

## Increased Late Sodium Current Contributes to the Electrophysiological Effects of Chronic, but Not Acute, Dofetilide Administration

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**Background**—Drugs are screened for delayed rectifier potassium current ( $I_{Kr}$ ) blockade to predict long QT syndrome prolongation and arrhythmogenesis. However, single-cell studies have shown that chronic (hours) exposure to some  $I_{Kr}$  blockers (eg, dofetilide) prolongs repolarization additionally by increasing late sodium current ( $I_{Na-L}$ ) via inhibition of phosphoinositide 3-kinase. We hypothesized that chronic dofetilide administration to intact dogs prolongs repolarization by blocking  $I_{Kr}$  and increasing  $I_{Na-L}$ .

**Methods and Results**—We continuously infused dofetilide (6–9  $\mu\text{g}/\text{kg}$  bolus+6–9  $\mu\text{g}/\text{kg}$  per hour IV infusion) into anesthetized dogs for 7 hours, maintaining plasma levels within the therapeutic range. In separate experiments, myocardial biopsies were taken before and during 6-hour intravenous dofetilide infusion, and the level of phospho-Akt was determined. Acute and chronic dofetilide effects on action potential duration (APD) were studied in canine left ventricular subendocardial slabs using microelectrode techniques. Dofetilide monotonically increased QTc and APD throughout 6.5-hour exposure. Dofetilide infusion during  $\geq 210$  minutes inhibited Akt phosphorylation.  $I_{Na-L}$  block with lidocaine shortened QTc and APD more at 6.5 hours than at 50 minutes (QTc) or 30 minutes (APD) dofetilide administration. In comparison, moxifloxacin, an  $I_{Kr}$  blocker with no effects on phosphoinositide 3-kinase and  $I_{Na-L}$  prolonged APD acutely but no additional prolongation occurred on chronic superfusion. Lidocaine shortened APD equally during acute and chronic moxifloxacin superfusion.

**Conclusions**—Increased  $I_{Na-L}$  contributes to chronic dofetilide effects in vivo. These data emphasize the need to include time and  $I_{Na-L}$  in evaluating the phosphoinositide 3-kinase inhibition–derived proarrhythmic potential of drugs and provide a mechanism for benefit from lidocaine administration in clinical acquired long QT syndrome. (*Circ Arrhythm Electrophysiol.* 2016;9:e003655. DOI: 10.1161/CIRCEP.115.003655.)

**Key Words:** dofetilide ■ dogs ■ lidocaine ■ long QT syndrome ■ PI3-kinase

QT interval prolongation associated with life-threatening Torsade de Pointes (TdP) is a potential risk during treatment with some antiarrhythmic and noncardiac drugs.<sup>1–3</sup> Because of this proarrhythmic effect, many noncardiovascular drugs have been withdrawn from the market, and the development of other compounds has been aborted late in development programs.<sup>2–6</sup> Most drugs that prolong the QT interval preferentially inhibit the rapid component of the delayed rectifier potassium current  $I_{Kr}$  (or hERG, the  $\alpha$ -subunit of  $I_{Kr}$  channels). Because  $I_{Kr}$  blocking drugs prolong action potential duration (APD) and the QT interval and may induce TdP,

testing the impact of new compounds on  $I_{Kr}$  has become an important step in drug development.<sup>7,8</sup> However, there are data showing absence of a direct association between hERG block and TdP: that is, some potent hERG inhibitors do not cause TdP whereas some weak hERG inhibitors do.<sup>9</sup>

The absence of a direct association between hERG inhibition and TdP can be explained at least partially by the effects of hERG inhibitors on other ionic currents, which may counter some effects of hERG block.<sup>10–12</sup> Hence, block of outward, repolarizing current can be counteracted by blocking an inward current during the action potential plateau.

Received August 17, 2015; accepted March 1, 2016.

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The Data Supplement is available at <http://circep.ahajournals.org/lookup/suppl/doi:10.1161/CIRCEP.115.003655/-/DC1>.

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*Circ Arrhythm Electrophysiol* is available at <http://circep.ahajournals.org>

DOI: 10.1161/CIRCEP.115.003655

### WHAT IS KNOWN

- Drugs that blocking phosphoinositide 3-kinase signaling prolong action potential duration and cause early afterdepolarizations in cardiomyocytes via increase in late sodium current,  $I_{Na-L}$ .
- Chronic exposure to some  $I_{Kr}$  blockers (eg, dofetilide) prolongs repolarization additionally by increasing late sodium current ( $I_{Na-L}$ ) via inhibition of phosphoinositide 3-kinase in single cardiomyocytes.

### WHAT THE STUDY ADDS

- Intravenous dofetilide administration to intact dogs immediately prolongs repolarization by an action attributable to  $I_{Kr}$  block and after 3 hours prolongs repolarization further by an effect attributable to phosphoinositide 3-kinase inhibition and increased  $I_{Na-L}$ .
- The extent of repolarization prolongation is far greater with combined  $I_{Kr}$  block/ $I_{Na-L}$  increase (as with dofetilide) than with  $I_{Kr}$  block alone (as with moxifloxacin).
- $I_{Na-L}$  blockers such as intravenous lidocaine and newer oral drugs may be indicated in drug-induced long QT syndrome.
- Screening of drugs for phosphoinositide 3-kinase inhibition/ $I_{Na-L}$  activation would seem necessary to evaluate their potential for clinical proarrhythmia.

Alternatively, increasing an inward plateau current concurrently with blocking a repolarizing current may synergize to increase the duration of repolarization and the risk of TdP more than with either intervention alone. The latter observation is consistent with recent studies showing that prolonged ( $\geq 5$  hours) but not acute application of tyrosine kinase inhibitors used in cancer chemotherapy—prolonged APD and caused early afterdepolarizations in canine ventricular myocytes. The mechanism was inhibition of phosphoinositide 3-kinase (PI3K) resulting in an increase in late sodium current,  $I_{Na-L}$ .<sup>13</sup> Yang et al<sup>14</sup> then showed that some  $I_{Kr}$  blocking antiarrhythmic drugs have a similar action to inhibit PI3K and augment  $I_{Na-L}$ . Particularly, chronic (5 hours) dofetilide exposure increased  $I_{Na-L}$  in mouse cardiomyocytes, human-induced pluripotent stem cell-derived cardiomyocytes, and Chinese hamster ovary cells transfected with the cardiac sodium channel Nav 1.5.

These data suggested to us that the prolonged repolarization and the potential for proarrhythmia in the intact canine heart might result, in part, from this mechanism. Consequently, we hypothesized that by augmenting  $I_{Na-L}$  through the PI3K pathway,<sup>14</sup> the continuous infusion of dofetilide should prolong repolarization to a much greater degree than a short-term infusion. To test this hypothesis, we compared the acute (50 minutes) and chronic (6.5 hours) electrophysiological effects of dofetilide infusion on canine heart in situ. Specifically, we investigated the effects of dofetilide on ventricular repolarization (QTc and local repolarization at left ventricular [LV] base and apex) and ventricular restitution properties using a

standard S1–S2 pacing protocol. Although we did not study arrhythmias per se, the study of restitution permitted us to consider proarrhythmic potential.

