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EXPERIMENTAL STUDIES

Differential Effects of Beta-Adrenergic Agonists and Antagonists in LQT1, LQT2 and LQT3 Models of the Long QT Syndrome

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OBJECTIVES To define the cellular mechanisms responsible for the development of life-threatening arrhythmias in response to sympathetic activity in the congenital and acquired long QT syndromes (LQTS).

METHODS Transmembrane action potentials (AP) from epicardial (EPI), M and endocardial (ENDO) cells and a transmural electrocardiogram were simultaneously recorded from an arterially perfused wedge of canine left ventricle. We examined the effect of beta-adrenergic agonists and antagonists on action potential duration (APD_{90}), transmural dispersion of repolarization (TDR) and the development of Torsade de Pointes (TdP) in models of LQT1, LQT2 and LQT3 forms of LQTS.

RESULTS I_{Ks} block with chromanol 293B (LQT1) homogeneously prolonged APD_{90} of the three cell types without increasing TDR. Addition of isoproterenol prolonged QT and APD_{90} of M but abbreviated that of EPI and ENDO, causing a persistent increase in TDR; Torsade de Pointes developed or could be induced only in the presence of isoproterenol. I_{Kr} block with d-sotalol (LQT2) and augmentation of late I_{Na} with ATX-II (LQT3) prolonged APD_{90} of M more than EPI and ENDO, causing increases in QT and TDR. TdP developed in the absence of isoproterenol. In LQT2 isoproterenol initially prolonged, then abbreviated, the APD_{90} of M but always abbreviated EPI, thus transiently increasing TDR and the incidence of TdP. In LQT3, isoproterenol always abbreviated APD_{90} of the three cell types, causing a persistent decrease in TDR and suppression of TdP. The arrhythmogenic as well as protective actions of isoproterenol were reversed by propranolol.

CONCLUSIONS Our data suggest that beta-adrenergic stimulation induces TdP by increasing transmural dispersion of repolarization in LQT1 and LQT2 but suppresses TdP by decreasing dispersion in LQT3. The data indicate that beta-blockers are protective in LQT1 and LQT2 but may facilitate TdP in LQT3. (J Am Coll Cardiol 2000;35:778–86) © 2000 by the American College of Cardiology

The congenital long QT syndrome (LQTS) is an hereditary disorder characterized by a prolonged QT interval in the electrocardiogram (ECG), commonly associated with an atypical polymorphic ventricular tachycardia known as Torsade de Pointes (TdP) (1–7). Genetic studies have identified five gene mutations on chromosomes 3, 7, 11 and 21 as responsible for the congenital long QT syndrome (8,9). Mutations in KvLQT1 and minK (KCNE1) are responsible for defects in the slowly activating delayed rectifier potassium current (I_{Ks}), which underlies the LQT1 and LQT5 forms of LQTS, whereas mutations in HERG and KCNE2 are responsible for defect in the rapidly activating component of the delayed rectifier potassium current (I_{Kr}), which underlies the LQT2 and LQT6 (10). A mutation in SCN5A causes an increase in late sodium current (late I_{Na}) which is responsible for LQT3. Congenital forms of LQTS have been shown to be exquisitely sensitive to increased sympathetic nervous system activity or to exogenously administered catecholamines. Indeed, an imbalance of sympathetic inputs to the heart was at one time thought to underlie the syndrome (1–4). Accordingly, beta-adrenergic blockade has long been accepted as front line therapy for LQTS.

Preliminary clinical reports suggest differences in the sensitivity of LQT1, LQT2 and LQT3 to beta-adrenergic stimuli (11). Experimental studies employing isolated guinea pig myocytes also suggest differences in the effect of isoproterenol on action potential duration (APD) in pharmacologic models that mimic LQT2 (dofetilide) and LQT3 (anthopleurin A) (12).

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Abbreviations and Acronyms

APD	= action potential duration
APD_{90}	= action potential duration measured at 90% repolarization
APD_{100}	= APD measured at full repolarization
BCL	= basic cycle length
EAD	= early afterdepolarization
ECG	= electrocardiogram
$I_{cl}(ca)$	= calcium activated chloride current
I_{kr}	= rapidly activating delayer rectifier current
I_{ks}	= slowly activating delayed rectifier current
IN_{a-Ca}	= Na^+/Ca^{2+} exchange current
late I_{Na}	= late sodium current
LQTS	= long QT syndrome
PES	= programmed electrical stimulation
S1	= basic stimuli
S2	= premature stimuli
TdP	= Torsade de Pointes
TDR	= transmural dispersion of repolarization

Recent studies from our laboratory examined the electrophysiologic, electrocardiographic and pharmacologic characteristics of the LQT1, LQT2 and LQT3 syndromes using arterially-perfused canine left ventricular wedge preparations, in which we are able to simultaneously record transmembrane activity from epicardial, M and endocardial or Purkinje sites together with a pseudo-ECG along the same axis, permitting correlation of transmembrane and ECG activity (13–18). The wedge preparation is capable of developing and sustaining a variety of arrhythmias, including TdP. The pharmacologic models mimicked the clinical congenital syndromes with respect to prolongation of the QT interval, T wave morphology, rate dependence of repolarization and response to antiarrhythmic drugs (14–18). Using this model, we recently demonstrated the determining role of beta-adrenergic stimuli in the development of transmural dispersion of repolarization (TDR) and TdP in a model of LQT1 (15). This study uses the wedge preparation to define the role of beta-adrenergic stimuli and elucidate their cellular mechanisms under conditions mimicking the LQT1, LQT2 and LQT3 forms of the congenital LQTS.

