Dose-Dependent Inhibition of Stretch-Induced Arrhythmias by Gadolinium in Isolated Canine Ventricles
Evidence for a Unique Mode of Antiarrhythmic Action

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Transient diastolic dilatation of the isolated canine left ventricle predictably elicits arrhythmias. To test the hypothesis that such arrhythmias may be mediated by sarcolemmal stretch-activated channels, we attempted to inhibit stretch-induced arrhythmias with gadolinium (Gd³⁺), a potent stretch-activated channel blocker. In experiments with six isolated canine hearts, left ventricular volume was increased for 50 msec during early diastole and then returned to initial volume by a computerized servopump. The stretch volume was adjusted to yield a probability of eliciting a stretch-induced arrhythmia of 95±2% before treatment with Gd³⁺. When Gd³⁺ (1–10 μM) was administered, dose-dependent suppression of stretch-induced arrhythmias was observed. The probability of a stretch-induced arrhythmia was reduced to 13±10% (p<0.05) with 10 μM Gd³⁺. Washout of Gd³⁺ completely reversed this effect. Since Gd³⁺ is known to be a calcium channel antagonist, we compared the effect of Gd³⁺ on stretch-induced arrhythmias with that of verapamil and nifedipine. These calcium channel blockers produced no demonstrable inhibition of stretch-induced arrhythmias when administered at concentrations (1 μM) that substantially depressed left ventricular pressure development. Thus, our results indirectly implicate stretch-activated channels in the genesis of stretch-induced arrhythmias and provide preliminary evidence for a potential new mode of antiarrhythmic drug action—blockade of stretch-activated channels. (Circulation Research 1991;69:820–831)

The concept that myocardial stretch modulates the electrophysiological properties of the heart ("mechanoelectrical feedback") is well established. This phenomenon was initially observed in isolated cardiac tissues, where changes in muscle load or length altered the membrane potential and action potential duration. In intact ventricles, volume and pressure loading were found to modulate action potential duration and, in some circumstances, produce transient depolarizations that could promote arrhythmias by a mechanism of triggered activity. Ventricular dilatation also shortens the effective refractory period in a regionally nonuniform manner, which may be the substrate for reentrant tachyarrhythmias. Studies of chronically infarcted, isolated canine ventricles suggest that the electrophysiological consequences of volume loading and the associated increased propensity to develop ventricular arrhythmias may be greater under pathological conditions. The possible role of mechanoelectrical feedback in the initiation of serious ventricular arrhythmias remains unproven but is intriguing, since it might explain the high prevalence of ventricular ectopy and sudden cardiac death in patients with regionally or globally dilated ventricles.

To study this problem, our laboratory has recently developed a model of stretch-induced arrhythmias in isolated canine ventricles in which timing, duration, and amount of left ventricular (LV) dilatation as well as prestretch volume can be independently altered. With transient diastolic stretch, ventricular arrhythmias, including ectopic beats, couplets, and occasionally ventricular tachycardia, are reproducibly elicited. As the amount of stretch is increased, the probability of eliciting a stretch-induced arrhythmia rises sharply,
approaching 100% with total volumes well within the physiological (or pathophysiological) range. The mechanism of these stretch-induced arrhythmias is unknown, but the role of ionic channels activated by stretch or tension increase within the sarcolemmal membrane has been proposed to explain mechanoelectrical feedback effects previously described in both frog and mammalian ventricle.24

Stretch-activated channels have been described in various types of tissues,25-30 where they appear to be important in mechanotransduction at the cellular level.31 The existence of stretch-activated channels in neonatal rat myocytes has been recently reported.29 These cardiac stretch-activated channels open in bursts, are nonselective for sodium and potassium, and have a conductance of 100 pS with a reversal potential 31 mV positive relative to resting membrane potential. Stretch-activated channels with these characteristics could explain how critically timed myocardial stretch modulates action potential duration and how diastolic stretch might produce transient depolarizations and trigger ventricular arrhythmias.24

Recently, Yang and Sachs30 demonstrated dose-dependent block of stretch-activated channels by gadolinium (Gd3+) in *Xenopus* oocytes. In their experiment, stretch-activated current was produced by applying negative suction to the recording pipette of a patch clamp; Gd3+ produced a concentration-dependent reduction in channel open time and unitary current and reversibly inhibited channel opening. Gd3+ blocked these stretch-activated channels at a lower concentration than two other lanthanides, lanthanum and lutetium, and is considered the most potent blocker of mechanoreceptor transducers.30 To test the hypothesis that sarcolemmal stretch-activated channels may play a role in the genesis of stretch-induced arrhythmias, the effect of Gd3+ was tested in isolated blood-perfused canine hearts subjected to transient diastolic dilatations of known volume. Since calcium channel block has been demonstrated with Gd3+ in noncardiac cells32,33 and lanthanum selectively blocks calcium current in isolated bullfrog atrial cells,34 additional experiments were performed with specific inhibitors of calcium channels. We found that Gd3+ produced dose-dependent inhibition of stretch-induced arrhythmias, whereas specific blockade of calcium channels was totally ineffective; thus, these findings provide additional indirect support for the hypothesis that stretch-activated channels play an important role in the initiation of such arrhythmias and suggest that cardiac stretch-activated channel blockers may provide a unique antiarrhythmic action.

