Ryanodine and Caffeine Prevent Ventricular Arrhythmias During Acute Myocardial Ischemia and Reperfusion in Rat Heart

Francis T. Thandroyen, Joy McCarthy, Karen P. Burton, and Lionel H. Opie

This study investigates the possible role of oscillatory release of calcium from sarcoplasmic reticulum in the genesis of ventricular arrhythmias during acute myocardial ischemia and reperfusion in isolated rat hearts. We used ryanodine and caffeine, which are known to modulate the oscillatory release of calcium from sarcoplasmic reticulum. During 30 minutes of left main coronary artery ligation, all 13 control hearts developed ventricular premature beats (number of beats, 225 ± 51) and ventricular tachycardia (duration, 123 ± 21 seconds); five hearts developed ventricular fibrillation. In a separate series of experiments, reperfusion after 15 minutes of coronary artery ligation caused ventricular fibrillation to occur within 15 seconds in all 12 hearts. Ryanodine (10^-7 to 10^-5 M) abolished ventricular arrhythmias during coronary artery ligation and prevented reperfusion ventricular fibrillation. Ryanodine (10^-7, 10^-5, and 10^-3 M) caused 15%, 23%, and 74% decreases in the maximal rate of rise of left ventricular pressure development and 20%, 32%, and 85% decreases in the maximal rate of fall of left ventricular pressure development, respectively, prior to coronary artery ligation. During acute myocardial ischemia, ryanodine 10^-7 M maintained and 10^-3 M impaired left ventricular function; 10^-7 M caused left ventricular failure. Coronary perfusion rate did not increase during ischemia. Antiarrhythmic activity occurred independent of preservation of high energy phosphates, reduction in tissue lactate, or tissue cyclic adenosine monophosphate in the ischemic myocardium. Caffeine 10^-7 M decreased the incidence of ventricular arrhythmias during ischemia and upon reperfusion; protection occurred coincident with development of diastolic contracture. Caffeine increased ischemic tissue cyclic adenosine monophosphate content and worsened tissue energy status. Our findings suggest that oscillatory release of calcium from the sarcoplasmic reticulum may play an important role in ventricular arrhythmogenesis during acute myocardial ischemia and reperfusion. Impairment of left ventricular mechanical function appears likely to preclude the use of ryanodine and caffeine in vivo as ventricular antiarrhythmic agents. (Circulation Research 1988;62:306–314)

In 1943, Bozler1 showed that a large excess of calcium ions caused oscillatory afterpotentials and rhythmic changes of pressure in the ventricle of turtle hearts. Bozler2 considered that oscillatory afterpotentials could underlie the development of abnormal automaticity in cardiac muscle. In 1968, Kaumann and Aramendia1 reported that verapamil, an inhibitor of transsarcolemmal calcium influx, prevented the occurrence of ventricular fibrillation in the dog heart with coronary artery ligation. Subsequently, several studies2–7 demonstrated that calcium channel antagonists protect against ventricular fibrillation during acute myocardial ischemia and following reperfusion of the acutely ischemic myocardium. In a recent communication, Steenbergen and coworkers8 showed cytosolic free calcium to be increased within 10 minutes of acute global ischemia in the perfused rat heart. Definitive evidence has been presented that cytosolic calcium is increased within 10 minutes of reperfusion of the acutely ischemic cat heart.9 Thus, one postulate is that intracellular calcium "overload" may play an important role in the genesis of ventricular fibrillation. When cardiac cells are subjected to calcium overload, phasic oscillations in intracellular calcium activity, presumably from the sarcoplasmic reticulum, may cause afterdepolarizations by activating the nonspecific transient inward current.10,11 Oscillatory afterpotentials underlie abnormal automaticity, which is a major electrophysiological mechanism of ventricular fibrillation.12 It follows that phasic release of calcium from the sarcoplasmic reticulum may play a role in the genesis of ventricular arrhythmias. Indirect support for the latter concept stems from the important studies of Hajdu and Leonard,13 and Kahn and coworkers,14 who showed that ryanodine, an inhibitor of calcium release from the sarcoplasmic reticulum, prevented ventricular arrhythmias induced by digitalis toxicity in cat and dog hearts. Ryanodine and caffeine are two pharmacological probes used in the evaluation of mechanisms that underlie intracellular calcium oscillations. Substantial evidence has recently been presented that ryanodine inhibits release of calcium from sarcoplasmic reticulum.15–17 For example, in skinned cardiac cells of rat ventricle, ryanodine inhibits calcium-induced calcium release evoked by elevation of free calcium at the...
sarcolemmal surface and also abolishes spontaneous release of calcium under conditions associated with calcium loading of the sarcoplasmatic reticulum. Ryanodine does not interfere with transsarcolemmal calcium influx by way of the slow channel or Na-Ca exchange. Caffeine initially enhances the release of calcium from sarcoplasmic reticulum and subsequently inhibits calcium uptake, thereby depleting this intracellular organelle of releasable calcium. Caffeine is less specific than ryanodine because by inhibiting phosphodiesterase activity, it increases intracellular cyclic adenosine monophosphate and may thereby augment transsarcolemmal influx of calcium. We demonstrate that ryanodine and caffeine abolish ventricular arrhythmias during acute myocardial ischemia and prevent ventricular fibrillation upon reperfusion of the acutely ischemic myocardium.