METHODS

Arterially perfused wedge of canine left ventricle. Dogs weighing 20–25 kg were anticoagulated with heparin and anesthetized with pentobarbital (30–35 mg/kg, IV). The chest was opened via a left thoracotomy, the heart excised, placed in a cardioplegic solution consisting of cold (4°C) or room temperature Tyrode's solution containing 8.5 mmol/L $[K^+]o$. Transmural wedges with dimensions of approximately 2 × 1.5 × 0.9 cm to 3 × 2 × 1.5 cm were dissected from the left ventricle. The tissue was cannulated via a small (diameter ~100 μm) native branch of left descending coronary artery and perfused with cardioplegic solution. Unperfused tissue, readily identified by its maintained red appearance (erythrocytes not washed away) was carefully removed using a razor blade. The preparation was then placed in a small tissue bath and arterially perfused with Tyrode's solution of the following composition (mmol/L): 129 NaCl, 4 KCl, 0.9 NaH_2PO_4, 20 $NaHCO_3$, 1.8 $CaCl_2$, 0.5 $MgSO_4$, 5.5 glucose, buffered with 95% O_2 and 5% CO_2 (37 ± 1°C). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co., Niles, Illinois). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Inc., Sarasota, Florida) and maintained between 40 and 50 mm Hg by adjustment of the perfusion flow rate. The preparations remained immersed in the arterial perfusate, which was allowed to rise to a level 2 to 3 mm above the tissue surface when possible. To facilitate impalement with the floating microelectrodes, in some experiments the bath solution was brought to a level just shy of the top of the wedge and the chamber was covered to the extent possible so as to avoid a temperature gradient between the top and lower segments of the wedge. Preparations displaying significant ST segment elevation or depression, or in which temperature gradients were evident along the length of the preparation, were excluded from the study.

Recordings of a transmural ECG and transmembrane action potentials. The ventricular wedges were allowed to equilibrate in the tissue bath until electrically stable, usually 1 h, and stimulated using bipolar silver electrodes insulated except at the tips and applied to the endocardial surface (S1).

A transmural ECG was recorded using 3 M KCL-Agar electrodes (1.1 mm, internal diameter). The electrodes were 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 vector as the transmembrane recordings (Epicardium: "positive" pole). The electrical field of the preparation as a whole was measured using this technique. Thus, the electrocardiographic registration represents a pseudo-ECG of that part of the left ventricle. To differentiate it from local electrogram activity, we refer to it as an ECG in the remainder of the text.

Transmembrane action potentials were simultaneously recorded from the epicardial, M and endocardial sites using three to four separate intracellular floating microelectrodes (direct current resistance: 10 to 20 M_; 2.7 mol/L KCl). Epicardial and endocardial action potentials were recorded from the epicardial and the endocardial surfaces of the preparations at positions approximating the transmural axis of the ECG recording. M cell action potentials were recorded at the site along the same axis at which APD was longest.

All amplified signals were digitized, stored on magnetic media and CD and analyzed using Spike 2 (Cambridge Electronic Design, Cambridge, United Kingdom).

Table 1. Effects of Chromanol 293B, d-Sotalol and ATX-II on the QT Interval, APD_{90} and Transmural Dispersion of Repolarization in Perfused Wedge Preparations

	QT	APD_{90} (Endo)	APD_{90} (M Cell)	APD_{90} (Epi)	TDR
Control	308 ± 12	266 ± 22	282 ± 14	227 ± 18	43 ± 10
Chromanol 293B (30 μM)	387 ± 11*	343 ± 19*	360 ± 12*	300 ± 10*	47 ± 9
Control	310 ± 13	265 ± 21	285 ± 12	228 ± 16	44 ± 9
d-Sotalol (100 μM)	383 ± 15*	345 ± 23*	359 ± 14*	272 ± 15*	74 ± 12*
Control	312 ± 14	268 ± 20	287 ± 12	228 ± 15	45 ± 9
ATX-II (100 nM)	552 ± 39*	494 ± 43*	530 ± 36*	375 ± 30*	142 ± 29*

APD_{90} = action potential duration at 90% repolarization; Endo = endocardial cell; Epi = epicardial cell; QT = QT interval; TDR = transmural dispersion of repolarization.
*$p < 0.0005$ vs. control.

Study protocols. The I_{Ks} blocker, chromanol 293B (30 μmol/L), was used to create a model of LQT1 (15), the I_{Kr} blocker, d-sotalol (100 μmol/L), was used to mimic the LQT2 syndrome (14) and ATX-II (20 nmol/L), an agent that augments late I_{Na}, was used to generate a model of LQT3 (14,18). The validity of these pharmacologic models as surrogates for the congenital syndromes was previously demonstrated in myocyte (12), perfused wedge (14–18) and in vivo studies (19,20).

We routinely examined:

1) the influence of isoproterenol (50–100 nmol/L), a beta-adrenergic agonist, on the QT interval, the APD and TDR (LQT1: n = 10, LQT2: n = 8, LQT3: n = 8),
2) the dose-dependent effects of propranolol (0.1, 0.3, 1.0, 3.0 μmol/L) in the absence of isoproterenol (LQT1: n = 8, LQT2: n = 6, LQT3: n = 6), and
3) the effects of propranolol (0.5–1.0 μmol/L) to suppress the influence of isoproterenol (LQT1: n = 10, LQT2: n = 8, LQT3: n = 8).

Control measurements were obtained after approximately 1 h of equilibration. The chromanol 293B, d-sotalol and ATX-II data were collected for a period of up to 1 h starting 1 h after addition of the respective drug. The effect of isoproterenol was recorded at 2 and 10 min, approximating the maximal and steady state influence of the catecholamine, respectively. A steady-state was achieved within 5–7 min. Propranolol data were recorded after 20 min of exposure to each concentration of drug.

Action potential duration was measured at 90% repolarization (APD_{90}). Transmural dispersion of repolarization (TDR) was defined as the difference between the longest and the shortest repolarization time (activation time + APD_{90}) of transmembrane action potentials recorded across the wall (M cell − epicardial cell). The QT interval was defined as the time between QRS onset and the point at which the final downslope of the T wave crossed the baseline. In all figures, graphic correlation of transmembrane and electrocardiographic activity was achieved by dropping a dotted line from the point of full repolarization of each action potential (APD_{100}-approximated by eye) to the ECG trace.

The development of spontaneous as well as programmed electrical stimulation (PES)-induced TdP was assessed in the absence of any drugs (control), in the presence of chromanol 293B, d-sotalol or ATX-II alone and after further addition of isoproterenol (2 min and 10 min), propranolol or a combination of the two agents. Programmed electrical stimulation-induced arrhythmias were evaluated using single extrastimuli (S2) applied to the epicardial surface of the wedge.

Statistics. Statistical analysis of the data was performed using a Student *t* test for paired data or Analysis of Variance coupled with Scheffe's test, as appropriate. Data are expressed as mean ± SD values, except for those shown in the figures, which are expressed as mean ± SEM values. Significance was defined as a $p < 0.05$.

RESULTS

Effect of chromanol 293B, d-sotalol and ATX-II on QT interval, APD and dispersion of repolarization. Table 1 shows effects of chromanol 293B (LQT1 model), d-sotalol (LQT2 model) and ATX-II (LQT3 model) alone on the QT interval, the APD_{90} of the endocardial, M and epicardial cells and TDR at a BCL of 2,000 msec. The APD_{90} of the endocardial cell was always between that of the M cell and epicardial cell. Chromanol 293B (30 μmol/L) produced a homogeneous prolongation of APD_{90} of the three cell types, thus prolonging the QT interval with no major change in the width of the T wave or TDR (Fig. 1, 4A and 4B). d-Sotalol (100 μmol/L) produced a preferential prolongation of APD_{90} of the M cell, thus causing an increase in the QT interval and TDR (Fig. 2, 4C and 4D). ATX-II (20 nmol/L) also preferentially prolonged the APD_{90} of the M cell, although the APD_{90} of all transmural sites prolonged more than with d-sotalol, resulting in a delay in the appearance of the T wave and a dramatic increase in the QT interval and TDR (Fig. 3, 4E and 4F).

Differential effect of isoproterenol in the LQT1, LQT2 and LQT3 models. The influence of isoproterenol in the LQT1 model is illustrated in Figure 1. Shown are superimposed action potentials recorded simultaneously from M

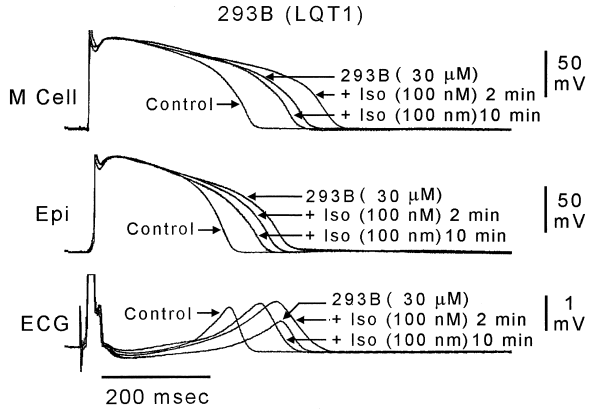

Figure 1. Effect of isoproterenol in the LQT1 model. Shown are superimposed action potentials recorded simultaneously from M and epicardial cells, together with a transmural ECG. BCL = 2,000 ms. Chromanol 293B (30 μmol/L) produced a homogeneous prolongation of APD of both cell types with no major change in the width of the T wave or in TDR. Isoproterenol (Iso, 100 nmol/L) in the continued presence of 293B dramatically prolonged the APD of the M cell (2 min) and maintained it prolonged as the effect approached a steady state (10 min), whereas the epicardial action potential was always abbreviated, resulting in a persistent increase in TDR and in a widening of the T wave, as commonly seen in LQT1 patients. APD = action potential duration; LQT1 = long QT syndrome 1; TDR = transmural dispersion of repolarization.

and epicardial cells, together with a transmural ECG at a BCL of 2,000 msec. In all cases, the peak of the T wave in the ECG was coincident with the repolarization of epicardium, whereas the end of the T wave was coincident with the repolarization of the M region. Thus, TDR across the ventricular wall was defined as the difference in the repolarization time between the M cell and epicardial cell. In the presence of 30 μmol/L chromanol 293B, which alone produced a homogeneous prolongation of APD with no major change in the width of the T wave or in TDR, isoproterenol (100 nmol/L) dramatically prolonged the QT interval and the APD of the M cell, but abbreviated that of the epicardial cell, resulting in a persistent increase in TDR and in a widening of the T wave, as commonly seen in LQT1 patients. Composite data of the influence of isoproterenol in the LQT1 model is shown in Figure 4, A and B.

