Effects of a K⁺ Channel Opener to Reduce Transmural Dispersion of Repolarization and Prevent Torsade de Pointes in LQT1, LQT2, and LQT3 Models of the Long-QT Syndrome

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Background—This study examines the effects of nicorandil, a K⁺ channel opener, on transmural dispersion of repolarization (TDR) and induction of torsade de pointes (TdP) under conditions mimicking the LQT1, LQT2, and LQT3 forms of the congenital long-QT syndrome (LQTS).

Methods and Results—Transmembrane action potentials of epicardial, M, and endocardial cells were recorded simultaneously from an arterially perfused wedge of canine left ventricle together with a transmural ECG. Chromanol 293B (30 μmol/L) was used to block If (LQT1 model). Isoproterenol (50 to 100 nmol/L) was used to mimic an increase in β-adrenergic tone, d-sotalol (100 μmol/L) to block If (LQT2 model), and ATX-II (20 nmol/L) to augment late IKS (LQT3 model). Isoproterenol + chromanol 293B, d-sotalol, and ATX-II produced preferential prolongation of the action potential duration at 90% repolarization (APD90) of the M cell, an increase of TDR, and spontaneous as well as stimulation-induced TdP (LQT1, 3/6; LQT2, 3/6; LQT3, 5/6). Nicorandil (2 to 20 μmol/L) abbreviated the QT interval and APD90 of the 3 cell types in the 3 models. High concentrations (10 to 20 μmol/L) completely reversed the effects of 293B + isoproterenol and those of d-sotalol to increase APD90 and TDR and to induce TdP in LQT1 and LQT2 models. Nicorandil 20 μmol/L reversed only 50% of the effect of ATX-II and failed to completely suppress TdP in the LQT3 model (5/6 to 3/6).

Conclusions—Our data suggest that K⁺ channel openers may be capable of abbreviating the long QT interval, reducing TDR, and preventing spontaneous and stimulation-induced TdP when congenital or acquired LQTS is secondary to reduced If or IKS but less so when it is due to augmented late IKS. ( Circulation. 2000;102:706-712.)

Key Words: long-QT syndrome | arrhythmia | genes | nicorandil | cells

Recent studies have identified 5 forms of the congenital long-QT syndrome (LQTS) caused by mutations in ion channel genes located on chromosomes 3, 7, 11, and 21.1–3 Mutations in KCNQ1 and KCNE1 are responsible for defects in the slowly activating component of the delayed rectifier potassium current (If), which underlies the LQT1 and LQT5 forms of the LQTS, whereas mutations in HERG and KCNE2 are responsible for defects in the rapidly activating component of the delayed rectifier potassium current (If) responsible for LQT2 and LQT6. Mutations in SCN5A alter the function of the sodium channel (Ina) responsible for LQT3.

Linkage of gene mutations to ion channel dysfunction is a first step in the formulation of a gene-specific approach to therapy in congenital LQTS. Identification of agents that exert differential actions in the various genotypes of LQTS is a next step. Schwartz and coworkers4 demonstrated that the sodium channel blocker mexiletine is more effective in shortening the QT interval in LQT3 patients with sodium channel defect than in either LQT1 or LQT2 patients with potassium channel defects. Compton et al5 showed that potassium infusion abbreviated the QT interval in LQT2 patients with an IKS defect. More recently, Shimizu and coworkers6 reported that nicorandil, a K⁺ channel opener, abbreviated the QT interval and monophasic action potential duration prolonged by epinephrine infusion in LQT1 patients with the IKS defect. However, shortening of the QT interval by these interventions is not necessarily congruent with their efficacy to decrease arrhythmic risk and sudden cardiac death.

We have developed an arterially perfused canine left ventricular wedge preparation in which we are able to simultaneously record transmembrane action potentials from epicardial, midmyocardial (M), and endocardial sites along the transmural surface using floating glass microelectrodes together with a transmural pseudo-ECG.7–14 The wedge is capable of developing and sustaining a variety of arrhyth-
mias, including torsade de pointes (TdP).9,11–14 In the present study, we use this preparation to examine the effect of nicorandil, a K+ channel opener, to decrease transmural dispersion of repolarization (TDR) and suppress TdP under control conditions as well as conditions mimicking the LQT1, LQT2, and LQT3 forms of congenital LQTS.

Methods

Arterially Perfused Wedge of Canine Left Ventricle

The methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused canine left ventricular wedge preparation, as well as the viability and electrical stability of the preparation, are detailed in previous studies.7–14 Time controls have demonstrated the electrical stability of the wedge preparations for a period of >6 hours.