As a further test of the hypothesis that dofetilide increases  $I_{Na-L}$  by interaction with a PI3K pathway, we performed separate experiments examining the effect of dofetilide to inhibit phosphorylation of the PI3K target, Akt. Here, myocardial biopsies were taken during 6-hour intravenous dofetilide infusion, and the level of phospho-Akt was determined.

To obtain further mechanistic data, acute and chronic dofetilide effects on myocardial repolarization were studied in vitro in LV endocardial slabs and compared with effects of moxifloxacin, an  $I_{Kr}$  blocker<sup>15</sup> that does not interact with PI3K.<sup>14</sup> To test for a contribution of  $I_{Na-L}$  in dofetilide and moxifloxacin effects on repolarization, the action of the  $I_{Na-L}$  blocker lidocaine was studied during acute and chronic exposure to the compounds.

## Methods

Experimental protocols complied with the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health publication No. 85-23, revised 1996) and were approved by the Stony Brook University Institutional Animal Care and Use Committee.

### In Situ Experiments

#### Surgical Preparation

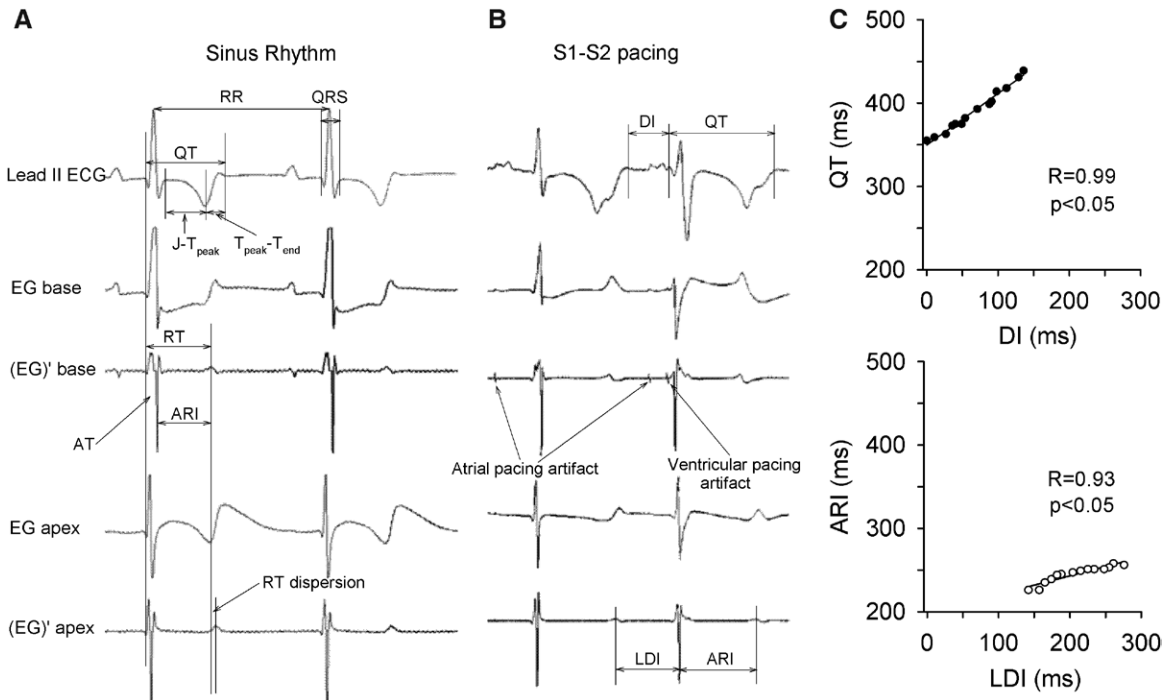
Eleven male mongrel dogs weighing 22 to 28 kg were anesthetized with propofol (6–8 mg/kg IV) followed by inhalational isoflurane 2.5 to 3.5% and oxygen. Three peripheral intravenous catheters were inserted for administration of fluids and drugs. A thoracotomy was performed via the fifth left intercostal space and the pericardium incised. We sewed a bipolar silver stimulating electrode to the left atrial appendage and unipolar surface recording electrodes to the anterobasal and apical LV epicardium. For ventricular stimulation, silver wire bipolar plunge electrodes were placed 4-mm deep to the epicardium via a 21-gauge needle at sites 10 mm from each surface recording electrode. The chest was closed, and the body temperature (recorded via an esophageal thermistor probe) was maintained between 36°C and 37°C by covering the animals, heating pad, and external heating. Femoral arterial pressure was recorded via microtip catheter pressure transducers (Millar Instruments, Inc, Houston TX).

#### Electrocardiographic Recordings

ECG leads I and II and local electrograms were monitored throughout each experiment (EMKA system, version 1.8, Paris, France). Figure 1A and 1B defines the intervals measured in ECG and electrogram. ECG and electrogram parameters were measured from 10 consecutive complexes at every experimental time point, and averaged values were analyzed. Rate correction of the QT, J-T<sub>peak</sub>, and T<sub>peak</sub>-T<sub>end</sub> intervals (QTc, J-T<sub>peak</sub>c, and T<sub>peak</sub>-T<sub>end</sub>c) was done using Bazett formula.

#### Pacing Protocol

The pacing protocol was performed at baseline and 30 and 380 minutes after starting dofetilide administration. To mimic ventricular premature beats arising from apex or base during sinus rhythm, we delivered ventricular premature stimuli (S2) from either site after a train of 15 basic drive stimuli delivered during atrial pacing (S1 at a cycle length [CL] of 5% to 10% <sinus CL). To broaden the range of diastolic intervals investigated, the first S2 was delivered 70 ms after the atrial stimulus (Figure 1B) and the S1–S2 interval was then shortened in 10-ms steps until S2 stimulus artifact merged with the end of the ECG T wave. To prevent retrograde P waves from interfering with QT measurements of ventricular paced beats, we paced the left atrium and LV simultaneously. During basal pacing, we recorded apical activation–recovery intervals (ARIs), and during



**Figure 1.** Interval measurements and construction of restitution curves. **A** and **B**, Intervals measured in ECGs and local electrograms (EGs) during sinus rhythm and S1–S2 pacing protocol, respectively. Lead II ECG, epicardial EGs recorded at LV base and apex and their first derivatives (EG') are shown. Intervals were measured as follows: RR, between the peaks of 2 successive R complexes; QT, from the Q-wave onset to the point at which the subsequent T wave merged with the isoelectric line (end of T wave); J-T<sub>peak</sub>, from end of QRS to peak of T wave; T<sub>peak</sub>-T<sub>end</sub>, from peak of T wave to end of T wave; QRS, from Q-wave onset to the point at which the S wave merged with the ST segment; activation–recovery intervals (ARIs), between the time of maximum negative slope of the local QRS complex and the time of maximum positive slope of the local EG T wave<sup>16</sup>; activation times (AT), between the onset of the Q wave and the time of maximum negative slope of the local QRS; repolarization times (RT), sum of ARI and AT; apicobasal repolarization dispersion, the difference in RT measured at the apex and base; diastolic interval (DI), from end of a T wave to onset of the after Q wave; local DI (LDI) between the maximum positive slope of the local T wave and the maximum negative slope of the following local QRS. **C**, Examples of QT and ARI restitution curves constructed by plotting QT vs preceding DI (**top**) and ARI vs LDI (**bottom**): linear regressions were performed for each slope.

apical pacing we recorded basal ARIs. The QT of each S2 versus the preceding diastolic interval and the ARI of each S2 versus the preceding local diastolic interval were then used to construct restitution curves (Figure 1C).

#### Drug Administration Protocol for Electrophysiological Experiments

In 8 dogs, we infused dofetilide (Sigma-Aldrich, St Louis, MO), whereas continuously monitoring QTc. When QTc prolongation was  $\approx 20\%$ , we stopped the infusion: this dofetilide dose was referred to as the bolus. We then infused dofetilide at rate=bolus/h during 7 hours. The total dofetilide dose varied among dogs (6- to 9- $\mu\text{g}/\text{kg}$  bolus followed by a 6- to 9- $\mu\text{g}/\text{kg}$  per hour infusion). Blood samples for dofetilide plasma measurements were taken before and after 30 and 390 minutes of dofetilide infusion. In 3 control experiments, dofetilide vehicle corresponding to the highest dofetilide dose was infused ( $\leq 100 \mu\text{L}$  of dimethyl sulfoxide [DMSO] during 7 hours infusion). QT interval and heart rate were recorded every 10 minutes. Fifty and 390 minutes after starting dofetilide infusion (and at comparable times in control experiments), 2 mg/kg lidocaine (Henry Schein Animal Health, OH) was infused intravenously  $>2$  minutes at least 5 minutes after the pacing protocol. We have previously shown that immediately after a 2 mg/kg lidocaine bolus, its plasma concentration can reach 11 to 12  $\mu\text{g}/\text{mL}$  and this decreases to  $\approx 5 \mu\text{g}/\text{mL}$  3 minutes and to  $<1 \mu\text{g}/\text{mL}$  45 minutes after the end of the bolus.<sup>17</sup> Therapeutic lidocaine plasma levels are in the range 1.4 to 6.0  $\mu\text{g}/\text{mL}$ .<sup>18</sup> Therefore, to avoid identifying possible nonspecific effects of lidocaine, its effects were determined 3 minutes after the end of a bolus. Blood samples for measurements of potassium serum level were taken every hour throughout the experiment (Akt phosphorylation experiments,

measurement of plasma dofetilide levels, and microelectrode techniques are available in the Data Supplement).

#### In Vitro Experiments

##### Protocols

Experiments were started after 3 hours of equilibration in control Tyrode solution. We have demonstrated that this time is required for full recovery and display of stable electrophysiological characteristics of endocardial slabs.<sup>19</sup> Action potentials (APs) were recorded for 30 to 40 minutes to ensure their stability, and preparations were then superfused with either dofetilide (0.2  $\mu\text{mol}/\text{L}$ ; n=6 preparations) or moxifloxacin (100  $\mu\text{mol}/\text{L}$ ; n=4 preparations) or as control preparations (n=6 preparations) for 7 hours. The CL and dofetilide concentration used in this study were chosen specifically to avoid early after depolarizations, which we have reported at longer CLs and higher dofetilide concentrations.<sup>20</sup>

Dofetilide, 0.2  $\mu\text{mol}/\text{L}$ , corresponds to 88 ng/mL, which is higher than the therapeutic plasma concentration range. However, we used dofetilide, 0.2  $\mu\text{mol}/\text{L}$  because of experiments in isolated ventricular tissue in which dofetilide, 1.0  $\mu\text{mol}/\text{L}$ , induced a nearly maximum APD prolongation at 30 minutes of exposure.<sup>21</sup> Thus, we reasoned that dofetilide, 0.2  $\mu\text{mol}/\text{L}$ , should produce a suitable acute APD prolongation that is far from saturation. This concentration resulted in a 31% APD prolongation at 30 minutes (compared with a 21% QTc prolongation seen at 30 minutes in our in situ experiments).

Lidocaine (20  $\mu\text{mol}/\text{L}$  for 20 minutes) was added to the superfusate after the effects of the compounds reached a plateau (30 minutes for dofetilide and 60 minutes for moxifloxacin) and again, 390

minutes after starting superfusion with the compounds. This lidocaine concentration does not affect  $V_{max}$  of canine ventricular AP.<sup>22</sup> Moreover, as we used the same CL (1000 ms) in all experiments, there was no concern about the potential impact of rate-dependent effects of lidocaine on  $V_{max}$  or on repolarization. In the control experiments, dofetilide vehicle, DMSO was added to the superfusate (9  $\mu$ L/L). Dofetilide was dissolved in DMSO and moxifloxacin (Selleckchem, Houston, TX) was dissolved in water to achieve a stock solution of 20 mg/mL.

## Data Analysis

Data are expressed as mean $\pm$ SE. Continuous parameters were analyzed with 1-way or 2-way ANOVA for repeated measures. Data analyses were performed by SigmaPlot (version 12.5 Systat Software, Inc, San Jose, CA). Data were checked for normality and equal variance before ANOVA. Post hoc analyses were performed with the Bonferroni correction.  $P < 0.05$  was considered statistically significant.