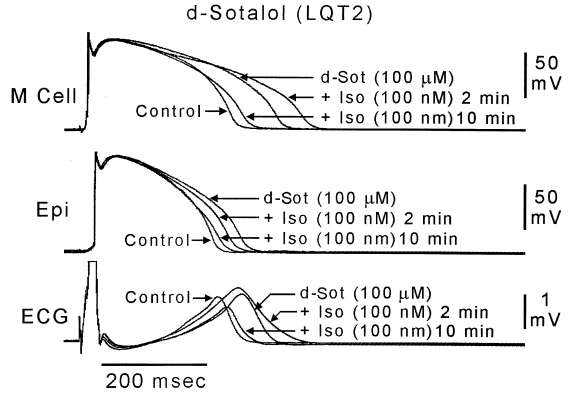

Figure 2. Effect of isoproterenol in the LQT2 model. d-Sotalol (d-Sot, 100 μmol/L) produced a preferential prolongation of the M cell APD and an increase in TDR. Isoproterenol (Iso, 100 nmol/L) in the continued presence of d-Sot initially prolonged (2 min) and then abbreviated the APD of the M cell to the control level (10 min), whereas the APD of epicardial cell was always abbreviated, resulting in a transient increase in TDR. APD = action potential duration; LQT2 = long QT syndrome 2; TDR = transmural dispersion of repolarization.

Figure 2 illustrates the influence of isoproterenol in the LQT2 model. After a preferential prolongation of the M cell action potential and an increase in TDR by 100 μmol/L d-sotalol, isoproterenol (100 nmol/L) initially prolonged (2 min) and then abbreviated the QT interval and the APD of the M cell to the control level (10 min), whereas the APD of the epicardial cell was always abbreviated, resulting in a transient increase in TDR. Composite data of the influence of isoproterenol in the LQT2 model is shown in Figure 4, C and D.

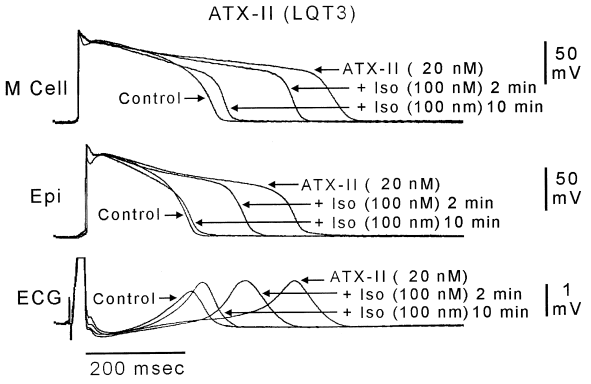

Figure 3. Effect of isoproterenol in the LQT3 model. ATX-II (20 nmol/L) produced a marked prolongation of APD of the M cell, more than that of the epicardial cell and a dramatic increase in TDR. Isoproterenol (Iso, 100 nmol/L) in the continued presence of ATX-II always abbreviated the APD of both cells, resulting in a persistent decrease in TDR. APD = action potential duration; LQT3 = long QT syndrome 3; TDR = transmural dispersion of repolarization.

The effect of isoproterenol in the LQT3 model is illustrated in Figure 3. ATX-II 20 nmol/L alone produced a dramatic prolongation of the M cell action potential and a more modest prolongation of the APD of epicardium, resulting in a dramatic increase in TDR. Isoproterenol (100 nmol/L) produced a persistent abbreviation of the QT interval and the APD of both cells, resulting in a persistent decrease of TDR. Composite data of the influence of isoproterenol in the LQT3 model is shown in Figure 4, E and F.

In four preparations, we examined the influence of isoproterenol (100 nmol/L) alone on transmembrane and ECG activity. Isoproterenol always abbreviated the APD_{90} of the three cell types, thus abbreviating the QT interval

