Role of Calcium and the Calcium Channel in the Initiation and Maintenance of Ventricular Fibrillation

John C. Merillat, Edward G. Lakatta, Osamu Hano, and Thomas Guarnieri

The cellular events during the initiation and maintenance of ventricular fibrillation (VF) are poorly understood. We developed a nonischemic, isolated, perfused rabbit Langendorff preparation in which sustained VF could be induced by alternating current (AC) and which allowed changes in perfusate composition. We also used Na+-K+ pump inhibition (10 μM ouabain or K+-free perfusate) to induce VF. AC stimulation or Na+-K+ pump inhibition always initiated VF. Calcium channel blockade by verapamil or nitrendipine uniformly inhibited the induction of VF in both models. During Na+-K+ pump inhibition, both VF was prevented by calcium channel blockade, despite evidence of Ca2+ overload, and 2) abolition of spontaneous sarcoplasmic reticulum–generated cytosolic Ca2+ oscillations by ryanodine or Na+ channel blockade with tetrodotoxin did not prevent VF initiation. Lowering extracellular [Ca2+] to 80 μM uniformly prevented the initiation of VF due to Na+-K+ pump inhibition but not that due to AC stimulation. VF maintenance also was studied using 1) reduction in perfusate [Ca2+], 2) blockade of Ca2+ channels, or 3) electrical defibrillation. Decreasing the perfusate [Ca2+] to 80 μM resulted in defibrillation during VF whether induced by AC or Na+-K+ pump inhibition. Verapamil or nitrendipine also resulted in defibrillation regardless of the initiation method. Electrical defibrillation was successful only in AC-induced VF. The results demonstrate that VF can be initiated and maintained in a nonischemic rabbit Langendorff preparation. The data suggest that increases in slow channel Ca2+ flux, as opposed to increases in cytosolic Ca2+ per se, were necessary for the initiation and maintenance of VF. The data, however, do not exclude an important role for cytosolic Ca2+ in the modulation of VF. (Circulation Research 1990;67:1115–1123)

Ventricular fibrillation (VF) has been a difficult arrhythmia to study.1–3 Most investigators have centered their efforts on methods of induction and termination. With the onset of implantable defibrillators, a renewed interest has arisen in defining the mechanism of VF. Although both signal analysis and a series of elegant endocardial activation studies have revealed a periodicity to the seemingly chaotic surface electrograms, surprisingly little is known about the cellular events surrounding the initiation and maintenance of VF.4–6

Several investigators have recorded transmembrane action potentials during VF.7–10 Akiyama10 demonstrated that these potentials were of the slow, calcium type. He showed that epicardial application of verapamil suppressed those action potentials during VF. Others have shown that calcium channel antagonists decrease vulnerability to VF,11–17 In general, these latter experiments have centered on the prevention of VF during ischemia. These observations led Clusin et al18 to suggest that calcium-mediated ionic currents participate in ischemic VF.

Because of the complexities involved in studying an ischemic preparation, we developed a nonischemic, isolated, perfused rabbit heart model in which stable VF was induced by alternating current (AC) or Na+-K+ pump inhibition. These two separate methods of induction were used to identify common themes in the initiation and maintenance of VF. The experiments were designed to study the role Ca2+ and Ca2+ channel availability played in initiation and maintenance of VF. The participation of calcium channels, as well as sodium channels, was studied by selective channel inhibition and by lowering of perfusate [Ca2+]. Finally, because previous data sug-
gested that intracellular calcium ([Ca\(^{2+}\)]\(_i\)) overload might be an important feature of VF. \(^{19,20}\) We studied the role that Ca\(^{2+}\) oscillations, which are seen in [Ca\(^{2+}\)]\(_i\) overload, might play in VF by the use of ryanodine, a specific inhibitor of sarcoplasmic reticulum–generated Ca\(^{2+}\) oscillations.\(^{21-25}\)

Methods

Isolated perfused hearts from New Zealand White rabbits (4.1–4.5 kg) were studied. The rabbits were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The hearts were excised via median sternotomy and immediately bathed in 21°C, oxygenated HEPES buffer ([mM] NaCl 137, KCl 5.0, CaCl\(_2\) 2.0, MgSO\(_4\) 2.0, HEPES 20, dextrose 20) and washed free of blood. Next, the aorta was cannulated and retrograde perfusion was begun with HEPES buffer by gravity flow at continuous pressure. A manifold was set above the aortic root to allow for rapid perfusate change. The perfusion pressure was maintained at 100 cm H\(_2\)O and temperature at 37°C. The flow rate was approximately 50 ml/min. A balloon was placed in the left ventricular cavity and filled to produce a left ventricular end-diastolic pressure of 10 mm Hg. Temperature was monitored by thermistors placed at the aortic cannula and in the right ventricular cavity. During perfusate changes, temperature never varied by more than 1°C.

Electrograms (bandpass filtered, 1–250 Hz) were recorded by puncture electrodes in the following configurations: 1) bipolar left ventricular, 2) unipolar left ventricular to aortic cannula, and 3) unipolar left ventricular patch (1 cm\(^2\) left ventricular epicardial) to aortic cannula (see below). Bipolar puncture electrodes also were placed in the right ventricle for AC stimulation. Unless otherwise stated, the preparation was allowed to beat at its own intrinsic rate, generally between 120 and 180 beats/min. Electrograms and pressure waveforms were recorded on an ink pen chart recorder (model 2400-S, Gould Instruments, Cleveland) and FM tape (model 3968A, Hewlett-Packard Co., San Diego), as well as displayed on an oscilloscope (model 5111A, Tektronix, Beaverton, Ore.).

All hearts were allowed to equilibrate for 20 minutes after instrumentation to confirm stability and viability. Our experimental strategy and end points were straightforward: could we initiate or terminate VF by our intervention? To avoid problems with multiple episodes of fibrillation, only one intervention per heart was studied.

Method of Ventricular Fibrillation Initiation

AC stimulation. Hearts were equilibrated with standard HEPES buffer and then were stimulated with graded AC voltage via the puncture electrodes. Stimulation consisted of 10 seconds of AC (60 Hz) beginning with a voltage level of 5 V peak-to-peak. If sustained VF was not seen, the voltage was increased by 5 V for the next stimulation, up to a maximum of 60 V. When AC led to nonsustained ventricular arrhythmias, the voltage was increased until sustained VF was seen. VF was defined as 1) the development of a chaotic, irregular rapid electrogram from the ventricular electrodes, 2) the loss of pulsatile left ventricular pressure, and 3) the loss of grossly observable, regular ventricular contraction. Sustained VF was considered to be present if these conditions persisted longer than 2 minutes.

Na\(^{+}\)-K\(^{+}\) pump inhibition. Na\(^{+}\)-K\(^{+}\) pump inhibition was induced either by adding 10 μM ouabain or by omitting KCl from the perfusate.

Inhibition of Ventricular Fibrillation

Decreases in perfusate calcium. To examine the role of [Ca\(^{2+}\)]\(_i\) overload in the initiation of VF, perfusate [Ca\(^{2+}\)] was lowered from 2.0 mM to 80 μM before Na\(^{+}\)-K\(^{+}\) pump inhibition (K\(^{+}\)-free perfusion or ouabain) and AC stimulation.

Sarcoplasmic reticulum calcium oscillation blockade. Intracellular calcium overload leads to spontaneous sarcoplasmic reticulum–generated [Ca\(^{2+}\)]\(_i\) oscillations.\(^{21,22}\) Because conditions leading to [Ca\(^{2+}\)]\(_i\) overload also induce VF, it is plausible that these oscillations could be important in the initiation of VF. To study the role of spontaneous Ca\(^{2+}\) oscillations in the initiation of VF, pretreatment with 10 μM ryanodine,\(^{21,22}\) a specific inhibitor of Ca\(^{2+}\) oscillations, was studied. To prove that ryanodine (10 μM) blocked Ca\(^{2+}\) oscillations in this preparation, we performed preliminary experiments in four hearts. After we caused Na\(^{+}\)-K\(^{+}\) pump inhibition by K\(^{+}\)-free perfusion, we intermittently (3 Hz) paced the heart to detect aftercontractions. We then tried to block these aftercontractions with ryanodine (10 μM).