## Results

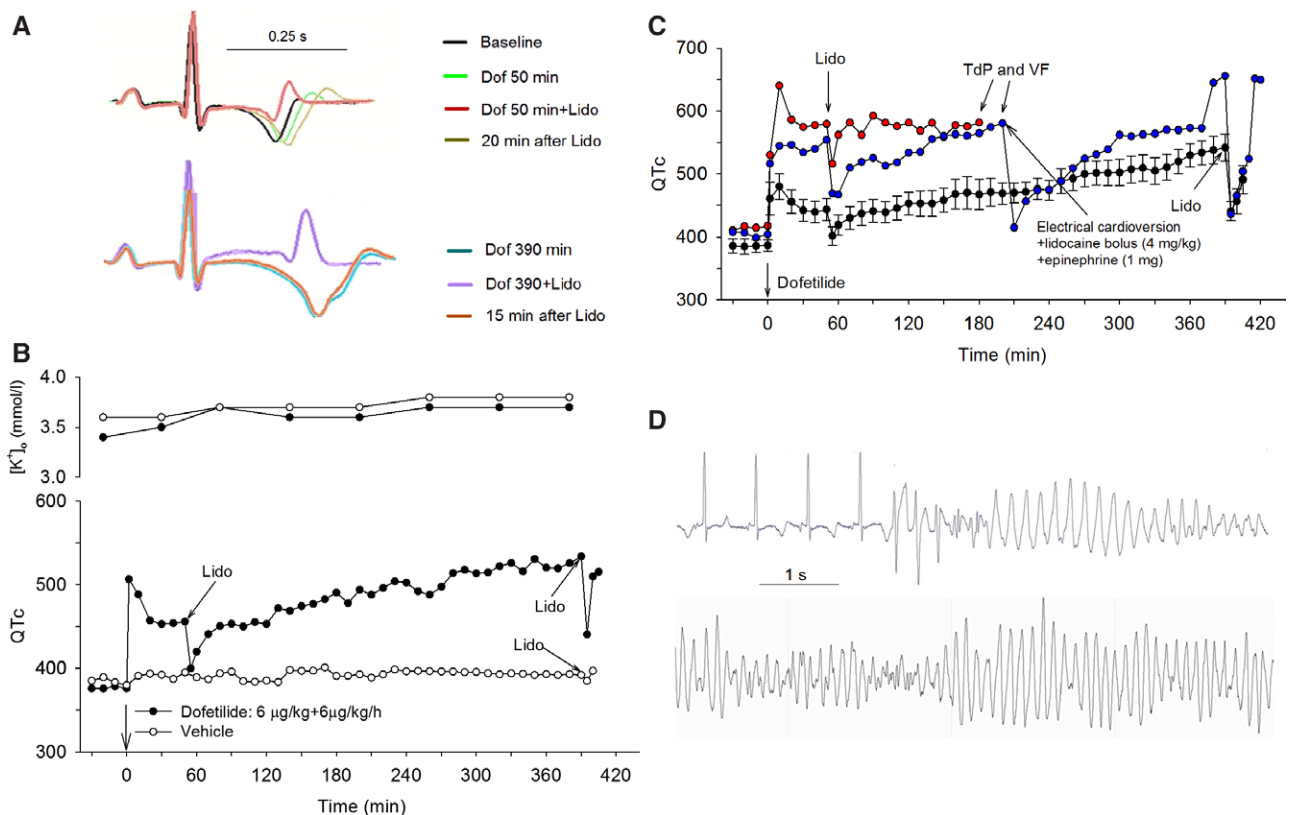
### In Situ Experiments

#### Dofetilide and Lidocaine Effects on Repolarization

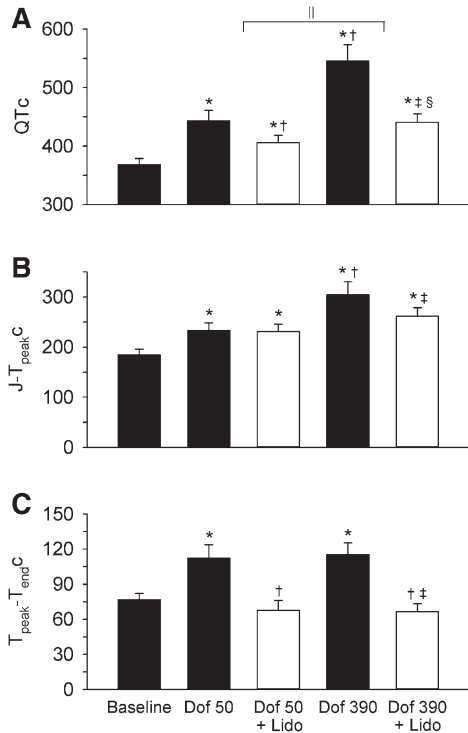
Six of the 8 dogs in this protocol developed no arrhythmias, and data could be collected from them during the entire experiment. Their dofetilide plasma concentrations were  $2.4 \pm 0.2$  ng/mL and

$5.2 \pm 0.6$  ng/mL ( $P < 0.05$ ,  $n = 6$ ), respectively, 30 minutes and 390 minutes after administering the 6- to 9- $\mu$ g/kg bolus and during the 6 to 9  $\mu$ g/kg per hour infusion. These plasma concentrations are in the therapeutic range for dofetilide.<sup>23</sup> After anesthesia and thoracotomy, systolic and diastolic blood pressures were  $88 \pm 5$  and  $54 \pm 3$  mmHg, and there were no significant changes in these values during the course of the experiments.

Figure 2A shows representative ECG data from 1 experiment demonstrating the lesser impact of lidocaine on dofetilide effects on repolarization in acute (top) than chronic (bottom) settings. Figure 2B demonstrates the time course of serum potassium concentrations (top) and QTc (bottom) throughout 1 dofetilide and 1 control experiment. Dofetilide-prolonged QTc within 30 minutes after starting the infusion and then QTc monotonically increased during the next 6 hours of infusion. Lidocaine boluses injected at 50 and 390 minutes of dofetilide infusion shortened QTc toward baseline values at both time points, although the QTc was longer after the second than the first lidocaine administration (Figure 2B, bottom). In the control experiment, QTc was stable throughout and the lidocaine boluses resulted in minimal QTc shortening. Throughout both experiments, serum potassium concentration remained stable.



**Figure 2.** Effects of acute and chronic dofetilide infusion on cardiac repolarization in situ. **A**, Lead II ECG traces are superimposed at baseline, before and at the end of the lidocaine bolus, and 15 to 20 minutes after lidocaine administration during acute (top) and chronic (bottom) dofetilide administration. **B**, Time course of serum potassium concentration (top) and QTc interval (bottom) throughout 1 dofetilide experiment and 1 control experiment. Arrows indicate QTc at the beginning of each lidocaine bolus (Lido). **C**, Time course of QTc interval throughout all experiments with dofetilide infusion. In 6 dogs, complete experiments were performed permitting data analysis throughout the 7-hour dofetilide infusion (Mean $\pm$ SEM, black circles). In 2 dogs (red and blue circles) Torsade de Pointes (TdP) and ventricular fibrillation (VF) developed  $\approx$ 3 hours after starting dofetilide infusion (example of ECG for red dog is shown in **D**). Both the dogs manifested more prominent QTc prolongation than the remaining 6 dogs. In 1 dog, defibrillation was achieved by electric shock and infusion of lidocaine and epinephrine (blue circles).



**Figure 3.** Summary data showing effects of acute (50 minutes dofetilide infusion, Dof 50) and chronic (390 minutes dofetilide infusion, Dof 390) dofetilide infusion and effects of lidocaine boluses injected at these time points. All values were recorded at baseline, before lidocaine bolus, and 3 minutes after the end of each lidocaine bolus. **A**, Effects of acute and chronic dofetilide and lidocaine boluses on QTc. \* $P < 0.05$  vs baseline, † $P < 0.05$  vs Dof 50, ‡ $P < 0.05$  vs Dof 390, § $P < 0.05$  vs Dof 50+Lido, ||magnitudes of QTc shortening induced by first (Dof 50+Lido) and second (Dof 390+Lido) lidocaine boluses are different ( $P < 0.05$ ). **B** and **C**, Effects of acute and chronic dofetilide and lidocaine boluses on early repolarization ( $J-T_{peak-c}$ ) and late repolarization ( $T_{peak}-T_{end-c}$ ), respectively. \* $P < 0.05$  vs baseline, † $P < 0.05$  vs Dof 50, ‡ $P < 0.05$  vs Dof 390 ( $n = 6$ ).

Figure 2C shows time course of QTc interval throughout all dofetilide experiments. Summary data for 6 dogs that had no arrhythmias (black circles) show QTc changes similar to those in the experiment presented in Figure 2B. In the 2 dogs (red and blue circles) that developed arrhythmias, these occurred  $\approx 3$  hours after starting dofetilide infusion (Figure 2D). Because of the arrhythmias and premature death in 1 dog and the interference of ectopy with the ECG and electrogram measurements and additional pharmacological intervention in the other, data from both the dogs were excluded from the overall experimental analysis.