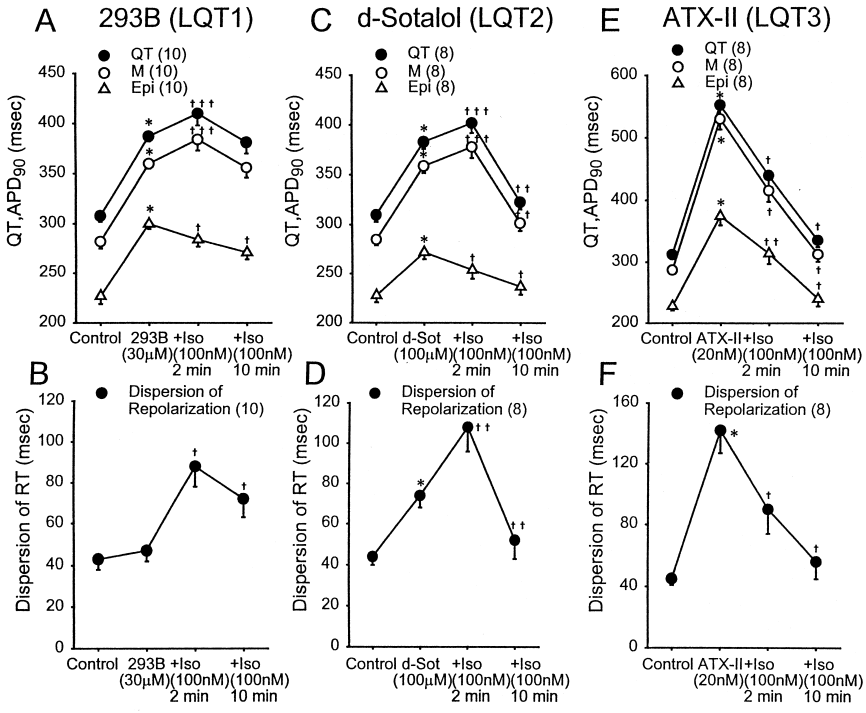

Figure 4. Composite data of the effect of isoproterenol in the LQT1, LQT2 and LQT3 models. In LQT1, isoproterenol (Iso) produced a persistent prolongation of the APD_{90} of the M cell and of the QT interval (at both 2 and 10 min), whereas the APD_{90} of the epicardial cell was always abbreviated, resulting in a persistent increase in TDR **(A and B).** In LQT2, isoproterenol initially prolonged (2 min) and then abbreviated the QT interval and the APD_{90} of the M cell to the control level (10 min), whereas the APD_{90} of epicardial cell was always abbreviated, resulting in a transient increase in TDR **(C and D).** In LQT3, isoproterenol produced a persistent abbreviation of the QT interval and the APD_{90} of both M and epicardial cells (at both 2 and 10 min), resulting in a persistent decrease in TDR **(E and F).** *$p < 0.0005$ vs. control; †$p < 0.0005$; ††$p < 0.005$; †††$p < 0.05$, vs. 293B, d-Sotalol (d-Sot) or ATX-II. APD_{90} = action potential duration measured at 90% repolarization; LQT1, 2, 3 = long QT syndrome 1, 2, 3; TDR = transmural dispersion of repolarization.

without any major changes in TDR or the development of TdP.

Effect of propranolol on the QT interval, APD and dispersion of repolarization. In all three models, therapeutic concentrations of propranolol (0.1 to 1 μmol/L) had little or no effect on the QT interval, APD_{90} of the three cell types or TDR. Higher concentrations (3 μmol/L) preferentially abbreviated the QT interval and APD_{90} of the M cell, resulting in a significant decrease of TDR, most likely due to the effect of this concentration of propranolol to block the late I_{Na}, which is intrinsically larger in the M cell than in the other cell types (21).

Effect of propranolol to suppress the influence of isoproterenol. Figure 5 illustrates composite data of the effects of propranolol (1 μmol/L) to suppress the influence of isoproterenol in the three models. Once again, 1 μmol/L propranolol had little to no effect on the QT interval, APD_{90} or TDR in the three models. In LQT1 (Fig. 5, A and B) and LQT2 (Fig. 5, C and D), propranolol completely suppressed the effect of isoproterenol (at both 2 and 10 min) to persistently or transiently prolong the M cell APD_{90} and thus to increase TDR. In LQT3 (Fig. 5, E and F), propranolol completely suppressed the protective effect of isoproterenol (at both 2 and 10 min) to abbreviate the M cell APD_{90} and to decrease TDR.

Effect of isoproterenol and propranolol on development of torsade de pointes. Spontaneous as well as stimulation-induced TdP were observed in the three models (Fig. 6). Under control conditions and in the presence of chromanol 293B alone (LQT1 model), neither spontaneous nor PES-induced TdP were observed. In this model, TdP could be induced only after addition of isoproterenol (at both 2 and 10 min). Propranolol (0.5 or 1 μmol/L) completely inhibited the effect of isoproterenol to induce spontaneous or to permit the induction of stimulation-induced TdP in the LQT1 model (Fig. 7A). In the LQT2 and LQT3 models, TdP occurred in the presence of d-sotalol and ATX-II alone (Fig. 6, A and B). In the LQT2 model, isoproterenol transiently increased the incidence of both spontaneous and stimulation-induced TdP (2 min), due in part to a transient exaggeration of TDR. At 10 min, the effect of isoproterenol reversed, approaching control values. Propranolol completely blocked isoproterenol's action to increase the incidence of TdP (Fig. 7B). In contrast to the LQT1 and LQT2 models, isoproterenol suppressed both spontaneous and stimulation-induced TdP (at both 2 and 10 min) in the LQT3 model. In

