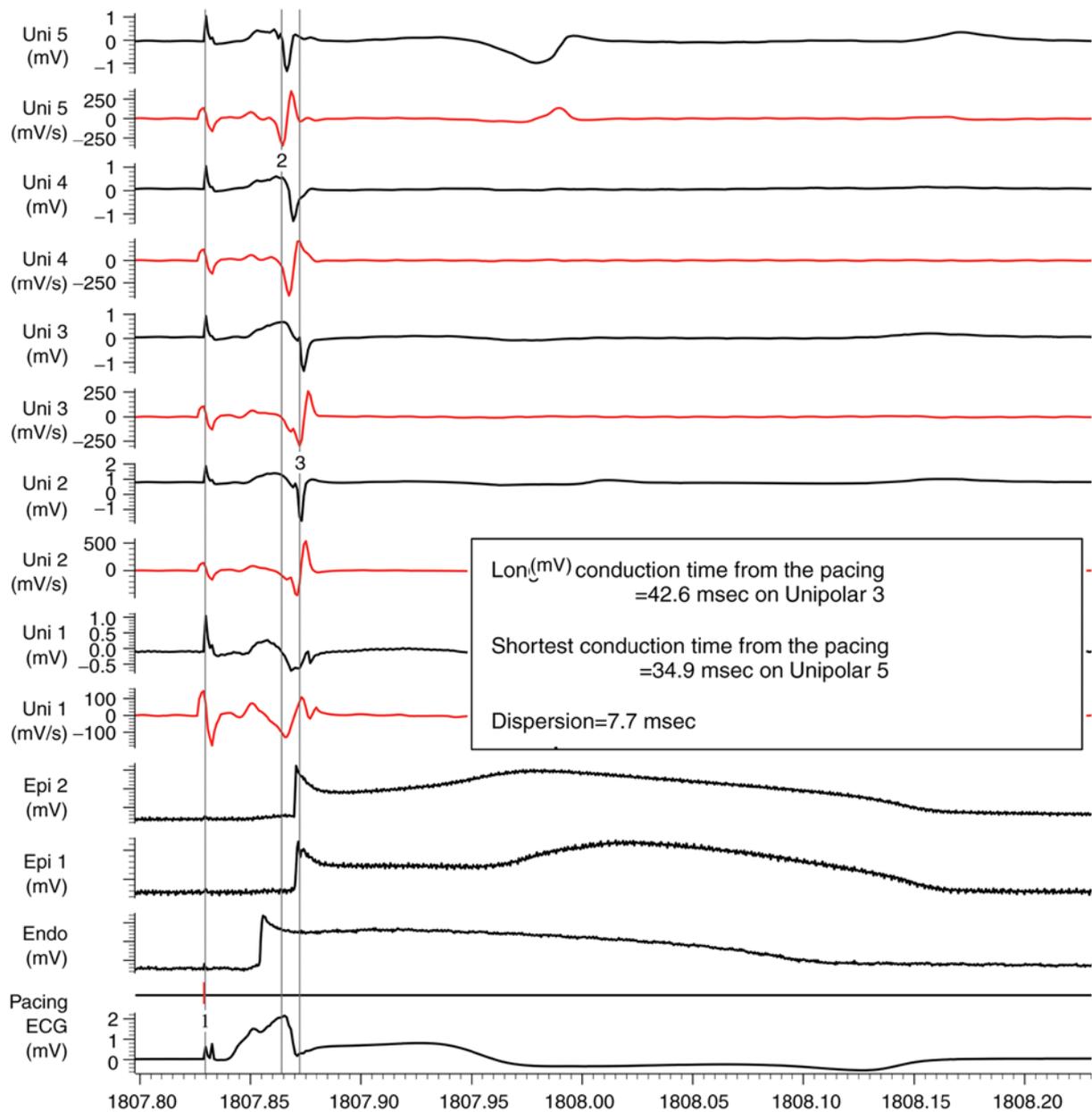


Figure 2. Transmural conduction time was defined as the time from endocardial pacing to the maximal negative deflection (dV/dt) of the signal at the unipolar electrode. APs, action potentials; ECG, electrocardiogram; Endo, endocardial; Epi, epicardial; Uni, unipolar electrode.

the before and after the provocative agents. For statistical analyses, the Statistical Package for the Social Sciences 25.0 software (IBM corp.) was used. Data are described as mean \pm standard error of the mean (SEM). A two-tailed analysis was used, and a P-value of <0.05 indicated statistical significance.

Results

The transmural pseudo-ECG and endocardial/epicardial APs were recorded in the coronary-perfused canine left ventricular wedge preparations (n=18). All preparations were endocardially stimulated at a cycle length of 1,000 msec. The transmural conduction time and the dispersion were measured. As indicated in Fig. 2, the longest conduction time on Unipolar 3 was 42.6 msec and the shortest conduction time on Unipolar 5 was

34.9 msec. Thus, the transmural conduction time dispersion was 7.7 msec in the preparation.

Coronary-perfusates of I_{to} agonist, calcium channel blocker and ACh were used to induce a state similar to the genetic defects and conditions known to be associated with ERS. In the present study, the ERS model was induced in 14/18 preparations. Under baseline conditions, the ECG did not show a marked J wave and augmentation of the AP notch was not seen in the epicardial sites (Fig. 3A). In the ECG, marked J waves with ST-segment elevation were induced by the I_{to} agonist NS5806 (9-10 μ M), Ca^{2+} channel blocker verapamil (2 μ M), and ACh (2 μ M), secondary to augmentation of the AP notch in the epicardium but not in the endocardium. For example, the maintained dome at the epicardial 1 electrode and loss of the dome at the epicardial 2 electrode were identified (Fig. 3B). It resulted in phase 2 re-entry (P2R) (Fig. 3B).

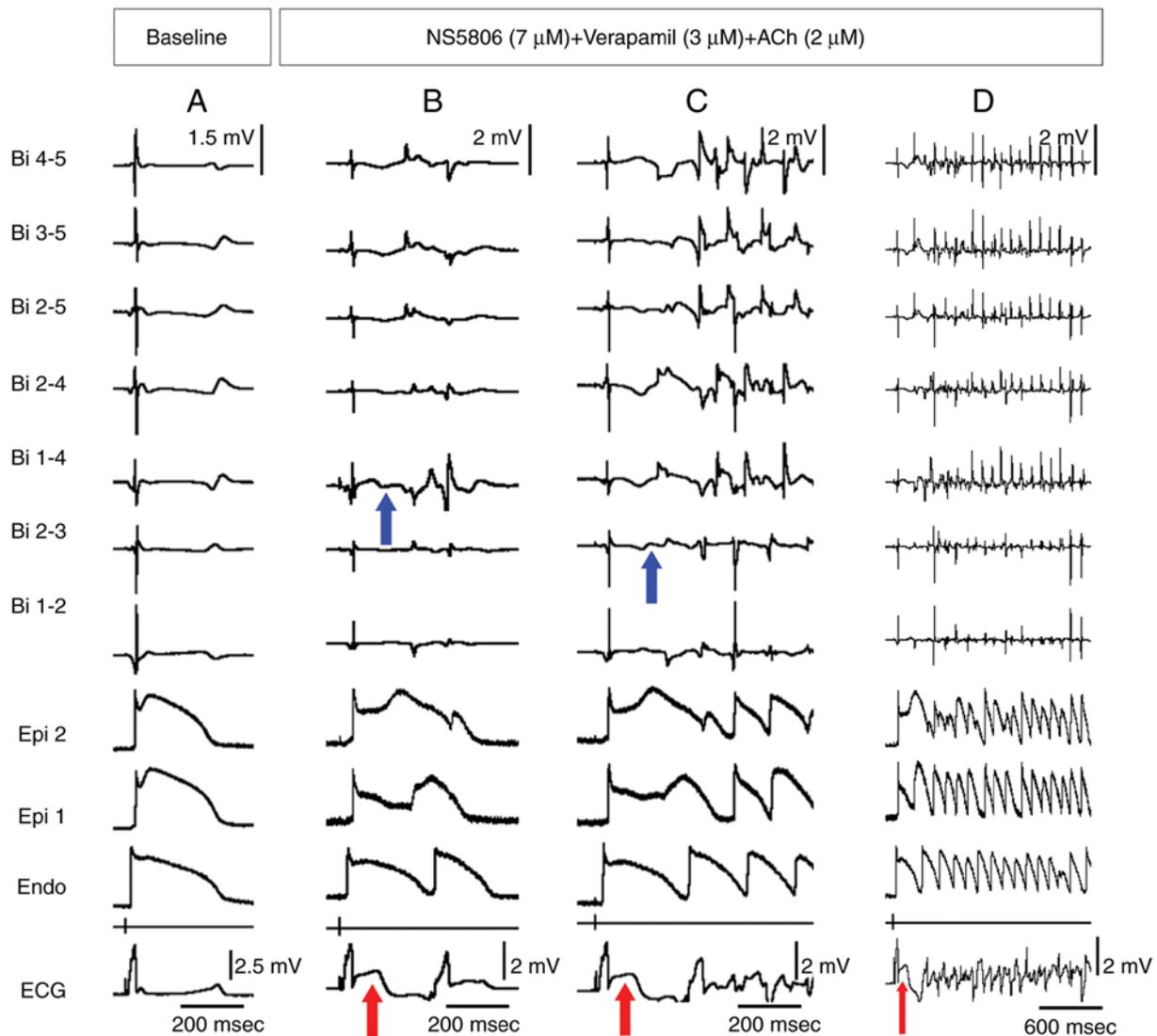


Figure 3. Experimental model of ERS created using a combination of the Ito agonist (NS5806), calcium channel blocker (verapamil) and ACh to mimic the genetic defects and conditions known to be associated with ERS. (A) Baseline. (B) Recorded during stimulation of the endocardium at a BCL of 1,000 msec at 20 min after the addition of the provocative agents to the coronary perfusate. Note the appearance of a pronounced ST-segment elevation (red arrows) in the ECG, low amplitude fractionated bipolar electrogram activity (Bi 1-4, Bi 2-3; blue arrows) secondary to epicardial AP maintained dome and loss-of-dome and phase 2 reentry. The epicardial reentry induced a single echo to the endocardium. (C) Then, the P2R propagated to the endocardium and precipitated pVT. The pVT sustained for 1-2 sec in the beginning. (D) The pVT continued for more than 30 sec. APs, action potentials; Bi, bipolar electrode; ECG, electrocardiogram; Endo, endocardial; Epi, epicardial; pVT, polymorphic ventricular tachycardia; ACh, acetylcholine.

Successful propagation of the P2R beat generated VT/VF on the electrograms in Fig. 3C. The pVT sustained for 1-2 sec in the beginning and sustained for more than 30 sec (Fig. 3D). The pVTs developed in 14/18 preparations. Slight 'delayed' phase 0 upstrokes of the transmembrane AP occurred in the preparations. Increases in the conduction delay were 3.2 ± 2.9 and 1.8 ± 2.8 msec for the epicardial glass microelectrodes 1 and 2, respectively.

The transmural conduction time, during which the impulse propagated from the endocardial pacing site to each epicardial site (two epicardial glass microelectrodes and five epicardial unipolar electrodes), increased significantly after the I_{to} agonist NS5806 (9-10 μ M), Ca^{2+} channel blocker verapamil (2 μ M) and ACh (2 μ M) were perfused (Fig. 4; Table I). The mean increase in the conduction time before and after the perfusion of provocative agents was 2.6 ± 0.4 msec. However,

dispersion of the transmural conduction time did not exhibit any significant difference (7.16 ± 0.93 msec vs. 7.76 ± 1.21 msec; $P=0.240$; Fig. 5).

Discussion

ERS is associated with the gain-of-function mutations in the pore-forming subunit of the ATP-sensitive potassium channel (*KCNJ8*) (10,12), loss-of-function mutations in the $\alpha 1$, $\beta 2$, and $\alpha 2\delta$ subunits of the cardiac L-type calcium channel (*CACNA1C*, *CACNB2* and *CACNA2D1*) (11), and loss-of-function mutations in the sodium channel activity (*SCN5A*) (18). Moreover, the greater vulnerability of the inferior wall of LV to early repolarization has been ascribed to the presence of a higher density of the transient outward current (I_{to}) (9). Both the electrocardiographic and arrhythmic features of ERS are

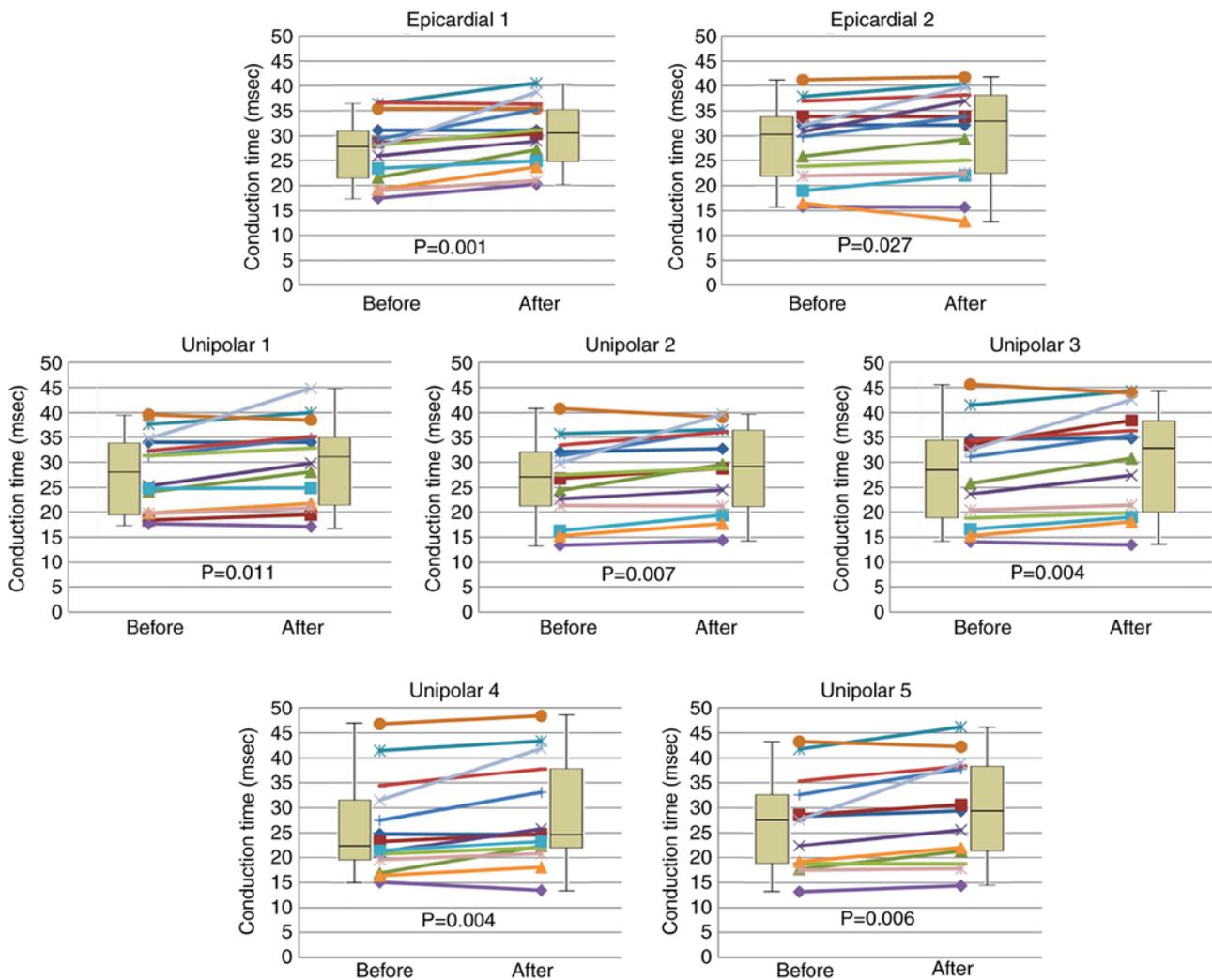


Figure 4. Serial plotting of the transmurals conduction time during which the impulse propagates from the endocardial pacing site to each epicardial site (two epicardial glass microelectrodes and five epicardial unipolar electrodes) before and after the provocative agents [NS5806 (9-10 μ M), verapamil (2 μ M) and acetylcholine (2 μ M)] were perfused. The yellow boxes are boxplots of the conduction time. The transmurals conduction time increased significantly after the Ito agonist verapamil and acetylcholine were perfused. The mean difference in the conduction time before and after perfusion was 2.6 ± 0.4 msec.

enhanced by vagal influences (19-24). Thus, a pharmacological approach was used in the present study to produce ERS-related conditions and genetic defects. Several agents were used, such as NS5806 for increased I_{to} current, verapamil for the loss-of-function mutation in the calcium channel activity and ACh for increased vagal tone (4,8,17).

According to the depolarization abnormality theory of BrS, the syndrome develops due to a difference in the conduction time between the RVot (25) and RVa, and a 200 msec difference in the conduction time is necessary (26). This may be regarded as a longitudinal conduction time difference; however, the longitudinal conduction time is different from the transmurals conduction time reported in the present study (endocardial to epicardial conduction time). Therefore, it may be illogical to explain the arrhythmogenicity of BrS using the results of the present transmurals conduction time study. However, in certain examples of ERS reported in a study by Haïssaguerre *et al* (14), the epicardial depolarization wavefront did not cross the J wave onset. This means that the local conduction delay is shorter and, thus, local conduction time dispersion is small.

Therefore, the result of the present study demonstrated JWS without a conduction delay, which was previously classified by Haïssaguerre *et al* (14). It was identified that arrhythmia reported in the present study was induced spontaneously, without conduction time heterogeneity. Di Diego *et al* (27) reported that there was no significant conduction delay between the RVot and RVa in their canine whole heart Langendorff model of BrS.

In the present experiment, the I_{to} agonist NS5806 (9-10 μ M), Ca^{2+} channel blocker verapamil (2 μ M) and Ach (2 μ M) induced prominent J waves due to the transmurals voltage gradients. In brief, K^{+} efflux by the I_{to} agonist and Ca^{2+} influx by the Ca channel blocker were involved in epicardial repolarization due to the dense distribution of I_{to} in the epicardium. It was hypothesized that the P2R was induced by regional AP loss of dome and maintained dome in the epicardium (Fig. 3B). This phenomenon is associated with heterogeneous shortening of the epicardial action potential duration (APD). Eventually, transepical heterogeneity induced a transmurals voltage gradient and precipitated VT/VF. Koncz *et al* (9) reported the

Table I. Transmural conduction time during which the impulse propagates from the endocardial pacing site to each epicardial site (2 epicardial glass microelectrodes and 5 epicardial unipolar electrodes) before (n=14) and after (n=14) the provocative agents were perfused.

Electrode	Transmural conduction time before treatment, msec	Transmural conduction time after treatment, msec	P-value
Glass electrode 1	27.1±1.7	30.3±1.7	0.001
Glass electrode 2	28.3±2.1	30.2±2.5	0.027
Unipolar 1	27.9±1.9	30.1±2.3	0.011
Unipolar 2	26.4±2.1	28.9±2.2	0.007
Unipolar 3	27.7±2.6	30.4±2.8	0.004
Unipolar 4	25.7±2.5	28.5±2.8	0.004
Unipolar 5	26.6±2.6	29.5±2.9	0.006
Dispersion	7.16±0.93	7.76±1.21	0.240

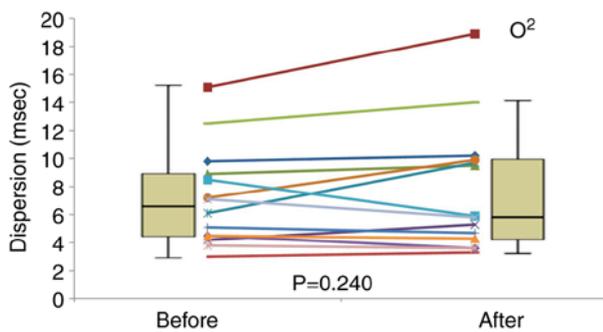


Figure 5. Comparison of the dispersion of transmural conduction time. Conduction time dispersion was defined as the difference between the longest and shortest conduction time. The transmural conduction time increased significantly. However, dispersion of the transmural conduction time did not exhibit a significant difference (7.16±0.93 msec vs. 7.76±1.21 msec; P=0.240).

heterogeneous shortening of APD₉₀ in the same model. According to our previous study, the QT interval was only prolonged by 13 msec, and the value did not contribute to the occurrence of ventricular arrhythmia (28). Moreover, the results of the present study indicated that there was no significant difference in the QT interval when compared to the baseline state.

Assuming that the ERS is generated by delayed depolarization or early repolarization, one of the key factors is a voltage gradient (8,13,14). In all theories, a great enough voltage gradient is required to induce reentrant arrhythmia. In the present study, the mean increase in the transmural conduction time after perfusing the provocative agents was only 2.6±0.4 msec, which was notably smaller than the 200 msec suggested by Wilde *et al* (26). This would be insufficient to create a steep transmural voltage gradient to generate arrhythmia. However, in the present experiment, the transmural dispersion of repolarization (TDR) was very long. For example, the TDR in Fig. 3B was 200 msec. The epicardial 1 electrode had a short APD and the endocardial electrode had a long APD, which induced a large TDR (200 msec). In the example presented in Fig. 3, the phase 2 voltage gradient between the endocardium and epicardium is very steep. This was sufficient to create a huge J wave including ST elevation (red arrows in Fig. 3).

If the J wave in ERS is generated due to temporal difference in the AP, a sufficient temporal delay of the AP should exist between the endocardium and epicardium. Therefore, the degree of temporal difference (conduction velocity difference in other words) should be, at least the same as, or larger than, the length of the J wave. When this concept was applied to the present experiment, it may have been possible to induce a very small J wave about 2.6 msec long. However, it was near impossible to induce large J waves, as revealed in Fig. 3B, which was recognized just before the development of VT/VF because there was not a sufficiently delayed phase 0 upstroke in the transmembrane AP recording, in any of the preparations. The delay was not enough to cause a steep transmural voltage gradient required to generate a reentrant arrhythmia.

As aforementioned, epicardial heterogeneity serves an important role in the occurrence of ventricular arrhythmias (29,30). If a sufficient temporal difference in the AP exists at the local epicardium sites, it may serve a role in the formation of arrhythmia substrates. Typically, an epicardial temporal difference in the AP increases the dispersion of the conduction time (31). The transmural conduction time, despite being very short, during which the impulse propagated from the endocardial pacing site to each epicardial site (two epicardial glass microelectrodes and five epicardial unipolar electrodes), increased significantly in the present experiments. However, dispersion of the transmural conduction time did not exhibit a significant difference (Fig. 5). Thus, this finding indicated that there was a homogeneous conduction delay.

Srinivasan *et al* (32) addresses that interventricular dispersion of the repolarization generates a voltage gradient for arrhythmias (33). However, we hypothesize that this theory also has an insufficient probability of being able to explain the creation of a steep transmural voltage gradient (34,35).

The present study supports the concept that ECG patterns of ERS and pVT can be reproduced by difference in the local repolarization, without heterogeneous conduction delay. However, the current results do not suggest that local depolarization heterogeneity is not associated with the underlying mechanism of ERS, because multiple factors (including autonomic nerve tones) seemed to be involved in

the development of ERS. A whole-heart global conduction, such as between RVot and RVa, was not demonstrated in the present study. Therefore, the role of global depolarization heterogeneity cannot be excluded when explaining the mechanism underlying ERS. The results of the present study may be used as a basis to establish the appropriate pharmacologic treatments for ERS. For example, several agents such as phosphodiesterase III inhibitors and flavonoids are being studied (36,37).

The wedge preparation model with a pharmacologically induced ERS phenotype is representative of a denervated heart. Thus, the model is in an autonomically denervated state. The canine wedge preparation model cannot reflect the global physiology of the whole heart. Notably, the use of whole heart Langendorff models would overcome this limitation.

Polymorphic ventricular tachycardia was induced in the canine wedge preparation ERS model without regional conduction velocity heterogeneity. This suggests that local depolarization heterogeneity would not greatly contribute to the generation of ERS with no conduction delay. However, the role of global conduction delay in ERS cannot be denied. Hence, demonstration of global conduction delay of action potentials using a whole heart Langendorff model or human heart is needed in further studies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NY was a major contributor in writing the manuscript and contributed to the design, literature search, experimental studies, data acquisition, data analysis, manuscript preparation, manuscripts editing, manuscript review, approval of the final version of the manuscript and agreed to all aspects of the work. HKJ contributed to the literature search, experimental studies, data analysis, statistical analysis, manuscripts editing, manuscript review, approval of the final version of the manuscript and agreed to all aspects of the work. KHL contributed to the literature search, statistical analysis, manuscript review, approval of the final version of the manuscript and agreed to all aspects of the work. HWP contributed in the conception of the work, manuscript review, approval of the final version of the manuscript and agreed to all aspects of the work. JGC contributed in the conception of the work, definition of intellectual content, manuscripts editing, manuscript review,

approval of the final version of the manuscript and agreed to all aspects of the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (38) and all experiments were approved by the Animal Care and Use Committee at Chonnam National University, Gwangju, Republic of Korea (approval number, CNU IACUC-H-2017-33).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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