Gadolinium Decreases Stretch-Induced Vulnerability to Atrial Fibrillation

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**Background**—Atrial fibrillation (AF) is frequently associated with atrial dilatation caused by pressure or volume overload. Stretch-activated channels (SACs) have been found in myocardial cells and may promote AF in dilated atria. To prove this hypothesis, we investigated the effect of the SAC blocker gadolinium (Gd³⁺) on AF propensity in the isolated rabbit heart during atrial stretch.

**Methods and Results**—In 16 isolated Langendorff-perfused rabbit hearts, the interatrial septum was perforated to equalize biatrial pressures. Caval and pulmonary veins were occluded. Intra-atrial pressure (IAP) was increased in steps of 2 to 3 cm H₂O by increasing the pulmonary outflow fluid column. Vulnerability to AF was evaluated by 15-second burst pacing at each IAP level. At baseline, IAP needed to be raised to 8.8±0.2 cm H₂O (mean±SEM) to induce AF. A dose-dependent decrease in AF vulnerability was observed after Gd³⁺ 12.5, 25, and 50 μmol/L was added. AF threshold increased to 19.0±0.5 cm H₂O with Gd³⁺ 50 μmol/L (P<0.001 versus baseline). Spontaneous runs of AF occurred in 5 hearts on a rise of IAP to 13.8±3.3 cm H₂O at baseline but never during Gd³⁺. Atrial effective refractory period shortened progressively from 78±3 ms at 0.5 cm H₂O to 52±3 ms at 20 cm H₂O (P<0.05). Gd³⁺ 50 μmol/L had no significant effect on effective refractory period.

**Conclusions**—Acute atrial stretch significantly enhances the vulnerability to AF. Gd³⁺ reduces the stretch-induced vulnerability to AF in a dose-dependent manner. Block of SAC might represent a novel antiarrhythmic approach to AF under conditions of elevated atrial pressure or volume. (*Circulation. 2000;101:2200-2205.)*

**Key Words:** stretch ■ channels ■ fibrillation ■ gadolinium ■ mechanics

The prevalence of atrial fibrillation (AF) increases with age and is associated primarily with hemodynamic or mechanical disorders of the heart (ie, hypertension, mitral valve disease, cardiac failure).1 Because of the clinical association of AF with atrial enlargement, it has long been assumed that atrial dilatation and stretch play an important role in the occurrence of AF.2 Mechanoelectrical feedback, ie, electrophysiological changes in response to mechanical perturbations or changes in hemodynamic loading, has been well established in ventricular myocardium. It has been recognized in isolated heart preparations,3 in situ hearts,4 and in humans.5 Ventricular stretch leads to a shortening of action potential duration (APD) and effective refractory period (ERP).5–6 Acute ventricular dilatation may induce premature depolarizations and triggered activity.5,6 The presence of mechanoelectrical feedback has been confirmed at the atrial level. Atrial stretch caused by ventricular contraction modulates the atrial flutter cycle length in humans.7 In the experimental setting, acute atrial dilatation has recently been shown to facilitate the induction and maintenance of AF.8 This vulnerability has been attributed to concurrent reductions in APD and ERP,8,9 resulting in decreased wavelength of atrial excitation.10

Stretch-activated ion channels (SACs) have been found in cardiac tissue of various species, including humans,11–13 and have been shown to produce potentially arrhythmogenic electrophysiological changes. Nonselective SACs pass Ca²⁺ as well as Na⁺ and K⁺, whereas others selectively carry K⁺ or Cl⁻.11,12 Experimental evidence indicates that SACs play an important role in promoting arrhythmias during stretch. Gadolinium (Gd³⁺), a potent blocker of SACs, suppressed the occurrence of stretch-induced depolarizations in ventricular myocardium6 and atrial tissue.14 The influence of SAC blockade on the intact atrium and on the propensity toward stretch-induced AF has not been explored. The aim of the present study was to evaluate in an isolated heart model whether the SAC blocker Gd³⁺ influences the inducibility and maintenance of AF during acute stretch. Because calcium channel block has been reported with Gd³⁺,15 additional experiments were performed with the L-type calcium channel blocker verapamil to rule out nonspecific AF suppression.
Methods

Isolated Heart Preparation

After approval by the Institutional Animal Care and Use committee, 16 New Zealand White rabbits of either sex weighing 3.0 to 3.7 kg were anesthetized with 50 mg/kg sodium pentobarbital IV. After administration of 500 IU heparin IV, hearts were rapidly removed through a midline sternal incision, and contractions were arrested by immersion in ice-cold perfusion fluid. The aorta was cannulated to perfuse the heart in a modified Langendorff apparatus at a pressure of 60 mm Hg and a temperature of 37°C. The composition of the perfusion fluid was (in mmol/L): NaCl 140, KCl 3.0, CaCl2 2.6, MgCl2 0.6, HEPES 10, and glucose 12. It was bubbled with 100% oxygen, and the pH was adjusted to 7.4.

