Cellular Mechanisms Underlying the Development of Catecholaminergic Ventricular Tachycardia

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Background—Mutations in the ryanodine 2 receptor (RyR2) gene have been identified in patients with catecholaminergic polymorphic ventricular tachycardia. We examined the cellular basis for the ECG features and arrhythmia mechanisms using low-dose caffeine to mimic the defective calcium homeostasis encountered under these conditions.

Methods and Results—A transmural ECG and action potentials were recorded simultaneously from epicardial, M, and endocardial cells in arterially perfused canine ventricular wedge preparations. Caffeine alone produced no change (10 to 100 μmol/L) or a slight abbreviation (300 μmol/L) of the QT interval and no change in transmural dispersion of repolarization. Isoproterenol (100 nmol/L) alone induced sustained monomorphic ventricular tachycardia (VT) that originated in the epicardium in 3 of 14 wedge preparations. Isoproterenol in the presence of caffeine (100 to 300 μmol/L) induced epicardial VT in 9 of 16 wedge preparations. Delayed afterdepolarization–induced triggered beats that originated in the epicardium were associated with an increased Tpeak−Tend interval and transmural dispersion of repolarization. Bidirectional VT developed in 11 of 16 wedge preparations as a consequence of alternation in the origin of ectopic activity between endocardial, M, and epicardial regions. Single extrastimuli delivered during sustained epicardial VT induced a rapid polymorphic VT/ventricular fibrillation (VF) in 3 of 9 wedges. Spontaneous polymorphic VT was observed in 3 of 16 preparations. Propranolol (1.0 μmol/L) or verapamil (1.0 μmol/L) completely suppressed ectopic activity that arose from the epicardium and prevented induction of polymorphic VT.

Conclusions—Our data suggest delayed afterdepolarization–induced extrasystolic activity serves to trigger catecholamine-induced VT/VF under conditions of defective calcium handling. Epicardial origin of the ectopic beats increases transmural dispersion of repolarization, thus providing the substrate for the development of reentrant tachyarrhythmias that underlie rapid polymorphic VT/VF. (Circulation. 2005;111:2727-2733.)

Key Words: antiarrhythmia agents • arrhythmia • catecholamines • electrocardiography • tachyarrhythmias

Catecholaminergic or familial polymorphic ventricular tachycardia is a rare, autosomal-dominant inherited disorder that predominantly affects children or adolescents with structurally normal hearts. It is characterized by bidirectional ventricular tachycardia (BVT), polymorphic VT (PVT), and a high risk of sudden cardiac death (30% to 50% by the age of 20 to 30 years). Recent molecular genetic studies have identified mutations in genes that encode for the cardiac ryanodine receptor 2 (RyR2) or calsequestrin 2 (CASQ2) in patients with this phenotype. Several lines of evidence point to delayed afterdepolarization–induced triggered activity as the mechanism underlying monomorphic VT or BVT in these patients. These include the identification of genetic mutations that involve Ca2+ regulatory proteins, a similarity of the ECG features to those associated with digitalis toxicity, and the precipitation by adrenergic stimulation. The cellular mechanisms underlying the various ECG phenotypes and the transition of monomorphic VT to PVT or ventricular fibrillation (VF) are not known. The present study was designed to create an experimental model of catecholaminergic polymorphic VT (CPVT) to elucidate the cellular basis of the ECG features and the mechanism underlying the arrhythmias responsible for sudden cardiac death.

Methods

Arterially Perfused Canine Ventricular Wedge Preparations

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 cold (4°C) cardioplegic solution that contained 8.5 mmol/L [K+]. Transmural wedges with dimensions of 2×1.5×0.9 cm to 3×2×1.5 cm were dissected from either the right or left ventricle. The tissue was cannulated via a small native branch of left or right coronary artery and 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 (35.5±0.5°C). The perfusate was delivered to the artery by a roller pump (Cole Parmer, Inc., Vernon Hills, IL).
Parmer Instrument Co). Perfusion pressure was maintained between 40 and 50 mm Hg by adjustment of the perfusion flow rate.

