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Dimethyl Lithospermate B, an Extract of Danshen, Suppresses Arrhythmogenesis Associated With the Brugada Syndrome

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Abstract

Background–Dimethyl lithospermate B (dmLSB) is an extract of Danshen, a traditional Chinese herbal remedy, which slows inactivation of I_{Na}, leading to increased inward current during the early phases of the action potential (AP). We hypothesized that this action would be antiarrhythmic in the setting of Brugada syndrome.

Methods and Results–The Brugada syndrome phenotype was created in canine arterially perfused right ventricular wedge preparations with the use of either terfenadine or verapamil to inhibit I_{Na} and I_{Ca} or pinacidil to activate $I_{K\text{-}ATP}$. AP recordings were simultaneously recorded from epicardial and endocardial sites together with an ECG. Terfenadine, verapamil, and pinacidil each induced all-or-none repolarization at some epicardial sites but not others, leading to ST-segment elevation as well as an increase in both epicardial and transmural dispersions of repolarization (EDR and TDR, respectively) from 12.9±9.6 to 107.0±54.8 ms and from 22.4±8.1 to 82.2±37.4 ms, respectively (P<0.05; n=9). Under these conditions, phase 2 reentry developed as the epicardial AP dome propagated from sites where it was maintained to sites at which it was lost, generating closely coupled extrasystoles and ventricular tachycardia and fibrillation. Addition of dmLSB (10 μmol/L) to the coronary perfusate restored the epicardial AP dome, reduced EDR and TDR to 12.4±18.1 and 24.4 ±26.7 ms, respectively (P<0.05; n=9), and abolished phase 2 reentry-induced extrasystoles and ventricular tachycardia and fibrillation in 9 of 9 preparations.

Conclusions–Our data suggest that dmLSB is effective in eliminating the arrhythmogenic substrate responsible for the Brugada syndrome and that it deserves further study as a pharmacological adjunct to implanted cardioverter/defibrillator usage.

Keywords

action potentials; arrhythmia; antiarrhythmia agents; sudden death; reentry

The Brugada syndrome is a familial disease with an autosomal dominant mode of inheritance. It is characterized by ST-segment elevation in the right precordial leads and episodes of syncope

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Disclosures
None.

and sudden cardiac death. To date, >100 mutations in SCN5A, the gene that encodes for the α-subunit of the cardiac sodium channel, have been linked to the syndrome (for review, see Antzelevitch et al,[1] Priori et al,[2] Antzelevitch,[3] Balser,[4] and Tan[5]). All known mutations result in a loss of sodium channel function.

Using the canine arterially perfused right ventricular wedge, our laboratory elucidated the mechanisms responsible for ST-segment elevation in the right precordial leads and the generation of lethal arrhythmias.[6,7] The prominent transient outward current (I_{to})-mediated notch in the action potential (AP) of the right ventricular epicardium plays a pivotal role in the arrhythmogenesis of the syndrome. A negative shift in the balance of currents active at the end of phase 1, namely, sodium current (I_{Na}), calcium current (I_{Ca}), and I_{to}, can result in all-or-none repolarization at the end of phase 1 (loss of the dome) in some areas of the epicardium but not the endocardium, leading to ST-segment elevation. As the dome propagates from regions where it is maintained to regions where it is lost, phase 2 reentry develops and gives rise to an extrasystole that precipitates polymorphic ventricular tachycardia and fibrillation (VT/VF). I_{to} block, via its actions to restore the AP dome and suppress phase 2 reentry and VT/VF, has been suggested as a therapeutic strategy for the Brugada syndrome.[6-10]

In the present study we explore a novel therapeutic strategy for the Brugada syndrome by selectively enhancing sodium current with dimethyl lithospermate B (dmLSB), a minor component of the root extract from *Salvia miltiorrhiza*. Previous studies with dmLSB have demonstrated a slowing of inactivation of I_{Na} without an increase in persistent late I_{Na}.[11] We hypothesized that this action of dmLSB will prevent loss of the epicardial AP dome and be effective in preventing phase 2 reentry and VT/VF in the Brugada syndrome. To test this hypothesis, we examined the actions of dmLSB in 3 distinct pharmacological models of the Brugada syndrome.

Methods

The detailed methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused canine right ventricular wedge preparation, as well as the viability and electric stability of the preparation, have been previously reported.[6,12] Experiments demonstrating that activity recorded from the cut surface of the perfused wedge preparation is representative of cells within the respective layers of the wall throughout the wedge have also been reported in a number of previous studies.[12-14]

Briefly, transmural wedge preparations with dimensions of ≈2×1×0.9 to 3.0×1.5×1.2 cm were dissected from the right ventricle of male and female random-source 20- to 35-kg canines. The preparations were cannulated via a small (diameter ≈100 to 150 μm) coronary artery (a descending branch of the right coronary artery) and perfused with cardioplegic solution (Tyrode's containing 12 mmol/L KCl). Unperfused tissue was carefully removed with a razor blade. The preparations were then placed in a small tissue bath and arterially perfused with Tyrode's solution. The temperature of the coronary perfusate was maintained at 35±0.5°C. The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co, Niles, Ill). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Inc, Sarasota, Fla) and maintained between 40 and 50 mm Hg by adjustment of the perfusion flow rate.