Briefly, dogs weighing 20 to 25 kg were anticoagulated with heparin and anesthetized with pentobarbital (30 to 35 mg/kg IV). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4°C) or room-temperature Tyrode’s solution containing 8.5 mmol/L KCl. Transmural wedges with dimensions of approximately 2.5×0.75×2 to 2.0×1.5 cm were dissected from the left ventricle. The tissue was cannulated via a small (diameter ~100 μm) native branch of the left anterior descending coronary artery and perfused with cardioplegic solution. Unperfused tissue, readily identified by its maintained red appearance (erythrocytes not washed away), was carefully removed with 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): NaCl 129, KCl 4, NaH2PO4 0.9, NaHCO3 20, CaCl2 1.8, MgSO4 0.5, and glucose 5.5, buffered with 95% O2 and 5% CO2 (37°C). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Inc) and maintained between 40 and 50 mm Hg by adjustment of the perfusion flow rate.

Recordings of a Transmural ECG and Transmembrane Action Potentials

The ventricular wedges were stimulated with bipolar silver electrodes insulated except at the tips and applied to the endocardial surface (S1). A transmural ECG was recorded by use of 3 mol/L KCl-agar electrodes (1.1 mm ID). 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, + pole). The electrical field of the preparation as a whole was measured with this technique. Thus, the ECG 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 recorded simultaneously from the epicardial, M, and endocardial sites by use of 3 to 4 separate intracellular floating microelectrodes (DC resistance, 10 to 20 MΩ; 2.7 mol/L KCl). Epicardial and endocardial action potentials were recorded from the epicardial and 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 action potential duration (APD) was longest. All amplified signals were digitized, stored on magnetic media and WORM-CD, and analyzed with Spike 2 (Cambridge Electronic Design).

Study Protocols

The LQT1 blocker chromanol 293B (30 μmol/L) was used to create a model of LQT1.9,14 and isoproterenol (50 to 100 nmol/L) was used to mimic increased β-adrenergic tone. The LQT2 blocker d-sotalol (100 μmol/L) was used to create a model that mimics LQT2.8,13,14 ATX-II (20 nmol/L), an agent that augments late Ica, was used to mimic LQT3.8,12–14 The validity of these pharmacological models as surrogates for the congenital syndromes has been demonstrated in myocardite,15 wedge,9,11–14 and in vivo studies.16–17

We examined (1) the dose-dependent effects of nicorandil (2, 5, 10, and 20 μmol/L) on the QT interval, the APD, and the TDR and (2) the effects of nicorandil to suppress the development of spontaneous as well as programmed electrical stimulation (PES)–induced TdP.

Control measurements were generally obtained after 1 hour of equilibration. The chromanol 293B, d-sotalol, and ATX-II data were collected for a period of up to 1 hour starting 1 hour after addition of the respective drug, and nicardiplen data were recorded after 20 minutes of exposure to each concentration of drug. In our previous study, dramatic increases of TDR and induction of TdP were seen only in the presence of both isoproterenol and chromanol 293B in the LQT1 model.9 Therefore, isoproterenol was infused both in the presence of 293B and after each dose of nicorandil, and isoproterenol data were collected at 2 minutes after addition of isoproterenol, approximating the maximal influence of the catecholamine.

APD was measured at 90% repolarization (APD90). TDR was defined as the difference between the longest and the shortest repolarization times (activation time plus APD90) of transmembrane action potential records recorded across the wall (typically, M-cell minus epicardial repolarization time). 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, a graphic correlation of transmembrane and ECG activity was achieved by dropping a dotted line from the point of full repolarization of each cell type (M, ep, Ca) and trace it across the T wave to the ECG trace.

The development of spontaneous and PES-induced TdP was assessed in the absence of any drugs (control conditions): in the presence of chromanol 293B with or without isoproterenol, d-sotalol, or ATX-II; and after the further addition of nicorandil (2, 5, 10, and 20 μmol/L). PES-induced arrhythmias were evaluated by use of single extrastimuli (S2) applied to the epicardial surface of the wedge. The vulnerable window was defined as the range of S1–S2 intervals during which a single S2 could induce TdP.

Statistics

Statistical analysis of the data was performed with a Student’s t test for paired data or ANOVA 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 value of P<0.05.

Results

Effect of Chromanol 293B±Isoproterenol, d-Sotalol, and ATX-II on QT Interval, APD, and TDR

The Table shows the effects of chromanol 293B in the absence and presence of isoproterenol (LQT1 model), d-sotalol (LQT2 model), and ATX-II (LQT3 model) on the QT interval; the APD90 of the endocardial, M, and epicardial cells; and the TDR at a basic cycle length (BCL) of 2000 ms. Chromanol 293B (30 μmol/L) produced a homogeneous prolongation of APD90 of the 3 cell types, thus prolonging the QT interval with no major change in the width of the T wave or in TDR (Figure 1A). Isoproterenol (50 to 100 nmol/L) in the continued presence of chromanol 293B abbreviated the APD90 of epicardial and endocardial cells but prolonged that of the M cells, resulting in a significant increase in TDR and a widening of the T wave, as commonly observed in LQT1 patients (Figure 1B). D-sotalol (100 μmol/L) produced a preferential prolongation of APD90 of the M cell, thus causing an increase in the QT interval and TDR (Figure 2A). ATX-II (20 nmol/L) also prolonged the M cell APD90 more than that

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Effects of Nicorandil on QT Interval, APD, and TDR in the LQT1, LQT2, and LQT3 Models

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Figure 1 illustrates the effects of nicorandil in the LQT1 model in the absence and presence of isoproterenol. Composite data are shown in Figure 3. Nicorandil (2 to 20 μmol/L) dose-dependently abbreviated the QT interval and the APD90 of both cells; 20 μmol/L nicorandil completely reversed the effect of 293B in the absence and presence of isoproterenol to prolong the QT interval and APD90 and to increase the TDR.

Figure 2 illustrates the effects of nicorandil in the LQT2 and LQT3 models. Composite data are shown in Figure 4. Nicorandil (2 to 20 μmol/L) dose-dependently abbreviated the QT interval and the APD90 of both cells; 20 μmol/L nicorandil completely reversed the effect of d-sotalol in the LQT2 model (Figure 2A). In contrast, 20 μmol/L nicorandil reversed only 50% of the effect of ATX-II in the LQT3 model (Figure 2B). An incomplete reversal (≤50%) was observed in LQT3 preparations with both large and small TDR.

Figure 5 graphically illustrates the normalized dose-response relationships (semilog scale) of nicorandil on the APD90 of the M (Figure 5A) and epicardial (Figure 5B) cells, the QT interval (Figure 5C), and TDR (Figure 5D) in the 3 models. Nicorandil 20 μmol/L totally reversed the effect of the drugs in LQT1 and LQT2 but reversed only 50% of the effect of ATX-II (LQT3).

Effect of Nicorandil on TdP in the LQT1, LQT2, and LQT3 Models

Under control conditions and in the presence of chromanol 293B alone, neither spontaneous nor PES-induced TdP was observed other than an occasional extrasystole. Spontaneous TdP developed in 1 of 6 LQT1+isoproterenol, 2 of 6 LQT2, and 3 of 6 LQT3 preparations (Figures 6A and 7). A single extrastimulus reproducibly induced TdP in 3 of 6 LQT1+isoproterenol, 3 of 6 LQT2, and 5 of 6 LQT3 preparations (Figures 6B and 7). In the LQT1 and LQT2 models, relatively low concentrations of nicorandil (5 μmol/L) partially suppressed both spontaneous and PES-induced TdP and reduced the vulnerable window (LQT1+isoproterenol, 32±9 ms; LQT2, 33±10 ms; LQT3, 55±11 ms) in conjunction with a decrease in TDR (LQT1+isoproterenol, 84±17 ms; LQT2, 74±9 ms; LQT3, 55±11 ms). Higher concentrations of nicorandil (10 to 20 μmol/L) completely suppressed spontaneous and PES-induced TdP in these models (Figure 7A and 7B). In contrast, 5 μmol/L nicorandil did not prevent TdP or reduce the vulnerable window (LQT1+isoproterenol, 32±9 ms; LQT2, 33±10 ms; LQT3, 55±11 ms). Higher concentrations of nicorandil (10 to 20 μmol/L) failed to totally suppress TdP in this model (Figure 7C).

Discussion

Differential Effect of a K+ Channel Opener on Repolarization in LQT1, LQT2, and LQT3 Syndromes

LQTS is a hereditary disorder characterized by pathophysiological prolongation of the QT interval, polymorphic ventricular tachycardia (TdP), syncope, and sudden cardiac death.18–20 Several clinical studies have presented evidence in
support of gene-specific therapy in LQTS. However, previous studies involving canine wedge preparations have shown that sodium channel block with mexiletine is effective in decreasing TDR and in suppressing TdP equally in the 3 syndromes, even though abbreviation of the QT interval is more pronounced in LQT3 than in either LQT1 or LQT2. The results suggested that an abbreviation of the QT interval by specific interventions is not necessarily consistent with their effectiveness in reducing arrhythmic risk and that reduction of TDR is a more reliable marker.