**Materials and Methods**

**Isolated Heart Preparation**

Ten adult mongrel dogs (21-26 kg) were anesthetized with a combination of morphine sulfate (2 mg/kg i.m.) and α-chloralose (100 mg/kg i.v.). This method of anesthesia has minimal effect on LV contractility35 and electrophysiology.36 After mechanical ventilation was initiated, a median thoracotomy was performed. The brachiocephalic artery was cannulated and connected to a pressure transducer (model P-23ID, Gould, Cleveland, Ohio) to measure coronary perfusion pressure. The pericardium was incised and cradled. The subclavian artery and right atrium were then cannulated and connected to the arterial perfusion and venous return lines, respectively, of the perfusion system, and intravenous heparin (10,000 units) was administered. The remaining vascular connections of the heart were then ligated, cardiopulmonary bypass was initiated, and the heart was excised from the chest without intervening ischemia. The left atrium was then widely opened, the LV was vented, and the mitral leaflets were excised. The mitral annulus was sutured to an adaptor that was connected to the metal conduit of the servo-controlled ventricular volume pump (Figure 1). A compliant fluid-filled balloon attached to the distal end of this conduit was used to measure and control LV intracavitary volume. Negative pressure (~10 mm Hg) was applied to the LV vent to ensure satisfactory fit between the intracavitary balloon and the endocardial surface. Absolute LV volume can be measured to within 0.2 ml using this method,37 and volume changes are accurate to ±0.1 ml over the entire stroke of the piston. A micromanometer-tipped catheter (model SPC-330A, Millar, Houston, Tex.) positioned within the balloon measured the instantaneous LV pressure. A vent was also placed in the right ventricular apex. The atrioventricular node was ablated by suture ligation as previously described,38 and pacing electrodes were attached to the LV apex. A pair of electrodes was also placed on the epicardial surface of the anterior wall, one at the base and the other located ~2 cm more apically, to provide a ventricular electrogram. A spring-loaded epicardial contact electrode described and validated by Franz et al39 was used to record monophasic action potentials from the LV anterior wall at the midventricular level.

**Coronary Perfusion System**

The isolated canine heart was metabolically supported by the perfusion system schematically illustrated in Figure 1. This perfusion system replaces the support dog used in our earlier studies.24 Perfusate from the coronary sinus drained through the right ventricular vent into a reservoir (model BMR 1500, Bentley Corp., Anasco, Puerto Rico) via the venous return line. The perfusate collected in the venous reservoir was pumped through an arterial perfusion line by a peristaltic pump (model 1215, Harvard Apparatus, South Natick, Mass.). The coronary perfusion pressure was held constant at 80 mm Hg throughout the experiment by computerized servo-regulation of the peristaltic pump. En route to the coronary arteries, the perfusate passed through a hollow fiber dialyzer (Focus 70, National Medical Care, Rockleigh, N.J.), which maintained glucose
and electrolyte homeostasis, and a membrane oxygenator (model HF 5000, BARD, Billerica, Mass.), where the perfusate was oxygenated with a gaseous mixture of 95% O₂-5% CO₂ and warmed to 37°C using a thermocirculator (model 50-1932, Harvard Apparatus Limited, Edenbridge, Ky.). The dialysate was a physiologically buffered salt solution composed of (mM) CaCl₂, 2.5, NaCl 110, KCl 4.0, MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 26.0, glucose 5.5, and sodium acetate 5.0 bubbled with a gaseous mixture of 95% O₂-5% CO₂ to a pH of 7.36-7.40. The dialysate also contained epinephrine (50 pg/ml) to approximate the effects of the normal circulating catecholamines. Using this system, pharmacological agents can be added to the dialysate to deliver known concentrations of drug to the coronary circulation.

The perfusion system was primed with dialysate solution to which 70,000 molecular weight dextran (5 g/l) was added to maintain osmotic pressure. Several days before each experiment, packed red blood cells were prepared from canine whole blood. The blood cells were passed through a leukocyte filter (model RC100, Pall Biomedical Products Corp., East Hills, N.Y.) and added to the priming solution to yield a leukocyte and platelet-poor perfusate with a physiological (35-40%) hematocrit.

**Servo-Regulated Volume Pump**

The servo-regulated ventricular volume pump consisted of a hydraulic piston (2.5-in. bore, 2-in. stroke) driven by a linear motor (model VG 100-4, Vibration Test Systems, Aurora, Ohio). A resistive linear displacement transducer (model SLF-S-50-1, Waters, Wayland, Mass.) sensed the position of the piston; its output was calibrated to measure absolute balloon volume (linearity, ±0.1%). A specially designed electronic circuit continuously compared the voltage output of the volume sensor with that of a volume command signal; the resulting volume error signal was amplified (model 7560, Techron, Elkhart, Ind.) and provided the electrical current to the linear motor as required to clamp the piston to the desired volume. The volume command signal was provided by a hybrid high-speed digital microcomputer (Intel 80386, Santa Clara, Calif.), which calculated the desired volume and converted it to an analog voltage signal every 2 msec, using a 12-bit analog-to-digital converter. This closed-loop feedback system could track a 10-ml sine wave of 50 Hz with <1% error of amplitude and a phase lag of <10°.

**Experimental Protocol**

To define the electrical and mechanical properties of the ventricles, steady-state recordings of monophasic action potential, LV pressure, dP/dt, and volume were acquired at each stage with LV volume held constant at 20 ml and the ventricle paced at 2 Hz. The absolute refractory period was also determined, using the method of Lerman et al.⁷ To determine absolute refractory period, a premature pacing stimulus was introduced after an eight-beat train of regularly timed pacing stimuli. The interval preceding the premature stimulus was reduced in steps of 2-20 msec until refractoriness occurred. The absolute refractory period was defined as the longest interval that failed to capture after increasing the stimulus strength stepwise to 10 mA.