To apply graded stretch to the atria, the heart was prepared according to a model previously described. Briefly, the caval and pulmonary veins were ligated. A Y-shaped manometer was inserted into the superior caval vein and a pulmonary vein to measure biatrial pressure. The interatrial septum was perforated to ensure pressure into the superior caval vein and a pulmonary vein to measure biatrial pressure changes caused by ventricular contractions, ventricular pressure and degree of atrial dilatation were controlled by adjustment of the height of the pulmonary outflow cannula. To avoid atrial pressure changes caused by ventricular contractions, ventricular fibrillation was induced by burst pacing through a bipolar hook electrode attached to the left ventricle. The AV node was crushed to prevent retrograde atrial activation.

Electrophysiological Measurements

Endocardial electrograms were recorded from the right and left midatrial free wall by bipolar 4F catheters introduced through the orifice of the inferior caval vein and a pulmonary vein. Signals were amplified by a differential DC-coupled preamplifier (model 1009, EP Technologies) and displayed and stored on a computer with custom data acquisition and analysis based on LabView software (National Instruments). To test the inducibility of AF, burst pacing was performed through bipolar epicardial hook electrodes attached to both atrial appendages. Stimuli of 1-ms pulse duration and 3-fold diastolic pacing threshold were applied for 15 seconds at 50 Hz.

Experimental Protocol

Intra-atrial pressure was increased progressively from 0 cm H2O in steps of 2 to 3 cm H2O. Hearts were allowed to adapt to each new pressure level for 2 minutes before a burst pacing sequence was delivered. AF was defined as inducible when a fast irregular rhythm was maintained >2 seconds after cessation of burst pacing. The ability of the atria to develop sustained AF was analyzed separately. Sustained AF was defined as a fast irregular rhythm that lasted for >60 seconds after cessation of burst pacing and could be terminated only by pressure lowering. The pressure level was increased until sustained AF was induced or a pressure level of 30 cm H2O was reached.

Drug Interventions

The dose-dependence of Gd3+ effects on AF inducibility was studied in 6 hearts. Gd3+ was added to the perfusate in serial concentrations of 12.5, 25, and 50 μmol/L, allowing 15 minutes for equilibration. The AF response to burst stimulation with increasing atrial pressure was assessed at each concentration and after a 20-minute washout period.

The effect of Gd3+ on AF inducibility was studied in a total of 16 experiments. The probability of AF induction and the duration of AF were determined at each pressure step before and after application of Gd3+.

Because Gd3+ has been reported to block calcium channels and to determine whether calcium channel block could interfere with AF vulnerability during stretch, the effect of calcium channel blockade with verapamil was examined in 5 studies. Measurements were obtained before and 15 minutes after administration of verapamil 1 μmol/L. After a 20-minute washout period, Gd3+ 50 μmol/L was applied to compare the effects of both substances. In isolated hearts, verapamil 1 μmol/L achieved marked block of Ca2+ channels that could be expected to exceed the Ca2+ channel blocking effect reported with Gd3+ doses up to 80 μmol/L.

The effect of SAC blockade on atrial refractoriness was evaluated in 8 hearts. The free right atrial midwall was paced in close proximity to the recording electrode at twice diastolic threshold strength. After a 10-beat train at 250-ms basic cycle length, a premature stimulus was introduced during electrical diastole. The coupling interval was shortened in 1-ms decrements until it failed to induce a propagated response, defining the ERP. Measurements were performed at increasing atrial pressure levels during baseline and after a 15-minute period of perfusion with Gd3+ 50 μmol/L. Again, atria were given 2 minutes to adapt to each pressure level before ERP determination.

Data Analysis

Data are presented as mean±SEM unless indicated otherwise. The probability of AF induction at different atrial pressures was analyzed by logistic regression analysis applying the function y = 1/[1 + exp(-(x-a)/b)], where a and b are fitting parameters determined by an iterative procedure with a convergence criterion of a change of <0.01% for the 2 parameters. A dose-response relationship was calculated for atrial pressures associated with 50% AF inducibility (P50). P50 values for different drug concentrations were compared by t test. AF durations and ERPs at different degrees of atrial dilatation were statistically evaluated with the paired t test. Values of P<0.05 defined statistical significance.