The wedge preparations were equilibrated in the tissue bath until electrically stable, usually 1 to 2 hours. The preparations were continuously stimulated at a basic cycle length (BCL) of 2000 ms with the use of bipolar silver electrodes insulated except at the tips and applied to the endocardial surface. To induce loss of the epicardial AP dome and phase 2 reentry, the preparations were paced at BCLs ranging from 200 to 5000 ms. A transmural ECG was

recorded with electrodes consisting of 2 AgCl half cells placed in the Tyrode's solution bathing the preparation, 1.0 to 1.5 cm from the epicardial and endocardial surfaces of the preparation, along the same axis as the transmembrane recordings (epicardium: "+" pole). Transmembrane APs were simultaneously recorded from 2 epicardial and 1 endocardial site with the use of floating microelectrodes (DC resistance=10 to 20 mol/LΩ) filled with 2.7 mol/L KCl, each connected to a high-input impedance amplifier. Impalements were obtained from the epicardial and endocardial surfaces of the preparation at positions approximating the transmural axis of the ECG recording. When 2 simultaneous epicardial impalements were recorded, the one with the longer action potential duration (APD) was designated Epi 1, and the other was designated Epi 2.

Isolation of dmLSB From the Root Extract of *S miltiorrhiza*

Dried roots of *S miltiorrhiza* (6 kg) were soaked in MeOH for 7 days at room temperature. After filtration, the extract was concentrated under the reduced pressure to give 470 g of a dark syrupy MeOH extract. This was suspended in H_2O and sequentially partitioned with *n*-hexane, EtOAc, and BuOH. This process yielded 69 g in the *n*-hexane fraction, 52 g in the EtOAc fraction, 69 g in the BuOH fraction, and a water-soluble residue. Half of the EtOAc fraction (26 g) was subjected to octadecyl silica gel column (6.0 × height 60 cm) chromatography. The column was eluted in a stepwise gradient manner with 300-mL aliquots of MeOH in H_2O (0% to 100%), which delivered 4 fractions: fraction 1 (3.2 g), fraction 2 (13 g), fraction 3 (2.4 g), and fraction 4 (7.0 g). Among these fractions (fraction 1 to fraction 4), fraction 2 was the most potent and was further purified by Sephadex LH-20 column chromatography with the use of 20% MeOH in CH_2Cl_2, which finally delivered 110 mg of dmLSB and 2.4 g of LSB. Moreover, LSB was easily converted to dmLSB by simple methylation of LSB in MeOH with the use of *p*-toluenesulfonic acid as catalyst. The chemical structure of dmLSB was elucidated with the use of ^{1}H-NMR and ^{13}C-NMR data.[15] A stock solution of 20 mmol/L dmLSB was prepared in 100% dimethyl sulfoxide. The wedge preparations were exposed to dmLSB for a period of 30 minutes.

Statistical Analysis

Statistical analysis was performed with the use of 1-way ANOVA or Kruskal-Wallis ANOVA on ranks in combination with a Tukey test, as appropriate. Incidence data were analyzed with a Fisher exact test. All data are reported as mean±SD.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

An inward shift in the balance of current active during the early phases of the right ventricular AP underlies the ECG and arrhythmic manifestations of the Brugada syndrome. In the right ventricular wedge preparation, such a shift can be achieved with the use of either agents that inhibit inward depolarizing current or agents that activate outward repolarizing current. We created the Brugada phenotype using both approaches. Verapamil was used to block inward calcium channel current (I_{Ca}), pinacidil to activate adenosine triphosphate (ATP)-sensitive outward potassium current ($I_{K\text{-}ATP}$), and terfenadine to block both inward sodium current (I_{Na}) and I_{Ca}. Figure 1 shows tracings recorded from arterially perfused wedge preparations before and after the addition of terfenadine, verapamil, or pinacidil. Each agent increased the magnitude of the epicardial AP notch, leading to the appearance of a more pronounced ECG J wave. The characteristics of the AP notch are summarized in Figure 2 and Table 1. Terfenadine, verapamil, or pinacidil significantly increased the phase 0 to phase 2 interval as well as the notch index, which approximates the area of the notch. dmLSB (10 μmol/L) in the

continued presence of terfenadine, verapamil, or pinacidil significantly reduced the notch magnitude, phase 0 to phase 2 interval, and notch index toward control values.

Figure 3 and Table 2 show APD measured at 90% repolarization (APD_{90}) at steady state (BCL=2000 ms) for each of the 3 Brugada syndrome models. Pinacidil abbreviated APD_{90}, whereas terfenadine and verapamil tended to prolong it, largely secondary to accentuation of the AP notch. Consistent with its effect to increase I_{Na} during the early but not late phases of the AP, dmLSB tended to abbreviate APD_{90}, secondary to diminution of the AP notch.

Heterogeneous loss of the epicardial AP dome occurred in the presence of terfenadine, verapamil, or pinacidil, generating both local epicardial dispersion of repolarization (EDR) and transmural dispersion of repolarization (TDR) between the briefest epicardial response and that of endocardium. EDR and TDR values for each of the 3 Brugada syndrome models are summarized in Figure 4 and Table 3. With each treatment as well as with the combined data, EDR and TDR increased compared with control, although the increase in TDR did not reach statistical significance in the case of pinacidil. The addition of dmLSB (10 μmol/L) resulted in a significant decrease in EDR and TDR toward control values.

Figures 5 and 6 illustrate heterogeneous loss of the epicardial AP dome and phase 2 reentry in each of the 3 Brugada syndrome models. Phase 2 reentry occurred as the dome propagated from regions of the epicardium where it was maintained to regions at which it was lost. This mechanism generated closely coupled extrasystoles in all 9 preparations as well as polymorphic VT in 6 of 9 wedge preparations. Addition of dmLSB (10 μmol/L) abolished phase 2 reentry and all arrhythmic activity in 9 of 9 preparations ($P<0.05$ versus terfenadine, verapamil, or pinacidil; Table 3).

The effects of dmLSB (0.5 to 20 μmol/L) alone in the canine arterially perfused right ventricular wedge are illustrated in Figure 7. There was a concentration-dependent reduction in the size of the epicardial AP notch, although this did not reach statistical significance. APD and transmural dispersion were largely unaffected by dmLSB.

Discussion

Our study demonstrates for the first time that delaying the inactivation of I_{Na} by as little as 20 ms can prevent the ECG and arrhythmic manifestations of the Brugada syndrome in our experimental models irrespective of the mechanism responsible for precipitating the disease. dmLSB is shown to prevent the development of all-or-none repolarization, phase 2 reentry, and the resultant closely coupled extrasystoles and polymorphic tachycardia in 3 different experimental models that mimic the Brugada syndrome. These data suggest that dmLSB may be a viable pharmacological alternative for the treatment of patients with the Brugada syndrome, as an adjunct to the use of implanted cardioverter/defibrillators (ICDs),[16] or as an alternative in cases in which ICDs are not feasible or affordable.

dmLSB has previously been reported to slow the inactivation kinetics of I_{Na} by increasing the proportion of the slowly inactivating component, raising the possibility that it would prolong APD and QT interval, like ATX-II.[17] Previous studies in rat ventricular myocytes demonstrate that inactivation of I_{Na} was complete within 50 ms after 10 μmol/L dmLSB, resulting in no increase in late I_{Na}.[11] In the present study, dmLSB alone had no significant effect on APD_{90} or TDR up to a dose of 20 μmol/L, suggesting no proarrhythmic effects of the drug (Figure 7).

A delicate balance of inward and outward currents determines the voltage at the end of epicardial AP phase 1. The 3 principal currents active at this point are I_{Na}, I_{Ca}, and I_{to}. Any manipulation resulting in a negative shift in the balance of these 3 currents at the end of epicardial AP phase 1 can result in an accentuated J wave as the epicardial AP notch becomes

accentuated or ST-segment elevation as all-or-none repolarization at the end of epicardial phase 1 occurs. Terfenadine blocks late I_{Na} and I_{Ca} with an IC_{50} in canine ventricular myocytes of 1.3 and 1.1 μmol/L, respectively (A.C. Zygmunt, PhD, and C. Antzelevitch, PhD, unpublished data, 2001). Terfenadine also produces both tonic and use-dependent block of I_{Na}, as was demonstrated in canine atrial myocytes.[18] These effects of terfenadine make the epicardial AP notch more prominent by shifting the end of epicardial phase 1 to more negative voltages and delaying the onset of phase 2 (Figure 1A). This model of the Brugada syndrome was described and characterized in 2004.[7] Similarly, verapamil inhibits I_{Ca} and late I_{Na} with an IC_{50} in canine ventricular myocytes of 0.31 and 0.21 μmol/L, respectively (A.C. Zygmunt, PhD, and C. Antzelevitch, PhD, unpublished data, 2001), resulting in a more prominent AP notch in the epicardium (Figure 1B). Verapamil has been shown in one clinical case to create a Brugada-like phenotype.[19] This model of the Brugada syndrome has also been previously described.[7] Pinacidil activates the normally quiescent ATP-sensitive potassium current ($I_{K\text{-}ATP}$), resulting in a more pronounced epicardial AP notch (Figure 1C). The pinacidil model of the Brugada syndrome was first described in 1999.[6] In all 3 models, the AP notch was significantly diminished after addition of dmLSB (10 μmol/L; Figures 1 and 2) because of the drug's ability to slow the inactivation kinetics of I_{Na}, resulting in a positive shift in the balance of currents active at the end of epicardial AP phase 1. This was quantified by taking the amplitude difference between phases 1 and 2 (notch magnitude) as well as the interval between phase 0 and phase 2. The notch magnitude was normalized to the amplitude of phase 2 to account for the variable amplitude of the floating microelectrode impalement recordings.

The terfenadine-, verapamil-, or pinacidil-induced outward shift of current active during phase 1 of the AP leads to all-or-none repolarization at the end of phase 1 (loss of the dome) at some epicardial sites but not others, generating a local EDR as well as a TDR (Figure 4). These dispersions of repolarization and refractoriness create a vulnerable window for the generation of reentrant arrhythmias. Propagation of the epicardial AP dome from regions in which it is maintained to regions in which it is lost generates a closely coupled phase 2 reentrant extrasystole that captures the vulnerable window leading to the development of closely coupled extrasystoles and polymorphic VT (Figures 5 and 6). dmLSB (10 μmol/L) significantly reduces both TDR and EDR in our models of the Brugada syndrome by preventing heterogeneous loss of the epicardial AP dome and resulting AP abbreviation, secondary to an outward shift in the balance of currents active during phase 1 of the AP. This action of the drug effectively eliminated both the trigger and the substrate for reentry in 9 of 9 preparations tested.