Our electromechanical stimulation protocol is diagrammed in Figure 2. Each sequence was initiated by a train of eight paced beats at a frequency of 2 Hz. Pacing was performed at twice diastolic threshold using an electrically isolated 2-msec square wave of constant current triggered by the computer. During this priming period, LV volume was held constant at

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**Figure 1. Diagram of the experimental preparation.** Pacer, a constant-current computer-controlled stimulator; ECG, electrocardiogram; MAP, monophasic action potential signal; LVDT, a resistive linear volume displacement transducer.
a specified initial value ($V_i$) of 20 ml, which is a typical end-systolic volume for these ventricles. The ventricle was subjected to either a transient stretch or no stretch (control) beginning 300 msec after the final pacing stimulus. As judged from the monophasic action potential recordings, repolarization was complete (i.e., electrical diastole) at this point in the cardiac cycle in all studies. Although isovolumic relaxation was not always complete at the onset of stretch, we chose to apply stretch early in diastole, since the arrhythmia induction is similar for early versus late diastolic stretch and our ability to distinguish between escape beats and stretch-induced arrhythmias is better when the stretch is applied early. The computer was programmed to alternate between control and stretch sequences. The stretch consisted of a ramp increase in LV volume of specified amount ($\Delta V$), which was delivered over a fixed interval of 100 msec. The maximum stretch volume ($V_i + \Delta V$) was maintained for 50 msec, and then the volume was returned to $V_i$ over 100 msec. Maximum LV volume during the stretch never exceeded the normal range of end-diastolic volumes for these ventricles. The response to stretch (or no stretch) was monitored for 2,000 msec. Pacing was then resumed, and the next sequence was initiated.

The minimum volume increment ($\Delta V$) that produced a stretch-induced arrhythmia $\geq$90% of the time was determined and used as the stretch stimulus during subsequent pharmacological interventions. In six studies, GdCl$_3$ was added to the perfusate in serial concentrations of 1, 3, and 10 $\mu$M, allowing 20 minutes for Gd$^{3+}$ to equilibrate. The probability of a stretch-induced arrhythmia was measured at each concentration and at 30-minute intervals during washout of Gd$^{3+}$. Washout was continued until the probability of eliciting a stretch-induced arrhythmia either returned to baseline or showed no change compared with the previous determination. In four additional isolated heart preparations, calcium channel blockade was investigated; in these studies, measurements were obtained before and 20 minutes after 1 $\mu$M verapamil ($n=3$) or 1 $\mu$M nifedipine ($n=1$) was added to the dialysate.

**Data Analysis**

From 10 consecutive steady-state beats at a paced frequency of 2 Hz, the LV peak isovolumetric pressure ($P_{max}$) and maximum rate of LV pressure rise ($dP/dt_{max}$) were determined as indexes of LV contractility. LV end-diastolic pressure was considered the pressure at the time of the electrocardiographic R wave. Monophasic action potential duration at 20%, 70%, and 90% repolarization was also determined from these steady-state beats as described in detail elsewhere. The stretch–response data were analyzed using a computerized method to detect QRS complexes during the 2,000-msec monitoring period. From the control sequences, the timing of ventricular escape complexes was determined relative to the final pacing stimulus. The longest time interval that excluded 95% of the ventricular escape complexes ($t_{95}$) was then computed using a minimum of 20 control sequences obtained during each experimental condition. Stretch-induced arrhythmias were then defined as electrocardiographic QRS complexes that followed a stretch and arose at a time when there was at least 95% confidence that it was not a ventricular escape complex (i.e., earlier than $t_{95}$). The probability of a stretch-induced arrhythmia was then computed for each experimental condition as the number of stretch-induced arrhythmias divided by the total number of stretch sequences. At least 20 stretch sequences were used for all determinations.

**Data Acquisition**

The ventricular electrogram, monophasic action potential, pressure, $dP/dt$, volume, and the coronary perfusion pressure were recorded on a multichannel recorder (model TA 2000, Gould). Data were digitized on-line at 500 Hz by a 12-bit analog-to-digital converter (Labmaster TM-100, Scientific Solutions Inc., Solon, Ohio) and stored for subsequent data analysis. The tracings of the LV electrogram, monophasic action potential, pressure, and volume were also continuously displayed in real time on the video monitor of the microcomputer.

**Statistical Considerations**

Analysis of variance for a repeated-measures design was used to compare the data obtained from the various experimental stages. When a significant treatment effect was indicated by the $F$ statistic, the Newman-Keuls multiple comparison test was applied to determine which means differed. A value of $p<0.05$ indicated that the observed differences were statistically significant.
Results

Hemodynamic Effects of Gd³⁺

The hemodynamic effects of Gd³⁺ are summarized in Table 1. Gd³⁺ had a complex dose-dependent effect on LV contractility, as determined by Pmax and dP/dtmax measured at a volume of 20 ml. Compared with measurements obtained before Gd³⁺ (baseline), Pmax and dP/dtmax were significantly lower at a Gd³⁺ concentration of 3 μM. As the concentration of Gd³⁺ was further increased to 10 μM, Pmax increased such that there was no longer significant depression of this contractility index relative to baseline; however, dP/dtmax remained significantly depressed. After washout of Gd³⁺, these indexes of LV contractility returned toward baseline values. LV end-diastolic pressure was at the lower limits of normal before Gd³⁺. There was no statistically significant change in LV end-diastolic pressure at any dose of Gd³⁺ or after washout; thus, LV compliance remained relatively constant throughout our study. The pressure at peak stretch volume was unaffected by Gd³⁺, indicating that modulation of stretch-induced arrhythmias by this agent was not due to an altered hemodynamic response to stretch.

Since our hemodynamic parameters did not entirely return to baseline after washout of Gd³⁺, we studied the stability of contractile function in one ventricle that beat at constant load for 6 hours, to exclude time-dependent deterioration of the preparation. Over this time interval, which was several hours longer than the time required to complete the Gd³⁺ protocol, Pmax and dP/dtmax varied by less than 5%, reflecting stable LV systolic performance; moreover, only a small increase (<2 mm Hg) in LV end-diastolic pressure was observed, indicating that ventricular compliance is also relatively stable with this preparation.

Electrophysiological Effects of Gd³⁺

The effects of Gd³⁺ on ventricular refractoriness and action potential duration are shown in Table 2. Ventricular refractoriness was assessed in terms of the absolute refractory period. The mean value of absolute refractory period increased significantly with both 3 and 10 μM Gd³⁺. The monophasic action potential duration, measured at 20%, 70%, and 90% repolarization, also increased (p<0.05) at the two highest Gd³⁺ concentrations. Unlike the effects of Gd³⁺ on ventricular contractility and stretch-induced arrhythmias (see below), this prolongation of absolute refractory period and monophasic action potential duration were not reversible when Gd³⁺ was washed out. The ventricular recovery time, as measured by t50, tended to increase as the concentration of Gd³⁺ was raised; however, these changes were not statistically significant (Table 2).

The monophasic action potential recordings diminished in amplitude, and resting potential increased over time in our study; given these technical shortcomings, a comprehensive analysis of monophasic action potential morphology (e.g., resting potential, amplitude, and upstroke velocity) was not performed.

Table 1. Effects of Gadolinium on Left Ventricular Contractility and Compliance in Isolated Canine Left Ventricle

<table>
<thead>
<tr>
<th>[Gd³⁺]</th>
<th>0 μM</th>
<th>1 μM</th>
<th>3 μM</th>
<th>10 μM</th>
<th>Washout</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>dP/dtmax (mm Hg/sec)</td>
<td>870±296</td>
<td>762±299</td>
<td>429±227*</td>
<td>545±246*</td>
<td>601±249*</td>
<td>5.68</td>
<td>0.0032</td>
</tr>
<tr>
<td>Pmax (mm Hg)</td>
<td>84.1±26.9</td>
<td>77.6±27.5</td>
<td>49.9±16.9*</td>
<td>61.3±19.1</td>
<td>66.5±16.0</td>
<td>3.46</td>
<td>0.0264</td>
</tr>
<tr>
<td>Ped (mm Hg)</td>
<td>6.3±4.6</td>
<td>5.0±2.5</td>
<td>8.6±11.0</td>
<td>7.7±7.8</td>
<td>10.0±11.0</td>
<td>0.63</td>
<td>0.6462</td>
</tr>
<tr>
<td>Pp (mm Hg)</td>
<td>55.0±18.1</td>
<td>65.1±19.9</td>
<td>52.2±13.7</td>
<td>51.7±12.7</td>
<td>48.2±14.3</td>
<td>1.12</td>
<td>0.3753</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=6 ventricles. dP/dtmax maximum rate of left ventricular pressure rise; Pmax left ventricular peak isovolumetric pressure; Ped left ventricular end-diastolic pressure; Pp peak pressure elicited by stretch stimulus. All measurements were made with a constant left ventricular volume of 20 ml during steady-state pacing at 2 Hz. F statistics and corresponding p values were determined by analysis of variance for repeated-measures design.

*p<0.05 vs. 0 μM Gd³⁺ by the Newman-Keuls multiple comparison test.

Table 2. Electrophysiological Effects of Gadolinium in Isolated Canine Left Ventricle

<table>
<thead>
<tr>
<th>[Gd³⁺]</th>
<th>0 μM</th>
<th>1 μM</th>
<th>3 μM</th>
<th>10 μM</th>
<th>Washout</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARP (msec)</td>
<td>173±19</td>
<td>184±30</td>
<td>209±29*</td>
<td>224±42*</td>
<td>214±31</td>
<td>6.10</td>
<td>0.0022</td>
</tr>
<tr>
<td>MAPD50 (msec)</td>
<td>75±19</td>
<td>60±33</td>
<td>76±41</td>
<td>112±42</td>
<td>110±57</td>
<td>4.99</td>
<td>0.0059</td>
</tr>
<tr>
<td>MAPD70 (msec)</td>
<td>142±22</td>
<td>152±21</td>
<td>176±13*</td>
<td>200±18*</td>
<td>193±25*</td>
<td>10.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>MAPD90 (msec)</td>
<td>180±29</td>
<td>189±44</td>
<td>209±27</td>
<td>244±32*</td>
<td>247±71*</td>
<td>4.56</td>
<td>0.0088</td>
</tr>
<tr>
<td>t50 (msec)</td>
<td>973±737</td>
<td>1,068±812</td>
<td>1,042±630</td>
<td>1,206±579</td>
<td>728±111</td>
<td>1.08</td>
<td>0.3920</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=6 ventricles. ARP, absolute refractory period; MAPD50, MAPD70, and MAPD90, monophasic action potential duration at 20%, 70%, and 90% repolarization, respectively; t50, time interval that excludes 95% of ventricular escape beats. All measurements were made with a constant left ventricular volume of 20 ml during steady-state pacing at 2 Hz. F statistics and corresponding p values were determined by analysis of variance for repeated-measures design.