Figure 2. Dose-dependent effects of nicorandil (Nic; 2, 5, 10, and 20 μmol/L) on transmembrane and ECG activity in LQT2 and LQT3 models. Both d-sotalol (A) and ATX-II (B) produced a preferential prolongation of APD of M cell more than that of epicardial (Epi) cell, thus prolonging QT interval with a major increase in TDR. Nicorandil (2 to 20 μmol/L) dose-dependently abbreviated QT interval and APD of both cells; 20 μmol/L nicorandil completely reversed effect of d-sotalol to prolong QT interval and APD and to increase TDR in LQT2 model (A), whereas 20 μmol/L nicorandil reversed only 50% of effect of ATX-II in LQT3 model (B).

Figure 3. Composite data of dose-dependent effect of nicorandil (Nic; 2, 5, 10, and 20 μmol/L) on QT interval (Q), APD90 in M (E) and epicardial (Epi) cells, and TDR time (RT) in LQT1 model with (C and D) or without (A and B) isoproterenol (Iso). In both cases, 2 to 20 μmol/L nicorandil dose-dependently abbreviated QT interval and APD90 of both cells; 20 μmol/L nicorandil completely reversed effect of chromanol 293B alone (A and B) and in presence of isoproterenol (C and D) to prolong QT interval and APD90 and to increase TDR. *P<0.0005 vs control (C); †P<0.05, ††P<0.005, †††P<0.0005 vs 293B or 293B+isoproterenol.

Figure 4. Composite data of dose-dependent effect of nicorandil (Nic; 2, 5, 10, and 20 μmol/L) on QT interval (Q), APD90 in M (E) and epicardial (Epi) cells, and TDR time (RT) in LQT2 and LQT3 models. In both models, 2 to 20 μmol/L nicorandil dose-dependently abbreviated QT interval and APD90 of both cells; 20 μmol/L nicorandil completely reversed effect of d-sotalol (d-Sot) to prolong QT interval and APD90 and to increase TDR. *P<0.0005 vs control (C); †P<0.05, ††P<0.005, †††P<0.0005 vs d-sotalol or ATX-II.
In the present study, nicorandil was found to be much more effective in preventing TdP in LQT1 and LQT2 than in LQT3. On a percentage basis, nicorandil-induced QT abbreviation and TDR reduction was greater in LQT1 and LQT2. On an absolute basis, the drug-induced QT abbreviation and TDR reduction were similar in the 3 models. In the case of LQT1 and LQT2 but not LQT3, modest concentrations of nicorandil were often able to reduce TDR below the level necessary for the development of TdP (70 to 80 ms). Thus, the key to the effectiveness of nicorandil in LQT1 and LQT2 appears to be the ability of the drug to diminish the width of the vulnerable window created by the TDR. These findings suggest that K\(^+\) channel openers may be of therapeutic value in LQT1 and LQT2 but perhaps less so in LQT3.

The ionic basis for the differential effects of the K\(^+\) channel opener in the 3 forms of congenital LQTS may be due to differences in input resistance that result from loss versus gain of ion channel function. The loss of function in LQT1 and LQT2 (reduced \(I_{Ks}\) and \(I_{Kr}\)) results in an increase in membrane resistance, which makes it easier for additional current from any source to modulate membrane potential at the level of the plateau. The opposite is true in LQT3. A smaller membrane resistance is encountered in LQT3 because of a gain of function (augmented late \(I_{Na}\)). As a consequence, a further increase in net outward current, such as with \(I_{K-ATP}\) activation, is expected to have a relatively small influence on the plateau to abbreviate APD. Conversely, because mem-

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**Figure 5.** Normalized data of effect of nicorandil on APD\(_{90}\) in M (A) and epicardial (Epi, B) cells, QT interval (C), and TDR (D) in chromanol 293B (LQT1) model with (○) or without (●) isoproterenol (Iso), d-sotalol (d-Sot: LQT2) model (◇), and ATX-II (LQT3) model (□). Effects of drugs are normalized so that 100% value represents maximum prolongation produced by these agents. Each panel shows dose-response relationship (semilog scale) for effect of nicorandil to reverse effect of drugs. In LQT1 and LQT2 models, 20 μmol/L nicorandil totally reversed effect of these drugs. In contrast, in LQT3 model, 20 μmol/L nicorandil reversed only 50% of effect of ATX-II.