The ventricular wedge preparations were allowed to equilibrate until electrically steady, usually 1 hour, and stimulated with bipolar silver electrodes insulated except at the tips and applied to the endocardial surface. A transmural pseudo-ECG was recorded by using 2 AgCl half-cells placed approximately 1.0 to 1.5 cm from the epicardial (+) and endocardial (−) surfaces of the preparation and along the same axis as the transmural recordings. Transm embrane action potentials (APs) were recorded simultaneously from the epicardial, M, and endocardial sites with 3 separate intracellular floating microelectrodes (DC resistance 10 to 20 MΩ; 2.7 mol/L KCl). Amplified signals were digitized, stored on magnetic media, and analyzed with Spike 2 (Cambridge Electronic Design).

Study Protocols and Data Analysis
Caffeine was used to mimic the sarcoplasmic reticulum defect, and isoproterenol was used to provide β-adrenergic stimulation. We evaluated the effect of caffeine and isoproterenol individually and in combination. To determine dose-response relationship, caffeine was infused at concentrations of 10, 30, 100, and 300 μmol/L. The effect of caffeine was measured after 10 to 15 minutes of exposure. Change in ECG and AP parameters were measured. The effect of isoproterenol was studied at a concentration of 100 nmol/L (n = 14). ECG and AP characteristics were evaluated together with the inducibility of tachycardia. The combination was studied with the following protocol (n = 16): caffeine (100 or 300 μmol/L) was introduced into the coronary perfusate; after 10 to 15 minutes, isoproterenol 100 nmol/L was added, and the development of spontaneous tachycardia was monitored. If sustained (>10 seconds) monomorphic tachycardia developed, single extrastimuli (S3) were delivered to the epicardial surface at progressively shorter S2−S3 intervals to induce PVT. In some experiments, we used 1000 μmol/L caffeine alone instead of caffeine plus isoproterenol, to reduce motion artifact. The effects of β-adrenergic blockade or calcium channel blockade were evaluated with propranolol 1.0 μmol/L and verapamil 1.0 μmol/L, respectively. Caffeine-treated CPVT model wedges were pretreated with propranolol or verapamil for 10 minutes before exposure to isoproterenol.

AP duration (APD) was measured at 90% repolarization (APD90). Transmural dispersion of repolarization (TDR) was defined as the difference between the longest and the shortest repolarization times (activation time plus APD90) of transmural APs recorded across the wall (typically, M-cell minus epicardial cell repolarization time). The QT interval was defined as the time interval between QRS onset and the point at which the line of maximal downslope of the T wave crossed the baseline.

Statistical Analysis
Statistical analysis was performed with Sigma Stat software (version 2.03). Friedman repeated-measures ANOVA was used for analysis of the dose-related effect of caffeine on AP and ECG variables. Wilcoxon signed rank test was used for comparison of ECG and AP variables after isoproterenol treatment or for comparison of TDR or Tp-e to Tp-end interval (Tp-e) secondary to change of activation sequence during epicardial VT. Data are expressed as median (25th, 75th percentile). A probability value <0.05 was considered significant.

Results
A low dose of caffeine, in the range of 10 to 100 μmol/L, did not produce a significant change in APD90 and QT intervals. Higher concentrations of the drug (300 μmol/L) decreased QT interval and epicardial and M-cell APD90. However, Tp-e and TDR remained unchanged because abbreviation of APD was homogeneous in the 3 layers (Figure 1). Over a concentration range of 100 to 300 μmol/L, caffeine reduced the magnitude of phase 1 of the AP, especially in right ventricular wedge preparations. Similar to previous reports,7 isoproterenol (100 nmol/L) decreased QT and AP duration in all 3 layers; however, Tp-e and TDR did not change significantly (data not shown).