Materials and Methods

Animals and Experimental Protocol

Long-Evans rats (weight 275–350 g) were anesthetized with ether, and their hearts were removed after injection of 100 U heparin into the femoral vein. The hearts were immediately arrested, mounted on an isolated Langendorff aortic retrograde perfusion system, and perfused at a constant filling pressure of 100 cm H₂O. The perfusate was modified Krebs-Henseleit solution of the following composition (mM): NaCl 125, NaHCO₃ 19, MgSO₄ 1.19, CaCl₂ 1.25, KCl 3.6, KH₂PO₄ 1.2, glucose 11.1, pH 7.4. The solution was bubbled with 95% O₂-5% CO₂ to maintain oxygen tension between 475 and 550 mm Hg; temperature was maintained at 37°C. For the study of arrhythmias induced by ischemia, the left main coronary artery was ligated within 2 mm of where it emerges adjacent to the left atrium. With this procedure, coronary flow was reduced by at least 25%, and 30–40% of the left ventricle was rendered ischemic. The duration of coronary artery ligation was 30 minutes. The electrocardiogram, heart rate, and coronary flow rate were monitored before and during coronary artery ligation. For the study of arrhythmias induced by reperfusion, the left main coronary artery was ligated for 15 minutes, and reflow was established by cutting the ligature across a polyvinyl sheath that encompassed the coronary artery.

Left Ventricular Pressure Measurement

Left ventricular pressure measurements were recorded in a separate series of experiments by way of an intraventricular balloon attached to a 1-mm diameter hollow cannula and connected to a P23Db Statham transducer. Tracings were made with a Hewlett-Packard recorder. Left ventricular pressure was measured by computer analysis. Isovolumic loading conditions were...
Table 1. Influence of Ryanodine and Caffeine on Heart Rate, Coronary Flow Rate, and Ventricular Arrhythmias During Coronary Artery Ligation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ryanodine 10^-9 M</th>
<th>Ryanodine 10^-8 M</th>
<th>Ryanodine 10^-7 M</th>
<th>Caffeine 10^-3 M</th>
<th>Caffeine 10^-2 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>314±5</td>
<td>280±5</td>
<td>275±9</td>
<td>275±8</td>
<td>322±11</td>
<td>271±12</td>
</tr>
<tr>
<td>CFR (ml/min)</td>
<td>8.1±0.5</td>
<td>8.7±0.2</td>
<td>9.2±0.4</td>
<td>9.6±0.3</td>
<td>12.4±0.7*</td>
<td>15.2±0.7*</td>
</tr>
<tr>
<td>VPB (incidence)</td>
<td>13/13</td>
<td>5/13*</td>
<td>3/14*</td>
<td>0/10*</td>
<td>5/12*</td>
<td>1/12*</td>
</tr>
<tr>
<td>VT (incidence)</td>
<td>13/13</td>
<td>2/13*</td>
<td>1/14*</td>
<td>0/10*</td>
<td>1/14*</td>
<td>0/14*</td>
</tr>
<tr>
<td>VF (incidence)</td>
<td>5/13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HR, heart rate; CFR, coronary flow rate; VPB, ventricular premature beat; VT, ventricular tachycardia; VF, ventricular fibrillation.

