An early repolarization (ER) pattern in the ECG is characterized by a J-point elevation with or without ST-segment elevation as well as notching or slurring of the terminal part of the QRS complex. ER is a relatively common ECG finding, long considered to be benign. Recent studies point to an association of ER with the development of ventricular tachycardia (VT) and fibrillation (VF) leading to sudden cardiac death.1–4 ER has been divided into 3 subtypes. Type 1 involves an ER pattern localized to the lateral leads. In type 2, the ER pattern is present in the inferior and inferolateral leads, and in type 3, it is present globally in the inferior, lateral, and anterior or right precordial leads. Individuals with type 1 rarely develop VT/VF. Type 2 is associated with a higher level of risk, and individuals displaying a type 3 ER have the highest risk for developing life-threatening arrhythmias, including electric storms.4

Clinical Perspective on p 142

Hypothermia is known to contribute to the development of prominent J waves leading to VT/VF.1–4 Current guidelines recommend mild therapeutic hypothermia to prevent neurological damage following a cardiac arrest.7,8 The extent to which hypothermia contributes to arrhythmogenesis and the mechanisms involved are not well defined. Recent reports point to an association between ER and the development of VT/VF in the setting of hypothermia.9,10 The present study was designed to test the hypothesis that ER, by causing outward shift in the balance of currents active during the early phases of the epicardial action potential (AP), exacerbates the response to hypothermia, thus leading to prominent J waves, phase 2 reentry, and VT/VF. In addition, we examine the

Background—Hypothermia has been reported to induce ventricular tachycardia and fibrillation (VT/VF) in patients with early repolarization (ER) pattern. This study examines the cellular mechanisms underlying VT/VF associated with hypothermia in an experimental model of ER syndrome and examines the effectiveness of quinidine, cilostazol, and milrinone to prevent hypothermia-induced arrhythmias.

Methods and Results—Transmembrane action potentials were simultaneously recorded from 2 epicardial and 1 endocardial site of coronary-perfused canine left ventricular wedge preparations, together with a pseudo-ECG. A combination of NS5806 (3–10 μmol/L) and verapamil (1 μmol/L) was used to pharmacologically model the genetic mutations responsible for ER syndrome. Acetylcholine (3 μmol/L) was used to simulate increased parasympathetic tone, which is known to promote ER. In controls, lowering the temperature of the coronary perfusate to induce mild hypothermia (32°C–34°C) resulted in increased J-wave area on the ECG and accentuated epicardial action potential notch but no arrhythmic activity. In the setting of ER, hypothermia caused further accentuation of the epicardial action potential notch, leading to loss of the action potential dome at some sites but not others, thus creating the substrate for development of phase 2 reentry and VT/VF. Addition of the transient outward current antagonist quinidine (5 μmol/L) or the phosphodiesterase III inhibitors cilostazol (10 μmol/L) or milrinone (5 μmol/L) diminished the ER manifestations and prevented the hypothermia-induced phase 2 reentry and VT/VF.

Conclusions—Hypothermia leads to VT/VF in the setting of ER by exaggerating repolarization abnormalities, leading to development of phase 2 reentry. Quinidine, cilostazol, and milrinone suppress the hypothermia-induced VT/VF by reversing the repolarization abnormalities. (Circ Arrhythm Electrophysiol. 2014;7:134-142.)

Key Words: arrhythmia ◼ phosphodiesterase 3 inhibitors ◼ ST segment elevation ◼ sudden cardiac death ◼ tachycardia, ventricular ◼ ventricular fibrillation

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hypothesis that pharmacological agents capable of producing an inward shift in the balance of current in the early phases of the epicardial AP, either by inhibiting transient outward current (Ito; quinidine) or augmenting Ica (cilostazol and milrinone), can protect against hypothermia-induced VT/VF in the setting of ER.

Methods
Arterially Perfused Wedge of Canine Left Ventricle
We used floating glass microelectrode techniques to record APs simultaneously from 2 epicardial (Epi1 and Epi2) and 1 endocardial site in coronary-perfused wedge preparations isolated from the inferior and lateral regions of the canine left ventricle (LV), together with pseudo-ECG recorded across the bath along the endocardial–epicardial axis. Detailed methods are provided in the Data Supplement. The Tyrode’s solution was warmed while passing through the heated coils to deliver the perfusate at 37°C. To simulate hypothermia, the solution was redirected to 2 coiled-perfusion lines in series immersed in beakers filled with water, before reaching the tissues. We lowered the temperature of the perfusate to 32°C.