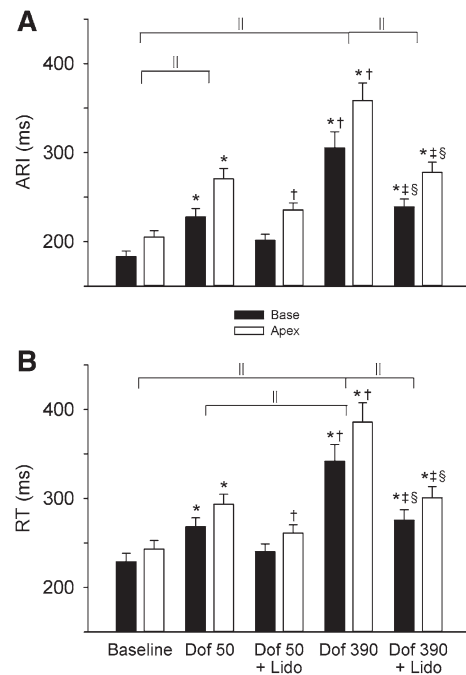
Summary data for dofetilide and lidocaine effects on QTc are shown in Figure 3A. Although acute and chronic dofetilide prolonged QTc, the effects of chronic infusion were significantly more prominent than acute ( $+49 \pm 4\%$  versus  $+21 \pm 2\%$ ,  $P < 0.05$ ). Lidocaine shortened the QTc that had been prolonged by both acute and chronic dofetilide by  $-8 \pm 1\%$  and  $-19 \pm 2\%$ , respectively ( $P < 0.05$ ). QTc after both lidocaine boluses remained longer than baseline, and QTc after the second bolus was longer than after the first. In contrast, in 3 control experiments, QTc was stable for 7 hours infusion and a lidocaine bolus injected at the end of the experiment shortened QTc  $< 2\%$  (eg, Figure 2B, bottom).

Lidocaine injected during either acute or chronic dofetilide infusion had no effects on QRS duration (QRS =  $68 \pm 1.3$  and  $68 \pm 0.8$  ms for 50 minutes dofetilide and dofetilide+lidocaine, respectively;  $64 \pm 0.9$  and  $63 \pm 1.6$  ms for 390 minutes dofetilide and dofetilide+lidocaine, respectively;  $n = 6$ ) and no effect on local activation times (at apex,  $39 \pm 2.6$  and  $40 \pm 2.3$  ms for 50 minutes dofetilide and dofetilide+lidocaine, respectively;  $38 \pm 2.4$  and  $39 \pm 1.0$  ms for 390 minutes dofetilide and dofetilide+lidocaine, respectively;  $n = 6$ ). Comparable results were seen for basal activation time (data not shown).

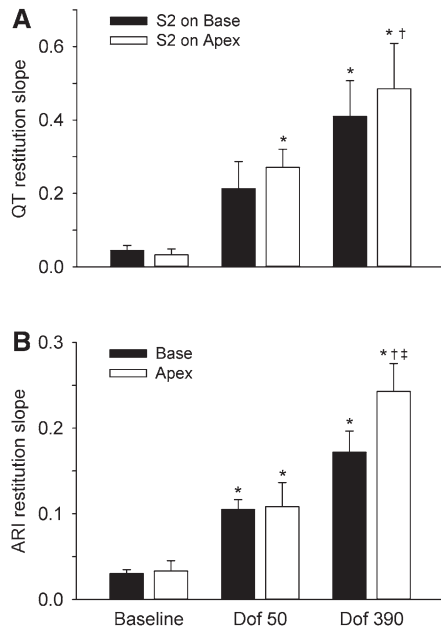
The major components of QTc ( $J-T_{peak-c}$  and  $T_{peak}-T_{end-c}$  intervals) respond differently to  $I_{Kr}$  and  $I_{Na-L}$  blockade.<sup>24</sup> Therefore, we determined dofetilide and lidocaine effects on both intervals (Figure 3B and 3C). Dofetilide effects on  $J-T_{peak-c}$  were qualitatively similar to its effects on QTc: both acute and chronic dofetilide lengthened the  $J-T_{peak-c}$  interval, but the chronic effects were significantly greater (Figure 3B). Lidocaine injected during acute dofetilide had no effects on  $J-T_{peak-c}$  but significantly abbreviated the  $J-T_{peak-c}$  that had been prolonged by chronic dofetilide.

Acute dofetilide also lengthened  $T_{peak}-T_{end-c}$ . This interval then remained unchanged during 6 hours of dofetilide infusion (Figure 3C). Lidocaine injected either at acute or chronic dofetilide shortened  $T_{peak}-T_{end-c}$  similarly.

Figure 4 summarizes dofetilide and lidocaine effects on local epicardial repolarization (ARI and repolarization time [RT]) at LV apex and base. Acute and chronic dofetilide lengthened ARI and RT at both sites with the effects of



**Figure 4.** Effects of acute (Dof 50) and chronic (Dof 390) dofetilide infusion and lidocaine boluses on local repolarization in left ventricular base and apex during sinus rhythm. **A** and **B**, Dofetilide and lidocaine effects on activation-recovery intervals (ARI) and local repolarization times (RT), respectively. For ARI and RT, differences between base and apex are significant at all time points (and are not indicated by symbols). \* $P < 0.05$  vs baseline at the same site, † $P < 0.05$  vs Dof 50 at the same site, ‡ $P < 0.05$  vs Dof 390 at the same site, § $P < 0.05$  vs Dof 50+Lido at the same site, ||differences in apico-basal dispersion are significant ( $n = 6$ ).



**Figure 5.** Effects of acute (Dof 50) and chronic (Dof 390) dofetilide on restitution slopes. **A**, Dofetilide effects on QT restitution slope: S2 were delivered either to left ventricular apex or base. \* $P < 0.05$  vs baseline at the same site of S2 delivery, † $P < 0.05$  vs Dof 50 at the same site of S2 delivery. **B**, Dofetilide effects on local activation–recovery intervals (ARI) restitution slopes at left ventricular apex and base. \* $P < 0.05$  vs baseline at the same site, † $P < 0.05$  vs Dof 50 at the same site, ‡ $P < 0.05$  vs base at Dof 390 ( $n = 6$ ).

chronic dofetilide being significantly greater. Lidocaine shortened ARI and RT prolonged by dofetilide at both time points. Importantly, dofetilide effects were heterogeneous: chronic (but not acute) dofetilide increased apicobasal dispersion of RT and lidocaine significantly attenuated this effect (Figure 4B).

#### Dofetilide Effects on Restitution

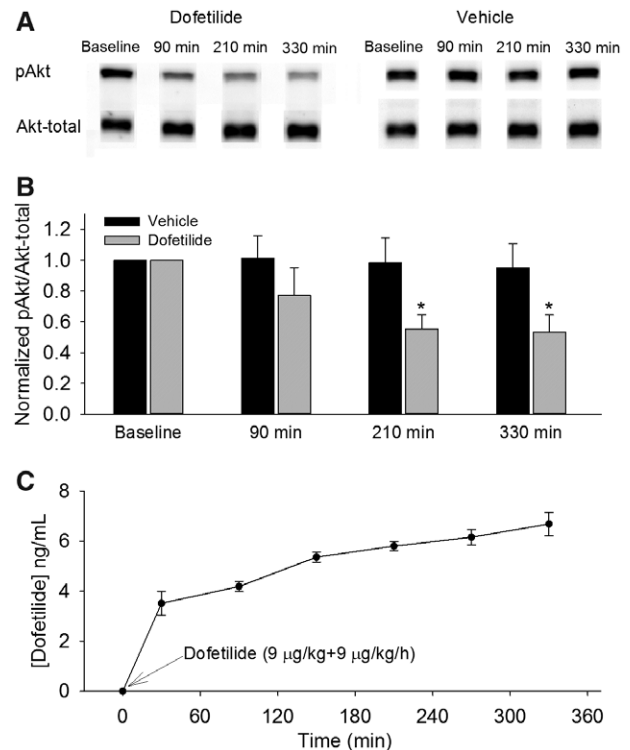
During the S1–S2 pacing protocol and chronic dofetilide administration, S2 delivery to the LV base significantly steepened the QT restitution slope (Figure 5A). When S2 were delivered to the LV apex, the slope of QT restitution increased during both acute and chronic dofetilide but the curve steepened more in chronic. Figure 5B shows dofetilide effects on restitution slopes of local ARI measured at LV base and apex. At baseline, the slopes of restitution curves were not different between base and apex. Acute dofetilide homogeneously steepened restitution at both sites. Chronic dofetilide steepened restitution significantly more than acute and this effect was heterogeneous: restitution slope increased more at apex than base.

#### Effect of Dofetilide on Akt Phosphorylation

Figure 6A and 6B shows inhibition of Akt phosphorylation by dofetilide. This effect became significant after  $\approx 3$  hours of dofetilide infusion. Figure 6C shows that after a rapid rise after the bolus, dofetilide plasma concentration monotonically increased throughout the experiments. Because of invasive nature of the biopsies and occurring frequent premature ventricular beats, QT interval was not measured in these experiments.

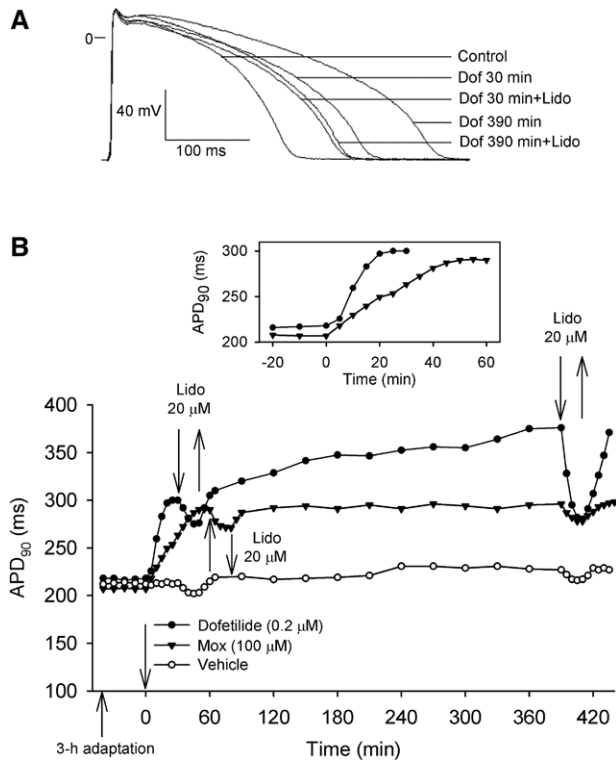
#### In Vitro Experiments

To gain further insight into mechanisms by which chronic dofetilide prolongs myocardial repolarization,



**Figure 6.** **A**, Representative Western blots showing phospho-Thr308 Akt and total Akt expression in left ventricular myocardium of 1 dog administered dofetilide (left) and 1 dog vehicle administered (right). **B**, Ratio of phospho-Thr308 Akt to Akt-total (pAkt/Akt-total) normalized to baseline during chronic dofetilide or vehicle infusion. \* $P < 0.05$  vs respective baseline ( $n = 3$  for Vehicle and Dofetilide groups). **C**, Time course of plasma dofetilide concentration in the experiments shown in **B** ( $n = 3$ ).

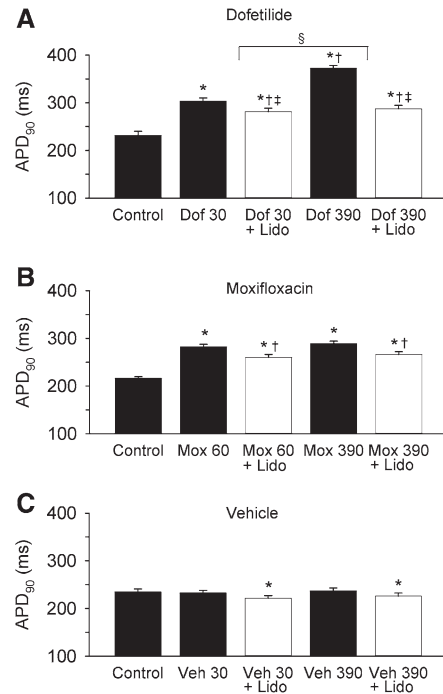
microelectrode experiments on ventricular endocardial transmembrane APs were performed. The major advantage of this design is that the concentration of dofetilide remains constant throughout all experiments. Figure 7A shows representative APs (phase 0 superimposed) at the major time points, and Figure 7B (top trace) depicts the time course of dofetilide effects on APD to 90% repolarization (APD<sub>90</sub>) in the same experiment. Dofetilide superfusion prolonged APD<sub>90</sub> to an initial plateau within 20 to 30 minutes (acute effect). APD<sub>90</sub> continued to prolong for the next 6 hours almost doubling the acute dofetilide effect (+62±4% versus +31±2%,  $P < 0.05$ ). Lidocaine applied 30 and 390 minutes after starting dofetilide superfusion shortened APD<sub>90</sub>, but the magnitude of lidocaine effect at 390 minutes was  $\approx 3\times$  that at 30 minutes (–23±1% versus –8±1%,  $P < 0.05$ ). The result was that both periods of lidocaine superfusion shortened APD<sub>90</sub> to approximately the same value (Figure 7B). In accordance with previous observations,<sup>22</sup> this lidocaine concentration (20  $\mu\text{mol/L}$ ) had no effects on AP  $V_{\text{max}}$  ( $V_{\text{max}}$  control=202±27; acute dofetilide=197±28, acute dofetilide+lidocaine 202±32, chronic dofetilide 208±27, chronic dofetilide+lidocaine 201±29 V/s;  $n = 6$ ). Figure 7B also shows a typical experiment with 100  $\mu\text{mol/L}$  of moxifloxacin, at concentration which induces APD prolongation in ventricular tissues comparable with that of dofetilide.<sup>25,26</sup> The moxifloxacin-induced prolongation of repolarization reached a plateau in  $\approx 60$  minutes, after which APD<sub>90</sub>



**Figure 7.** Effects of acute and chronic dofetilide superfusion on cardiac repolarization in vitro. **A**, For the experiment with dofetilide, transmembrane APs are superimposed at control, acute (Dof 30 minutes), and chronic (Dof 390 minutes) dofetilide superfusion and 20 minutes after lidocaine superfusion added to acute and chronic dofetilide. **B**, Representative data from 3 in vitro experiments: with dofetilide superfusion, with moxifloxacin superfusion, and 1 control experiment. Time course of action potential duration (APD) to 90% repolarization ( $APD_{90}$ ) throughout the experiments is shown. Inset shows early phases of drug effects with an expanded time scale (before the first lidocaine superfusion). Note, at acute exposure, lidocaine superfusion was started after effects of the compounds reached plateau, requiring 30 minutes for dofetilide and 60 minutes for moxifloxacin.