*p<0.01 versus control; tp<0.05 versus control.

established by setting left ventricular end-diastolic pressure at approximately 8 mm Hg. The heart rate was maintained constant at 280–310 beats/min by a pacing electrode inserted into the right atrium; pulse width was 0.2 msec, and voltage was 2–5 V.

Metabolic Profile

Hearts were freeze-clamped for biochemical analysis using Wollenberger tongs precooled in liquid nitrogen. Hearts were freeze-dried for approximately 48 hours in an Edwards-Modulyo freeze drier. The hearts were homogenized in 6% perchloric acid with an Ultra-turrex homogenizer. The extracts were centrifuged, and the supernatant neutralized with Tris-KOH-KCl buffer to pH 7.0. Adenosine triphosphate (ATP), phospho-creatine (PCR), and lactate were assayed spectrophotometrically at 340 nm in samples from nonischemic and ischemic myocardium.

Cyclic AMP (cAMP) was assayed as described by Tovey et al. The kit for the assay (Radiochemical Centre, Amersham, England) contained Tris-EDTA buffer, purified bovine muscle protein, [8H]cAMP, cAMP standard, and charcoal absorbent in freeze-dried form.

The metabolic profile was analyzed 15 minutes after coronary artery ligation (i.e., during the period of maximum vulnerability to ischemic arrhythmias) and within 10 seconds of reperfusion (i.e., immediately preceding the expected onset of reflow ventricular tachycardia and fibrillation).

Evaluation of Ventricular Arrhythmias

Ventricular tachycardia was defined as three or more consecutive, morphologically similar, rapid ventricular extrasystoles (Figure 1); ventricular fibrillation was defined as more than six consecutive ventricular complexes showing total irregularity of morphology (Figure 1).

Pharmacological Agents

Racemic chemicals were used unless otherwise indicated. Ryanodine was obtained as a powder from Penick, Lyndhurst, New Jersey; caffeine was obtained as a powder from Sigma Chemical, St. Louis, Missouri. Ryanodine or caffeine were perfused for 10 minutes prior to coronary artery ligation and continued during the subsequent period of ischemia and reperfusion.

Table 2. Influence of Ryanodine and Caffeine on the Maximum Rate of Left Ventricular Pressure Development

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV dP/dtmax (mm Hg/sec)</td>
<td>LV dP/dtmax (mm Hg/sec)</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>2,463±89</td>
<td>1,786±121</td>
</tr>
<tr>
<td>Ryanodine 10^-9 M (n=4)</td>
<td>2,322±59</td>
<td>1,888±138</td>
</tr>
<tr>
<td>Ryanodine 10^-8 M (n=5)</td>
<td>2,273±52</td>
<td>1,619±52</td>
</tr>
<tr>
<td>Ryanodine 10^-7 M (n=4)</td>
<td>2,239±126</td>
<td>1,640±99</td>
</tr>
<tr>
<td>Caffeine 10^-2 M (n=4)</td>
<td>2,374±71</td>
<td>1,841±24</td>
</tr>
</tbody>
</table>

* Number of experiments; *p<0.01 versus control; †p<0.05 versus control; ‡p<0.01 intervention versus baseline.

Baseline LV dP/dtmax recorded at 0 minutes; intervention LV dP/dtmax recorded at 15 minutes (i.e., after 10 minutes of drug perfusion and just prior to coronary artery ligation); (+) LV dP/dtmax, maximum rate of rise of LV pressure development; (-) LV dP/dtmax, maximum rate of fall of LV pressure development; LV, left ventricular.
Tests of Statistical Significance

Results are given as mean ± SEM. The number (n) of hearts perfused in each series ranged from 8 to 13. Probability (p) values were calculated with Student's t test using two-tailed values as corrected for unequal variances. Probability values less than 0.05 indicated significance except when multiple comparisons required Bonferroni's corrections. The Fisher's exact test was applied to assess the differences in the incidence of ventricular arrhythmias between groups. Two-way analysis of variance was used to detect possible differences in left ventricular mechanical function.

Results

Effect of Coronary Artery Ligation (Tables 1–4)

Acute left main coronary artery ligation caused a decrease in the coronary flow rate from 8.1 ± 0.5 to 4.9 ± 0.3 ml/min (p < 0.05) and in heart rate from 314 ± 5 to 265 ± 9 beats/min (p < 0.05) (Table 1). Within 5 minutes of coronary artery ligation, the maximal rate of rise of left ventricular pressure development decreased from 2,416 ± 144 to 1,321 ± 80 mm Hg/sec, and the maximal rate of fall of left ventricular pressure development fell from 1,720 ± 173 to 881 ± 104 mm Hg/sec, respectively (each p < 0.01). Left ventricular peak systolic pressure fell from 109 ± 5 to 68 ± 4 mm Hg at 5 minutes after coronary ligation. Lower left ventricular pressure measurements were recorded through the 30-minute period of acute myocardial ischemia, e.g., the maximal rate of rise and fall of left ventricular pressure development was 1,526 ± 106 and 922 ± 125 mm Hg/sec, respectively, at 30 minutes after coronary artery ligation (Tables 2 and 3). Metabolic evidence of ischemia was manifest since high energy phosphate stores (ATP and PCr) were reduced, and the end product of anaerobic metabolism, lactate, was increased in the ischemic myocardium (Table 4). There was an increase in the ischemic tissue level of cAMP, which hypothetically plays a role in the genesis of ventricular arrhythmias.27

During the 30-minute period of coronary ligation, all

<table>
<thead>
<tr>
<th>Coronary artery ligation (minutes)</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,597 ± 108</td>
<td>1,079 ± 115</td>
<td></td>
</tr>
<tr>
<td>1,584 ± 123</td>
<td>1,066 ± 67</td>
<td></td>
</tr>
<tr>
<td>965 ± 113*</td>
<td>493 ± 51*</td>
<td></td>
</tr>
<tr>
<td>322 ± 79*</td>
<td>133 ± 34*</td>
<td></td>
</tr>
<tr>
<td>356 ± 53*</td>
<td>99 ± 8*</td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,526 ± 106</td>
<td>992 ± 125</td>
<td></td>
</tr>
<tr>
<td>1,592 ± 104</td>
<td>957 ± 52*</td>
<td></td>
</tr>
<tr>
<td>957 ± 52*</td>
<td>425 ± 32*</td>
<td></td>
</tr>
<tr>
<td>344 ± 66*</td>
<td>143 ± 33*</td>
<td></td>
</tr>
<tr>
<td>322 ± 23*</td>
<td>102 ± 10*</td>
<td></td>
</tr>
</tbody>
</table>

Effect of Ryanodine (Tables 1–4, Figure 2)

Ryanodine 10−9 to 10−7 M prevented ventricular premature beats, ventricular tachycardia, and ventricular fibrillation during coronary artery ligation. There was neither improvement in the coronary perfusion rate during ischemia nor preservation of the tissue stores of high energy phosphates in the ischemic myocardium; furthermore, lactate accumulation still occurred. Anti-arrhythmic activity occurred despite accumulation of cAMP in the ischemic myocardium.

Ryanodine (10−9, 10−4, and 10−7 M) caused 15%, 23%, and 74% decreases in the maximal rate of rise of left ventricular pressure development and 20%, 32%, and 85% decreases in the maximal rate of fall of left ventricular pressure development, respectively, prior to coronary artery ligation. During acute myocardial ischemia, ryanodine 10−8 M maintained left ventricular function, 10−7 M was associated with impaired left ventricular function, and 10−6 M with mechanical failure (Tables 2 and 3, Figure 2). Ryanodine 10−1 M decreased heart rate (Table 1).

Effect of Caffeine (Tables 1–4, Figure 2)

Caffeine 10−3 M increased heart rate (280 ± 12 to 322 ± 11 beats/min, p < 0.01), while 10−2 M decreased heart rate (313 ± 8 to 271 ± 12 beats/min, p < 0.01); both concentrations increased coronary flow rates (8.9 ± 0.7 to 12.4 ± 0.7 ml/min and 10.5 ± 0.6 to 15.2 ± 0.7 ml/min, respectively, each p < 0.01) prior to coronary artery ligation. Caffeine 10−2 M produced a transient increase within a few seconds in both the maximal rate of rise and fall of left ventricular pressure development (Figure 2). However, the development of diastolic contracture followed; left ventricular end-diastolic pressure increased from 7.0 ± 3 to 66 ± 6 mm Hg (p < 0.01) (Tables 2 and 3, Figure 2).

Caffeine 10−2 to 10−1 M decreased the incidence of ventricular arrhythmias during ischemia (Table 1). Caffeine 10−2 M maintained higher coronary flow rates during coronary ligation, reduced tissue ATP content in nonischemic and ischemic myocardium, and increased tissue cAMP content in nonischemic and ischemic myocardium. A micromolar concentration of caffeine (10−8 M) was ineffective in protecting against ventricular premature beats (incidence 6/6) or ventricular tachycardia (6/6) during coronary artery ligation, while the incidence of ventricular fibrillation (2/6) did not fall significantly.

Effect of Reperfusion (Table 5)

Reflow after 15 minutes of coronary artery ligation resulted in an increase in coronary flow rates (10.9 ± 0.7 ml/min, p < 0.001 versus ligation, 4.9 ± 0.3 ml/min) to levels above that present prior to coronary artery ligation (8.1 ± 0.5 ml/min), presumably because of reactive hyperemia. Within 15 sec-
Table 3. Influence of Ryanodine and Caffeine on Left Ventricular Peak Systolic Pressure, Left Ventricular End-Diastolic Pressure, and Developed Pressure

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (n=6)</th>
<th>Ryanodine 10⁻⁹ M (n=4)</th>
<th>Ryanodine 10⁻⁸ M (n=5)</th>
<th>Ryanodine 10⁻⁷ M (n=4)</th>
<th>Caffeine 10⁻² M (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP (mm Hg)</td>
<td>110 ± 7</td>
<td>114 ± 7</td>
<td>109 ± 5</td>
<td>107 ± 10</td>
<td>109 ± 7</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>9 ± 0.9</td>
<td>6 ± 1</td>
<td>9 ± 2</td>
<td>7 ± 0.5</td>
<td>7 ± 0.3</td>
</tr>
<tr>
<td>DP (mm Hg)</td>
<td>101 ± 7</td>
<td>108 ± 4</td>
<td>100 ± 4</td>
<td>100 ± 9</td>
<td>102 ± 3</td>
</tr>
</tbody>
</table>

Baseline LV pressure recorded at 0 minutes; intervention LV pressure recorded at 15 minutes (i.e., after 10 minutes of drug perfusion and just prior to coronary artery ligation); LVP, peak left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; DP, developed pressure (LVP-LVEDP); LV, left ventricular.

*p<0.01 versus control; †p<0.01 intervention versus baseline.

Table 4. Influence of Ryanodine and Caffeine on Tissue Metabolic Status 15 Minutes After Coronary Artery Ligation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nonischemic myocardium</th>
<th>Ischemic myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP (μmol/g)</td>
<td>PCr (μmol/g)</td>
</tr>
<tr>
<td>Nonligated hearts</td>
<td>4.2 ± 0.1</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>Controls (n=11)</td>
<td>3.3 ± 0.1</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Ryanodine 10⁻⁹ M (n=5)</td>
<td>3.2 ± 0.1</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Ryanodine 10⁻⁸ M (n=7)</td>
<td>3.5 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Ryanodine 10⁻⁷ M (n=8)</td>
<td>2.9 ± 0.1</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Caffeine 10⁻³ M (n=6)</td>
<td>3.4 ± 0.1</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Caffeine 10⁻² M (n=5)</td>
<td>2.6 ± 0.2†</td>
<td>3.5 ± 0.2</td>
</tr>
</tbody>
</table>

ATP, adenosine 5'-triphosphate; PCr, phosphocreatine.

*p<0.01 versus nonischemic myocardium; †p<0.05 versus control.

Discussion

The isolated perfused rat heart subjected to left main coronary artery ligation and subsequent reperfusion provides a reproducible model of spontaneous ventricular arrhythmias. All hearts developed ventricular extrasystoles and ventricular tachycardia during acute myocardial ischemia and ventricular fibrillation upon reperfusion.

Ryanodine

Ryanodine prevents severe ventricular arrhythmias during acute myocardial ischemia and abolishes reperfusion ventricular fibrillation. Such protection was not due to either a reduction in heart rate or an increase in the preceding period of coronary occlusion but not upon reperfusion; the myocardial energy profile showed deterioration of high energy phosphate and glycogen stores.

Table 4. Influence of Ryanodine and Caffeine on Tissue Metabolic Status 15 Minutes After Coronary Artery Ligation
Table 3. (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Coronary artery ligation (minutes)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVP (mm Hg)</td>
<td>LVEDP (mm Hg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DP (mm Hg)</td>
</tr>
<tr>
<td>73 ± 5</td>
<td>10 ± 1</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>74 ± 10</td>
<td>12 ± 2</td>
<td>62 ± 10</td>
</tr>
<tr>
<td>66 ± 4</td>
<td>26 ± 1*</td>
<td>40 ± 4*</td>
</tr>
<tr>
<td>44 ± 3*</td>
<td>29 ± 0.5*</td>
<td>15 ± 3*</td>
</tr>
<tr>
<td>79 ± 3</td>
<td>66 ± 3*</td>
<td>13 ± 1*</td>
</tr>
</tbody>
</table>

in coronary flow rate. In two previous studies, ryanodine prevented ventricular arrhythmias induced by toxic doses of digitalis in cat and dog models.13,14

Ventricular Function

All three concentrations of ryanodine, which prevented ventricular arrhythmias, decreased the maximal rate of rise in left ventricular pressure development and the maximal rate of fall in left ventricular relaxation prior to coronary artery ligation. During acute myocardial ischemia, ryanodine $10^{-7}$ M maintained while $10^{-7}$ M caused marked impairment of mechanical function. Similar alterations in left ventricular mechanical function have been shown in isolated papillary muscle in rat, cat, and dog.15 The negative inotropic effect is reported to be due to a decrease in calcium released from internal stores, namely the saroplastic reticulum.16 The impairment in relaxation might be due to a decrease in calcium uptake by the saroplastic reticulum as a consequence of calcium loading of this intracellular organelle. For example, when isolated saroplastic reticulum vesicles were exposed to ryanodine, they accumulated calcium apparently by inhibition of calcium efflux.24

Energy Status

Depletion of high energy phosphate stores in the ischemic myocardium is believed to be one major factor responsible for reduced saroplastic reticulum calcium ATPase activity.25 Impaired calcium uptake by the saroplastic reticulum during diastole may predispose to intracellular calcium overload. The ventricular antiarrhythmic action of ryanodine was not contingent upon preservation of myocardial high energy phosphate stores, which might have improved intracellular calcium homeostasis by maintaining energy dependent enzyme pumps, such as the saroplastic reticulum calcium ATPase or sarcolemmal Na⁺,K⁺-ATPase. In contrast, calcium antagonist agents that inhibit trans-