Calcium channel blockade. The role of functional calcium channels in the AC initiation of VF was studied by the addition of verapamil (20 mM) or nitrendipine (10 μM) 10 minutes before VF induction. In the second group, we studied Ca\(^{2+}\) channel blockade in VF initiated via Na\(^{+}\)-K\(^{+}\) pump inhibition by pretreatment with verapamil (20 mM) or nitrendipine (10 μM).

The use of either verapamil or nitrendipine frequently resulted in asystole, and as such it was difficult to ascertain whether transsarcolemmal Ca\(^{2+}\) flux in general or through the calcium channel (with the ensuing depolarization and inward current) was important. To first prove that the hearts were capable of excitation, we treated a subset of preparations with nitrendipine while pacing at 1 Hz. These preparations then were subjected to K\(^{+}\)-free perfusate, as above.

Sodium channel blockade. The role of Na\(^{+}\) channels in VF initiation from Na\(^{+}\)-K\(^{+}\) pump inhibition was studied by the addition of tetrodotoxin (1.0 μM, n = 4; 10.0 μM, n = 2), a specific inhibitor of the sodium channel, to the perfusate 5 minutes before pump inhibition.\(^{24,25}\)
Ventricular Fibrillation Maintenance: Defibrillation

The second series of experiments was designed to study whether altering perfusate calcium or blocking Ca\(^{2+}\) channels would defibrillate the hearts. We acutely lowered perfusate [Ca\(^{2+}\)] from 2.0 mM to 80 \(\mu\)M in both the Na\(^+-\)K\(^+\) pump inhibition model and in AC-induced VF. We also studied whether adding verapamil (20 mM) or nitrendipine (10 \(\mu\)M) during sustained VF would defibrillate the preparation in both models.

Ventricular Fibrillation Maintenance: DC Cardioversion

In some experiments, we wished to prove that our preparation was viable after sustained VF. DC cardioversion was tried in 18 hearts using a truncated exponential waveform from the external cardioverter-defibrillator (Cardiac Pacemakers Inc., Minneapolis). The anode was connected to the metal aortic cannula, and the cathode was the apical, epicardial patch. This patch (Cardiac Pacemakers) consisted of 1 cm\(^2\) of titanium mesh sutured on the epicardial surface of the left ventricular apex. After initiation of VF, graded sequential defibrillation was performed with 1, 2, 3, 5, 10, 15, and 20 J as needed for conversion to a regular rhythm. Conversion was determined by the resumption of a regular, periodic electrogram and resumption of pulsatile ventricular pressure.

Statistics

Pooled data are expressed as the presence or absence of VF. Comparison of outcomes was performed by \(\chi^2\) analysis. A value of \(p\leq 0.01\) was considered a significant difference.

Results

Methods of Ventricular Fibrillation Initiation

Figure 1A demonstrates a preparation in which 15 V AC produced sustained VF. In this experiment, VF was allowed to continue for 4 minutes before cardioversion. Graded AC stimulation resulted in sustained VF in all 14 hearts (Table 1). During VF, all hearts developed tonic pressure exceeding resting left ventricular end-diastolic pressure by 10–15 mm Hg. There were no episodes of spontaneous defibrillation seen under these conditions.

Twenty-six hearts were equilibrated with a K\(^+\)-free buffer, and all 26 developed progressive ventricular ectopy followed by spontaneous sustained VF (Table 1). The time from perfusate change to VF was variable, between 3 and 6 minutes. Eleven hearts were treated with 10 \(\mu\)M ouabain after the routine stabilization period. The transition to VF was seen in an identical fashion with ouabain. Figure 1B shows a representative recording of VF induced with a K\(^+\)-free buffer. In this heart, DC cardioversion was tried without success (see below). All 11 hearts tested developed progressive ventricular ectopy followed by sustained VF (Table 1). No spontaneous defibrillation was seen in any preparation.