remained unchanged. Lidocaine applied either at 60 minutes or at 390 minutes of moxifloxacin superfusion induced similar  $APD_{90}$  shortening. In a control experiment (Figure 7B, bottom trace),  $APD_{90}$  remained constant throughout the 430-minute period. Lidocaine applied at times consistent with those for the acute and chronic dofetilide experiment shortened  $APD_{90}$  but these effects were small. The lidocaine concentration ( $20 \mu\text{mol/L}$ ) used in these experiments corresponds to  $5.4 \mu\text{g/mL}$ , which is similar to that measured 3 minutes after the end of a 2-mg/kg intravenous bolus in intact canine experiments.<sup>17</sup>

Summary data for the effects of acute and chronic dofetilide and moxifloxacin in isolated tissue experiments are shown in Figure 8A and 8B. Similar to in situ experiments (Figure 2), chronic dofetilide prolonged repolarization significantly more than acute. Lidocaine decreased  $APD_{90}$  during both acute and chronic dofetilide; however, the shortening during chronic dofetilide was more pronounced. Both periods of lidocaine exposure shortened  $APD_{90}$  to the same value. Unlike dofetilide, there were no differences between acute and chronic moxifloxacin effects as well as between 2 periods of lidocaine superfusion (Figure 8B). In control



**Figure 8.** Summary data showing effects on action potential duration ( $APD_{90}$ ) of dofetilide (**A**), moxifloxacin (**B**), and vehicle (**C**). Effects of 20 minutes lidocaine superfusion added to acute and chronic dofetilide or moxifloxacin or vehicle are shown as well. **A**, \* $P < 0.05$  vs Control, † $P < 0.05$  vs Dof 30, ‡ $P < 0.05$  vs Dof 390, §shortenings of  $APD_{90}$  induced by first (Dof 30+Lido) and second (Dof 390+Lido) periods of lidocaine superfusion are different ( $n=6$ ). **B**, \* $P < 0.05$  vs Control, † $P < 0.05$  vs Mox 60 and vs Mox 390, ( $n=4$ ), **C**, \* $P < 0.05$  vs Control, vs Veh 30 and vs Veh 390 ( $n=6$ ).

experiments,  $APD_{90}$  remained constant during the experiments and lidocaine induced small and similar decreases at both time points (Figure 8C).

## Discussion

The hypothesis we tested was that by augmenting  $I_{\text{Na-L}}$  through the PI3K pathway,<sup>14</sup> the chronic infusion of dofetilide should lead to a significantly greater prolongation of repolarization than a short-term infusion. This was borne out, in part, by the in vivo experiments, and further evidence was obtained in vitro. In vivo, QTc monotonically increased >6 hours of dofetilide infusion. As a result, QTc prolongation at the end of the experiments was more than twice that seen with acute (50 minutes) dofetilide. Lidocaine shortened QTc significantly more after chronic than acute dofetilide infusion, consistent with a greater contribution of  $I_{\text{Na-L}}$  to QTc prolongation with chronic dofetilide. Although ranolazine has been found to be more selective for  $I_{\text{Na-L}}$  than lidocaine,<sup>27</sup> its effects to block  $I_{\text{Kr}}$  at therapeutic concentrations render it a less appropriate choice than lidocaine.<sup>28</sup> Moreover, the lidocaine concentrations and doses we used were those having no effect on conduction or maximum upstroke velocity ( $V_{\text{max}}$ ) of action potential, emphasizing lidocaine effect on  $I_{\text{Na-L}}$ . A caveat here is that while remaining within the therapeutic concentration range dofetilide plasma concentration increased during the chronic infusion. Hence, the QTc results do not permit an unambiguous conclusion about the mechanism of its prolongation by

chronic dofetilide. Because  $I_{Kr}$  inhibition increases the role of  $I_{Na-L}$  in prolongation of repolarization,<sup>29,30</sup> more prominent QTc shortening induced by lidocaine after 6.5 hours of dofetilide infusion could arguably result from either greater  $I_{Kr}$  inhibition because of increased dofetilide concentration or increased  $I_{Na-L}$  through the PI3K pathway (or both). Another limitation for interpreting in vivo data results from the fact that dofetilide is lipophilic; and therefore its delayed effects may be contributed to by myocardial tissue accumulation. Nonetheless, the Akt assay showed that dofetilide infusion  $\geq 210$  minutes inhibits Akt phosphorylation. Because PI3K inhibition leads to an increase of  $I_{Na-L}$ ,<sup>13,14</sup> these results can be considered as independent evidence that prolonged dofetilide exposure engages a PI3K pathway to increase  $I_{Na-L}$  and the QT interval.

To gain additional insight into mechanisms of dofetilide-induced QTc prolongation, we measured its effects separately on 2 major components of QTc: J-T<sub>peak</sub>c (which we refer to here as early repolarization) and T<sub>peak</sub>-T<sub>end</sub>c (referred to as late repolarization). We did this because hERG potassium channel block prolongs both phases 2 and 3 of repolarization, whereas late sodium current block preferentially shortens phase 2.<sup>24</sup> In contrast, an increase in  $I_{Na-L}$  should preferentially prolong phase 2. We found that acute dofetilide prolonged both intervals, whereas chronic dofetilide further increased J-T<sub>peak</sub>c but not T<sub>peak</sub>-T<sub>end</sub>c. This would be consistent with a chronic effect of dofetilide to augment  $I_{Na-L}$ . The shortening of J-T<sub>peak</sub>c after chronic dofetilide by lidocaine is consistent with a recent clinical trial<sup>31</sup> and supports this suggestion. Complicating interpretation here is that the prolongation of T<sub>peak</sub>-T<sub>end</sub>c by dofetilide was reversed by lidocaine in the chronic as well as the acute setting. Because T<sub>peak</sub>-T<sub>end</sub>c is an index of total dispersion of repolarization,<sup>32</sup> these lidocaine effects may result mainly from its effect to decrease the dispersion of repolarization that had been increased by dofetilide. Finally, the effects of dofetilide and lidocaine shown here together with the effects of moxifloxacin discussed below suggest that further consideration might be warranted of J-T<sub>peak</sub>c and T<sub>peak</sub>-T<sub>end</sub>c measurements as a noninvasive means to estimate the influence of  $I_{Na-L}$ .

We performed the isolated tissue experiments to further test and clarify a potential contribution of increased  $I_{Na-L}$  in the prolongation of repolarization induced by long-term dofetilide infusion. A major benefit of this design was that even when dofetilide concentrations were completely stable, there still were 2 phases of dofetilide-induced APD prolongation: a rapid effect reaching a plateau in 30 minutes and a slow monotonic prolongation through the next 6 hours of superfusion. The rapid effect has been described in numerous studies and is a result of  $I_{Kr}$  inhibition. Fundamental to understanding the mechanisms of the second phase are the effects of lidocaine: the same lidocaine concentration applied either during acute or during chronic dofetilide superfusion shortened APD to the same value. This implies that APD lengthening additional to the acute effect and induced by 6 hours of dofetilide superfusion results from augmentation of  $I_{Na-L}$ . Results with moxifloxacin are consistent with this conclusion: in contrast to dofetilide, moxifloxacin has no chronic effects on APD; this is probably because of the absence of any interaction with PI3K.<sup>14</sup> Although moxifloxacin prolongs QT in clinical

situations, it has not been shown to cause TdP.<sup>33-35</sup> Our data suggest that the low proarrhythmic potential of moxifloxacin results from a lack of this chronic action on ventricular repolarization.