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Top panel: Control; left ventricular pressure (LVP) and rate of rise (+) and fall (−) of left ventricular pressure development (LV dP/dt) shown at 0 and 5 minutes, i.e., two time points prior to coronary artery ligation. At 15 minutes, left main coronary artery was ligated (CAL); LVP and LV dP/dt are within 1 minute and at 30 minutes of coronary artery ligation. In the series treated with ryanodine $10^{-7}$ M (middle panel) or caffeine $10^{-2}$ M (bottom panel), LVP and LV dP/dt were measured at 0 minutes. Drug was added at 5 minutes, and perfused for 10 minutes prior to coronary artery ligation (15 minutes) and throughout the duration of coronary artery ligation. LVP and LV dP/dt are shown within 1 minute and at 30 minutes of coronary artery ligation. Paper speed: slow, 0.25 mm/sec; fast speed: 100 mm/sec; time period marked 6.4 secs depicts paper speed of 2.5 mm/sec.
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The enhanced release of calcium from the sarcoplasmic reticulum. The development of diastolic contracture is in keeping with the subsequent action of caffeine in impairing calcium reuptake by the sarcoplasmic reticulum. Laser spectroscopy shows that as caffeine $10^{-2}$ M increases the oscillatory release of calcium in rat cardiac cells, the amplitude of each oscillation is decreased. At such millimolar concentrations, caffeine prevented ventricular arrhythmias during acute ischemia as well as during reperfusion of the ischemic myocardium. Protection against arrhythmias occurred despite the development of two potentially arrhythmogenic factors: depletion of ATP content and accumulation of myocardial cAMP content. Energy depletion may be due to increased utilization of ATP secondary to diastolic contracture, while cAMP accumulation presumably results from phosphodiesterase inhibition. Protection also occurred independent of alteration of heart rate or reduction in tissue lactate content. Therefore, it could be argued that the antiarrhythmic activity of caffeine was due to an action on calcium movements in and out of the sarcoplasmic reticulum.

**Table 5. Influence of Ryanodine and Caffeine on Ventricular Tachyarrhythmias, Tissue Energy Status, and Coronary Flow Rate on Reperfusion**

<table>
<thead>
<tr>
<th>VT (incidence)</th>
<th>VF (incidence)</th>
<th>CFR (ml/min)</th>
<th>ATP (µmol/g)</th>
<th>PCr (µmol/g)</th>
<th>Glycogen (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) CAL + reperfusion</td>
<td>12/12</td>
<td>12/12</td>
<td>10.9 ± 0.7</td>
<td>2.9 ± 0.1</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>2) CAL + reperfusion +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Ryanodine 10^{-9} M</td>
<td>5/9*</td>
<td>1/9†</td>
<td>10.0 ± 0.6</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>b. Ryanodine 10^{-8} M</td>
<td>4/10*</td>
<td>0/10†</td>
<td>9.7 ± 0.7</td>
<td>3.3 ± 0.2</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>c. Ryanodine 10^{-7} M</td>
<td>5/9*</td>
<td>0/9†</td>
<td>10.6 ± 0.6</td>
<td>3.1 ± 0.4</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>d. Caffeine 10^{-3} M</td>
<td>6/7</td>
<td>5/7</td>
<td>12.4 ± 0.5</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>e. Caffeine 10^{-2} M</td>
<td>8/9</td>
<td>3/9†</td>
<td>11.6 ± 0.7</td>
<td>2.0 ± 0.2†</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
</table>

VT, ventricular tachycardia; VF, ventricular fibrillation; CFR, coronary flow rate; ATP, adenosine 5'-triphosphate; PCr, phosphocreatine; CAL, coronary artery ligation.

*p<0.05 versus 1) Fisher's exact test.
†p<0.01 versus 1) Fisher's exact test.
‡p<0.01 versus 1).

Caffeine

To test further the hypothesis that oscillatory release of calcium from sarcoplasmic reticulum may play a role in ventricular arrhythmogenesis, experiments were conducted with caffeine. Ventricular function studies showed that caffeine $10^{-2}$ M caused a rapid and transient increase in the rate of rise of left ventricular pressure development, which is believed to be due to the enhanced release of calcium from the sarcoplasmic reticulum. The development of diastolic contracture is in keeping with the subsequent action of caffeine in impairing calcium reuptake by the sarcoplasmic reticulum.
ticity occurred in dogs with 1-day-old myocardial infarction. 37

Conclusion

The ability of ryanodine and caffeine to prevent ventricular arrhythmias during ischemia and reperfusion suggests that oscillatory release of calcium from the sarcoplasmic reticulum may play an important role in the genesis of ventricular arrhythmias during acute myocardial ischemia and reperfusion. Caffeine induces diastolic contracture coincident to protection of arrhythmias and, therefore, is not of value as an antiarrhythmic agent. Ryanodine prevents arrhythmias at concentrations that decrease left ventricular mechanical function. Hence, impairment of left ventricular mechanical function appears likely to preclude the use of ryanodine and caffeine in vivo as ventricular antiarrhythmic agents.

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