Results

Effect of Acute Stretch on AF Inducibility

In the undilated atrium at a pressure of 0 cm H2O, burst pacing did not induce AF. After an increase in atrial pressure, AF could be induced in each preparation. The initial AF response seen during a stepwise pressure increase was predominantly nonsustained, whereas sustained AF responses emerged at higher pressure levels. Figure 1 illustrates the effect of atrial pressure on AF inducibility and AF persistence. AF inducibility in response to increasing pressure followed a sigmoidal curve (Figure 2). On average, intraatrial pressure needed to be raised to 8.8 cm H2O (P50) to allow perpetuation of AF after cessation of burst pacing. A further increase of intra-atrial pressure to 11.6±0.6 cm H2O (P<0.01) was required to induce sustained AF that lasted >60 seconds. Sustained AF terminated promptly on pressure release. Figure 3 demonstrates how the average duration of AF episodes lengthened as intra-atrial pressure was increased. Spontaneous AF occurred in 5 hearts when intra-atrial pressure increased to 13.8±3.3 cm H2O. Premature depolarizations often preceded runs of AF (Figure 4).

Effect of Gd3+ on AF Inducibility

In 6 hearts exposed to successive doses of 12.5, 25, and 50 μmol/L Gd3+, the vulnerability to AF was progressively decreased (Figure 5A). P50 showed a linear correlation (r=0.99; P<0.005) with Gd3+ concentration over the 0 to 50 μmol/L dose range (Figure 5B). Gd3+ increased by 0.15 cmH2O per 1 μmol/L Gd3+. P50 differed significantly between baseline and data at increasing Gd3+ doses as well as among doses (P<0.01). Each experiment lasted ≈3 hours. Yet time-related changes in the preparation could not have accounted for the observed reduction in AF vulnerability, because the effect of Gd3+ was largely reversible after 20
minutes of washout ($P<0.01$ compared with Gd$^{3+}$ 25 and 50 μmol/L).

Gd$^{3+}$ 50 μmol/L was applied to all 16 hearts. In each preparation, the lowest atrial pressure that had enabled AF induction during baseline was no longer sufficient to maintain AF during Gd$^{3+}$. Instead, intra-atrial pressure had to be increased to significantly higher levels to obtain AF. P$_{50}$ for AF induction was shifted from 8.8±0.2 to 19.0±0.5 cm H$_2$O ($P<0.001$). Figure 2 illustrates the pressure-related decrease in AF inducibility. On average, atrial pressure needed to be elevated to 21.9±0.4 cm H$_2$O to obtain sustained AF after Gd$^{3+}$ (versus 11.6±0.6 cm H$_2$O at baseline; $P<0.001$). Gd$^{3+}$ 50 μmol/L markedly decreased the average duration of induced AF at pressures between 7.5 and 22.5 cm H$_2$O ($P<0.05$; Figure 3). Reduced inducibility of AF was not explained by changes in pacing thresholds (0.20±0.02 mA at baseline versus 0.21±0.02 mA with Gd$^{3+}$ 50 μmol/L; $P=\text{NS}$). Spontaneous AF was no longer observed during the stepwise increase in atrial pressure after Gd$^{3+}$ was added.

**Effect of Verapamil on Vulnerability to AF**

In 5 hearts, the effect of specific L-type calcium channel block was studied with verapamil. Pacing thresholds were not affected by verapamil (0.21±0.04 versus 0.20±0.03 mA at baseline). Verapamil 1 μmol/L did not inhibit AF induction during acute dilatation. P$_{50}$ was 6.3±0.1 cm H$_2$O at baseline and 4.9±0.2 cm H$_2$O after application of verapamil ($P=\text{NS}$). In the same preparations, Gd$^{3+}$ 50 μmol/L increased P$_{50}$ to 12.9±0.5 cm H$_2$O ($P<0.001$).

**Atrial Refractoriness**

The right atrial ERP progressively shortened with an increase in atrial pressure (Figure 6). On average, ERP shifted from 78±3 ms at 0.5 cm H$_2$O to 52±3 ms at 20 cm H$_2$O ($P<0.05$). After application of 50 μmol/L Gd$^{3+}$, this ERP-pressure relationship was maintained. ERP decreased from 73±3 ms at 0.5 cm H$_2$O to 54±2 ms at 20 cm H$_2$O. At each pressure step, ERP was unchanged from baseline (Figure 6).

**Discussion**

The major finding of this study is that Gd$^{3+}$, a SAC blocker, reduces the vulnerability to AF during acute atrial dilatation. The study shows for the first time that block of SAC counteracts the proarrhythmic effect of acute myocardial dilatation on the inducibility and maintenance of a sustained atrial arrhythmia.

**Figure 2.** Inducibility of AF by burst pacing as a function of intra-atrial pressure. Data points show success rate of AF induction in 16 hearts at baseline (○) and after Gd$^{3+}$ 50 μmol/L (●). Pressure-response curves were fitted by logistic regression. A 50% chance of AF induction was associated with pressure of 8.8 cm H$_2$O at baseline and pressure of 19.0 cm H$_2$O after Gd$^{3+}$ ($P<0.001$).

**Figure 3.** Duration of induced AF in 16 hearts as function of intra-atrial pressure (mean±SEM). