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Osadchii, Oleg E.

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Oleg E. Osadchii

Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200, Copenhagen N, Denmark, and Department of Health Science and Technology, University of Aalborg, Fredrik Bajers Vej 7E, 9220 Aalborg, Denmark

Corresponding author: Dr Oleg Osadchii, Department of Health Science and Technology, University of Aalborg, Fredrik Bajers Vej 7E, 9220 Aalborg, Denmark

E-mail: osadchiy@hst.aau.dk

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- **What is the central question of this study?**

  Premature ventricular activations during clinical electrophysiological testing are associated with slowed conduction. It is uncertain whether this change is attributed to the prolonged activation latency, or increased impulse propagation time, or both.

- **What is the main finding and its importance?**

  Prolonged activation latency at the stimulation site is the critical determinant of conduction slowing and associated changes in the ventricular response intervals in premature beats initiated during phase 3 repolarization in perfused guinea-pig heart. These relations are likely to have an effect on arrhythmia induction and termination, independently of the presence of ventricular conduction defects, or the proximity of the stimulation site to the re-entrant circuit.

Abstract

During cardiac electrophysiological testing, slowed conduction upon premature ventricular activation can limit the delivery of the closely coupled impulses from the stimulation site to the region of tachycardia origin. In order to examine the contributing factors, in this study, cardiac conduction intervals and refractory periods were determined from the left ventricular (LV) and the right ventricular (RV) monophasic action potential recordings obtained in perfused guinea-pig hearts. A premature activation induced

immediately after the termination of the refractory period was associated with conduction slowing. The latter was primarily accounted for by the markedly increased (+54%) activation latency at the LV stimulation site, with only negligible changes (+12%) noted in the LV-to-RV delay. The prolonged activation latency was acting to limit the shortest interval at which two successive action potentials can be induced in LV and RV chamber. The prolongation of the activation latency in premature beats was accentuated upon an increase in the stimulating current intensity, or during hypokalemia. This change was related to the reduced ratio of refractory period to the action potential duration, which allowed extrastimulus capture to occur earlier during phase 3 repolarization. Flecainide, Na^+ channel blocker, prolonged both the activation latency and the LV-to-RV delay, without changing their relative contributions into conduction slowing. In summary, these findings suggest that the activation latency is the critical determinant of conduction slowing and associated changes in the ventricular response intervals upon extrastimulus application during phase 3 of the action potential.

Introduction

Programmed ventricular stimulation that involves a premature extrastimulus application after a train of regular pulses is widely used for testing the inducibility, entrainment, and termination of cardiac arrhythmia in patients with a history of recurrent ventricular tachycardia or aborted sudden cardiac death (Wellens *et al.* 1985; Marchlinski *et al.* 1994; Josephson *et al.* 2014). The efficacy of electrophysiological testing is importantly determined by the ability to deliver closely coupled impulses to the region of tachycardia origin, which ensures that the premature action potential arrives at the reentrant circuit at a critical time (i.e. during the excitable gap) shortly after previous excitation (Mitchell *et al.* 1986; Liem *et al.* 1988). Importantly, the programmed stimulation protocol is usually applied at the ventricular sites remote from the arrhythmic focus. Specifically, the right ventricular endocardium is the preferred stimulation site (Wellens *et al.* 1985; Marchlinski *et al.* 1994), because it is easily accessible via transvenous catheterization, whereas tachycardia in most cases originates from the left ventricle (Horowitz *et al.* 1980; Bogun *et al.* 2008), because myocardial infarction and subsequent arrhythmogenic remodeling more often occur in the left ventricular chamber.

During electrophysiological testing, a premature activation initiated prior to the full repolarization in the previous beat is associated with significant conduction slowing (Ramza *et al.* 1990; Koller *et al.* 1995a), an effect that limits the shortest interval at which two successive electrical responses can be induced at the remote ventricular regions (Mitchell *et al.* 1986; Liem *et al.* 1988). It is not clear whether conduction slowing upon premature activation is primarily attributed to the prolonged stimulus-response latency, or slowed impulse propagation from the stimulation site, or both (Liem *et al.* 1988; Koller *et al.* 1995b). The relative importance of these factors have not been systematically addressed, because clinical electrophysiological recordings are usually limited to the right ventricular apex (Mitchell *et al.* 1986; Liem *et al.* 1988; Koller *et al.* 1995a), and the impulse propagation time at the remote myocardial regions is not assessed. Likewise, measuring the stimulus-response latency from the bipolar electrogram recordings in the right ventricle is a challenging task, because a large stimulus artifact from the adjacent stimulating electrode is usually superimposed on the initial part of the electrogram.

The purpose of the present study was to define the relative contributions of the activation latency and the impulse propagation time to the conduction slowing upon premature electrical activations induced in perfused guinea-pig hearts. The programmed stimulations were applied in the left ventricle, and cardiac conduction intervals were assessed by recording the monophasic action potentials at the adjacent (left ventricular) and the remote (right ventricular) epicardial sites. The activation latency and the interventricular conduction delay were measured at different coupling stimulation intervals, basic drive cycle lengths, stimulating current strengths, and upon interventions known to slow ventricular conduction, such as hypokalemia and Na^+ channel blocker administration.

Methods

Ethical approval

The present study complies with the European Community Guidelines for the Care and Use of Experimental Animals and was approved by the Animal Ethics Screening Committee of the Panum Institute (clearance number: 2010/561-1799). Male Dunkin-Hartley guinea-pigs (Charles River, Sulzfeld, Germany) weighing 400–500 g were allowed to acclimate to the housing conditions, with free access to food and tap water, for at least 7 days prior to entry into the study. The experimental procedures used in this study comply with the policies and regulations described in the journal guidelines for reporting animal experiments (Grundby, 2015).

Isolated, Langendorff-perfused heart preparations

The experiments on isolated, perfused hearts were performed as described previously (Osadchii *et al.* 2006; Soltysinska *et al.* 2011). The guinea-pigs were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and anticoagulated with heparin (1000 IU/kg i.p.). The chest was opened, the hearts were immediately excised, mounted on a Langendorff perfusion set-up (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) and perfused via the aorta at a constant flow (15 ml/min) with carefully filtered, warmed physiological saline solution saturated with 95%O_2 and 5%CO_2. The perfusion solution contained (in mM) 118.0 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 25 $NaHCO_3$; 1.2 KH_2PO_4; 1.2 $MgSO_4$; and 10.0 glucose, and had a pH of 7.4. In a subset of experiments with hypokalemic perfusions, KCl concentration in the perfusion solution was decreased from 4.7 mM to 2.5 mM. The aortic perfusion pressure (65-70 mm Hg) was measured with a ISOTEC pressure transducer and the coronary flow rate was determined using an ultrasonic flowmeter probe (Transonic Systems Inc., USA) placed just above the aortic cannula. The electrical activity of the heart preparations was assessed from the volume-conducted ECG as well as monophasic action potential recordings. Throughout the experiments, the heart preparations were kept immersed in the temperature-controlled, perfusate-filled chamber to minimize thermal loss. Aortic pressure, coronary flow rate, ECG and ventricular action potentials were continuously monitored using the 16-channel PowerLab system (ADInstruments, Oxford, UK).

Monophasic action potentials, refractory periods, and electrical stimulations

In order to slow the intrinsic beating rate and enable ventricular stimulations at variable pacing intervals, both atria were removed and the atrioventricular node was crushed mechanically with forceps prior to taking electrophysiological recordings. Epicardial monophasic action potentials (MAP) were obtained from the base of the left ventricular (LV) and the right ventricular (RV) lateral wall using spring-loaded pressure contact electrodes (Hugo Sachs Electronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). The action potential duration was measured at 90% repolarization (APD_{90}). For electrical stimulations, the bipolar needle electrodes were placed at LV epicardium close (within 3-4 mm) to the adjacent MAP recording electrode. The stimulations were performed with 2 ms rectangular pulses generated by a programmable stimulator (Hugo Sachs Electronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). In addition to LV stimulations utilized in the main part of the study, in a subset of experiments, the electrical stimulations were applied at RV epicardium, close to the RV MAP recording electrode.

The programmed stimulation protocol involved an application of the burst of 10 regular (S_1) pulses followed by a premature extrastimulus (S_2) generated at progressively reduced coupling intervals (S_1–S_2). In successive stimulations, the S_1-S_2 interval was reduced from 500 ms to 200 ms in steps of 100 ms, followed by further 5-10 ms decrements until the refractoriness was reached. The longest S_1-S_2 interval that failed to produce ventricular capture was normalized by subtracting the activation latency in S_1 beat, and taken as the effective refractory period (ERP). In parallel, the functional refractory period was determined as the shortest ventricular response interval (V_1-V_2) attained upon progressive reduction of the S_1-S_2 interval (Liem *et al.* 1988). The V_1-V_2 interval was measured as the time between the action potential upstroke in the last S_1 beat in a drive train and the upstroke of the following S_2-evoked action potential, both in LV and RV MAP-recording sites.

Ventricular conduction

Ventricular conduction intervals were assessed in the last S_1 beat in a basic drive train and in premature beats upon extrastimulus application, as illustrated in Figure 1. The activation latency at the LV stimulation site was measured as the time from the pacing stimulus artifact to the fastest upstroke of the following action potential. The LV-to-RV conduction delay was determined as the delay between the upstrokes of the LV action potential and the RV action potential. The total conduction time was calculated as a sum of the activation latency and the LV-to-RV delay. The minimal ventricular capture interval (e.g. the S_1-S_2 interval applied in the second beat in Fig. 1) is referred to throughout the text as the S_1-S_2 coupling stimulation interval exceeding the ERP by 5 ms.

Study groups

The main data were obtained from three series of experiments in which the electrical stimulation protocol was applied at LV epicardium. In the first series (n=9), ventricular APD_{90}, refractory periods, and the S_2 vs. S_1 conduction intervals were measured upon variations in parameters of programmed stimulations, namely, the basic drive train cycle length (550 ms vs. 300 ms) and the stimulating current intensity (two times vs. five times diastolic threshold). The second series (n=11) examined effects produced by hypokalemic (2.5 mM KCl) vs. normokalemic perfusion (4.7 mM KCl). These levels of hypokalemia (2.5 mM KCl) have been shown to produce significant effects on ventricular repolarization and conduction in perfused guinea-pig heart in previous studies (Osadchii *et al.* 2009; Osadchii, 2014a). The third series (n=10) assessed electrophysiological changes produced by Na^+ channel blocker (flecainide) administration. Ventricular pacing thresholds determined at baseline were re-evaluated after hypokalemia or flecainide infusion (both were maintained over 30 min), and the pacing output was adjusted as needed. Finally, in the additional set of experiments (n=7), ventricular conduction intervals determined in conditions of basal normokalemic perfusion during LV stimulations were compared to those obtained with RV stimulations. An overview of the experimental series and the used programmed stimulation protocols is given in Table 1.

Drug adminisrration

Flecainide (Sigma-Aldrich, Germany) was infused at a concentration of 1.5 μM, which is close to the maximum free (i.e. protein-unbound) therapeutic plasma levels (Conard & Ober, 1984). For precise dosing, drug infusions were performed at a rate of 0.3 ml/min using a calibrated infusion pump, while perfusing the hearts with normokalemic saline solution at a constant coronary flow rate (see above).

Data analysis

The data were analysed using Chart 5-Pro for Windows software (ADInstruments). Data are expressed as mean $\pm$ standard deviation of the mean. One-way ANOVA followed by the Tukey–Kramer test was used for multiple comparisons, and Student's paired t-tests were used to compare two data sets. *P* values less than 0.05 were considered to be significant.

Results

Ventricular conduction intervals in S_2 vs. S_1 beats during programmed stimulations

Figure 1 shows representative LV and RV monophasic action potential recordings and the measurements of ventricular conduction intervals during programmed LV stimulation, and Figure 2 shows the summary data that illustrate changes of the activation latency, LV-to-RV delay, and total conduction time at variable S_1-S_2 intervals. With a S_1-S_1 cycle length of 550 ms, LV and RV APD_{90} were 163$\pm$6 ms and 165$\pm$6 ms, respectively. In the last regular beat in a basic drive train (indicated as "R" in Fig. 2), the activation latency at the LV stimulation site was 12$\pm$2 ms, and the LV-RV delay was 16$\pm$2 ms, thus yielding the total conduction time of 28$\pm$2 ms. Upon extrastimulus application, ventricular conduction intervals were not changed when premature beats were evoked in late diastole, i.e. at S_1-S_2 intervals greater than 200 ms (Fig. 2, panels A, B and C). In contrast, extrastimulus application at shorter coupling intervals close (within 20 ms) to ERP (indicated as the vertical dashed line) was associated with prolongation of the total conduction time, the amount of which was progressively increasing upon increasing the S_2 prematurity (Fig. 2, panel C). At the shortest S_1-S_2 interval that enabled LV capture (S_1-S_2 of 172$\pm$8 ms), the total conduction time in S_2 beat was increased by 30% compared to its value during S_1 pacing. This increase was almost entirely accounted for by the markedly prolonged activation latency at the LV stimulation site (Fig. 2, panel A), whereas the LV-to-RV delay showed only negligible changes (Fig. 2, panel B). Specifically, at the minimal ventricular capture interval, the activation latency was increased by 54%, whilst an increment in LV-to-RV delay amounted only 12%, compared to the values determined in S_1 beat (Fig. 2, panel D).

In a subset of experiments, ventricular conduction intervals determined upon LV stimulations were compared to those obtained when the stimulation protocol was applied at RV epicardium (Fig. 3). At the shortest S_1-S_2 interval that enabled RV capture (S_1-S_2 of 171$\pm$6 ms), the % increase in the activation latency in S_2 vs. S_1 beat (+61%) was much greater compared to the increment in RV-to-LV delay (+11%), thus replicating the relationships observed with the LV stimulations. No difference in the activation latency, interventricular conduction delay, or total conduction time was observed with RV vs. LV stimulations, either in S_1- or S_2-evoked beats (Fig. 3).

V_1-V_2 to S_1-S_2 relations

A prolongation of ventricular conduction upon extrastimulus application can be acting to limit the value of the functional response interval in cardiac cells. In Figure 4, these effects are addressed by plotting the V_1-V_2 response intervals obtained during programmed LV stimulations as a function of the S_1-S_2 interval.

The measurements illustrated in Figure 4A suggest that the V_1-V_2 interval (i.e., the time between the upstrokes of S_1- and S_2-induced action potentials) at the LV site would be equal to the S_1-S_2 interval if the activation latency is the same in S_2 vs. S_1 beat (i.e., L_1=L_2). Likewise, the RV response interval is equal to the S_1-S_2 interval if the LV-to-RV delay is the same in S_2 vs. S_1 beat (i.e., D_1=D_2). Figure 4B shows that these relations are valid when extrastimulus is applied at the long S_1-S_2 intervals. Indeed, with S_1-S_2 ranged from 550 ms to 200 ms, the V_1-V_2 responses both in LV and RV chamber fall on the identity line, indicating that any reduction in S_1-S_2 interval is followed by a proportional decrease in V_1-V_2 response interval. This is explained by no change in either the activation latency or the LV-to-RV delay in S_2 vs. S_1 beats when extrastimulus is applied in late diastole (Fig. 2, panels A and B). Nevertheless, the V_1-V_2 vs. S_1-S_2 relations are considerably changed over a range of short (less than 200 ms) coupling stimulation intervals (Fig. 4, panel C). In this setting, the V_1-V_2 responses deviate from the identity line, with the V_1-V_2 vs. S_1-S_2 difference being progressively increased upon shortening of the S_1-S_2 interval. As a result, the minimum V_1-V_2 intervals (i.e., the functional refractory periods) attained at the LV and RV MAP recording sites during programmed stimulation (LV: 179$\pm$8 ms; RV: 181$\pm$8 ms) were found to significantly exceed the minimum S_1-S_2 interval that enabled ventricular capture (172$\pm$8 ms). Importantly, despite the markedly increased V_1-V_2 vs. S_1-S_2 difference at the LV site (suggesting a prolonged activation latency in S_2 vs. S_1 beats), the V_1-V_2 responses in RV chamber remained closely aligned to those from LV (Fig. 4, panel C), indicating no significant S_2 vs. S_1 difference in LV-to-RV delay. Thus, it follows that an increase in the activation latency at the stimulation site, rather than in the LV-to-RV delay, is the main factor that limits the minimal ventricular response interval in RV chamber during LV stimulations.

Effects of variations in parameters of programmed stimulation

S_1-S_1 cycle length

Figure 5 shows effects of varying basic drive train cycle length on the outcomes of programmed LV stimulations applied at twice diastolic threshold current intensity. S_1-S_1 shortening from 550 ms to 300 ms was associated with proportional reductions in both LV APD_{90} and ERP, translating to no change in ERP-to-APD_{90} ratio (panels A, D, and G), and the repolarization time point for the earliest S_2 capture (87% repolarization time with both S_1-S_1 cycle lengths). In connection with this, a similar S_2 vs. S_1 % increase in all conduction intervals was determined with both S_1-S_1 cycle lengths (panels C, F and I). Nevertheless, it is noteworthy that independently of the S_1-S_1 cycle length used, an increase in the total conduction time in S_2 vs. S_1 beats (panel H) was primarily attributable to the prolonged activation latency (panel B), with only marginal changes in LV-to-RV delay (panel E).

S_1-S_2 stimulating current strength

Figure 6 shows effects of varying stimulus intensity on the outcomes of programmed LV stimulations applied at S_1-S_1=550 ms. An increase in the stimulating current strength from two to five times diastolic threshold had no effect on LV APD_{90}, but decreased the ERP in S_1 beat, leading to the reduced ERP-to-APD_{90} ratio (panels A, D, and G). In S_1 beat, the activation latency at the LV stimulation site was reduced when using a higher stimulus intensity (panel B). The reduced ERP-to-APD_{90} ratio allowed the S_2 capture to occur earlier during repolarization phase (77% vs. 87% repolarization time with five times vs. two times diastolic threshold, respectively). The increased S_2 prematurity contributed to the prolonged activation latency in S_2 beats (panel B), resulting in markedly accentuated % increase in the activation latency in S_2 vs. S_1 beats (panel C), when using a higher stimulus intensity. The S_2 vs. S_1 changes in the total conduction time (panels H and I) followed those in the activation latency (panels B and C). An increase in the stimulating current strength, however, had no effect on the LV-to-RV delay either in S_1 or S_2 beats (panel E), or its % increment upon premature ventricular activation (panel F).

Effects of hypokalemia

Figure 7 illustrates electrophysiological effects produced during hypokalemic as compared to normokalemic perfusion. Hypokalemia increased LV APD_{90}, while reducing the ERP in S_1 beat, thus contributing to the reduced ERP-to-APD_{90} ratio (panels A, D, and G). Consequently, the S_2 capture occurred earlier during repolarization phase of a preceding action potential (82% vs. 90% repolarization time with hypokalemia vs. baseline, respectively). Increased S_2 prematurity contributed to the prolonged activation latency in S_2 beats in hypokalemia (panel B), resulting in accentuated % increase in the activation latency in S_2 vs. S_1 beats (panel C). The LV-to-RV delay in hypokalemic hearts was proportionately increased in both S_1 and S_2 beats (panel E), translating to the same S_2 vs. S_1 % difference, when compared to baseline (panel F). Accordingly, the S_2 vs. S_1 % increase in the total conduction time (panel I) in this setting was entirely accounted for by an increment in the activation latency in S_2 beat (panel C).

Effects of flecainide

Figure 8 illustrates electrophysiological effects produced by flecainide, class Ic Na^+ channel blocker. Flecainide prolonged both LV repolarization and refractoriness, with an increase in ERP being greater than that in APD_{90} (panels A and D). As a result, the ERP-to-

APD_{90} ratio was increased upon drug administration (panel G), and the S_2 capture occurred later during final repolarization phase (93% vs. 88% repolarization time with flecainide vs. baseline, respectively). The activation latency, the LV-to-RV delay, and the total conduction time were proportionately increased in S_1 and S_2 beats by flecainide (panels B, E, and H), translating to the same S_2 vs. S_1 % differences, when compared to baseline (panels C, F, and I). Overall, even though flecainide significantly prolonged all conduction intervals (panels B, E, and H), the relative S_2 vs. S_1 increase in the total conduction time in flecainide-treated heart preparations remained to be primarily determined by the prolonged activation latency (+46%), with only small changes in LV-to-RV delay (+13%), similar to the S_2 vs. S_1 relations noted at baseline.

Discussion

Main findings

This study suggests that during programmed stimulations, independently of the S_1-S_1 cycle length used, slowed conduction in premature beats initiated shortly after the termination of ERP is primarily accounted for by the markedly increased activation latency at the LV stimulation site, with only negligible changes occurring in LV-to-RV delay. Increased activation latency is acting to limit the minimal ventricular response intervals in LV and RV chamber. The prolongation of the activation latency in premature beats is further accentuated upon interventions (such as an increase in the stimulating current intensity, or hypokalemia) that reduce the ERP-to-APD_{90} ratio, and hence permit extrastimulus capture earlier during phase 3 repolarization. Na^+ channel blocker such as flecainide prolongs both the activation latency and the LV-to-RV delay in the regular and premature beats, without changing their relative contributions into the increment in total conduction time in S_2 vs. S_1 beats.

Changes in the activation latency in S_2 vs. S_1 beats

Extrastimulus applied in late diastole, i.e. when excitability is fully recovered in cardiac cells, initiates an action potential with a fast upstroke which rapidly propagates from the stimulation site to the adjacent MAP-recording site in LV chamber. This accounts for only a brief (about 12 ms) activation latency. In contrast, extrastimulus applied at the shorter S_1-S_2 intervals close to ERP, i.e. prior to the completion of repolarization and full recovery of Na^+ channels from inactivation, typically initiates a slowly rising action potential (Ramza *et al.* 1990), or even a graded response (subthreshold depolarization) which can propagate few mm away from the stimulation site and induce a regenerative activation in the distal cells with recovered excitability (Hoffman *et al.* 1957; Gotoh *et al.* 1997; Karagueuzian & Chen, 2001). As both slowly rising action potentials and graded responses are associated with decreased conduction velocity, the activation latency is markedly prolonged in S_2 beats.

These changes are amplified upon decreasing the takeoff potential for initiation of the premature ventricular responses, consistent with progressively reduced Na^+ channel availability at incomplete repolarization levels (Weidmann, 1955). Accordingly, the prolongation of the activation latency in S_2 beats is accentuated upon interventions that decrease the ERP-to-APD_{90} ratio and therefore allow the ventricular capture to occur at the earlier repolarization time points; in the present study, this was observed with increasing the S_1-S_2 stimulating current intensity, or upon hypokalemic perfusion. With hypokalemia, the contributing mechanism appears to be related to the contrasting changes in APD_{90} vs. ERP, that is, whilst APD_{90} is prolonged, presumably through inhibition of I_{Kr}, the rapid component of the delayed rectifier K^+ current (Sanguinetti & Jurkiewicz, 1992), the ERP is shortened, most likely owing to the hypokalemia effects on the recovery of Na^+ channels from inactivation in final repolarization phase (Kern *et al.* 1978; Li *et al.* 1992). With increased stimulating current strength, a reduction in ERP is attributable to the increased amount of depolarized myocytes upon extrastimulus application, and therefore improved source-to-sink ratio (Kleber & Rudy, 2004), which facilitates initiation of the propagated responses at less complete repolarization levels.

With flecainide, class Ic Na^+ channel blocker, changes in the activation latency in premature beats are likely to be determined by an interplay of several factors. Whilst flecainide can moderately prolong APD_{90} owing to its inhibitory effect on I_{Kr} (Wang *et al.* 1996; Melgari *et al.* 2015), it produces more prominent ERP lengthening in connection with its I_{Na} blocking effects, which is leading to the increased ERP-to-APD_{90} ratio. The latter postpones the initiation of the earliest S_2 response towards a later repolarization time point, an effect that can be expected to prevent a prolongation of the activation latency in S_2 vs. S_1 beats, assuming a greater Na^+ channel availability at more negative membrane potentials (Weidmann, 1955). This voltage-dependent change, nevertheless, is balanced by the drug-induced I_{Na} block, which is leading to the reduced number of Na^+ channels recovered from inactivation at a given level of the membrane potential throughout the repolarization phase (Glaaser & Clancy, 2006). As a result, flecainide effects during programmed stimulations appear to be mostly related to the increase in S_1-S_2 interval at which the activation latency starts to be prolonged upon S_2 applications with increasing proximity to a preceding action potential, whereas the magnitude of the S_2 vs. S_1 increment in the activation latency is not significantly changed (Fig. 8, panel C).

Changes in the LV-to-RV delay in S_2 vs. S_1 beats

The velocity of the action potential upstroke (V_{max}) is thought to be the primary determinant of the conduction time in premature ventricular beats (Weidmann, 1955; Ramza *et al.* 1990). When a premature activation is initiated in incompletely repolarized cells prior to the full recovery of Na^+ channels, the V_{max} is reduced and the conduction is slowed. The present study, nevertheless, suggests that these changes are minimized in the setting of the prolonged activation latency. An increase in the activation latency ensures that cellular repolarization can proceed for 18-20 ms after the extrastimulus application, until it is interrupted by a premature action potential (Fig. 1 and Fig. 2, panel A). As the moment for initiation of the premature activation is postponed towards a later repolarization time point whereby more Na^+ channels have recovered from inactivation, the expected V_{max} reduction in S_2 beat is minimized, which translates to only marginal (+12%, Fig. 2, panel D) increase in the LV-to-RV delay, compared to its value in the regular beat.

Ventricular conduction is known to be slowed upon drug-induced Na^+ channel blockade, as well as in the setting of hypokalemia that is acting by increasing the ventricular excitation threshold secondary to hyperpolarization of the cardiac myocyte sarcolemma, an effect attributable to the suppression of I_{K1}, the inward rectifier K^+ current (Weiss *et al.* 2017). In this study, even though hypokalemia and flecainide were found to significantly increase the LV-to-RV delay in both S_1 and S_2 beats (panel E in Figs. 7 and 8), the % increment in the LV-to-RV delay in S_2 vs. S_1 beats remained the same (panel F in Figs. 7 and 8). The latter can be partly explained by a prolongation of the activation latency in S_2 beats in both settings (panel B in Figs. 7 and 8), with a subsequent effect on timing of the premature activation in the late repolarization phase.

Overall, the aforementioned changes imply that an increase in the activation latency upon premature ventricular activation may represent an intrinsic regulation that prevents a significant slowing of the conduction velocity with which the initiated depolarization wave spreads away from the stimulation site.

Clinical implications

The conventional clinical electrophysiological techniques do not allow to discriminate whether conduction slowing upon premature activation is attributed to the prolonged activation latency, or slowed impulse propagation away from the stimulation site, or both. The endocardial electrogram recordings performed during electrophysiological testing are invariably limited to the RV chamber (Wellens *et al.* 1985; Mitchell *et al.* 1986; Liem *et al.* 1988; Koller *et al.* 1995a), and hence do not permit the evaluation of the interventricular conduction delay. The monophasic action potential recordings in cardiac patients are technically complicated and therefore not used in the daily clinical work. With other techniques, for example, the pace mapping which is designed to locate the arrhythmia source by pacing at multiple endocardial sites, ventricular conduction time is assessed by measuring the interval between the pacing stimulus artifact and the onset of the QRS complex on the surface ECG (Brunckhorst *et al.* 2003). Although this can provide a crude measure of slow conduction, the contributing physiological factors remain uncertain. In this regard, the present study suggests that a significant part of the large-scale conduction delay measured during clinical testing may be related to the increased activation latency at the stimulation site, rather than conduction slowing away from it, at least during premature activations initiated during phase 3 repolarization.

Another practical aspect of this study is related to the impact of the activation latency on the ventricular response interval. It is generally assumed that ERP reduction is invariably leading to the reduced minimal interval for successive ventricular activations, which results in the faster excitation rate during induced tachyarrhythmia, and renders it less responsive to antiarrhythmic drugs (Opthof *et al.* 1991; Wu *et al.* 2002; Chen *et al.* 2003). However, with interventions that prolong the activation latency in S_2 beats (e.g. hypokalemia, or increased stimulating current intensity), the ERP reduction would likely to be associated with no change in the minimal ventricular response interval (i.e., the functional refractory period), thus eliminating the possibility for accelerated excitation frequency. These relationships, therefore, challenge the value of assessments aimed at predicting the activation frequency during tachyarrhythmia based on measuring the ERP alone.

Impact of hypokalemia and flecainide

Importantly, cardiac electrophysiological changes produced by hypokalemia or flecainide are likely to be of clinical relevance. Indeed, hypokalemia represents the most common electrolyte disorder seen in cardiac patients, which typically develops as a side effect of diuretic therapy, or may be caused by neurohormonal derangements in heart failure (Weiss *et al.* 2017). Hypokalemia occurs in about 16% of patients admitted to the hospital (Jensen *et al.* 2015), and it plays an important role in cardiac arrhythmogenesis by facilitating the arrhythmic triggers, such as the early and delayed afterdepolarizations, and by causing conduction slowing and repolarization derangements, which precipitate re-entry (Weiss *et al.* 2017). With flecainide, an increase in the risk of arrhythmic death has been found in patients with healed myocardial infarction enrolled in the CAST trial (CAST Investigators, 1989). The flecainide-induced arrhythmia is thought to be attributed to the multiple mechanisms, including non-uniform conduction slowing in myocardial regions bordering the post-infarction scar (Ranger & Nattel, 1995), and amplified spatial repolarization gradients (Osadchii, 2014b). Flecainide, nevertheless, remains one of the first line treatments used for the rhythm control in atrial fibrillation in patients with structurally normal hearts (Apostolakis *et al.* 2013). This agent is also considered as a promising treatment option in patients with catecholaminergic polymorphic ventricular tachycardia (Salvage *et al.* 2018). Overall, these clinical findings, in connections with the results of the present study, strongly suggest that both hypokalemia and flecainide treatment should be considered among those factors which can influence the outcomes of ventricular conduction assessments and associated arrhythmogenicity during programmed stimulations in cardiac patients.

Activation latency in the premature beats: the contributing mechanisms

The activation latency measured at the LV stimulation site has a complex mechanism, and includes both the time needed to initiate the action potential, and the time taken for the action potential to propagate from the stimulating electrodes to the adjacent MAP-recording electrodes. Importantly, the S_2 application is known to produce a greater conduction slowing close to the pacing site than in distant myocardial regions (Koller *et al.*1995a, 1995b; Karagueuzian & Chen, 2001), an effect that can be partly attributed to the impaired source-sink relationships (Kleber & Rudy, 2004). With point stimulation, the amount of depolarized cells (i.e., the source) is small, and the initiated depolarization wavefront is very convex, which contributes to the dissipation of the excitatory current over a large downstream myocardial area (i.e., the sink). The resulting source-sink mismatch then may account for the low conduction velocity with which the impulse spreads away from the pacing site. Upon subsequent ventricular activation, the area of depolarized cells is progressively increased, and the wavefront curvature is reduced, thus improving the source-sink ratio, and reducing the degree of conduction slowing at the distant sites. With these considerations, it can be assumed that upon S_2 application in late diastole, a significant part of the activation latency is attributed to the local conduction slowing in the immediate vicinity of the stimulating electrode, rather than to the time needed to initiate a premature action potential. These relations, nevertheless, can be significantly changed upon S_2 application during phase 3 repolarization (i.e., in close proximity to ERP) in the preceding S_1 beat (Fig. 1). In the setting of the reduced Na^+ channel availability in incompletely repolarized cardiac cells (Weidmann, 1955), the S_2 application evokes a depolarizing graded response that slowly attains the threshold voltage for generating action potential upstroke (Hoffman *et al.* 1957; Gotoh *et al.* 1997; Karagueuzian & Chen, 2001). Consequently, the initiation of the propagating action potential is significantly delayed. Therefore, when S_2 is applied shortly after the termination of ERP, the prolonged time for initiation of the premature action potential is likely to be an independent factor that contributes to the activation latency at the stimulation site.

Limitations

In this study, action potential recordings were performed at only two sites in the LV and RV epicardium, thus providing no information on the pathway of electrical activation during programmed stimulation, and precluding the assessments of the conduction velocity. Spatiotemporal mapping studies that involve optical action potential recordings at multiple ventricular sites are warranted in order to assess more precisely the relative contribution of the activation latency at the stimulation site and the LV-to-RV delay into the mechanism of conduction slowing in premature beats.

Conclusions

During cardiac electrophysiological testing, prolonged activation latency at the stimulation site should be considered as the critical determinant of conduction slowing and associated changes in the ventricular response intervals in premature beats initiated shortly after the termination of effective refractory period.

Additional information

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Figure 1. Assessments of the activation latency, LV-to-RV conduction delay, and total conduction time from monophasic action potential recordings obtained during programmed stimulations.

Representative monophasic action potentials from the left ventricular (LV, upper trace) and the right ventricular (RV, lower trace) epicardium were recorded during S_1-S_2 stimulations applied in LV chamber. Conduction intervals were assessed in the last regular (S_1) beat in a drive train, and in a premature beat upon an application of extrastimulus (S_2). The activation latencies in the regular beat (L_1) and in the premature beat (L_2) were measured from the moment of S_1 and S_2 application, respectively, to the fastest upstroke of the following action potential (vertical dotted line). The LV-to-RV conduction delays in the regular beat (D_1) and in the premature beat (D_2) correspond to the delays between the upstrokes of the LV action potential and the RV action potential upon S_1 and S_2 application, respectively. The total conduction time in the regular beat (CT_1) is a sum of L_1 and D_1, and the total conduction time in the premature beat (CT_2) is a sum of L_2 and D_2. Note that S_2 application at the shortest coupling interval that allows ventricular capture is associated with markedly increased activation latency (L_2 is greater than L_1), but no significant change in the LV-to-RV delay (D_2 is similar to D_1), compared to the values determined in S_1 beat.

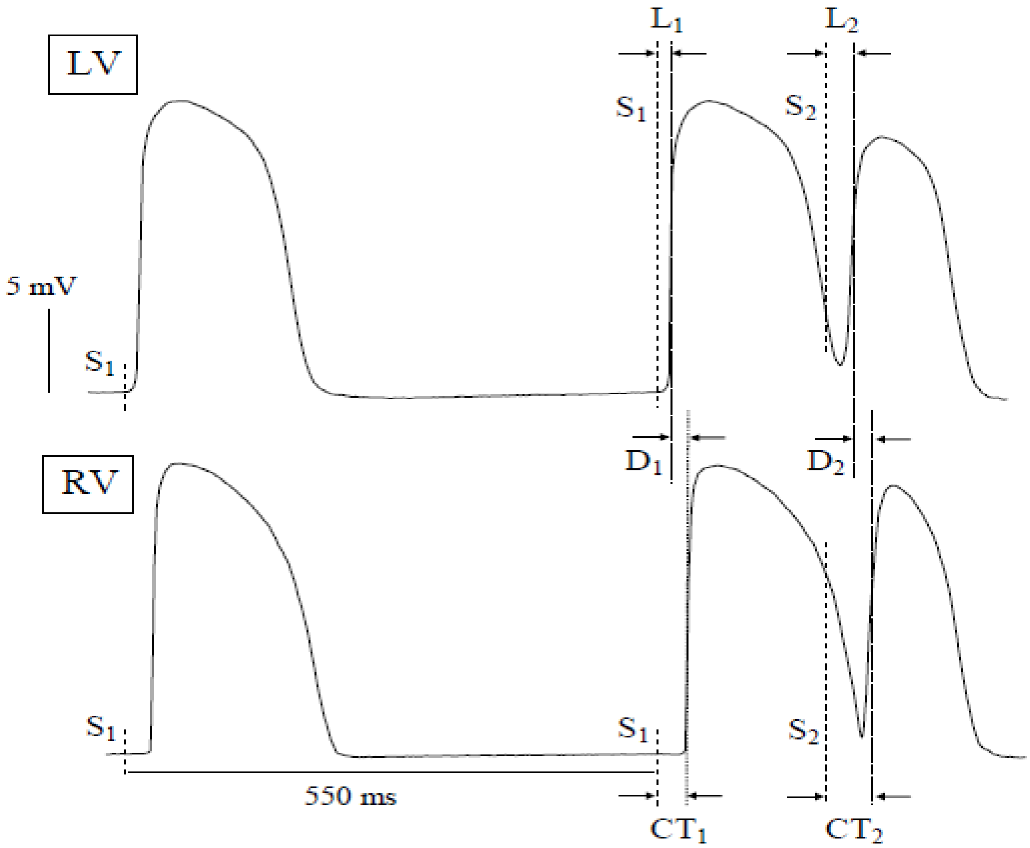

Figure 2. Dynamics of the activation latency, LV-to-RV delay, and total conduction time at variable S_1-S_2 coupling stimulation intervals.

During programmed stimulations, the LV activation latency (panel A), LV-to-RV delay (panel B), and total conduction time (panel C) were measured upon progressive shortening of the S_1-S_2 interval towards the minimal values slightly exceeding the effective refractory period (ERP, the vertical dashed line). "R" denotes to the values of conduction intervals measured in the last regular beat in a drive train prior to extrastimulus application. Panel D shows % increase in LV activation latency and LV-to-RV delay in S_2 beats evoked at the shortest ventricular capture interval, compared to the values determined in S_1 beats. *P<0.05.

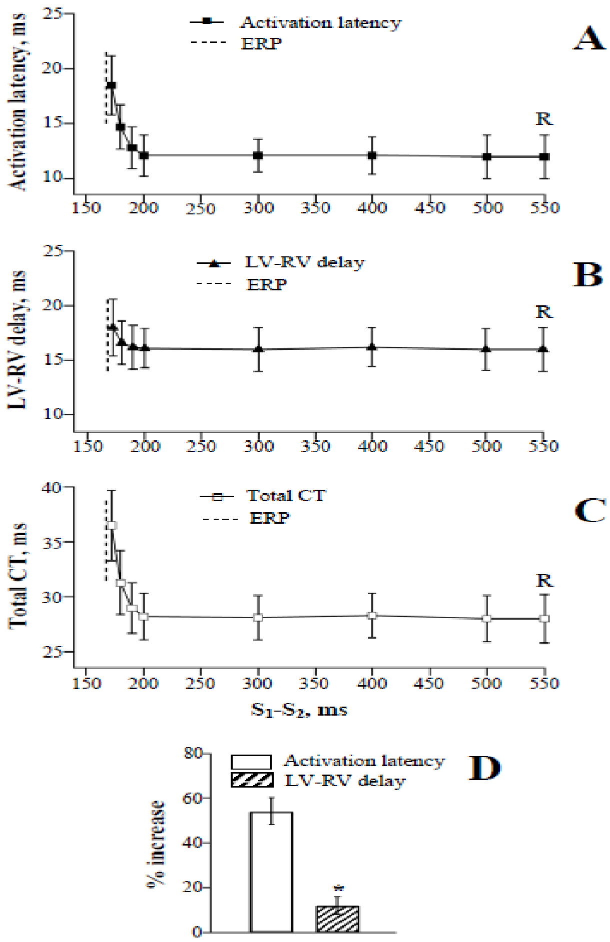

Figure 3. Ventricular conduction intervals determined during programmed stimulations applied at LV vs. RV epicardium.

Programmed stimulations (stim) were applied with S_1-S_1 cycle length of 550 ms at twice diastolic threshold current intensity, either in LV (open bars) or RV chamber (hatched bars).

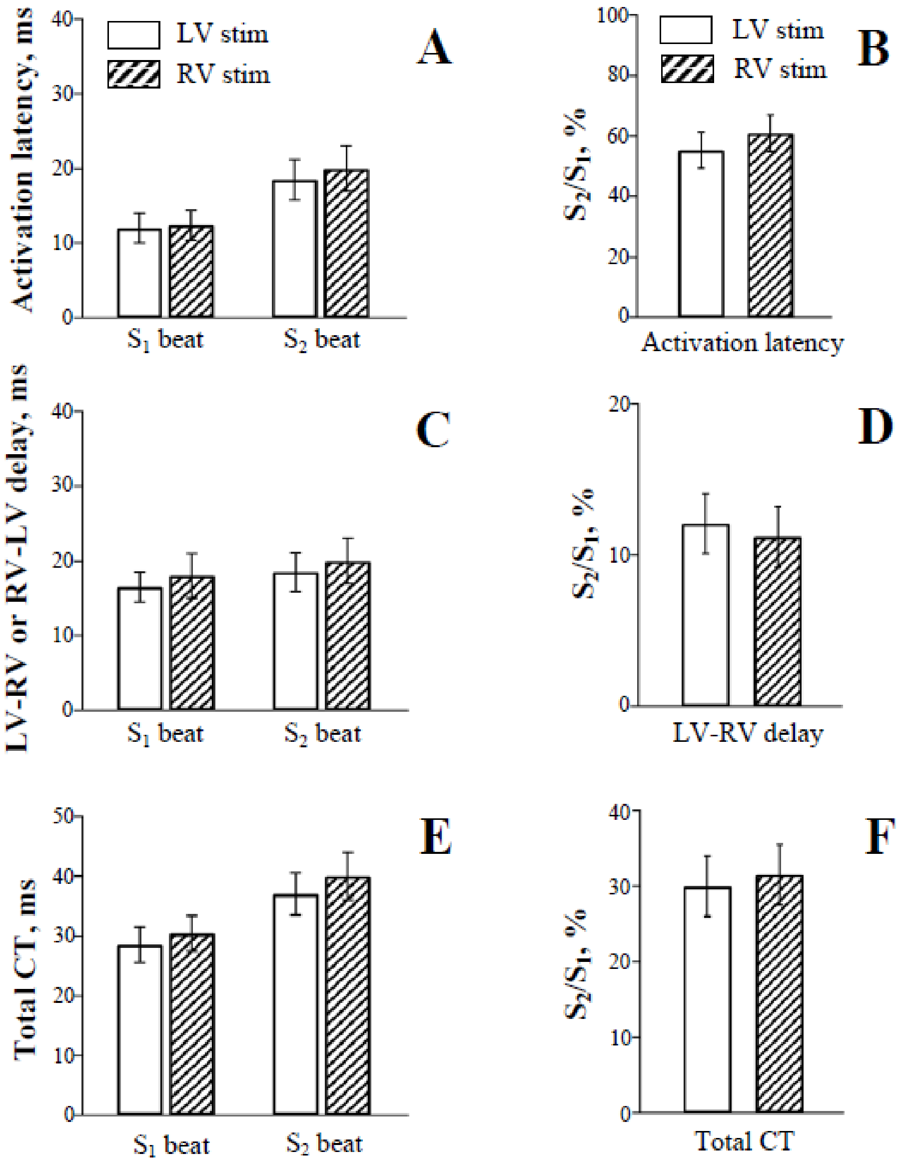

Figure 4. Relations between ventricular V_1-V_2 response intervals and S_1-S_2 coupling stimulation intervals.

V_1-V_2 intervals were determined in LV and RV MAP-recording sites (panel A), and then plotted vs. S_1-S_2 intervals (panels B and C). L_1 and L_2 are the activation latencies in S_1 beat and S_2 beat, respectively. D_1 and D_2 are the LV-to-RV conduction delays in S_1 beat and S_2 beat, respectively. The dashed line in panels B and C is the identity line whereby V_1-V_2=S_1-S_2.

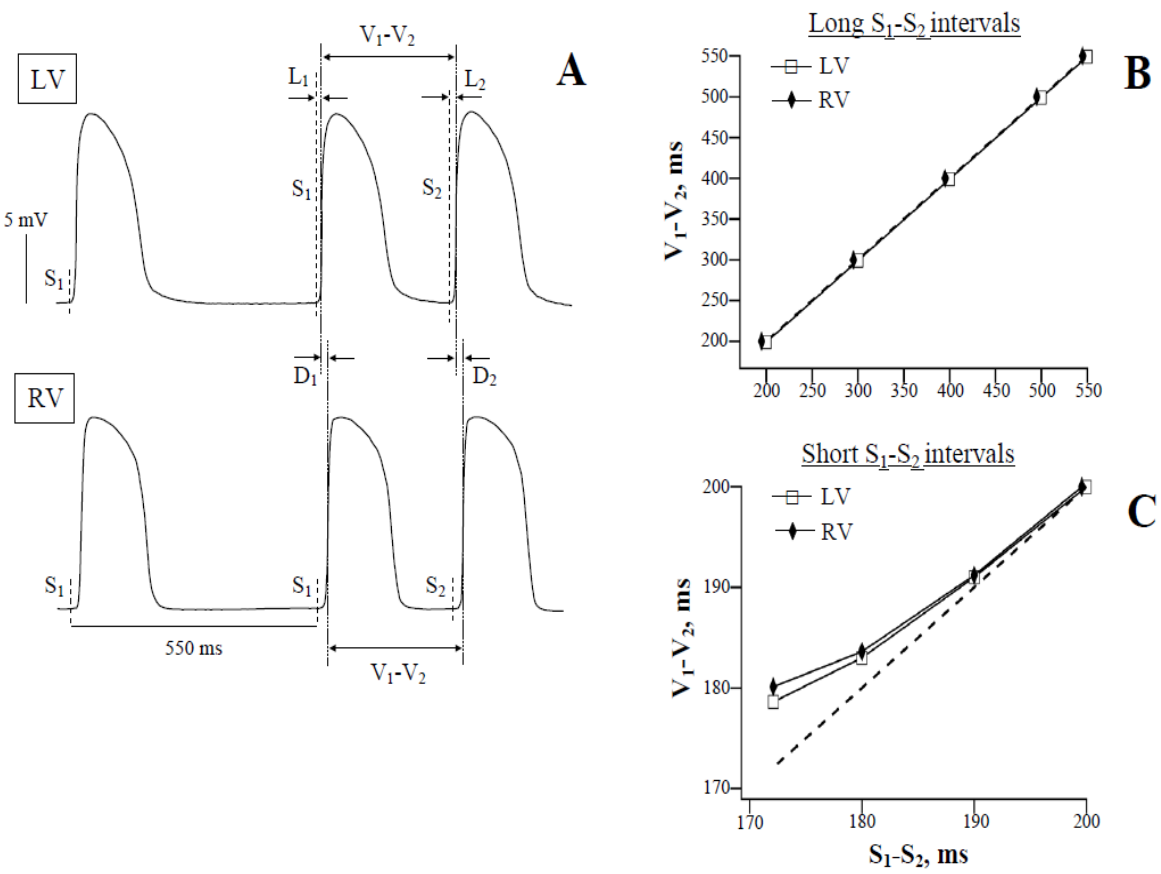

Figure 5. Effects of changes in the basic drive train cycle length on the ventricular action potential duration, effective refractory period, and conduction intervals during programmed stimulations.

Programmed stimulations were applied at twice diastolic threshold current intensity with the S_1-S_1 cycle length of either 550 ms (open bars) or 300 ms (hatched bars). The left set of panels shows changes in left ventricular (LV) action potential duration (APD_{90}, panel A), effective refractory period (ERP, panel D), and ERP-to-APD_{90} ratio (panel G) in the last S_1 beat in a drive train. The middle set of panels shows changes in LV activation latency (panel B), LV-to-RV delay (panel E), and total conduction time (CT) (panel H) determined in the last S_1 beat in a drive train (first pair of bars) and in S_2 beat evoked at the shortest ventricular capture interval (second pair of bars). The right set of panels shows S_2 vs. S_1 % increase in the activation latency (panel C), LV-to-RV delay (panel F), and total conduction time (panel I) determined with the two S_1-S_1 cycle lengths. **P*<0.05. The same figure design is used in Figs. 6-8.