**Figure 6.** Polymorphic ventricular tachycardia displaying features of TdP in LQT2 (A) and LQT3 (B) models of arterially perfused canine left ventricular wedge preparations. A, Spontaneous TdP induced in LQT2 (d-sotalol) model. Shown are action potentials simultaneously recorded from Purkinje and epicardial (Epi) cells together with a transmural ECG. Preparation was paced from endocardial surface at a BCL of 1000 ms (S\(_1\)). First grouping shows spontaneous ventricular premature beat that failed to induce TdP, and second grouping shows spontaneous premature beat that succeeded. Premature response appears to originate from deep subendocardium (M or Purkinje cells). B, PES-induced TdP in LQT3 (ATX-II) model. Shown are action potentials simultaneously recorded from M and epicardial (Epi) cells together with a transmural ECG. Preparation was paced from endocardial surface at a BCL of 2000 ms (S\(_1\)). ATX-II produced significant TDR (first grouping). Single extrastimulus (S\(_2\)) applied to epicardial surface initiated TdP (second grouping).

**Figure 7.** Incidence of spontaneous and PES-induced TdP in LQT1 (A), LQT2 (B), and LQT3 (C) models. TdP was induced only in presence of both chromanol 293B and isoproterenol (Iso) in LQT1 model. In LQT1 and LQT2 models, higher concentrations of nicorandil (10 to 20 μmol/L) completely suppressed both spontaneous and PES-induced TdP, whereas even highest concentration of nicorandil (20 μmol/L) failed to totally suppress TdP in LQT3 model.
brane resistance is increased in LQT1 and LQT2, an increase in $I_{K,ATP}$ is expected to produce greater modulation of the plateau, resulting in a greater abbreviation of APD.

The extent to which our pharmacological models mimic the 3 forms of congenital LQTS is difficult to quantify and is subject to modulation by a number of factors, including sympathetic activity. These models have been shown to mimic their clinical counterparts with respect to the ECG signature, rate dependence of QT interval, and response to a variety of drugs. Exceptions to these rules are encountered in the wedge model as they are in the clinic. It is important to keep in mind that individual mutations in $KCNQ1$, HERG, or $SCN5A$ can cause quantitatively different changes in ion channel characteristics as well as differences in modulation by extrinsic forces. The various mutations responsible for the LQTS phenotype are similar only in their ability to qualitatively enhance or augment function. Thus, qualitatively, the pharmacological models represent reasonable surrogates for both congenital and acquired LQTS.

**Effect of a K+ Channel Opener on TdP**

TdP is an atypical polymorphic ventricular arrhythmia most often associated with QT prolongation in both congenital and acquired forms of LQTS. Recent in vivo studies from El-Sherif et al.,6,16 and Vos and coworkers,17 perfused wedge studies from our group,7 simulation studies,21 and clinical observations using monophasic action potential recordings6,22 present evidence in support of the hypothesis that an early afterdepolarization (EAD)–induced triggered activity initiates TdP but that the arrhythmia is maintained by a reentrant mechanism. Our previous studies have demonstrated that sodium channel block with mexiletine suppresses TdP by decreasing TDR in the LQT1, LQT2, and LQT3 syndromes.7,8 Similarly, in the present study, nicorandil reduced the vulnerable period for induction of TdP by reducing TDR. When TDR was not reduced below the lower limit of the vulnerable window (TDR > 70 to 80 ms), spontaneous and stimulation-induced TdP was not suppressed, providing further support for reentry as the basis for the maintenance of TdP.

Nicorandil as well as 2 other $I_{K,ATP}$ openers, pinacidil and cromakalim, are known to shorten APD and to suppress EADs induced by cesium chloride,23-25 clofilium,26 or Bay K 8644.27 Recent clinical studies that used monophasic action potential recordings have reported the effect of nicorandil to abolish EADs.6,28 Thus, $I_{K,ATP}$ openers may prevent spontaneous TdP by suppressing the EAD-induced triggered activity responsible for the initiating premature beat.

A problem with K+ channel openers is that they can cause a drop in blood pressure and elicit reflex sympathetic activity that may be arrhythmogenic in LQTS. Nicorandil is unique among K+ channel openers because it exerts less of a hypotensive effect than other K+ channel openers, including cromakalim and pinacidil.29 The present study examines the dose-dependent effects of nicorandil over a concentration range of 2 to 20 μmol/L. Oral dosing of nicorandil leads to blood levels in the range of 0.2 to 0.3 μmol/L, whereas intravenous injection can raise plasma levels to 4 μmol/L. Although we must exercise great caution in the interpretation of these findings, the data thus far available suggest that intravenous but not orally administered nicorandil may be of therapeutic value in suppressing repetitive episodes of TdP in patients with LQT1 and LQT2 syndromes but less so in those with LQT3.30

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