Although all mutations thus far associated with the Brugada syndrome have been linked to SCN5A, mutations in this gene account for ≈20% of Brugada syndrome cases,[20] suggesting the likelihood that genetic defects linked to other ion channel currents active during the early phases of the AP may be involved, including I_{to}, $I_{K\text{-}ATP}$, I_{Kr}, I_{Ks}, or I_{Ca}. The effectiveness of dmLSB in preventing Brugada syndrome induced in these 3 diverse pharmacological models suggests that this strategy may be effective in patients with Brugada syndrome induced by various etiologies.

Previous studies from our laboratory suggest block of I_{to} as a therapeutic strategy for the Brugada syndrome (for review, see Antzelevitch and Fish[10]). Both I_{to} block and delay in the inactivation of I_{Na} with dmLSB result in a positive shift in the balance of currents active at the end of epicardial AP phase 1, making loss of the epicardial AP dome and phase 2 reentry unlikely.

ICD implantation is the mainstay of therapy for the Brugada syndrome. Although feasible, implantation is challenging in infants and is not an adequate solution for patients residing in regions of the world where an ICD is unaffordable. A pharmacological solution is desirable as an alternative to device therapy in these cases as well as in minimizing the firing of the ICD

in patients with frequent events.[1,10,16] Our data suggest that dmLSB is effective in eliminating the arrhythmogenic substrate responsible for the Brugada syndrome and that it deserves further study as a pharmacological adjunct to ICD usage.

Study Limitations

As with all in vitro experimental pharmacological models of human disease, caution must be exercised in extrapolating the results to the clinic. Although our models closely resemble the clinical syndrome with respect to ECG and arrhythmic manifestations, the full extent to which the models predict the behavior of the various congenital forms of the Brugada syndrome remains to be established.

Acknowledgments

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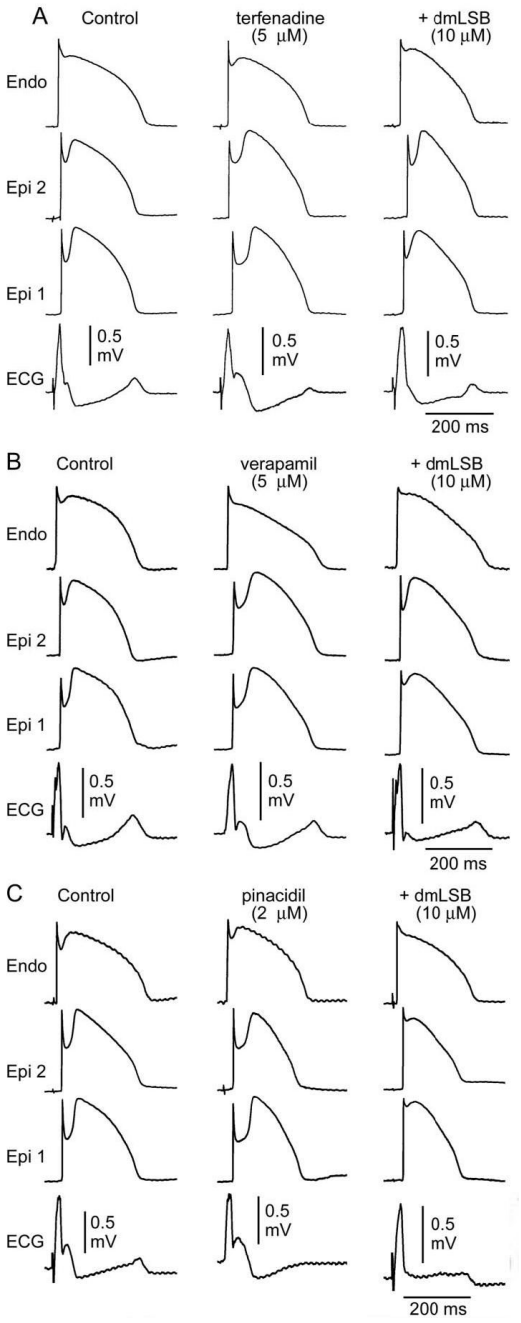

Figure 1.

Recordings from canine arterially perfused right ventricular wedges. Transmembrane APs recorded from 2 epicardial sites and 1 endocardial site together with a simultaneous ECG are shown. A, Control followed by terfenadine (5 μmol/L) ±dmLSB (10 μmol/L). B, Control followed by verapamil (5 μmol/L) ±dmLSB (10 μmol/L). C, Control followed by pinacidil (2 μmol/L) ±dmLSB (10 μmol/L). BCL=2000 ms.

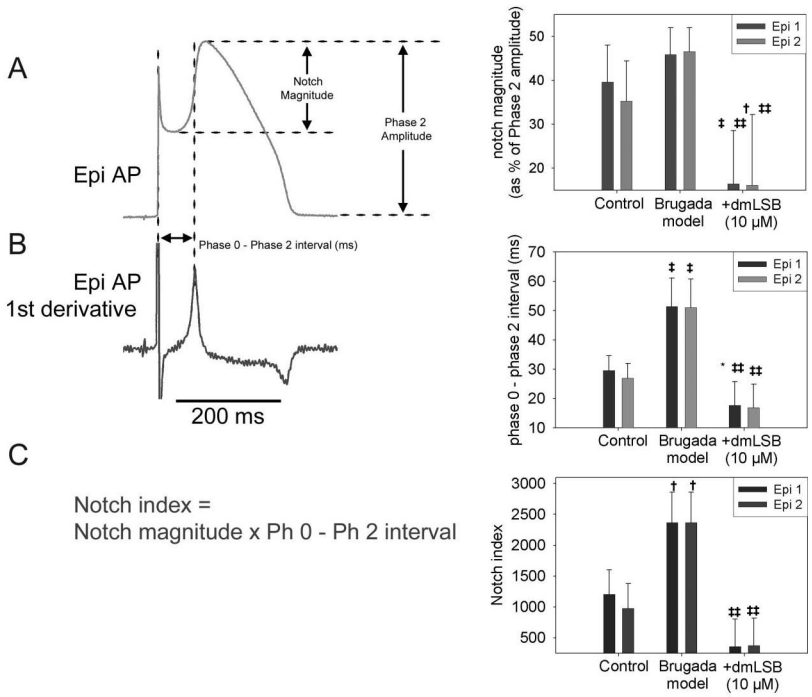

Figure 2.

Epicardial notch parameters during control and Brugada syndrome model (terfenadine, verapamil, or pinacidil) ±dmLSB (10 μmol/L). A, Notch magnitude [100 – (100 × phase 1 amplitude / phase 2 amplitude)]. B, Phase 0 to phase 2 (Ph 0 -Ph 2) interval (time between the first 2 peaks of the derivative of the AP). C, Notch index (notch magnitude × Ph 0 – Ph 2 interval). BCL=2000 ms; n=9. Values are mean±SD. $^{*}P<0.05$ vs control; †$P<0.01$ vs control; ‡$P<0.001$ vs control; $^{**}P<0.05$ vs Brugada syndrome model; ††$P<0.01$ vs Brugada syndrome model; ‡‡$P<0.001$ vs Brugada syndrome model.

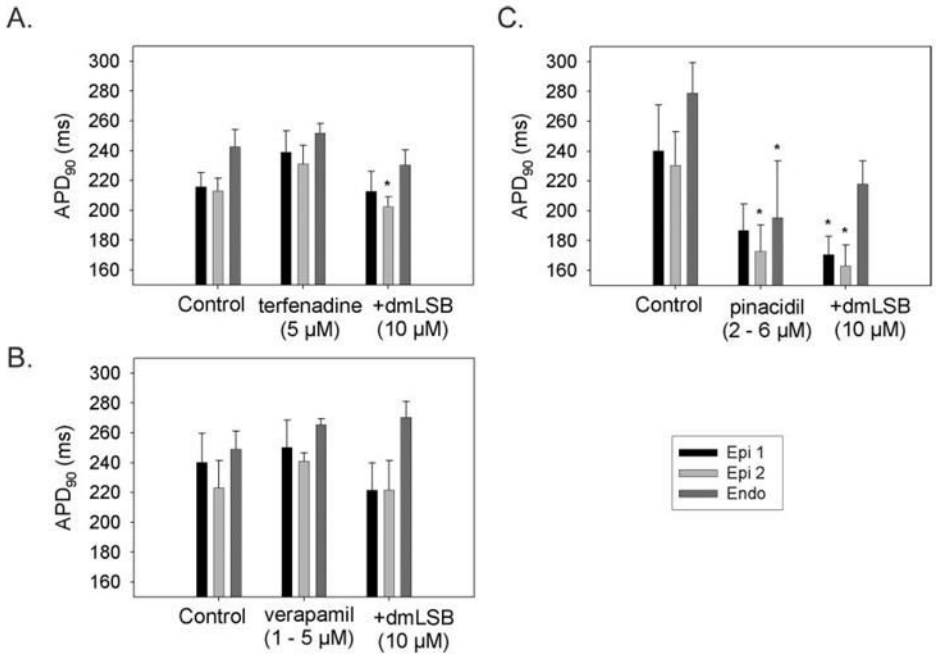

Figure 3.

APD_{90} during control and after application of terfenadine (A; n=3), verapamil (B; n=3), and pinacidil (C; n=3) with and without dmLSB (10 μmol/L). BCL=2000 ms. $^{*}P<0.05$ vs control. Endo indicates endocardial.

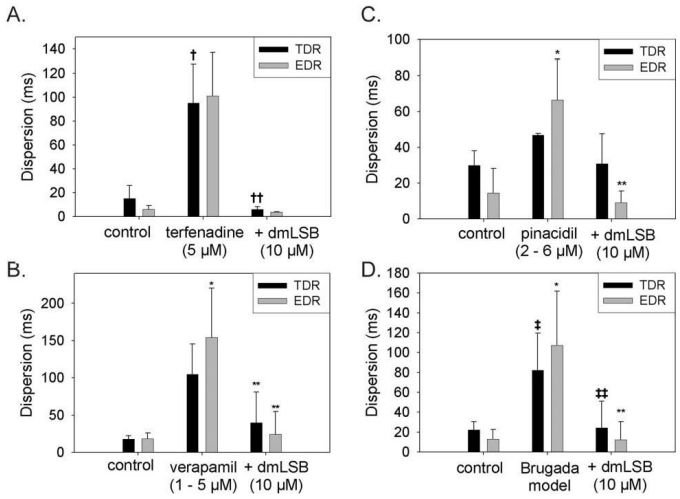

Figure 4.

Terfenadine (5 μmol/L; A; n=3), verapamil (1 to 5 μmol/L; B; n=3), and pinacidil (2 to 6 μmol/L; C; n=3) induce heterogeneous loss of the epicardial AP dome, producing both EDR and TDR. D, Combined data for all 3 models (Brugada model, n=9). Addition of dmLSB (10 μmol/L) reduces dispersion in all 3 models. Values are mean±SD. $^{*}P<0.05$ vs control; †$P<0.01$ vs control;‡$P<0.001$ vs control; $^{**}P<0.05$ vs terfenadine, verapamil, pinacidil, or Brugada syndrome model; ††$P<0.01$ vs terfenadine, verapamil, pinacidil, or Brugada syndrome model; ‡‡$P<0.001$ vs terfenadine, verapamil, pinacidil, or Brugada syndrome model.

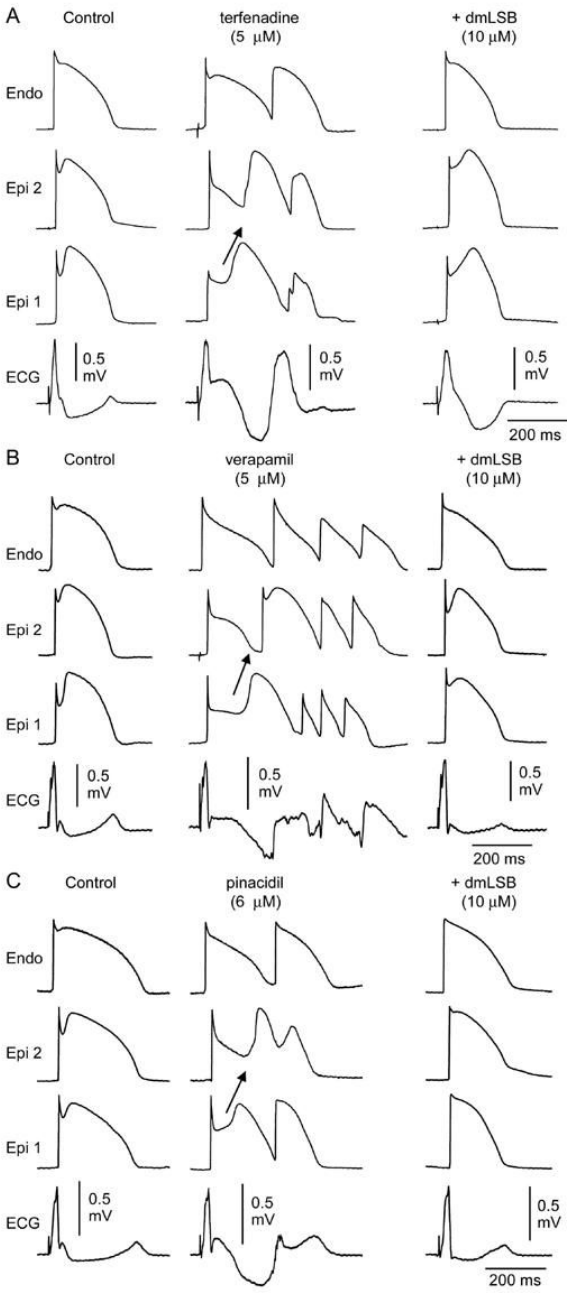

Figure 5.

Phase 2 reentry induced in 3 separate models of the Brugada syndrome. Terfenadine (5 μmol/L; A), verapamil (5 μmol/L; B), or pinacidil (6 μmol/L; C) induces heterogeneous loss of the epicardial AP dome and ST-segment elevation. Phase 2 reentry occurs as the dome is propagated from Epi 1 to Epi 2, triggering either a closely coupled extrasystole or polymorphic VT. In all 3 models, addition of dmLSB (10 μmol/L) normalizes the ST segment and abolishes phase 2 reentry and resultant arrhythmias. Endo indicates endocardial.

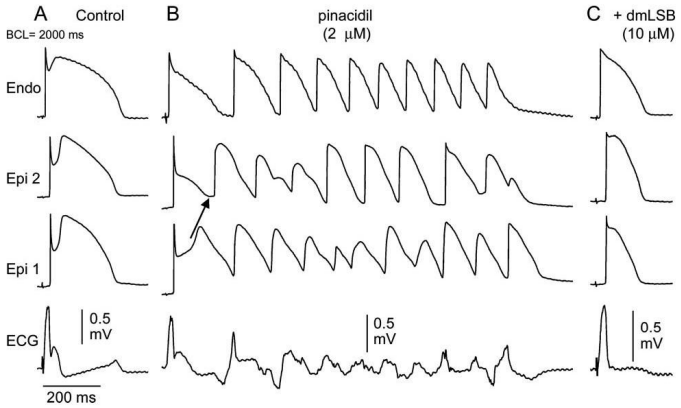

Figure 6.