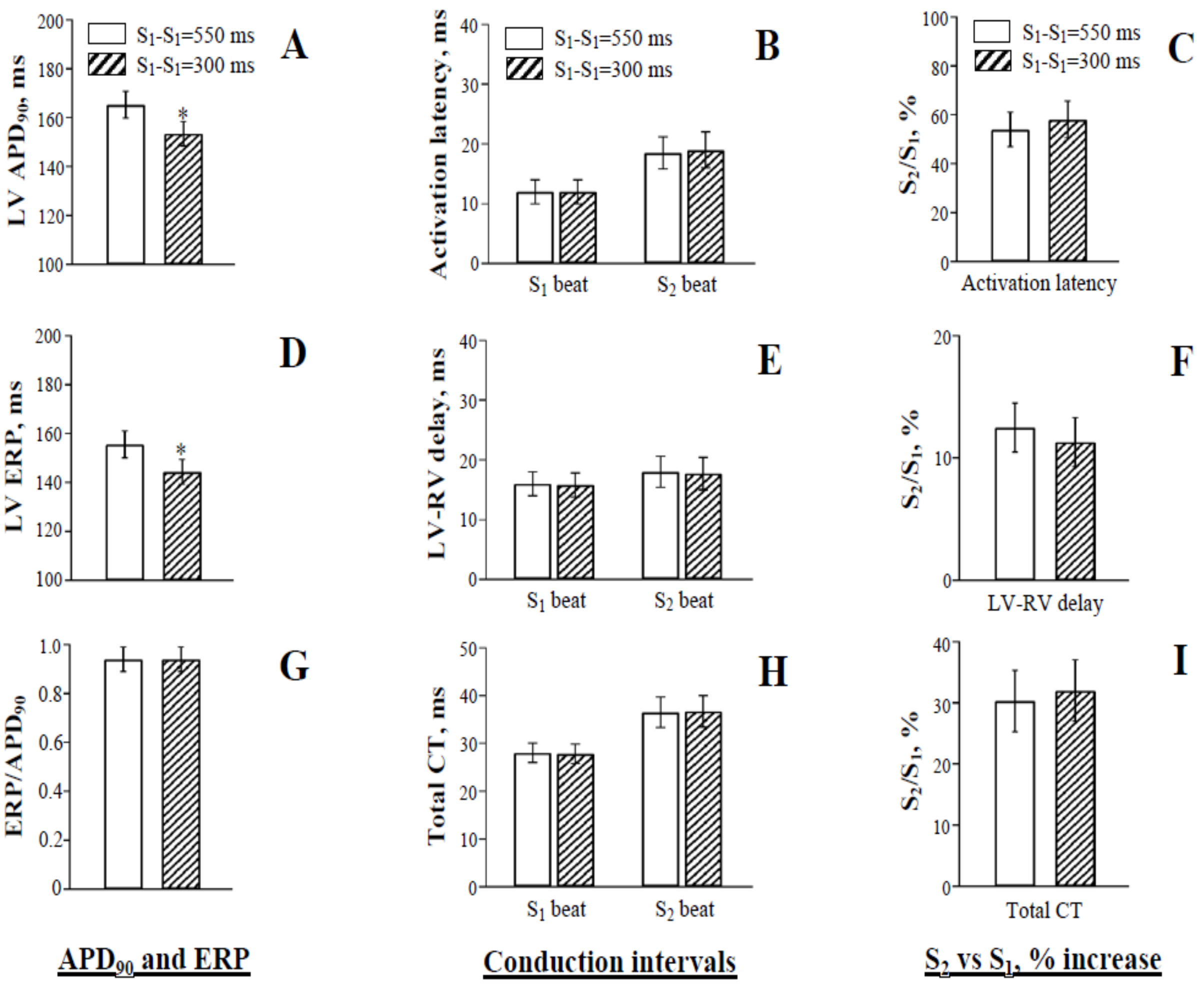

Figure 6. Effects of changes in the stimulating current intensity on the ventricular action potential duration, effective refractory period, and conduction intervals during programmed stimulations.

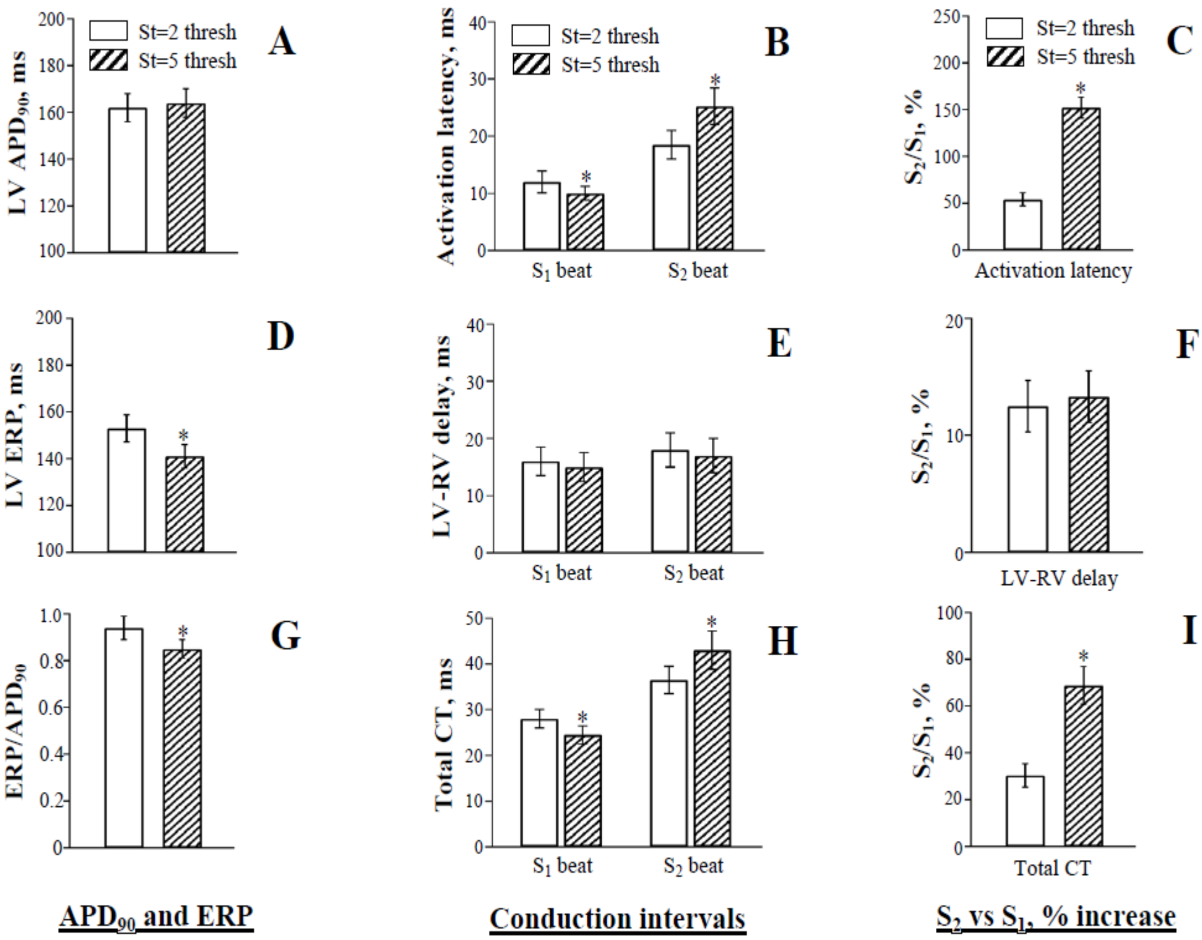

Figure 7. Effects of hypokalemia (HypoK) on the ventricular action potential duration, effective refractory period, and conduction intervals during programmed stimulations.

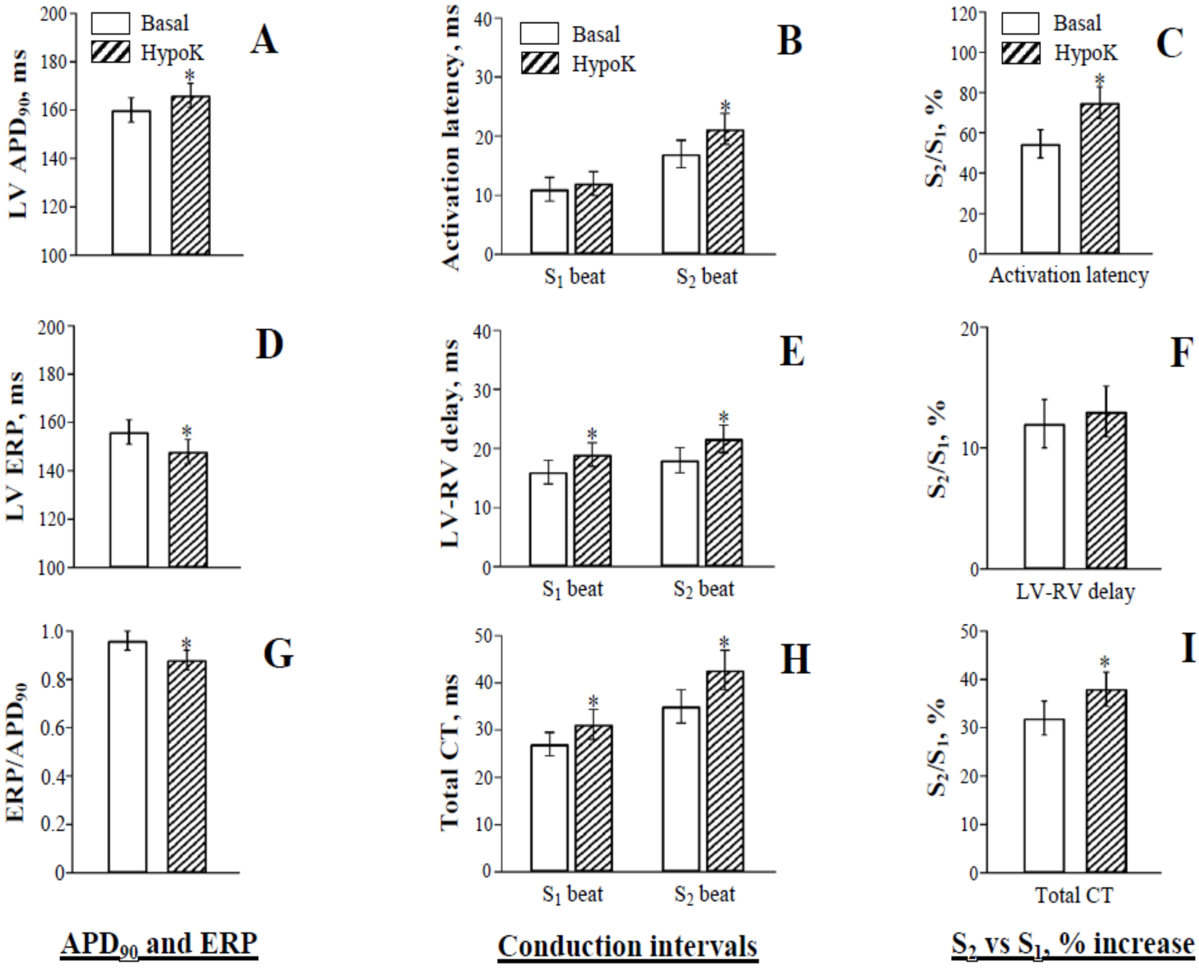

Figure 8. Effects of flecainide on the ventricular action potential duration, effective refractory period, and conduction intervals during programmed stimulations.

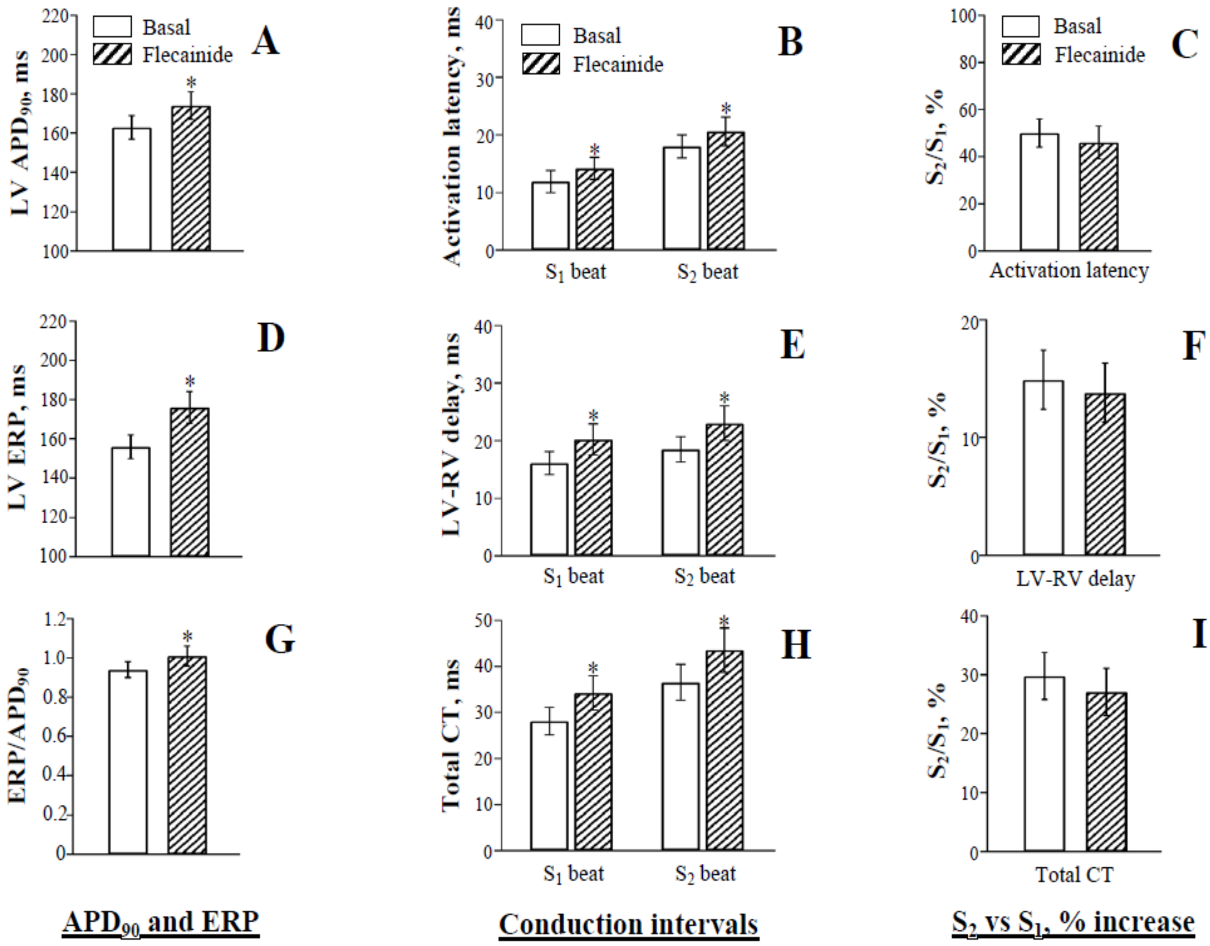

Table 1. An overview of experimental series and the programmed stimulation protocols used in the study.

Experimental series	Sample size	Stimulation site	Basic drive cycle length	Stimulating current strength
Testing effects produced	n=9	LV epicardium	S_1-S_1=550 ms	2 times diastolic threshold
by variations in parameters			S_1-S_1=300 ms	2 times diastolic threshold
of programmed stimulations			S_1-S_1=550 ms	5 times diastolic threshold
Testing effects produced by	n=11	LV epicardium	S_1-S_1=550 ms	2 times diastolic threshold
hypokalemia (2.5 mM KCl)				
vs. normokalemia (4.7 mM KCl)				
Testing effects produced	n=10	LV epicardium	S_1-S_1=550 ms	2 times diastolic threshold
by flecainide (1.5 μM)				
Testing effects of LV vs.	n=7	LV epicardium	S_1-S_1=550 ms	2 times diastolic threshold
RV stimulations		RV epicardium	S_1-S_1=550 ms	2 times diastolic threshold