Caffeine alone in concentrations as high as 300 μmol/L did not induce any arrhythmias. Isoproterenol alone frequently caused premature beats, accelerated ectopic rhythms, or monomorphic VT. Tachycardias induced by isoproterenol usually displayed a QRS axis and morphology similar to that evoked by endocardial stimulation, which suggests that the VT originates in the subendocardial Purkinje network. VTs of endocardial origin were observed in 7 of 14 preparations in the presence of 100 mmol/L isoproterenol. In 3 of these 7 cases of VT, the origin of ectopic activity shifted spontaneously from an endocardial site to an epicardial or deep subepicardial site, with a shift of the QRS from positive to negative (Figure 2).

Simulation of a leaky RyR2 was accomplished by pretreatment of the wedge preparations with caffeine concentrations of 100 (n = 10) or 300 (n = 6) μmol/L. The response to adrenergic stimulation was evaluated by addition of isoproterenol (100 nmol/L). The combination of caffeine and
isoproterenol produced VT of endocardial origin in 15 of 16 wedge preparations, 9 of which shifted to an epicardial site of origin that displayed inversion of the QRS. In 4 cases, the site of origin of the VT appeared to shift to the midmyocardium, with the QRS displaying neither upright nor inverted morphologies. The incidence of VT under these various conditions is presented in Figure 2.

Mechanisms of VT

The salient ECG features of the VT induced in the CPVT models used in the present study suggest epicardium or the subepicardial M-cell layer as the origin of the ectopic activity. When caffeine was infused at a higher concentration (1000 μmol/L), brief episodes of rapid pacing induced nonsustained rhythms that displayed inverted QRS waves, and the AP recorded from the epicardium displayed DAD and triggered activity (Figure 3). Similar DAD-induced triggered activity, although more enduring, was observed with a combination of low-dose caffeine (300 μmol/L) and isoproterenol (100 nmol/L; Figure 8A).

VT responses of epicardial origin displayed a markedly increased T-wave amplitude, Tp-e, and TDR compared with those elicited with endocardial stimulation (Figure 4). Reversal of the transmural sequence of activation as a consequence of the epicardial origin of the VT led to a significant prolongation of Tp-e and TDR from 34 (33, 36) to 52 (44, 60) ms, respectively. Single epicardial extrastimuli delivered during sustained epicardial VT induced rapid PVT in 3 of 9 preparations (Figure 5). Epicardial extrastimuli delivered during VT of endocardial or midmyocardial origin did not induce PVTs in 6 preparations. Figure 6 presents the Tp-e values recorded with VTs of endocardial, midmyocardial, and epicardial origin. PES-induced PVT was observed only in the 3 cases of epicardial VT (Figure 6, right; n=3) that displayed the largest Tp-e values. Inability to induce PVT in endocardial/midmyocardial VTs (Figure 6, left; n=6) or in epicardial VTs with less accentuated increases in Tp-e (Figure 6, middle; n=5) provides support for the hypothesis that an increase in TDR underlies the development of PVT and VF.

In 11 of 16 wedge preparations, the combination of caffeine (100 to 300 μmol/L) and isoproterenol (100 nmol/L) gave rise to BVT as a consequence of alternation in the origin of ectopic activity, most typically between endocardium and epicardium (Figure 7). BVT also resulted from alternation of ectopic foci between other layers of myocardium. Changes in the T wave, TDR, and Tp-e were most remarkable when the alternation occurred between endocardium and epicardium (Figure 7A). Although a 2:1 pattern of alternation was observed most frequently, a variety of transitional rhythms were also recorded. Figure 7B catalogues the various alternating sequences encountered. Spontaneous PVT was observed in 3 of 16 preparations.

Figure 2. Incidence of spontaneous tachycardia in wedge model of CPVT. Bar graph plots incidence of slow monomorphic VT observed with caffeine alone, isoproterenol alone, or combination thereof. Numbers on each bar represent (number of VTs)/(number of tested wedge preparations) and its percentage. Endo VT indicates VT of endocardial origin; Epi VT, VT of epicardial origin; and M VT, VT of midmyocardial origin.

Figure 3. DAD and triggered activity recorded from epicardium of right ventricular wedge preparation after caffeine (1000 μmol/L). Each tracing shows simultaneously recorded APs from epicardial cells and Purkinje fibers (A) or subepicardial M cells (B) together with transmural ECG. After termination of rapid pacing at cycle length of 200 ms, 3 triggered beats followed by DAD appear in epicardium. A, To further localize origin of triggered beat, electrodes were moved from Purkinje to subepicardial M cell layer, where DADs were not observed, which indicates that epicardium is an important source of ectopic beats in caffeine-treated CPVT model. Epi indicates epicardial; PF, Purkinje fibers.
Effect of Propranolol or Verapamil

Propranolol (1.0 μmol/L) suppressed the development of ectopic ventricular tachyarrhythmias in 4 of 4 wedge preparations. In 2 preparations, a spontaneous rhythm with a cycle length of 350 and 460 ms (endocardial origin) persisted. Verapamil (1.0 μmol/L, n=4) suppressed VT in all cases. A slow escape rhythm (700 to >2000 ms) was observed in all 4 preparations (Figure 8).

Discussion

CPVT is a clinical syndrome, characterized by stress-induced VT and sudden cardiac death in the absence of structural heart disease. Although genetic mutations involving RyR2 and CASQ2 have been demonstrated, the electrophysiological consequences of these mutations and the

Figure 4. Development of epicardial VT is associated with increase in TDR. A, Tp-e and TDR in left ventricular wedge preparation during endocardial pacing at basic cycle length of 2000 ms are 36 and 34 ms, respectively. B, Reversal of transmural sequence of activation as consequence of focal ventricular rhythm arising from epicardium causes Tp-e and TDR to increase to 57 and 52 ms, respectively. C through E, Comparison of Tp-e and TDR values during endocardial pacing (caffeine only) and during VT (caffeine plus isoproterenol). Tp-e and TDR increased significantly during epicardial VT compared with levels recorded during endocardial pacing at 2000 ms (C, D) or during endocardial VT (E). Data are expressed as median (25th, 75th percentile). n=7. *P<0.05. Endo indicates endocardial; Epi, epicardial.

Figure 5. Rapid PVT induced by single extrastimulus during sustained episode of slow epicardial VT in right ventricular wedge preparation. APs were recorded simultaneously from subendocardial Purkinje, M, and epicardial (Epi) cells together with transmural ECG. Perfusion of isoproterenol (100 nmol/L) in presence of caffeine (300 μmol/L) produced slow monomorphic epicardial VT. Single extrastimulus (S2) applied to epicardial surface at S1-S2 interval of 100 ms initiated rapid PVT.

Figure 6. Relationship between Tp-e and development of programmed electrical stimulation–induced VT. Tp-e was compared in 3 groups of monomorphic tachycardia. Endo VT indicates VT of endocardial origin; Epi VT, VT of epicardial origin; M VT, VT of midmyocardial origin. Bars show median values.
cellular mechanisms of VT/VF have not been elucidated. We have developed an experimental model of a leaky RyR2 calcium release channel using low-dose caffeine in arterially perfused canine ventricular wedge preparations. In this simulated model of RyR2 dysfunction, epicardium was a frequent source of ectopic beats in response to adrenergic stimulation. These epicardial ectopic beats play a crucial role in the genesis of various ECG phenotypes and, by amplifying TDR, provide an important electrophysiological substrate in the degeneration of VT to VF.

Genetic and Molecular Pathogenesis in CPVT

Several point mutations in hRyR2 have been reported in individuals with CPVT, and recently their functional consequences were published. Jiang et al. demonstrated in HEK293 cells that the mutation R4496C showed an increased open probability of RyR2 at low Ca\textsuperscript{2+} concentrations and more frequent spontaneous Ca\textsuperscript{2+} oscillations than cells transfected with wild-type RyR2. This suggests that in patients with CPVT, enhanced basal activity of mutated RyR2 could influence the properties of spontaneous Ca\textsuperscript{2+} release and, under sympathetic stimulation, could cause lethal cardiac arrhythmias. This biophysical property of the RyR2 mutation is very similar to the effect of low-dose caffeine. George et al. reported that Ca\textsuperscript{2+} release was augmented in HL-1 cells expressing mutant hRyR2 after RyR activation or \beta-adrenergic stimulation. The present experiments in canine wedge preparations are intended to extend these subcellular derangements in Ca\textsuperscript{2+} handling to find the critical pathophysiological link that is operating at a cellular level.

Mechanism of VT/VF

The development of tachyarrhythmias in CPVT is extremely sensitive to exercise or infusion of catecholamine. Leenhardt et al. described the typical succession of ECG patterns at the initiation of exercise. A typical sequence of arrhythmia evolution includes premature beats that increase progres-
sively to bigeminy, polymorphic doublets, and then a bidirectional tachycardia or irregular PVT. In the present experiments in canine wedge preparations, epicardium served as an important source of ectopic beats under the simulated leaky RyR condition. These ectopic beats, which are most likely to result from triggered activity induced by DADs, appear to arise from discrete regions of epicardial surface. More detailed systematic mapping studies are needed to identify why some regions of epicardium develop DAD, whereas others do not. The ionic basis for this predominant distribution of ectopic beats in epicardium is not clear. It has been shown that changes in repolarization rate or the presence of a prominent phase 1, a property of epicardial cells, can modulate Ca$^{2+}$ influx via I$_{Ca,L}$. In addition, our recent data suggest that sarcoplasmic reticulum Ca$^{2+}$ content is much larger and cell shortening is faster in epicardial cells. The greater Ca$^{2+}$ influx via I$_{Ca,L}$ coupled with a greater sarcoplasmic reticulum Ca$^{2+}$ content in epicardium may contribute to the greater propensity for Ca$^{2+}$ overload–induced DAD and triggered activity.

The present data suggest that adrenergic stimulation induces ectopic discharges by accelerating pacemaker activity or inducing DADs in subendocardial Purkinje fibers and by inducing DAD-mediated ectopic activity in epicardium. Although DAD-induced triggered activity arises most frequently in epicardium, it may contribute to ectopy in any region of the myocardium, including the conduction system. These mechanisms likely account for the relatively slow VT that arises from various regions of the ventricular wall, as well as the BVT and other alternating rhythms. The epicardial origin of the ectopic activity, because it reversed the transmural sequence of activation, greatly amplified the TDR, thus creating the substrate for development of reentrant activity, which most likely underlies the much more rapid PVT. The latter is more likely to be life-threatening, in that the relatively slow DAD-induced VT should be hemodynamically well tolerated and less likely to degenerate to VF.

The importance of the epicardial origin of the ectopic beats in the creation of vulnerable electrophysiological substrate for development of VT/VF has been demonstrated recently both in experimental models and in the clinic. The reversed sequence of activation significantly increases TDR and creates a vulnerable window for reentry. In the present study, relatively slow epicardial tachycardias developed spontaneously on isoproterenol infusion, and rapid PVTs were inducible from these epicardial VTs.

The present data also show the concordance between TDR and T$_{max}$ measurements, which we and others have suggested as an index of TDR. This parameter, when measured in precordial leads, may be useful in the clinic, because TDR measurements cannot be obtained noninvasively. The threshold T$_{max}$ value for induction of PVT in this model is estimated to be \( \approx 70 \text{ ms} \). The average T$_{max}$ (78.3 \pm 10.1 \text{ ms}; Figure 6, right) in the 3 VTs in which PVT was induced by single extrastimuli was longer than that obtained from the noninducible preparations. Accentuation of TDR and T$_{max}$ occurs as a consequence of the preferential abbreviation of the epicardial APD.