Measurements of AP Parameters
The epicardial AP notch magnitude (phase 1 magnitude/phase 0 amplitude×100), phase 0 to phase 2 interval (time between the first 2 peaks of the derivative of the AP), as well as the notch index (notch magnitude/phase 0–phase 2 interval), which approximates the area of the notch, were measured in AP recordings as previously described (Figure 1).11

J-Wave Area Calculations
The area of the J wave was calculated as follows: The start of J wave was defined using derivative of the ECG signal. In case of clear separation, it was set at the time when this derivative is zero which corresponds to the notch between R wave and J wave. When this separation was not clearly visible, this time was set at the moment when the negative derivative attains its maximal value (ie, minimal rate of decline) after the maximal downslope of the R wave. The J-wave area was expressed as millivolt×millisecond (Figure 1).

Statistical Analysis
Results are presented as mean±SEM throughout the article. Statistical comparisons were made using Student t test for effect of hypothermia on action potential duration at 90% of repolarization, maximum epicardial dispersion of repolarization, transmural dispersion of repolarization, notch index, notch magnitude, notch duration, and J wave area and effect of hypothermia on these parameters in the setting of ER (Table). One-way repeated measures ANOVA was used for effects of quinidine, cilostazol, and milrinone (Table), followed by pairwise comparisons corrected by Bonferroni method.

Results
Effects of Hypothermia in LV Wedge Preparation
In an initial series of 7 experiments, we examined the effects of hypothermia in coronary-perfused canine LV wedge preparations. Lowering the temperature to 32°C to 34°C caused prolongation of AP durations and accentuation of the AP notch in the epicardium but not endocardium, thus augmenting the amplitude of the J wave (Figure 2, Table). Under baseline condition, lowering the temperature to 32°C failed to cause loss of the epicardial AP dome or to induce arrhythmic activities.

Effects of Hypothermia in ER-Induced LV Wedge Preparation
Because vagal influences are known to accentuate the electrocardiographic and arrhythmic manifestations of ER,12–14 in a second series of experiments, we induced an ER phenotype using a combination of the Ito activator NS5806 (3–10 μmol/L), the Ca2+ channel blocker verapamil (1 μmol/L), and acetylcholine (3 μmol/L) added to the coronary perfusate. The combination accentuated the AP notch in epicardium but not endocardium, thus leading to augmentation of the electrocardiographic J wave but no arrhythmia (Figure 3A). Hypothermia caused a further increase of J-wave area, notch magnitude, and notch index, leading to all-or-none repolarization at the end of phase 1 of the epicardial AP. Loss of the epicardial AP dome at some sites but not others resulted in a prominent increase in epicardial dispersion of repolarization and transmural dispersion of repolarization. The voltage gradient between the abbreviated

Figure 1. A and B, Method of quantitation of action potential (AP) and J-wave parameters. C, Measurement of dispersion of AP repolarization. D, Measurement of dispersion of repolarization when the AP dome is lost at 1 epicardial (Epi) site but not the other. APD90 indicates action potential duration at 90% repolarization; EDR, epicardial dispersion of repolarization; Endo, endocardial; and TDR, transmural dispersion of repolarization.
### Table. Effect of Hypothermia, Quinidine, Cilostazol, and Milrinone

<table>
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<th></th>
<th>Endo APD&lt;sub&gt;90&lt;/sub&gt;, ms</th>
<th>Epi1 APD&lt;sub&gt;90&lt;/sub&gt;, ms</th>
<th>Epi2 APD&lt;sub&gt;90&lt;/sub&gt;, ms</th>
<th>EDR, ms</th>
<th>TDR, ms</th>
<th>Notch Index* (%) of Ph&lt;sub&gt;0&lt;/sub&gt;</th>
<th>Notch Magnitude (% of Ph&lt;sub&gt;0&lt;/sub&gt;)</th>
<th>Notch Duration, ms</th>
<th>J-Wave Area, mV×ms</th>
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<tr>
<td>Control (37°C)</td>
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<td>152.4±21.0†</td>
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<td>3823.4±217.7†</td>
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<td>†Quinidine (5 μmol/L) (32°C)</td>
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<td>352.0±16.3§</td>
<td>346.9±16.4**</td>
<td>21.5±11.0†</td>
<td>1783.8±361.3†</td>
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<td>6397.9±897.4†</td>
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<td>14.9±11.1†</td>
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<td>67.5±5.2†</td>
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<td>109.8±16.2†</td>
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<td>†Milrinone (5 μmol/L) (32°C)</td>
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<td>68.4±10.5**</td>
<td>23.2±7.7</td>
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</table>

EDR and TDR are measured in case of single stimulated beat with loss of the action potential dome at Epi2 site but not Epi1. Results are mean±SEM. Basic cycle length=1000 ms. n=7 for effect of hypothermia on APD<sub>90</sub>, maximum EDR, TDR, notch index, notch magnitude, notch duration, and J-wave area; n=12 for effect of hypothermia on these parameters in the setting of early repolarization; n=5 for effect of quinidine; n=5 for effect of cilostazol; n=5 for effect of milrinone. APD<sub>90</sub> indicates action potential duration at 90% of repolarization; EDR, epicardial dispersion of repolarization; and TDR, transmural dispersion of repolarization.

*Notch index=notch magnitude×(Ph<sub>0</sub>–Ph<sub>2</sub> interval) which approximates the area of the notch.

†P<0.01 32°C vs 37°C.
‡Provocative agents=NS5806 3–10 μmol/L+verapamil 1 μmol/L+acetylcholine 3 μmol/L.
§P<0.05.
||P<0.05.
¶P<0.01 quinidine 5 μmol/L or cilostazol 10 μmol/L or milrinone 5 μmol/L vs NS5806 3–10 μmol/L+verapamil 1 μmol/L+acetylcholine 3 μmol/L (32°C).
#P<0.01 NS5806 3–10 μmol/L+verapamil 1 μmol/L+acetylcholine 3 μmol/L (37°C) vs control (37°C).
**P<0.05.
epicardial AP and the relatively normal endocardial AP produced a prominent ST-segment elevation. A prominent AP duration gradient developed between sites displaying a normal AP dome and adjacent sites where the dome was lost, thus creating a vulnerable window within epicardium as well as between epicardium and endocardium across the ventricular wall. Propagation of the dome from regions at which it was maintained to regions, at which it was lost, caused local re-excitation via a phase 2 reentry mechanism, leading to the development of closely coupled extrasystoles and polymorphic VT/VF (Figure 3).

Similar results were obtained in 12 experiments. Composite of the AP and electrocardiographic parameters are presented in Figure 3 and the Table.
Effect of Quinidine, Cilostazol, and Milrinone to Suppress and Prevent Hypothermia-Induced Arrhythmogenesis

Quinidine has been shown to restore transmural electric homogeneity and abort arrhythmic activity in the J-wave syndromes. In another series of 5 experiments, we tested the hypothesis that quinidine could prevent the hypothermia-induced VT/VF developing in the setting of ER attributable to its effect to reduce the $I_{to}$. VT/VF was first induced by exposure of the LV wedge to hypothermia+NS5806 ($3–10 \mu\text{mol/L}$)+verapamil ($1 \mu\text{mol/L}$)+acetylcholine ($3 \mu\text{mol/L}$). Temperature was then restored to $37^\circ\text{C}$, at which point the arrhythmia subsided. Quinidine ($5 \mu\text{mol/L}$) was then added to the coronary perfusate, and hypothermia was reinduced. Quinidine ($5 \mu\text{mol/L}$) diminished the AP notch and J wave at $37^\circ\text{C}$ and prevented loss of the epicardial AP dome and development of the repolarization abnormalities, thus preventing the development of phase 2 reentry and VT/VF when temperature was reduced to $32^\circ\text{C}$ (Figure 4). A similar effect of quinidine to prevent VT/VF was observed in 5 of 5 preparations exposed to hypothermia (Figure 4 and Table). In 2 experiments, quinidine ($5–10 \mu\text{mol/L}$) was added during the VT/VF episode at $32^\circ\text{C}$. In both cases, the drug suppressed all arrhythmic activity and restored electric homogeneity throughout the preparation.

In a final series of experiments, we examined the effectiveness of the phosphodiesterase III inhibitors cilostazol and milrinone.

![Figure 4](http://circep.ahajournals.org/DownloadedFrom)

Figure 4. Quinidine prevents hypothermia-induced ventricular tachycardia and fibrillation (VT/VF) in the setting of early repolarization. A. The first grouping shows recordings from 2 epicardial (Epi) and 1 endocardial (Endo) sites, together with a pseudo-ECG after exposure of the left ventricular wedge to NS5806 ($3–10 \mu\text{mol/L}$)+verapamil ($1 \mu\text{mol/L}$)+acetylcholine (ACh; $3 \mu\text{mol/L}$). In the second grouping, the coronary perfusate temperature was lowered to $32^\circ\text{C}$, leading to development of VT/VF. Temperature was then restored to $37^\circ\text{C}$, at which point the arrhythmia subsided (not shown). The third grouping was recorded after addition of quinidine ($5 \mu\text{mol/L}$) and reinduction of hypothermia. Quinidine ($5 \mu\text{mol/L}$) diminished the action potential (AP) notch and J wave at $37^\circ\text{C}$ and prevented loss of the Epi AP dome and development of the repolarization abnormalities, thus preventing the development of phase 2 reentry and VT/VF when temperature was reduced to $32^\circ\text{C}$. B to F, Composite data showing the effect of lowering coronary perfusate temperature to $32^\circ\text{C}$ on AP duration at 90% repolarization (APD$_{90}$), notch magnitude, notch index, and J-wave area in the presence and absence of quinidine. All recordings were obtained at a basic cycle length of 1000 ms. EDR indicates epicardial dispersion of repolarization; and TDR, transmural dispersion of repolarization. Results are mean±SEM. *$P<0.05$. †$P<0.01$ vs NS5806+verapamil+ACh combination. n=5 for B, C, D, E, and F.
milrinone to prevent hypothermia-induced VT/VF in the setting of ER. These agents are known to augment $I_{Ca}$ via their action to increase cAMP. Here again, we first demonstrated the ability of the combination of provocative agents and hypothermia (32°C) to accentuate ER, thus creating a large epicardial and transmural dispersion of repolarization giving rise to phase 2 reentry and VT/VF. We then restored temperature to 37°C, which reversed the repolarization abnormality and suppressed VT/VF. The addition of cilostazol (10 μmol/L) or milrinone (5 μmol/L) reduced the epicardial AP notch at 37°C and prevented the repolarization abnormality as well as the development of phase 2 reentry and
VT/VF when temperature was once again reduced to 32°C (Figures 5 and 6). Both agents prevented the hypothermia-induced increase in transmural dispersion of repolarization, epicardial dispersion of repolarization, epicardial AP notch magnitude, notch index, and J-wave area on pseudo-ECG. Cilostazol was successful in preventing the VT/VF in 5 of 7 preparations. Similarly, milrinone (5 μmol/L) prevented the hypothermia-induced VT/VF in 5 of 7 preparations (Figures 5 and 6, Table).

Discussion

Therapeutic hypothermia is today widely used to prevent tissue injury secondary to cardiac arrest, ischemic stroke, traumatic brain and spinal cord injury, and neurogenic fever.
following brain trauma. Subjects are generally cooled to a target temperature of 32°C to 34°C (90°F–93°F). In cases of cardiac arrest, the Hypothermia after Cardiac Arrest Study Group reported that 55% of 137 patients in the hypothermia group experienced favorable outcomes, compared with only 39% in the group that received standard care following resuscitation. Among the adverse events encountered with therapeutic hypothermia is the development of cardiac arrhythmias. Hypothermia’s adverse events increase in severity the lower a patient’s body temperature is reduced. The accepted medical standard is to drop <32°C (90°F).

The factors that predispose to hypothermia-induced arrhythmogenesis and the mechanisms involved are poorly understood. Recent studies report an association between ER and the development of hypothermia-induced VT/VF. The present study recapitulates this clinical phenomenon in an experimental model consisting of the canine LV coronary-perfused LV wedge preparation, provides a mechanistic understanding of the cellular basis for the development of VT/VF in the setting of ER and hypothermia, and identifies potential therapeutic agents that may be useful in suppressing or preventing the development of hypothermia-induced VT/VF.

Mechanism of Hypothermia-Induced Arrhythmias in Patients With ER Syndrome

The J-wave syndrome, which includes both Brugada and ER syndromes, has been associated with genetic defects that cause a gain of function of \( I_w \) and a loss of function of \( I_{Ca} \) among many others. Both syndromes are also known to be aggravated by increased vagal tone. In this study, we pharmacologically mimicked these genetic and autonomic factors to create ER using the \( I_w \) agonist NS5806 (3–10 μmol/L), \( I_{Ca} \) antagonist verapamil (1 μmol/L), and acetylcholine (3 μmol/L). Acetylcholine contributes to the generation of the ER syndrome phenotype by causing both indirect (via accentuated antagonism) and direct use-dependent inhibition of \( I_{Ca} \). Litovsky and Antzelevitch demonstrated in 1990 the ability of acetylcholine to accentuate the epicardial AP notch and to cause loss of AP dome.

Under baseline conditions, lowering temperature to 32°C caused the appearance of prominent J wave in the ECG attributable to accentuation of the epicardial AP notch. In the absence of provocative agents designed to generate an ER pattern in the ECG, no arrhythmic activity was observed in any of the preparations. After the generation of the ER phenotype, relatively mild hypothermia (32°C–34°C) caused all-or-none repolarization, leading to loss of the AP dome at some epicardial sites but not others. This resulted in dispersion of AP repolarization both within epicardium and between epicardium and endocardium. Propagation of the AP dome, from sites at which the dome was maintained to sites at which it was lost, caused local re-excitation via a phase 2 reentry mechanism, leading to the development of closely coupled extrasystoles and VT/VF.

Consistent with these observations is the demonstration by Nishida et al that vagal nerve stimulation accentuates the hypothermia-induced spike and dome AP morphology in canine epicardium.

Hypothermia is thought to accentuate the AP notch attributable to the difference in the Q10 for activation of \( I_w \) and \( I_{Ca} \). The greater slowing of \( I_{Ca} \) activation leaves \( I_w \) less opposed, thus accentuating phase 1 repolarization and the AP notch. Thus, an effective strategy to reduce the impact of hypothermia would be to either reduce \( I_w \) or augment \( I_{Ca} \), thus producing an inward shift in the balance of current during the early phase of the AP. In 3 experimental series, we tested this hypothesis by pretreating the preparations with either quinidine to block \( I_w \) or cilostazol and milrinone to augment \( I_{Ca} \).

Potential Therapeutic Interventions

In support of our hypothesis, quinidine (5 μmol/L) proved capable of both suppressing and preventing the hypothermia-induced VT/VF in all preparations in which the drug was tested. Cilostazol (10 μmol/L) and milrinone (5 μmol/L) were effective in preventing the hypothermia-induced VT/VF in most but not all preparations. These agents increase \( I_{Ca, sec} \) to an increase in cAMP. It is noteworthy that cilostazol was reported to be able to prevent VT/VF in a patient with ER syndrome for an extended period of time. A beneficial effect of milrinone has as yet not been reported.

Conclusions

The present study demonstrates the effect of hypothermia to accentuate repolarization abnormalities within the LV epicardium and that in the setting of ER, this effect of hypothermia is accentuated leading to the development of phase 2 reentry and VT/VF. We also provide support for the hypothesis that agents capable of producing an inward shift in the balance of current during the early phases of the AP can exert a protective and/or ameliorative effect. Quinidine, by virtue of its \( I_w \) inhibition, and cilostazol and milrinone, by virtue of their effects to augment \( I_{Ca} \), are effective in partially reversing the hypothermia-induced repolarization abnormalities, thus restoring electric homogeneity and abolishing the arrhythmogenic substrate.

Study Limitations

As with all data derived from experimental animal models, extrapolation of the data from in vitro models to the clinic must be done with great care.

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Disclosures
None.

References

CLINICAL PERSPECTIVE
The pathogenicity associated with the presence of an early repolarization (ER) pattern in the ECG has been mired in controversy. Although an ER pattern is considered to be largely benign, a growing number of case–control and population-based studies have demonstrated increased prevalence of ER pattern, particularly in inferior and inferolateral leads, among patients with idiopathic ventricular fibrillation (VF), thus providing clinical evidence in support of experimental studies showing a link between ER pattern in the ECG and life-threatening cardiac arrhythmias. The pathogenicity of ER has been elusive, in part, because it remains subclinical in the absence of environmental triggers or comorbidities. Several recent studies have shown that an ER pattern in the ECG before a myocardial infarction predisposes an individual to life-threatening arrhythmias during and after an acute myocardial infarction. Other studies point to hypothermia as a trigger. Current guidelines recommend mild therapeutic hypothermia to prevent neurological damage following a cardiac arrest. Recent reports, however, point to increased prevalence of hypothermia-induced ventricular tachycardia/VF in patients with an ER pattern. The present study examines the mechanisms involved as well as pharmacological strategies to minimize the risk of hypothermia-induced ventricular tachycardia/VF. Our experimental data obtained using coronary-perfused canine left ventricular wedge preparation in which ER is induced by pharmacologically mimicking the effect of genetic mutations responsible for ER syndrome indicate that hypothermia leads to ventricular tachycardia/VF in the setting of ER by exaggerating repolarization abnormalities, leading to development of phase 2 reentry. Quinidine, cilostazol, and milrinone all suppress the hypothermia-induced ventricular tachycardia/VF by reversing these repolarization abnormalities.
Cellular Mechanism Underlying Hypothermia-Induced Ventricular Tachycardia/Ventricular Fibrillation in the Setting of Early Repolarization and the Protective Effect of Quinidine, Cilostazol, and Milrinone

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SUPPLEMENTAL MATERIAL

Methods

All experiments were carried out in compliance with the *Guide for Care and Use of Laboratory Animals published by the National Institutes of Health* (NIH publication No 85-23, Revised 1996) and approved by the Institutional Animal Care and Use Committee. We used mongrel dogs weighing 20–35 kg of either sex. The chest was opened via a left thoracotomy and the heart was excised. Transmural wedge preparations with dimensions of up to 32 x 20 x 15 mm were dissected from the LV wall. The preparations were cannulated via a distal diagonal branch of the left anterior descending coronary artery, or a left marginal branch of the circumflex artery, or a branch of the posterior descending artery and perfused with cardioplegic solution (Tyrode’s containing 12 mmol/L KCl). Non-perfused regions of the tissue were removed using a razor blade. The preparations were then placed in a tissue bath and perfused with oxygenated Tyrode’s solution (mM): NaCl 129, KCl 4, NaH₂PO₄ 0.9, NaHCO₃ 20, CaCl₂ 1.8, MgSO₄ 0.5, glucose 5.5, pH 7.4. The perfusate was delivered using a peristaltic pump (Masterflex peristaltic pump, Cole Parmer Instrument Co, Niles, Illinois) at a constant flow rate at 12-14 mL/min warmed to 37±0.5°C.

The preparations were equilibrated in the tissue bath until electrically stable, usually 1 hour. Pacing stimuli were delivered to the endocardial surface basic cycle length of 1000 ms using bipolar silver electrodes insulated except at the tips. The temperature of the perfusate was controlled by a heating bath associated with a glass condenser, a tube internally coiled within a wide cylindrical housing. The Tyrode’s solution was warmed while passing through the heated coils to deliver the perfusate at 37°C. To simulate hypothermia, the solution was redirected to
two coiled-perfusion lines in series immersed in beakers filled with water, before reaching the tissues. We lowered the temperature of the perfusate to 32°C.

A transmural ECG was recorded using two electrodes consisting of AgCl half cells placed in the tissue bath, 1.0 to 1.5 cm from the Epi and Endo surfaces of the preparation, along the same axis as the transmembrane recordings (Epi electrode is connected to the positive input of the ECG amplifier). Transmembrane APs were simultaneously recorded from two Epi sites (Epi 1 and Epi 2; Epi1-Epi2 distance was approx. 10-20 mm) and one Endo site with the use of floating microelectrodes (DC resistance=10 to 20 MΩ) filled with 2.7 mol/L KCl, each connected to a high-input impedance amplifier. Impalements were obtained from the Epi and Endo surfaces of the preparation at positions approximating the transmural axis of the ECG recording. Spike 2 for Windows (Cambridge Electronic Design, Cambridge, UK) was used to record and analyze the ECG and the AP. NS 5806, cilostazol and milrinone were dissolved in dimethyl sulphoxide (DMSO); acetylcholine, verapamil HCl, quinidine, were dissolved in distilled water (10 mM stock). DMSO controls were performed to ensure the absence of an effect of the solvent.