*p<0.05 vs. 0 μM Gd³⁺ by the Newman-Keuls multiple comparison test.
**Effect of Transient Diastolic Stretch on Monophasic Action Potential**

The monophasic action potential recordings frequently demonstrated transient depolarizations that were coincident with the onset of stretch and increased in magnitude with increasing stretch volume. In the monophasic action potential recordings illustrated in Figure 3, the magnitude of the transient depolarization preceding the second action potential of the stretch sequence is quite small, although it is better appreciated when the tracings from the control and stretch sequences are compared. In some studies, the transient depolarizations were much more pronounced. The magnitude of these transient depolarizations not only varied remarkably from study to study, but their appearance was also greatly influenced by slight changes in the position of the epicardial contact electrode for a given study, raising the concern that motion artifact contributed, in part, to the genesis of these "transient depolarizations." For this reason, a detailed analysis of these transient depolarizations was not performed.

**Initiation of Stretch-Induced Arrhythmias**

Figure 3 demonstrates data recorded during typical control and stretch sequences. The arrows labeled S₉ indicate the timing of the final pacing stimulus in an eight beat pacing train. As LV volume is held constant (left tracing), the ventricular electrogram shows the late emergence of a ventricular escape beat approximately 1,800 msec after the last paced beat. In contrast, a transient 16-ml increase in ventricular volume (right tracing) results in the early appearance of a stretch-induced arrhythmia. The mean stretch volume (ΔV) required to elicit such a stretch-induced arrhythmia ≥90% of the time was 21.3±8.5 ml.

Ventricular electrograms from 20 consecutive control and stretch sequences are illustrated in Figure 4. In the controls (left tracings), two ventricular escape beats can be seen. Escape beats generally occurred late (e.g., eighth tracing, left), but occasionally early escape (e.g., seventh tracing, left) was observed. In the stretch sequences (right tracings), stretch-induced arrhythmias were elicited early after the final paced beat. To ensure that beats observed during stretch sequences were actually stretch-induced arrhythmias and not merely escape beats, t₀ₙ was determined from control sequences. This time is represented by the dashed lines. In this example, t₀ₙ was 1,403 msec. Given this information, we could determine with at least 95% confidence that QRS complexes arising earlier than t₀ₙ during stretch sequences were actually stretch-induced arrhythmias. In this example, all but one (18th tracing, right) of the 20 stretches yielded stretch-induced arrhythmias; thus, the probability was 95% in this study for these stretches of 22 ml.

**Figure 3.** Recordings of a stretch-induced arrhythmia (SIA). ECG, ventricular electrocardiogram as a function of time; MAP, monophasic action potential recording as a function of time; LV pressure, left ventricular pressure as a function of time; LV volume, left ventricular volume as a function of time. The arrows labeled S₉ indicate the timing of the final pacing stimulus in an eight-beat pacing train. In the control sequence (left tracings), the ECG (upper tracing) shows the late emergence of a ventricular escape beat ~1,800 msec after the last paced beat, as ventricular volume (bottom tracing) is held constant. In the stretch sequence (right tracings), a transient 16-ml increase in LV volume results in the early appearance of an SIA.

**Figure 4.** Representative escape beats and stretch-induced arrhythmias from 20 consecutive control and stretch sequences. In the controls (left tracings), t₀ₙ (dashed line) is the time interval that excludes ventricular escape beats in 95% of the electrocardiographic tracings (i.e., all but the seventh tracing). In the stretch sequences (right tracings), 19 of 20 electrocardiogram tracings display an early ectopic beat, occurring before t₀ₙ. Thus, the probability of a stretch-induced arrhythmia in this example was 95%. The stretch volume was 22 ml.
Inhibition of Stretch-Induced Arrhythmias by Gd³⁺

Figure 5 shows electrocardiographic tracings from 20 consecutive stretches before and after 10 μM Gd³⁺ was administered. The ventricular electrograms depicted in Figures 4 and 5 were obtained from the same ventricle. The values of t⁹⁵ obtained from control tracings at these Gd³⁺ concentrations are indicated by the vertical dashed line. Before Gd³⁺ (left tracings), 19 of 20 stretches (95%) elicited a stretch-induced arrhythmia. In the presence of 10 μM Gd³⁺ (right tracings), only one of 20 stretches produced an arrhythmia, whereas all other sequences were completely quiescent. Thus, in this example, 10 μM Gd³⁺ reduced the probability of producing a stretch-induced arrhythmia from 95% to 5%.

As shown in Figure 6, which illustrates the mean probability of a stretch-induced arrhythmia plotted as a function of Gd³⁺ concentration, Gd³⁺ produced dose-dependent inhibition of stretch-induced arrhythmias. By experimental design, early diastolic stretch elicited an arrhythmia 95±2% of the time before Gd³⁺ was administered. With each increase in Gd³⁺ concentration, there was a progressive reduction in the probability of a stretch-induced arrhythmia. A significant reduction in probability was demonstrated for both 3 and 10 μM Gd³⁺. This antiarrhythmic action of Gd³⁺ was reversible, as the probability after washout of Gd³⁺ was statistically indistinguishable from baseline.

Initiation of Stretch-Induced Arrhythmias in the Presence of Calcium Channel Block

A representative study demonstrating the lack of inhibition of stretch-induced arrhythmias with a calcium channel antagonist is illustrated in Figure 7. In each of the 20 consecutive stretch sequences (∆V=16 ml), obtained before (left tracings) and after (right tracings) administration of 1 μM verapamil, the ventricular electrogram displays an early ectopic beat; thus, the probability of a 16-ml stretch inducing an arrhythmia was 100% in this study, and verapamil did not show any ability to suppress such arrhythmias. In this example, the values of t⁹⁵ were 741 and 643 msec for the data obtained before and after verapamil, respectively, and many ectopic depolarizations are present late after the stretch was applied. Although the mean baseline value of t⁹⁵ was slightly lower for the Gd³⁺-treated ventricles, some of the Gd³⁺ studies were also performed in ventricles with values of t⁹⁵ in the 600–700-msec range, and a similar number of late ectopic depolarizations were present in some of the Gd³⁺ studies as well. Thus, the...
experiments with the calcium blockers were not done on hearts that were highly excitable compared with the experiments using Gd$^{3+}$. Although inhibition of stretch-induced arrhythmias by verapamil is not evident in this example, $P_{\text{max}}$ decreased from 98.7 to 60 mm Hg, and $dP/dt_{\text{max}}$ declined from 920 to 379 mm Hg/sec in this study, indicating a substantial negative inotropic effect had occurred.

The results of all calcium channel blocker studies are shown in Table 3. Neither verapamil nor nifedipine had any inhibitory effect on the initiation of stretch-induced arrhythmias at a concentration of 1 $\mu$M in any study. The presence of a significant calcium channel blocking effect was confirmed, however, by the marked deterioration in $P_{\text{max}}$ and $dP/dt_{\text{max}}$. In one study, the initiation of stretch-induced arrhythmias was unimpeded even after the concentration of verapamil was increased to 10 $\mu$M, a concentration that completely prevented electrical excitation with a 10 mA stimulus and virtually abolished excitation–contraction coupling of the sporadic spontaneous beats. Thus, calcium channel block, induced by agents that are considered highly specific, does not inhibit stretch-induced arrhythmias, although the depression of LV contractility was similar to or greater than that observed with Gd$^{3+}$.

### Discussion

The major finding of the study was that Gd$^{3+}$, a putative blocker of stretch-activated channels, produced dose-dependent inhibition of stretch-induced arrhythmias in the isolated canine ventricle. We also found that this trivalent lanthanide cation had a complex dose-dependent effect on LV contractility. At the intermediate dose (3 $\mu$M), the pressure-generating capability of the heart was significantly depressed. As the Gd$^{3+}$ concentration was increased to 10 $\mu$M, pressure generation improved slightly, although contractility never returned to the baseline level after washout. Lastly, Gd$^{3+}$ prolonged both the absolute refractory period and monophasic action potential duration at high concentration.

### Hypothesized Role of Stretch-Activated Channels in the Genesis of Arrhythmias

As first described by Guharay and Sachs in skeletal muscle, stretch-activated channels are present in many cell types, where they facilitate mechano-transduction at the cellular level. Such channels have been identified in isolated ventricular myocytes of the neonatal rat by Craelius et al.; in their study, stretch-activated currents were demonstrated in patch-clamp recordings after application of negative suction to the patch. The cardiac stretch-activated channel appears to be nonselective for the transport of Na$^+$ and K$^+$ ions with a reversal potential intermediate to the equilibrium potential of sodium and potassium channels at 31 mV positive relative to resting membrane potential. We have hypothesized that such stretch-activated channels may be expressed in adult myocardium, where they modulate cardiac electrophysiology and may be implicated in the genesis of stretch-induced arrhythmias. Figure 8 schematically illustrates how stretch-activated channels may produce arrhythmias such as the ones we have elicited in response to early diastolic stretch. The upper tracing (solid curve) represents theoretical action potentials of cardiac Purkinje fibers in the isovolumic, supported

### Table 3. Effects of Calcium Channel Antagonists on the Initiation of Stretch-Induced Arrhythmias and Left Ventricular Contractility in Four Isolated Canine Ventricles

<table>
<thead>
<tr>
<th>Ventricle</th>
<th>Treatment</th>
<th>$P_{\text{SIA}}$</th>
<th>$P_{\text{max}}$ (mm Hg)</th>
<th>$dP/dt_{\text{max}}$ (mm Hg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nifedipine (1 $\mu$M)</td>
<td>0.90</td>
<td>98.4</td>
<td>961</td>
</tr>
<tr>
<td>2</td>
<td>Verapamil (1 $\mu$M)</td>
<td>0.91</td>
<td>98.7</td>
<td>920</td>
</tr>
<tr>
<td>3</td>
<td>Verapamil (1 $\mu$M)</td>
<td>0.91</td>
<td>146.9</td>
<td>1,407</td>
</tr>
<tr>
<td>4</td>
<td>Verapamil (1 $\mu$M)</td>
<td>0.95</td>
<td>67.1</td>
<td>853</td>
</tr>
</tbody>
</table>

$P_{\text{SIA}}$, probability of eliciting a stretch-induced arrhythmia; $P_{\text{max}}$, left ventricular peak isovolumic pressure; $dP/dt_{\text{max}}$, maximum rate of left ventricular pressure rise; Pre, before treatment; Post, after treatment.
heart. The corresponding volume tracing is illustrated below as the solid horizontal line. A transient increase in ventricular volume in early diastole (lower tracing, dotted line) activates the stretch-activated channel at a time when the cell is fully repolarized. This produces a depolarizing current that is essentially unopposed, returning the membrane potential (upper tracing, dotted curve) toward the equilibrium potential of the stretch-activated channel \( E_a \) (upper dashed line). If this transient depolarization reaches threshold potential \( E_r \) (lower dashed line), an early action potential is triggered, resulting in a stretch-induced arrhythmia.

**Lanthanide Biochemistry**

Lanthanides have numerous useful applications in studies of biological systems.44 Of principal interest to this study is the dose-dependent block of stretch-activated channels by \( \text{Gd}^{3+} \), described by Yang and Sachs40 using patch-clamp techniques in *Xenopus* oocytes. It is likely that the stretch-activated channels subserve the function of osmotic regulation in these cells. When negative suction was applied to the patch, a stretch-activated current characterized by bursts of channel openings was observed. This stretch-activated current was inhibited in a dose-dependent fashion by the addition of \( \text{Gd}^{3+} \) to the recording pipette. The stretch-activated current was completely abolished with \( 10 \mu\text{M} \text{Gd}^{3+} \).