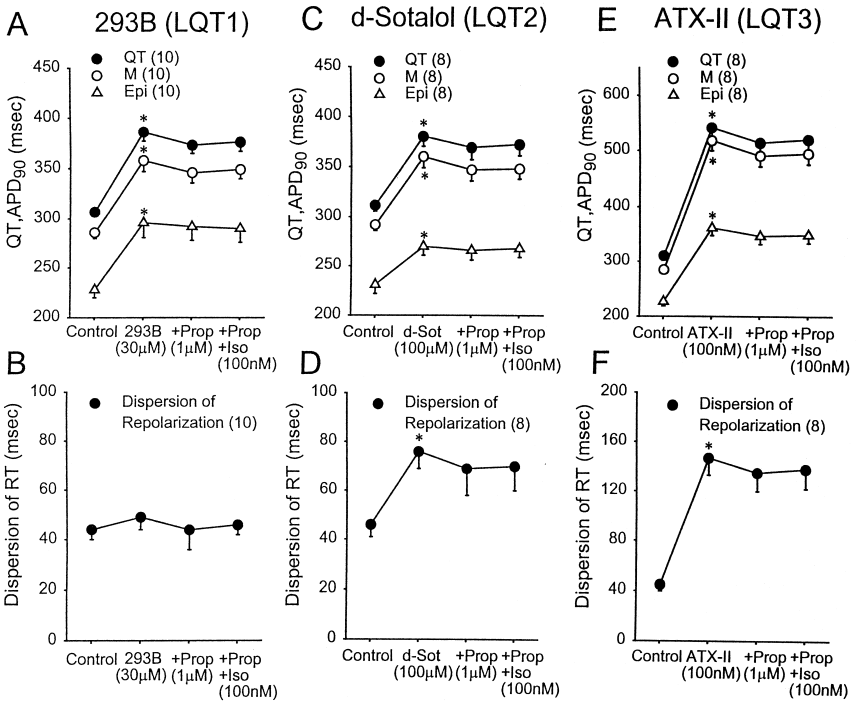

Figure 5. Composite data of the effect of propranolol (Prop, 1 μmol/L) to suppress the influence of isoproterenol (Iso, 100 nmol/L) on the QT interval **(solid circle),** the APD_{90} of M **(open circle)** and epicardial (Epi, **open triangle**) cells and TDR **(solid circle)** in the LQT1 **(A and B),** LQT2 **(C and D)** and LQT3 **(E and F)** models. In the LQT1 and the LQT2 models, propranolol completely prevented the influence of isoproterenol to persistently or transiently increase TDR. In the LQT3 model, propranolol completely suppressed the protective effect of isoproterenol to decrease TDR. The isoproterenol data reported here were recorded 10 min after addition of the catecholamine although similar results were obtained at 2 min in the presence of propranolol. *$p < 0.0005$ vs. control. APD_{90} = action potential duration measured at 90% repolarization; LQT1, 2, 3 = long QT syndrome 1, 2, 3; TDR = transmural dispersion of repolarization.

this case, propranolol reversed the protective effect of isoproterenol, causing an increase in the incidence of TdP (Fig. 7C).

DISCUSSION

Differential response of LQT1, LQT2 and LQT3 models to beta-adrenergic stimulation. Although it has long been appreciated that some forms of congenital and acquired LQTS are exquisitely sensitive to beta-adrenergic stimulation (1–7), the cellular basis for the arrhythmogenic actions of the sympathetic nervous system was poorly understood. Schwartz et al. (11) reported that cardiac events (sudden death and cardiac arrest) are most often associated with adrenergic factors, defined as physical and emotional stress, in patients with the LQT1 genotype than in those with either the LQT2 or LQT3. Wilde et al. (22) recently demonstrated that a sudden startle in the form of an auditory stimulus (alarm clock) is the predominant trigger of cardiac events in patients with the LQT2 syndrome. Relatively little is known about the effect of the sympathetic nervous system on TdP in LQT3, except that cardiac events usually occur at rest or during sleep (11,23).

Beta-adrenergic stimulation with isoproterenol is known to augment a number of currents, including Ca^{2+}-activated I_{Ks}, Ca^{2+}-activated chloride current ($I_{Cl[Ca]}$) (24) and Na^+/Ca^{2+} exchange current ($I_{Na\text{-}Ca}$). The response of APD and of the QT interval to beta-adrenergic stimulation largely depends on the shift in net outward current. An increase in net outward repolarizing current, due to a relatively large increase of I_{Ks} and $I_{Cl(Ca)}$ vs. $I_{Na\text{-}Ca}$ is thought to be responsible for the abbreviation of APD and QT interval in response to beta-adrenergic stimulation under normal conditions and for abbreviation of epicardial and endocardial cell APD under long QT conditions. A defect in I_{Ks} (especially in the M region) could offset this balance and account for failure of beta-adrenergic stimulation to abbreviate APD and QT interval in patients with the LQT1 genotype (5–7,25–27). Among the three transmural cell types, the M cell has been reported to have an intrinsically smaller I_{Ks} (28).

In the LQT1 model (reduced I_{Ks}), isoproterenol prolongs the APD of the M cell because I_{Ks} (outward current) is reduced to levels at which $I_{Na\text{-}Ca}$ (inward current) predominates. The presence of higher levels of I_{Ks} in epicardial and endocardial cells (even in the presence of chromanol 293B) accounts for the effect of the beta-agonist to abbreviate APD in these cells. The disparate effects of isoproterenol on the three cell types results in a persistent increase of TDR and in the development of spontaneous as well as

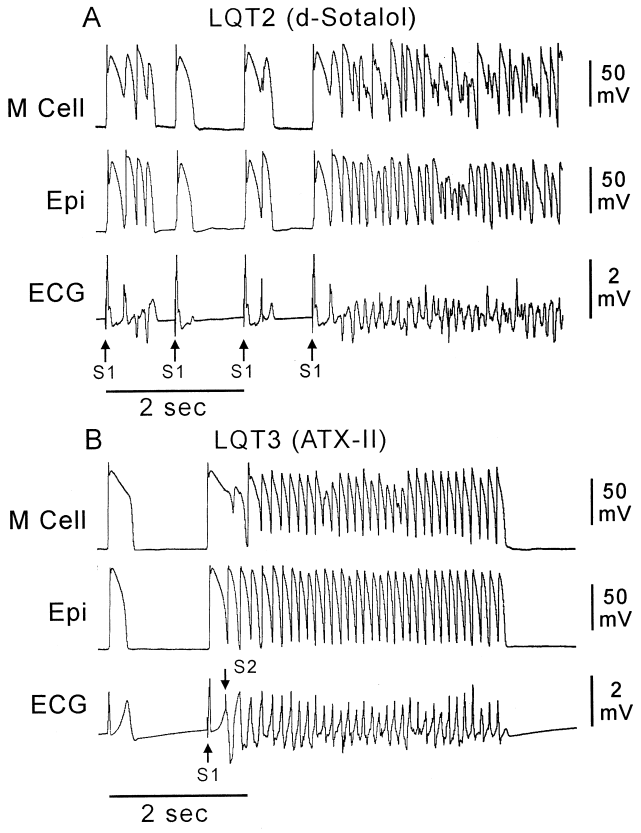

Figure 6. Polymorphic ventricular tachycardia displaying features of Torsade de Pointes (TdP) in the LQT2 **(A)** and the LQT3 **(B)** models of arterially-perfused canine left ventricular wedge preparations. Each trace shows action potentials simultaneously recorded from M and epicardial (Epi) cells together with a transmural ECG. The preparation was paced from the endocardial surface at a BCL of 1,000 or 2,000 msec (S1). **A:** Spontaneous TdP induced in the LQT2 (d-Sotalol) model. The long episode of TdP (fourth group) was preceded by salvos of three consecutive beats (first group) and an isolated ventricular premature beat (third group), similar to the clinical experience. **B:** Stimulation-induced TdP in the LQT3 (ATX-II) model. ATX-II produced very significant dispersion of repolarization (first grouping). A single extrastimulus (S2) applied to the epicardial surface initiated TdP (second grouping). LQT1, 2 = long QT syndrome 1, 2.

stimulation-induced TdP. Our findings in the LQT1 model are concordant with a high sensitivity of patients with the LQT1 syndrome to beta-adrenergic stimulation. Cardiac events in this syndrome often occur during exercise, when catecholamine levels are maximally elevated.

In the LQT2 model (reduced I_{Kr}), isoproterenol transiently prolongs the APD of the M cell, possibly due to a more rapid increase of $I_{Na\text{-}Ca}$ than of I_{Ks}. The constant abbreviation of epicardial and endocardial action potentials is most likely due to higher levels of I_{Ks} in these cell types. As a consequence, TDR and the incidence of TdP are only transiently increased. The persistent effect of isoproterenol to abbreviate epicardial and endocardial APD and its transient effect to prolong the APD of the M cells is also observed in strips of tissues isolated from the three regions of the wall following pretreatment with the I_{Kr} blocker, E-4031 (29). Effects similar to those of isoproterenol are observed in the M cell preparations in response to acceleration of the stimulation rate. These effects are eliminated after addition of ryanodine or in the presence of low $[Ca^{2+}]_o$, suggesting that the effects are secondary to intracellular Ca^{2+} loading. In isolated M cell, but not endocardial or epicardial, preparations pretreated with E-4031, acceleration of rate or addition of isoproterenol also transiently induce early afterdepolarization (EAD) activity (29). Our data are concordant with those of Priori et al. (12) who demonstrated an effect of isoproterenol to transiently prolong APD and induce EADs in guinea pig myocytes pretreated with the I_{Kr} blocker, dofetilide. Taken together, these findings suggest that beta-adrenergic stimulation contributes to precipitation of TdP in LQT2 by producing a transient but dramatic increase in TDR and causing induction of EAD-mediated extrasystoles. The transient nature of these changes following an increase in sympathetic activity may explain why cardiac events in LQT2 generally occur following a startle, especially from a sleep state (alarm clock, telephone bell or ambulance siren, etc.). Delays in APD adaptation during sudden arousal reaction is reported to occur not only in LQTS patients, but also in normal subjects (30). The presumption is that if TdP does not occur soon after the surge in sympathetic activity in LQT2 patients, it is unlikely to occur later (22).

In the LQT3 model (increased late I_{Na}), isoproterenol produces a persistent abbreviation of the APD of all three ventricular cell types. Preferential abbreviation of the M cell APD results in a persistent decrease of TDR and a decrease in the incidence of both spontaneous and stimulation-induced TdP. These effects of isoproterenol are likely due to an increase of I_{Ks} as well as a reduction of the electrogenic $I_{Na\text{-}Ca}$ due to a more positive M cell action potential plateau voltage. Our data suggest that beta-adrenergic stimulation may protect against cardiac events in patients with the LQT3 genotype. These findings are consonant with the observation that patients with the LQT3 syndrome, unlike those with LQT1 or LQT2, often have cardiac events at rest or during sleep when sympathetic tone is expected to be low (11,23).