This study demonstrates that in addition to quantitative differences in acute and chronic dofetilide effects on global and local repolarization, there is an important qualitative difference: chronic but not acute dofetilide prolongs repolarization heterogeneously: it significantly increases apicobasal RT dispersion. This is important because RT dispersion is a factor favoring ventricular arrhythmogenesis in acquired long QT syndrome. Lidocaine not only shortens repolarization prolonged by dofetilide but also reduces RT dispersion enhanced by chronic dofetilide, consistent with an important role of increased  $I_{Na-L}$  in the development of spatial heterogeneity of repolarization. We make this comment understanding that estimating dispersion of repolarization based on measuring differences between 2 sites is a simplification. Nevertheless, that both the QT interval on ECG and 2 separate local electrogram sites provide an outcome consistent with dispersion of repolarization measured across the entire heart with more exacting techniques<sup>32</sup> suggests our interpretation is valid.

Experimental and simulation studies suggest that ventricular electric restitution (the relationship between the duration of repolarization and preceding diastolic interval) is relevant to the stability of the heart and vulnerability to ventricular fibrillation and, therefore, can be used as a marker of proarrhythmia.<sup>36,37</sup> Drugs that steepen the restitution curve have been suggested to facilitate ventricular fibrillation, whereas drugs that reduce the slope of the restitution curve are suggested to prevent induction of arrhythmias.<sup>38,39</sup> In addition to the steepness of the restitution curve, spatial heterogeneity of restitution slopes can contribute to an arrhythmogenic substrate.<sup>37,40-42</sup> In this study, chronic dofetilide steepens restitution more than acute and only chronic dofetilide produces apicobasal heterogeneity in restitution slopes. These data suggest that chronic dofetilide may be more arrhythmogenic than acute. Supporting this interpretation is the observation that of the 8 dogs initially entered into the study, 2 died of TdP leading to ventricular fibrillation approximately halfway through the protocol.

In sum, this study shows that in addition to its acute effect to prolong repolarization, the additional prolongation seen with chronic dofetilide seems attributable to an increase in  $I_{Na-L}$ . Our results with myocardial biopsies showing that dofetilide inhibits Akt phosphorylation support the hypothesis that dofetilide interacts with the PI3K pathway to affect the increase in  $I_{Na-L}$ .<sup>14</sup> Importantly, electrophysiological changes induced by chronic dofetilide are more arrhythmogenic than the acute. These data support the suggestion that screening the arrhythmogenic potential of new drugs should include an evaluation of both time and  $I_{Na-L}$ .<sup>13,14</sup> They also provide a mechanistic basis for the clinical observation that QTc must be monitored for days after initiating dofetilide therapy to detect delayed prolongation of repolarization that may predispose to TdP. Finally, our results show a mechanism for benefit from lidocaine administration in clinical acquired long QT syndrome.



## Sources of Funding

The study was supported by the Institut de Recherches Servier and USPHS-NHLBI grant HL-094410 (Dr Cohen) and Department of Veterans Affairs merit award (Dr Lin). Dr Chauveau was supported by the French Federation of Cardiology.

## Disclosures

Dr Rosen was a consultant to Servier at the time of these studies. The other authors report no conflicts.

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## Increased Late Sodium Current Contributes to the Electrophysiological Effects of Chronic, but Not Acute, Dofetilide Administration

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*Circ Arrhythm Electrophysiol.* 2016;9:

doi: 10.1161/CIRCEP.115.003655

*Circulation: Arrhythmia and Electrophysiology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1941-3149. Online ISSN: 1941-3084

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## **SUPPLEMENTAL MATERIAL**

### **Akt phosphorylation experiments**

Six dogs were anesthetized and thoracotomy was performed as above. In three dogs we infused dofetilide (9 µg/kg+9 µg/kg/h) during 6 hours, in three control experiments dofetilide vehicle was infused. Myocardial biopsies (50-80 mg) were taken from left ventricular epicardium at baseline and at 90, 210, and 330 minutes after starting dofetilide or vehicle infusion and were snap frozen in liquid nitrogen. During biopsies, to decrease the risk of TdP, hearts were paced at a CL=500 ms. Blood samples for dofetilide plasma measurements were taken before dofetilide infusion and every hour during the infusion. Western blotting analysis of lysates prepared from biopsies were performed as described<sup>1</sup>. Briefly, frozen samples were homogenized and centrifuged to make tissue lysates. The protein concentration was measured by a Bradford assay (Bio-Rad). Equal amounts of heart extract proteins were subjected to SDS-PAGE, and the proteins were transferred onto polyvinylidene difluoride membranes. Membranes were blocked in 5% nonfat milk for 1 h and then incubated with antibodies to P-T308-Akt (Cell Signaling Technology, Beverly, MA) overnight at 4°C. After extensive washing, membranes were incubated with secondary antibody coupled to horseradish peroxidase (Amersham Pharmacia Biotech) for 1 h at room temperature. After immunoblotting, signals were visualized with an enhanced chemiluminescence kit (PerkinElmer Life Sciences) under FluorChem E system (Proteinsimple). The integrated density of bands was quantified using AlphaView SA software (Proteinsimple).

### **Measurement of plasma dofetilide levels**

Dofetilide plasma levels were quantified by LC/MS using dofetilide-d4 (Toronto Research Chemicals, North York, ON, Canada) as an internal standard. After the addition of the internal

standard, samples (100  $\mu$ L) were treated with 0.1 mM NaOH (10  $\mu$ L) and extracted with methyl tertiary-butyl ether (1 mL). The extracts were dried, redissolved in 10% methanol and applied to a Poroshell UHPLC column (2.1x50mm, 2.7 $\mu$ m) eluted by a gradient of 0.1% formic acid in methanol/0.1% formic acid in water running at 0.5 mL/minute running from 10% to 20% over 3.25 minutes. The eluent was then analyzed on an Agilent 6410 triple quadrupole mass spectrometer operated by the CUMC Irving Institute for Clinical and Translational Research Biomarkers Core.

### **Microelectrode techniques**

We studied tissue from 8 dogs, for which anesthesia and thoracotomy were performed as above. The heart was removed and immersed in Tyrode's solution equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> containing (in mmol/L): NaCl 131, NaHCO<sub>3</sub> 18, KCl 4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 0.5, NaH<sub>2</sub>PO<sub>4</sub> 1.8, and dextrose 5.5. Endocardial strips (~10x5x0.5 mm) were filleted with surgical blades parallel to the surface of the anterobasal left ventricular free wall. Preparations were placed in a 4-mL tissue bath, superfused (37°C, pH 7.35 $\pm$ 0.05) at a rate of 12 mL/min and allowed to equilibrate at a stimulus CL of 1000 ms (to approximate a resting canine sinus CL). All preparations were impaled with 3 mol/L KCl-filled glass capillary microelectrodes having tip resistances of 10 to 20 M $\Omega$ . The electrodes were coupled via an Ag/AgCl junction to an amplifier with high input impedance and input capacity neutralization. V<sub>max</sub> was obtained by electronic differentiation with an operational amplifier. Transmembrane AP and V<sub>max</sub> signals were digitized with an analog-to-digital convertor and stored to PC for subsequent analysis. For stimulation of preparations, standard techniques were used to deliver 2-ms square-wave pulses 2x threshold through bipolar Teflon-coated silver electrodes.

**Reference:**

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