The cell membrane is relatively impermeant to lanthanides; thus, it is believed that the effects of these trivalent cations on cardiac physiology are mediated at the level of the sarcolemma.44-47 By virtue of their high ion strength, lanthanides firmly bind multiple components of the cell membrane.44 The lanthanides displace \( \text{Ca}^{2+} \) from negatively charged surface molecules (e.g., sialic acid), depleting this source of activator calcium.46 A number of proteins that subserve both structural and ion transport functions have suitable binding sites for lanthanides, which preferentially bind to exposed carbonyl and, to a lesser extent, amine groups of these proteins.44 A number of investigators32-34 have demonstrated that lanthanides, including \( \text{Gd}^{3+} \), are potent calcium channel blockers. There is also indirect evidence that suggests that lanthanides inhibit the \( \text{Na}^+\text{-Ca}^{2+} \) exchange mechanism.45 Thus, \( \text{Gd}^{3+} \) is not a specific blocker of stretch-activated channels, but it is the most potent blocker of stretch-activated channels currently known, and blockade of calcium transport is the only known effect of lanthanides on cardiac cellular physiology.30

**Inhibition of Stretch-Induced Arrhythmias by \( \text{Gd}^{3+} \)**

\( \text{Gd}^{3+} \), in the range of 1–10 \( \mu\text{M} \), produced dose-dependent inhibition of stretch-induced arrhythmias in the isolated canine ventricle, as shown in Figure 6. This conclusion is considerably strengthened by the fact that this effect was reversed when \( \text{Gd}^{3+} \) was cleared from the perfusate by dialysis against \( \text{Gd}^{3+} \)-free solution. This is the first demonstration that stretch-induced arrhythmias can be pharmacologically modulated. Hennekes et al5 have demonstrated that caffeine and partial depolarization of cat papillary muscle with high external potassium (20 mM) can abolish the changes in action potential associated with quick release followed by restretch of the muscle. They also observed that transient length changes applied after repolarization resulted in afterdepolarizations that could reach threshold to trigger an extra action potential after administration of caffeine. Thus, our data with \( \text{Gd}^{3+} \) provide additional information regarding how mechanoelectrical feedback can be pharmacologically modulated.

**Failure to Inhibit Stretch-Induced Arrhythmias With Calcium Channel Antagonists**

To test the possibility that the inhibitory effect of \( \text{Gd}^{3+} \) on the initiation of stretch-induced arrhythmias was mediated by its known calcium channel blocking effect,32,33 we performed a series of studies evaluating the effects of specific calcium channel blockers. We were unable to demonstrate any inhibition of stretch-induced arrhythmias with either verapamil or nifedipine, at concentrations that significantly depressed ventricular contractility. As shown in Figure 7, \( 1 \mu\text{M} \) verapamil completely failed to suppress stretch-induced arrhythmias in this model. This was a reproducible finding in three studies with verapamil and in one ventricle treated with nifedipine (Table 3). Thus, it is unlikely that the calcium channel blocking action of \( \text{Gd}^{3+} \) is responsible for the inhibition of stretch-induced arrhythmias we observed. The studies with calcium channel blockers also provide evidence that the myocardial depressant effect of \( \text{Gd}^{3+} \) does not play a direct role in the inhibition of stretch-induced arrhythmias, since reduction in LV contractility with verapamil and nifedipine was not associated with any change in our ability to initiate such arrhythmias.

**Effect of \( \text{Gd}^{3+} \) on Myocardial Contractility**

To assess LV contractility, we measured the pressure-generating capability of the ventricle at a standard volume of 20 ml in terms of \( P_{\text{max}} \) and \( dP/dt_{\text{max}} \). \( \text{Gd}^{3+} \) had a complex, dose-dependent effect on LV contractility. It is interesting that the effects of \( \text{Gd}^{3+} \) on these two indexes of contractile function were somewhat discrepant. Both of these contractility indexes were depressed significantly by 3 \( \mu\text{M} \) \( \text{Gd}^{3+} \) (Table 1). Although the cellular mechanism of this myocardial depressant effect cannot be determined from our study, it is possible that the calcium channel blocking action of \( \text{Gd}^{3+} \) was responsible for this particular effect. The negative inotropic effects of \( \text{Ca}^{2+} \) channel block are well established.48 Our results with \( \text{Gd}^{3+} \) are consistent with earlier studies45-47,49-51 that also demonstrated a myocardial depressant effect of lanthanides, although we did not observe potentiation after washout of \( \text{Gd}^{3+} \) as Kawata et al47 have reported with both \( \text{La}^{3+} \) and \( \text{Gd}^{3+} \). This afterpotentiation was considerably more prominent in amphibian than mammalian myocardium, and higher concentrations of \( \text{La}^{3+} \) and \( \text{Gd}^{3+} \) (0.1 mM), which
nearly abolished excitation–contraction coupling during perfusion with these lanthanides, were used in the study of Kawata et al. At higher doses of Gd\(^{3+}\) (10 μM), we found that P\(_{max}\) actually improved slightly, such that the value was no longer significantly different from baseline. In contrast, dP/dt\(_{max}\) remained depressed relative to initial conditions. Chiu et al.\(^{52}\) have also observed that inotropic interventions may have divergent effects on the force–velocity characteristics of cardiac muscle. They found that isoproterenol greatly augmented the peak force development and its rate of rise during isometric twitches of isolated rabbit myocardium. In contrast, they found that caffeine greatly augmented the peak developed force but that the time to peak force development increased. Thus, the two indexes of LV contractile function we used probably reflect fundamentally different properties of the contractile process and do not necessarily change in parallel.