Pinacidil (2 μmol/L) induces heterogeneous loss of the epicardial AP dome. Phase 2 reentry occurs as the dome is propagated from Epi 1 to Epi 2, triggering an episode of polymorphic VT (B). Addition of dmLSB (10 μmol/L) abolishes phase 2 reentry and polymorphic VT (C). Endo indicates endocardial.

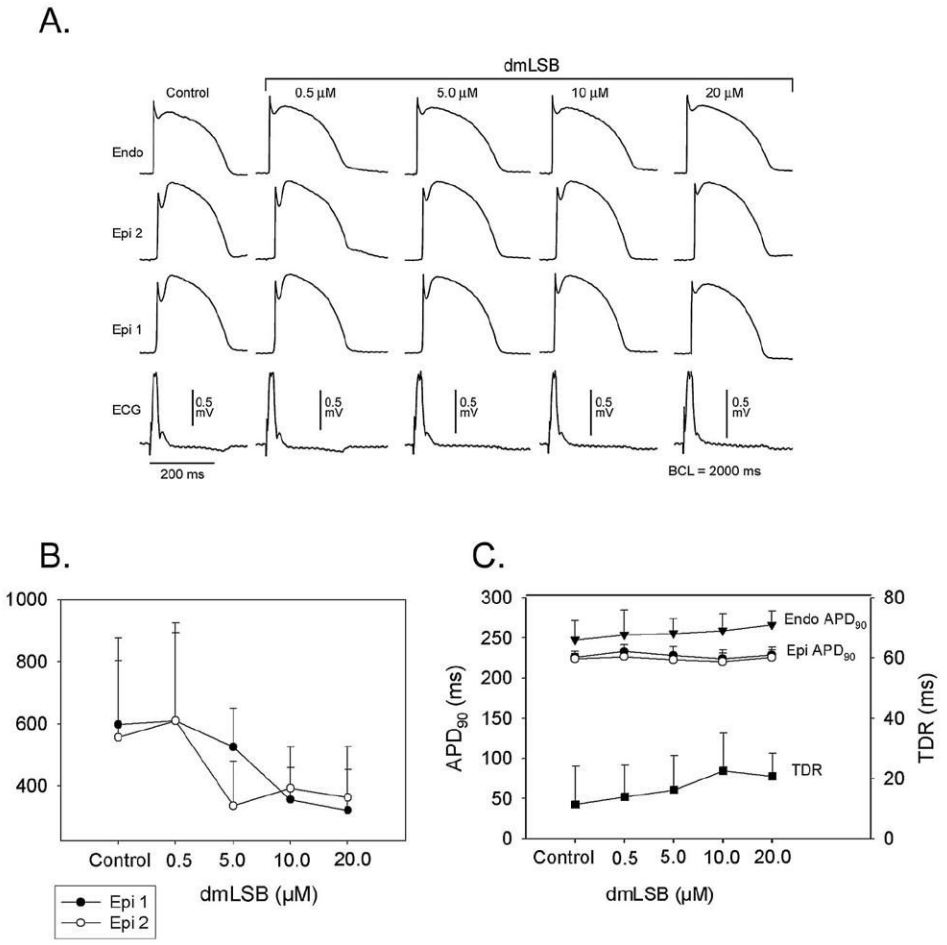

Figure 7.

Effect of dmLSB in the canine right ventricular wedge preparation. A, Transmural ECG and APs recorded from 2 epicardial sites and 1 endocardial site (Endo) in a canine arterially perfused wedge under control conditions and in the presence of dmLSB (0.5 to 20 μmol/L). BCL=2000 ms. B, Effect of dmLSB (0.5 to 20 μmol/L) on the epicardial AP notch index (notch magnitude × notch duration; see Figure 2 for complete definition). BCL=2000 ms; n=3. C, Effect of dmLSB (0.5 to 20 μmol/L) on TDR and APD_{90} in 2 epicardial and 1 endocardial AP. BCL=2000 ms; n=3.

TABLE 1.

Epicardial AP Notch Parameters at Steady State (BCL 2000 ms)