Differential effect of beta-adrenergic blockade in the LQT1, LQT2 and LQT3 genotype. Our data indicate that beta-blockade with propranolol totally suppresses the development of TdP in the LQT1 model, largely suppresses it in LQT2 and can induce the arrhythmia in LQT3. These distinctions are due to the fact that the substrate for reentry (augmented TDR) appears only after beta-adrenergic stimulation in LQT1, may be present in the absence of sympathetic stimulation, but is greatly amplified by beta-adrenergic stimulation in LQT2 and is importantly reduced following sympathetic stimulation in LQT3.

These experimental findings appear to closely mirror the clinical experience. Beta-blockade has long been considered

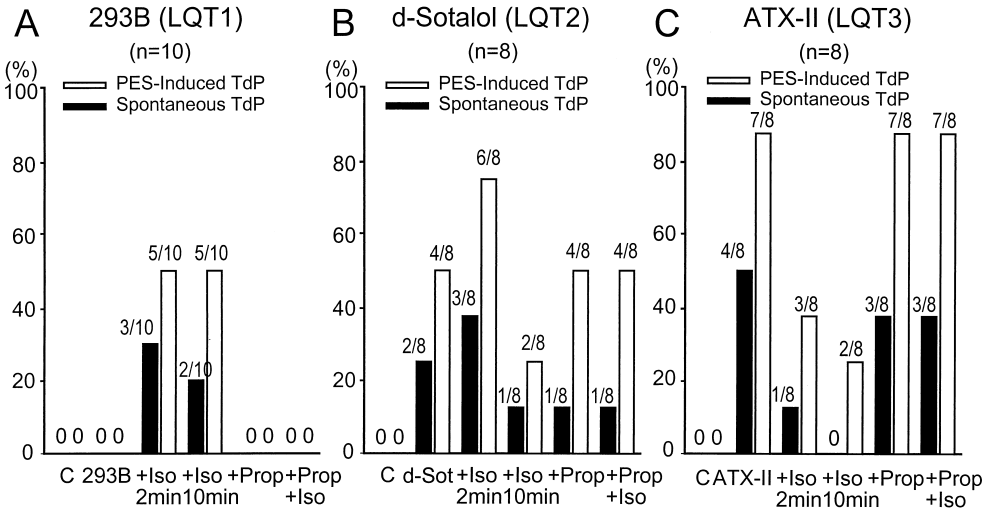

Figure 7. Incidence of spontaneous and programmed electrical stimulation (PES)-induced Torsade de Pointes (TdP) in the LQT1 **(A),** LQT2 **(B)** and LQT3 **(C)** models. Under control conditions **(C),** neither spontaneous nor PES-induced TdP were observed in any of the three models. In LQT1, TdP could be induced only after addition of isoproterenol (Iso) (at both 2 and 10 min). 0.5 or 1 μmol/L propranolol (Prop) completely suppressed the influence of isoproterenol to provoke both spontaneous and PES-induced TdP in this model. In the LQT2 and LQT3 models, TdP occurred in the presence of d-Sotalol (d-Sot) and ATX-II alone. In the LQT2 model, isoproterenol transiently increased the incidence of both spontaneous and PES-induced TdP (2 min) and then decreased it (10 min). Propranolol completely prevented these effects of isoproterenol. In contrast, isoproterenol always suppressed both spontaneous and PES-induced TdP (at both 2 and 10 min) in the LQT3 model. Propranolol reversed the protective effect of isoproterenol in LQT3, causing an increase in the incidence of TdP. LQT1, 2, 3 = long QT syndrome 1, 2, 3.

as a first-line therapy in patients with congenital LQTS (1–7). Patients with the LQT1 genotype are highly responsive to beta-adrenergic blocking agents (31). Beta-blockers are also effective in suppressing cardiac events in patients with the LQT2 genotype (22). The effectiveness of beta-blockers in patients with the LQT3 genotype is not well defined because of the rarity of the syndrome, although events generally occur at rest or during sleep, when heart rate is relatively slow (11). Both clinical (23) and experimental (12,14) studies have demonstrated that LQT3 displays a much steeper QT- and APD-rate relation than either LQT1 or LQT2. Thus, the substrate for reentry rapidly dissipates as heart rate is accelerated in LQT3 and reappears as heart rate is slowed. Our data of a protective effect of isoproterenol in LQT3, when coupled with the rate-slowing effect of the beta-blockers, suggest that beta-blockade might be contraindicated in LQT3. A test of this hypothesis clearly must await appropriate clinical trials.

Study limitations. Our interpretations of the data are based on the assumption that the 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. Such validation was provided in three previous studies employing the perfused wedge preparation (14,17,18).

The extent to which our pharmacologic models mimic the three forms of congenital long QT syndrome is also important. We believe that these pharmacologic models provide reasonable surrogates for the respective clinical syndromes because the behavior of the models mimics the clinical observations with respect to the electrocardiographic changes in the morphology and appearance of the T wave (32,33), rate dependence of the QT interval (23) and response to pharmacologic agents (23,31).

Finally, extrapolation of the experimental data to the clinic must be approached with great caution for obvious reasons. It is our hope that these findings will prove helpful in the design of clinical trials to test the hypotheses suggested by the study.

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