**Mechanism of BVT**

BVT is a rare form of tachycardia known to be associated with digitalis toxicity, aconitine poisoning, or familial periodic paralysis. Leenhardt et al described that the morphology of BVT typically showed a right bundle-branch block pattern with alternating right and left QRS-axis deviation. The mechanism of this alternating QRS complex polarity in CPVT is not clear. Available data suggest that the bidirectional change in QRS axis results from ectopic foci firing close to the common left bundle, which is accompanied by alternating conduction between the anterior and posterior fascicles. This mechanism best suits the morphological features of the BVT described by Leenhardt et al, ie, right bundle-branch block and alternating anterior or posterior fascicular block; however, the morphology of the BVT in CPVT does not always follow the same alternating bundle-branch block pattern, and it is also dependent on the location of the recording electrodes. This suggests that the BVT may not result from a single mechanism. The present wedge model does not contain bundle branches, yet an arrhythmia with similar characteristics was observed. The present data show that alternation in the transmural origin of the ectopic beats can generate a 2:1 pattern or more complex patterns, reproducing the ECG features of BVT. Alternation between epicardial and endocardial beats was the most typical form of BVT. In some cases, a gradual transition of the site of origin of the ectopic activity from epicardium to endocardium gave rise to relatively slow PVT (Figure 7B). This activity, presumably secondary to DAD-induced triggered beats, is distinctly different from the rapid PVT/VF precipitated by extrastimulation, which is most likely due to a reentrant mechanism.

**Drug Response**

The pharmacological approach to therapy in CPVT is not well characterized in the clinic because of the small number of patients. \( \beta \)-Adrenergic blockers are thought to be effective in most patients in preventing cardiac events. Poor treatment compliance appears to be the major reason for treatment failure. Caffeine has a complex mechanism of action, which includes (1) release of Ca$^{2+}$ from the sarcoplasmic reticulum, (2) inhibition Ca$^{2+}$ reuptake into the sarcoplasmic reticulum, (3) elevation of cellular cAMP via inhibition of phosphodiesterase, and (4) release of catecholamines from various sources. Thus, our canine ventricular wedge model may not precisely mimic a leaky RyR2. For example, the QT-interval abbreviation seen with 300 \( \mu \)mol/L caffeine does not conform with known clinical features of CPVT. The reason for selecting caffeine rather than ryanodine as a pharmacological tool to release Ca$^{2+}$ from intracellular stores was its fast and reversible effects on [Ca$^{2+}$]. Ryanodine, even at 10 \( \mu \)mol/L, progressively and irreversibly depressed contractility in our pilot experiments.
It is noteworthy that low concentrations (<500 μmol/L) of caffeine have been shown to have no effect on the L-type calcium current, which suggests that an increase in CAM is unlikely to mediate the effects of caffeine that we observed with the lower dose of the drug.9 Our experimental model was created with an acute drug treatment designed to simulate defective calcium homeostasis and is unable to simulate all possible alternative contributing mechanisms and adaptive changes that may attend congenital mutations of RyR2 or CASQ2.

The arterially perfused wedge preparation lacks an intact neural network. Although this is a limitation, patients with CPVT develop ventricular tachyarrhythmia not only during exercise or emotional stress but also after isoproterenol infusion. Our model most closely mimics isoproterenol infusion.

The epicardial ectopy that we observed is not specific to the CPVT model, because it is observed in the presence of isoproterenol alone. Epicardial ectopy is therefore a feature rather than the hallmark of the CPVT model. Epicardial, M, endocardial, and Purkinje cells all appear to have the potential to develop DAD and ectopic firing under these conditions. The incidence is higher in the epicardium than in other layers, including the M cell. In the clinic, CPVT patients show multifiform ectopic beats and relatively slow PVT in the early phase of exercise testing. These multiform premature ventricular contractions or slow PVT likely occurs as a consequence of DAD-induced simultaneous firing of multiple sites within the ventricle. The model presented demonstrates these features and goes on to define the conditions underlying the development of rapid PVT/VF (increased TDR), which could only be elicited in the presence of caffeine.

Our study does not provide definitive mapping data in support of a focal versus reentrant mechanism for the various VT activities observed in the model. Although the number of recordings obtained from the preparation is limited, our conclusions are based on the well-established characteristics of triggered versus reentrant arrhythmias. The monomorphic VTs observed in the CPVT model after caffeine with or without isoproterenol were relatively slow VTs, associated with the appearance of DADs. They were not induced or terminated reproducibly by programmed electrical stimulation, nor did they require a large dispersion of repolarization, a well-recognized substrate for reentry. These observations are consistent with a reentrant mechanism.

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