Relation Between Action Potential–Prolonging and Contractile Effects of Gd\(^{3+}\)

The increase in P\(_{max}\) as the concentration of Gd\(^{3+}\) was increased from 3 to 10 μM was associated with prolongation of the action potential (Table 2). The action potential prolongation is a curious finding, since calcium antagonists, including lanthanides, abbreviate the plateau currents of cardiac cells.\(^{46,53}\) It is possible that Gd\(^{3+}\) also blocked K\(^+\) current in our experiments and that this had a countervailing effect that prolonged the action potential, but we have no direct evidence to support this speculation. Other investigators have noted that action potential prolongation by class III antiarrhythmic agents\(^{54}\) and tetramethylammonium\(^{55}\) is associated with a positive inotropic effect. It has been suggested that prolongation of the action potential per se is responsible for this positive inotropic effect, perhaps by a mechanism of increased calcium entry through voltage-gated sarcolemmal calcium channels.\(^{54,55}\) The slight improvement in peak pressure generation observed in our study at high Gd\(^{3+}\) concentration may be mediated by a similar mechanism; however, it remains to be determined whether Gd\(^{3+}\) has any demonstrable K\(^+\) current block (the class III antiarrhythmic drug action).\(^{56}\) However, we can be relatively certain that the action potential–prolonging effect of Gd\(^{3+}\) does not modulate the initiation of stretch-induced arrhythmias, since the probability of eliciting an arrhythmia returned to baseline after washout of Gd\(^{3+}\) (Figure 6) despite the fact that prolongation of the monophasic action potential duration and absolute refractory period persisted.

Potential Limitations

The availability of a more specific blocker of stretch-activated channels would have been greatly desirable for this present study. Unfortunately, Gd\(^{3+}\) binds nonspecifically to excitable tissues and may have mediated its various effects by a number of different mechanisms. Therefore, we cannot be absolutely certain that the suppression of stretch-induced arrhythmias we observed was due to stretch-activated channel block. However, because of the results of our studies with verapamil and nifedipine, we can exclude an important contribution from the Ca\(^{2+}\) channel blocking effect of Gd\(^{3+}\). Since the stretch-activated channel is the only other channel that Gd\(^{3+}\) is known to block\(^{30}\) and since the stretch-activated channel hypothesis successfully explains all known mechanoelectrical feedback effects of cardiac tissue, we have interpreted these experimental results in terms of this unifying hypothesis and believe that our study provides additional indirect evidence for this mechanism of arrhythmogenesis. Additional experiments at the cellular and subcellular level will be required in the future to study this more directly.

A number of investigators have suggested that certain mechanoelectrical feedback effects may be caused by length-dependent increases in [Ca\(^{2+}\)].\(^{1-7,57}\) In support of this mechanism, Lab et al.\(^{57}\) have shown that both action potential and the intracellular calcium transient measured by aequorin luminescence are prolonged in lightly loaded contractions of papillary muscle when compared with isometric contractions. Our results with transient diastolic stretch in no way exclude the possible role of calcium in the initiation of stretch-induced arrhythmias. Indeed, as reviewed by Morris,\(^{58}\) measurements of the permeation of nonselective stretch-activated cation channels by Ca\(^{2+}\) provide evidence that such channels could act as a significant route of Ca\(^{2+}\) entry.

As illustrated in Figure 3, the stretch stimulus was relatively brief in duration, and not infrequently, the early ectopic depolarization arises during the release phase of the volume pulse. Thus, the relative importance of ventricular dilatation and shortening in the initiation of arrhythmias is obscured in such studies. One way to determine whether ventricular dilatation per se can trigger arrhythmias is to maintain the stretch for a longer period of time. Figure 9 shows such an experiment in which a three-beat run of ventricular tachycardia was initiated by a 16-mL stretch held for 500 msec. At the onset of the volume increase, a transient depolarization occurs in the monophasic action potential recording. This transient depolarization appears to trigger the arrhythmia, the first two beats of which clearly arise during the stretch. Thus, we can be certain that diastolic stretch is a sufficient stimulus to trigger arrhythmias. The possibility that sudden release from high ventricular volume may also trigger arrhythmias during diastole has not as yet been systematically evaluated.

In summary, we have developed a reproducible model of stretch-induced arrhythmias to study the apparently important relation between ventricular dilatation and serious ventricular arrhythmias.\(^{24}\) Innovations in the method used to metabolically support the isolated canine heart now enable careful evaluation of pharmacological effects in this model. In this initial pharmacological study, we investigated the effect of Gd\(^{3+}\) on the initiation of stretch-induced
arrhythmias because of its known stretch-activated channel blocking action. While stretch-activated channels have been previously demonstrated in neonatal myocytes, there has been no evidence, until the present study, that such channels are expressed in adult myocardium. We found that Gd³⁺ modulates both electrical and mechanical properties of the isolated canine heart. The finding of dose-dependent inhibition of stretch-induced arrhythmias by Gd³⁺ is consistent with our hypothesis that such arrhythmias may be mediated by stretch-activated channels. Our results with verapamil and nifedipine confirm that this inhibition of stretch-induced arrhythmias by Gd³⁺ was not due to its calcium channel blocking effect. Thus, this study provides preliminary evidence that stretch-activated channel block, with agents such as Gd³⁺, may have a novel antiarrhythmic action.

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