Brugada Syndrome Model	Epi 1 Notch Duration, ms	Epi 1 Notch Magnitude (as % of Phase 2 Amplitude)	Epi 1 Notch Index (Duration ×Magnitude)	Epi 2 NotchDuration, ms	Epi 2 Notch Magnitude (as % of Phase 2 Amplitude)	Epi 2 NotchIndex (Duration Magnitude)
Terfenadine (n=3)						
Control	29.5± 4.1	39.0± 9.0	170.4± 384.5	28.1± 0.9	40.3± 4.4	1131.3± 88.9
Terfenadine (5μ mol/L)	45.1± 6.6*no	45.2± 2.5	2046.9± 361.1*no	51.3± 10.9*no	48.9± 5.1	2538.9± 87.9
+dmLSB (10 μ mol/L)	18.6± 3.4	24.1± 7.7§	464.3± 237.4	23.8± 8.8§	22.2± 15.4§	597.5± 610.8§
Verapamil (n=3)						
Control	32.4± 0.9	44.9± 3.6	1790.0± 163.1	25.8± 4.1	34.5± 2.1	1685.6± 222.4
Verapamil (1-5 μ mol/L)	59.5± 10.2	49.0± 3.3	3034.4± 559.6*no	50.6±11.4*no	48.2± 2.5	2634.8±693.3
+dmLSB (10 μ mol/L)	18.4± 0.3§	8.5± 4.6‡//	1687.1± 68.0¶	21.1± 3.2¶	24.1± 18.4	1624.1± 595.8
Pinacidil (n=3)						
Control	26.7± 7.8	35.1± 10.6	1681.5± 197.6	26.8± 8.4	31.1± 15.6	1765.7± 266.8
Pinacidil (2-6μ mol/L)	49.6± 7.8	43.3± 10.3	2824.3± 787.7	51.3± 8.8*no	42.4± 7.1	2993.2±905.2
+dmLSB (10 μ mol/L)	16.0±15.5§	16.8± 18.1	1144.0±1027.8	5.6± 9.8	2.2± 3.7*no	527.8±914.1§
All (n=9)						
Control	29.6 ±5.1	39.6± 8.4	1204.9±136.8	26.9±4.8	35.3±9.1	978.1±398.4
Brugada model	51.4±9.6‡	45.9±6.1	2366.7± 602.9‡	26.9±4.8‡	35.3±9.1	2367.3±492.9†
+dmLSB (10 μ mol/L)	17.7 ± 8.1*no//	16.4 ± 12.1‡//	358.2± 353.4//	16.8±10.9//	16.2 ±16.1†//	373.6± 444.4//

*no P< 0.05 vs control.

†P< 0.01 vs control.

‡P< 0.001 vs control.

§P<0.05 vs terfenadine, verapamil, pinacidil, or Brugada model.

// P < 0.01 vs terfenadine, verapamil, pinacidil, or Brugada model.

¶P< 0.001 vs terfenadine, verapamil, pinacidil, or Brugada model.

TABLE 2.

APD90 at Steady State (BCL=2000 ms)

Brugada Syndrome Model	Epi 1 APD90, ms	Epi 2 APD90, ms	Endocardial APD_{90}, ms
Terfenadine (n=3)			
Control	215.7± 9.7	213.2± 8.6	242.7± 11.6
Terfenadine (5μ mol/L)	239.0±14.4	231.1± 12.4	251.8±6.4
+dmLSB (10 μ mol/L)	212.8±13.2	202.3±6.9*	230.5±9.9
Verapamil (n=3)			
Control	240.2±19.4	223.0±18.4	249.0±12.1
Verapamil (1-5 μ mol/L)	250.4 ±18.2	241.1± 5.4	265.4 ±4.2
+dmLSB (10μ mol/L)	221.7±18.4	221.6±19.8	270.4±10.7
Pinacidil (n=3)			
Control	240.3±30.8	230.4± 22.5	279.0± 20.4
Pinacidil (2-6μmol/L)	187.0± 17.5	172.9±17.6*	195.4± 38.3*
+dmLSB (10 μ mol/L)	170.6±12.2*	163.0± 14.1*	218.0± 15.4

*P< 0.05 vs control.

TABLE 3.

Maximal EDR and TDR and Incidence of Arrhythmias

Brugada Syndrome Model	EDR	TDR	Phase 2 Reentry	Polymorphic VT
Terfenadine (n=3)				
Control	5.9± 3.2	15.2± 10.8	0/3	0/3
Terfenadine (5 μ mol/L)	101.2±35.8	95.0± 32.7†	3/3	2/3
+dmLSB (10μ mol/L)	3.8 ±0.1	6.1± 2.3//	0/3	0/3
Verapamil (n=3)				
Control	18.3± 7.6	17.9± 4.5	0/3	0/3
Verapamil (1-5μ mol/L)	154.0± 66.1*	104.8± 40.5*	3/3	3/3
+dmLSB (10μ mol/L)	24.4±30.3§	39.6± 41.3	0/3	0/3
Pinacidil (n=3)				
Control	14.6± 13.6	29.8± 8.3	0/3	0/3
pinacidil (2-6 μ mol/L)	66.4± 22.7*	46.8±1.0	3/3	1/3
+dmLSB (10 μ mol/L)	9.1± 6.6§	30.9± 16.8	0/3	0/3
All (n=9)				
Control	12.9± 9.6	22.4± 8.1	0/9	0/9
Brugada model	107.2± 54.8*	82.2± 37.4‡	9/9*	6/9
+dmLSB (10 μ mol/L)	12.4± 18.1§	24.4 ±26.7¶	0/9§	0/9

*P<0.05 vs control.

†P < 0.01 vs control.

‡P< 0.001 vs control.

§P<0.05 vs terfenadine, verapamil, pinacidil, or Brugada model.

//P 0.01 vs terfenadine, verapamil, pinacidil, or Brugada model.

¶P 0.001 vs terfenadine, verapamil, pinacidil, or Brugada model.