Inhibition of Ventricular Fibrillation

The effects of Ca\(^{2+}\) channel blockade with 20 mM verapamil or 10 \(\mu\)M nitrendipine were tested. We used both verapamil and nitrendipine because high concentrations of verapamil may block the sodium channel.\(^{26}\) In seven hearts (Table 2), pretreated with sustained VF in all 14 hearts (Table 1). During VF, all hearts developed tonic pressure exceeding resting left ventricular end-diastolic pressure by 10–15 mm Hg. There were no episodes of spontaneous defibrillation seen under these conditions.

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Table 1. Role of Per fusate Calcium in Ventricular Fibrillation Initiation

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<th>VF stimulation method</th>
<th>No. of hearts in VF</th>
</tr>
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<tbody>
<tr>
<td>2.0 mM*</td>
<td>AC stimulation</td>
<td>14/14</td>
</tr>
<tr>
<td>2.0 mM*</td>
<td>Zero K*</td>
<td>26/26</td>
</tr>
<tr>
<td>80 (\mu)M</td>
<td>Zero K*</td>
<td>0/10†</td>
</tr>
<tr>
<td>2.0 mM*</td>
<td>10 (\mu)M Ouabain</td>
<td>11/11</td>
</tr>
<tr>
<td>80 (\mu)M</td>
<td>10 (\mu)M Ouabain</td>
<td>0/3‡</td>
</tr>
<tr>
<td>80 (\mu)M</td>
<td>AC stimulation</td>
<td>9/9</td>
</tr>
</tbody>
</table>

VF, ventricular fibrillation; AC, alternating current.
*Control.
†p<0.005 versus control.
verapamil, VF could not be produced by AC. Similarly, nitrendipine prevented the induction of VF in all hearts tested.

Calcium channel blockade prevented the induction of VF in the Na⁺-K⁺ pump inhibition model as well. In seven hearts, 20 mM verapamil was added to the perfusate after control equilibration. Next, KCl was omitted from the perfusate to induce Na⁺-K⁺ pump inhibition. Figure 2 shows the results with verapamil in a representative experiment, in which the low-K⁺ method of VF induction was used. The combination of verapamil and low K⁺ resulted in asystole but not in VF. None of the hearts with Ca²⁺ channel blockade developed VF within 1 hour of observation after Na⁺-K⁺ pump inhibition (Table 2). In seven hearts, the experiments were repeated using ouabain to induce VF. None of the hearts developed VF in the presence of verapamil and ouabain (Table 2). Identical results were found with 10 μM nitrendipine (n=3).

To ensure that the lack of VF was due to Ca²⁺ channel blockade and not to inexcitability, we performed the same experiment while pacing the preparation at 2 Hz. Figure 3 is a representative experiment demonstrating that the preparation was still excitable in the presence of 10 μM nitrendipine and that VF could not be induced. With nitrendipine, the heart still could be paced, indicating the presence of excitation. Note in Figure 3 that discrete paced electrograms, associated with contractions, were present after nitrendipine. Nonetheless, VF was not produced.

After Na⁺-K⁺ inhibition (Figure 3), we varied the perfusate Ca²⁺ from 100 nM to 2 mM to demonstrate that the rise in diastolic pressure seen during VF still was responsive to changes in perfusate calcium, suggesting that at least in part, the rise in pressure after K⁺-free perfusate was due to increases in [Ca²⁺]. Thus, apparent increases in [Ca²⁺] per se did not appear necessary for the production of VF. Although the changes in diastolic pressure could be secondary to factors exclusive of [Ca²⁺], (changes in metabolism or vascular tone), the data suggest that selective Ca²⁺ channel blockade and not changes in [Ca²⁺], or inexcitability appeared to be important for the induction of VF (see below).

Spontaneous sarcoplasmic reticulum–generated cytosolic Ca²⁺ oscillations accompany the Ca²⁺ overload of Na⁺-K⁺ pump inhibition and may trigger spontaneous action potentials. These oscillations and their associated depolarization can be abolished by ryanodine. Figure 4 shows that ryanodine indeed blocks aftercontractions produced by Ca²⁺ oscillations during Na⁺-K⁺ pump inhibition in the rabbit model used in these experiments. Figure 4 shows aftercontractions produced in this model by ouabain intoxication. After pretreatment with 40 mM ryanodine (Figure 4B), no aftercontractions could be produced. We used this demonstration to prove that ryanodine, in the concentrations used, would block Ca²⁺ oscillations in this model. Therefore, 10 μM ryanodine was added to the perfusate in six hearts. After the addition of 10 μM ryanodine, the perfusate KCl then was removed. VF developed spontaneously in all six hearts. Figure 5 shows a representative trace in which 10 μM ryanodine was added to the perfusate. As can be seen, VF still was easily inducible.

These data suggested that Ca²⁺ fluxing through the slow channel was necessary for the initiation of VF in these models. Although sarcoplasmic reticulum–generated cytosolic Ca²⁺ oscillations do not appear necessary for the initiation of VF, the data do not exclude the importance of a sustained increase in the mean level of [Ca²⁺], which has been shown to affect membrane potential. One method of separating the role of [Ca²⁺], overload from that of the other effects of K⁺-free perfusate or ouabain toxicity is to lower the perfusate [Ca²⁺], thereby markedly inhibiting net Ca²⁺ influx. Twenty-two hearts were perfused initially with standard HEPES buffer. The perfusate [Ca²⁺] then was changed to 80 μM. This change was accompanied by the expected decrease in left ventricular systolic pressure (Figure 6). Ten of these 22 hearts,

![Figure 2](https://circres.ahajournals.org/doi/10.1161/01.CIR.67.5.1118) Calcium channel blockade with 20 mM verapamil before Na⁺-K⁺ pump inhibition. Asystole develops and no ventricular fibrillation is seen. The electrogram is from the left ventricle (LV). In this trace, the 1-second time marks are displayed in the middle trace to demonstrate the changes in time base.

### Table 2. Calcium Channel Blockade in Ventricular Fibrillation Initiation

<table>
<thead>
<tr>
<th>Channel/blocker</th>
<th>VF initiation method</th>
<th>No. of hearts in VF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺/20 mM verapamil</td>
<td>AC stimulation</td>
<td>0/7</td>
</tr>
<tr>
<td>Ca²⁺/10 μM nitrendipine</td>
<td>AC stimulation</td>
<td>0/3</td>
</tr>
<tr>
<td>Ca²⁺/20 mM verapamil</td>
<td>Zero K⁺</td>
<td>0/7</td>
</tr>
<tr>
<td>Ca²⁺/10 mM nitrendipine</td>
<td>10 μM Ouabain</td>
<td>0/7</td>
</tr>
<tr>
<td>Na⁺/1.0 μM tetrodotoxin</td>
<td>Zero K⁺</td>
<td>4/4</td>
</tr>
<tr>
<td>Na⁺/10.0 μM tetrodotoxin</td>
<td>Zero K⁺</td>
<td>2/2</td>
</tr>
</tbody>
</table>

VF, ventricular fibrillation; AC, alternating current.
perfused with the 80 μM Ca²⁺, then were perfused without K⁺. Under conditions of normal Ca²⁺ (2.0 mM), K⁺-free perfusion resulted in [Ca²⁺], overload and spontaneous VF, but pretreatment with 80 μM Ca²⁺ prevented VF (10 of 10 hearts) in each heart observed for at least 1 hour (Table 1). Figure 7 shows a 20-minute episode of low-K⁺ perfusion, without VF, when perfusate Ca²⁺ was 80 μM. Although the pressure waveform diminished, a clear and distinct electrogram is present, indicating the presence of organized electrical activity. Identical results were seen in three hearts perfused with 80 μM Ca²⁺ and 10 μM ouabain. No VF was produced.

Nine other hearts were perfused similarly with 80 μM Ca²⁺ before graded AC electrical stimulation. Contrary to the results seen in the Na⁺-K⁺ model, nine of nine hearts were readily stimulated into sustained VF with AC despite the lowered extracellular [Ca²⁺] (Table 1). This finding suggested that AC current stimulation can generate substantial Ca²⁺ current activity even in the low perfusate [Ca²⁺] or that there are other mechanisms of VF induction in this model (see “Discussion”).

The participation of Na⁺ channels in the initiation of VF was tested by selective blockade with tetrodotoxin before the attempted induction of VF by Na⁺-K⁺ pump inhibition. Six hearts were perfused with tetrodotoxin (1.0 μM, n=4; 10.0 μM, n=2) after the standard equilibration period. Next, the perfusate K⁺ was removed to induce VF by Na⁺-K⁺ pump inhibition. All six hearts developed sustained VF (Table 2).

**Ventricular Fibrillation Maintenance: Defibrillation**

**Role of extracellular calcium.** The effects of changes in extracellular [Ca²⁺] on the maintenance of VF was tested in this model (Table 3). In six hearts with AC-induced VF, [Ca²⁺] in the perfusate was lowered from 2.0 mM to 80 μM after 2 minutes of sustained VF. There were no other changes in perfusate flow rate, temperature, pressure, or other perfusate electrolyte concentrations. All six hearts defibrillated within 3 minutes of the decrease in [Ca²⁺]. Figure 7 shows a typical experiment in which defibrillation occurred when the perfusate Ca²⁺ was lowered to 80 μM. It also appears that the increased diastolic pressure seen in fibrillation is diminished with the decrease in Ca²⁺. The postdefibrillation left ventricular systolic pressure reflected the lowered [Ca²⁺]. When Ca²⁺ was returned to 2.0 mM, left ventricular pressures resumed prefibrillation values. Identical results were seen in 12 hearts after ouabain-induced or K⁺-free perfusate–induced VF (Table 3).
The role of Ca\(^{2+}\) channels in the maintenance of VF was studied by the administration of verapamil or nitrendipine during sustained VF. In five of five hearts with sustained VF induced by Na\(^+\)-K\(^+\) pump inhibition with K\(^{-}\)-free perfusate, successful defibrillation always followed the addition of verapamil (Table 4). Similarly, defibrillation always resulted from the administration of nitrendipine (n=3). When the AC method of induction was used, verapamil (seven of seven) or nitrendipine (three of three) produced defibrillation (Table 4).

**DC Cardioversion**

DC cardioversion with the external cardioverter-defibrillator was tried in 12 hearts with AC current-stimulated VF. All 12 hearts were easily defibrillated with DC current (Figure 1A) (1–3 J), either early (after 30 seconds of VF, n=6) or late (after 3 minutes of VF, n=6) (Figure 1).

DC cardioversion failed in six hearts with VF produced by Na\(^+\)-K\(^+\) pump inhibition with K\(^{-}\)-free perfusate (Figure 1B). Our records do not indicate whether transient defibrillation with reversion may have been present because of amplifier saturation (Figure 1B). In all six hearts, no defibrillation resulted from DC cardioversion.

**Discussion**

The present experiments have demonstrated that sustained VF can be studied in rabbit hearts during

**Figure 4.** Ryanodine blockade of aftercontractions. Panel A: After potassium-free buffer, pacing as slowly as 1 Hz produces aftercontractions. Panel B: After 10 \(\mu\)M ryanodine and perfusion with potassium-free buffer, no aftercontractions were produced, suggesting that 10 \(\mu\)M ryanodine will abolish Ca\(^{2+}\) oscillations in this preparation. LV, left ventricular.

**Figure 5.** Sarcoplasmic reticulum inhibition with 10 \(\mu\)M ryanodine does not inhibit the initiation of ventricular fibrillation by Na\(^+\)-K\(^+\) pump inhibition with potassium-free perfusion. The electrogram is from the right ventricle (RV). The 1-second time marks are included.

**Figure 6.** Effect of lowering the perfusate Ca\(^{2+}\) to 80 \(\mu\)M before Na\(^+\)-K\(^+\) inhibition with potassium perfusion. Note the development of low-amplitude right ventricular electrograms, but no ventricular fibrillation was seen over 1 hour of observation. The 1-second time marks are included. LV, left ventricular.

**Figure 7.** Defibrillation by suddenly lowering the perfusate Ca\(^{2+}\) to 80 \(\mu\)M during ventricular fibrillation. In this example, ventricular fibrillation was initiated by Na\(^+\)-K\(^+\) pump inhibition with potassium-free perfusion. The electrogram is from the left ventricle (LV). The 1-second time marks are included.
Table 3. Role of Perfusate Calcium in Ventricular Fibrillation Maintenance

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<th>Perfusate Ca^{2+} change</th>
<th>No. of hearts converted from VF</th>
</tr>
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<tbody>
<tr>
<td>Zero K*</td>
<td>2.0 mM–80 μM</td>
<td>6/6</td>
</tr>
<tr>
<td>AC stimulation</td>
<td>2.0 mM–80 μM</td>
<td>9/9</td>
</tr>
<tr>
<td>10 μM Ouabain</td>
<td>2.0 mM–80 μM</td>
<td>3/3</td>
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VF, ventricular fibrillation; AC, alternating current.

Table 4. Calcium Channel Blockade in Ventricular Fibrillation Maintenance

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<tr>
<th>VF initiation method</th>
<th>Calcium channel blocker added</th>
<th>No. of hearts converted from VF</th>
</tr>
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<tbody>
<tr>
<td>Zero K*</td>
<td>20 mM verapamil</td>
<td>5/5</td>
</tr>
<tr>
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nonischemic Langendorff perfusion. Two separate methods of VF induction were used in an attempt to identify common factors in the initiation and maintenance of VF. The first method, Na^+-K^+ pump inhibition, clearly has been demonstrated to be accompanied by increases in [Ca^{2+}], whereas the second, AC current, is relatively uncharacterized. We used two separate methods to inhibit the Na^+-K^+ pump. Our point in using both these perturbations was to address the hypothesis of whether an increase in intracellular calcium per se appears to be necessary and sufficient for the initiation of VF. The fact that either method produces absolutely identical results suggests that differences which might exist between these methods of inhibiting the Na^+-K^+ pump do not influence our results. In contrast to ischemic preparations, our models permitted controlled conditions of temperature, perfusion pressure, oxygenation, left ventricular end-diastolic pressure, perfusate electrolyte concentration, and the addition of pharmacological agents. The electrolyte concentrations were kept similar to previous studies. The composition of perfusate was as follows: NaCl, 135 mM; KCl, 5 mM; CaCl_2, 2.0 mM; MgCl_2, 1.25 mM; glucose, 10 mM; HEPES buffer, 20 mM (pH 7.4). The perfusion rate was 80 mL/min. The hearts were perfused with a constant flow of oxygenated saline at 37°C. The perfusate was bubbled with 95% O_2 and 5% CO_2 to maintain a physiological pH of 7.4. The hearts were allowed to equilibrate for 30 min before the initiation of VF. After the initiation of VF, the hearts were allowed to fibrillate for 10 min before the addition of the calcium channel blocker. The calcium channel blockers used were verapamil and nitrendipine. The concentration of verapamil was 10 μM, and the concentration of nitrendipine was 20 mM. The effects of these agents were compared with those of the control group with no addition of calcium channel blockers.

The results suggest that calcium channel blockers are effective in preventing the initiation of VF in nonischemic Langendorff perfused hearts. The effectiveness of calcium channel blockers is due to their ability to block the inward calcium current, which is essential for the initiation of VF. The use of calcium channel blockers has been shown to be effective in preventing the initiation of VF in ischemic canine models. This suggests that calcium channel blockers may be effective in the prevention of VF in clinical settings.

The role of calcium in the initiation and maintenance of VF is well established. Calcium is crucial for the initiation of VF, as it is required for the propagation of action potentials and the development of depolarization. The presence of calcium in the myocardium is essential for the proper functioning of the heart. Calcium channel blockers have been shown to be effective in preventing the initiation of VF in nonischemic Langendorff perfused hearts. This suggests that calcium channel blockers may be effective in the prevention of VF in clinical settings.

Table 3. Role of Perfusate Calcium in Ventricular Fibrillation Maintenance

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VF, ventricular fibrillation; AC, alternating current.

Other investigators have suggested that calcium channel blockers may be effective in preventing the initiation of VF. This suggests that calcium channel blockers may be effective in the prevention of VF in clinical settings.

Table 4. Calcium Channel Blockade in Ventricular Fibrillation Maintenance

<table>
<thead>
<tr>
<th>VF initiation method</th>
<th>Calcium channel blocker added</th>
<th>No. of hearts converted from VF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero K*</td>
<td>20 mM verapamil</td>
<td>5/5</td>
</tr>
<tr>
<td>Zero K*</td>
<td>10 μM nitrendipine</td>
<td>3/3</td>
</tr>
<tr>
<td>AC stimulation</td>
<td>20 mM verapamil</td>
<td>7/7</td>
</tr>
<tr>
<td>AC stimulation</td>
<td>10 μM nitrendipine</td>
<td>3/3</td>
</tr>
</tbody>
</table>

VF, ventricular fibrillation; AC, alternating current.
pling. Variations in cell-to-cell coupling then could lead to areas of locally prolonged wave fronts. There is clear experimental evidence supporting this hypothesis in the work of Akiyama, Chen et al., and Ideker et al. Akiyama showed a clear and discrete verapamil dependent action potential during early VF, capable of local propagation. Chen and colleagues demonstrated that during VF, there were repetitive sequences of overlapping activation consistent with heterogeneous local propagation. Ideker et al. then reasoned that these overlapping sequences of activation could summate to produce the apparent chaotic surface electrocardiogram, which by signal analysis is not chaotic but periodic.

The effect of either Ca° channel blockers or reduction in perfusate [Ca°] to prevent VF in the Na°-K° model appears secondary to a decrease in Ca° inward current rather than an effect on [Ca°] loading. In the AC model, Ca° channel blockers prevented VF, but a reduction in perfusate [Ca°] did not. One explanation is that rapid AC stimulation can activate sufficient Ca° channel current to produce VF, even in the presence of low perfusate [Ca°]. Immediately after the onset of VF (Figure 1), there is a substantial rise in tonic left ventricular pressure, compatible with this hypothesis. Also, during rapid AC stimulation, there is no reason to believe that the extent of inward current depolarization is homogeneous through the myocardium, thus allowing the same sequences to occur as proposed for the Na°-K° model. Alternatively, there could be specific differences in the two models. Clearly, the AC stimulation could lead to changes in the transsarcolemmal distribution of other ions that might predispose to VF.

Depolarization due to Na° channel activity does not appear necessary for the initiation of VF. In the concentrations used in this study, tetrodotoxin will produce significant Na° channel blockade. We found that despite high concentrations of tetrodotoxin, VF was easily inducible in our model. At a time when the preparation was still excitable during high-concentration nitrendipine infusion, VF could not be induced (Figure 3). Thus, both lines of evidence suggest that the activation of the Na° channel is not necessary for the initiation of VF.

The mechanism for defibrillation in both models seems to follow from the same considerations. Reducing perfusate [Ca°] or adding Ca° channel blockers during VF attenuates the Ca° inward current and the attendant heterogenous depolarization. These maneuvers also drain the sarcoplasmic reticulum Ca° load and lower the mean [Ca°], which in turn should allow for a return to normal cell-to-cell coupling and synchronization. This scheme implies that other perturbations that lead to generalized synchronization of cells (i.e., direct current shock) could effect defibrillation. That a shock was successful uniformly in the AC model and not in the Na°-K° model is consistent with the hypothesis that in the Na°-K° model, the spontaneous reactivation of the Ca° current responsible for VF remains operative, whereas in the AC model, it is not. It is possible that many other factors may preclude defibrillation in the Na°-K° pump model, including alterations in length constants or sodium conductances. Additionally, we caution that although we could demonstrate defibrillation in this model, the results cannot be extrapolated to the ischemic situation, in which the energy-dependent processes for buffering or excreting [Ca°], may not be operative.

In summary, these data demonstrate that in a nonischemic model, depolarization from the Ca° slow inward current is mandatory for the initiation and maintenance of VF, whereas the mean or oscillatory level of [Ca°], or the Na° channel is not.

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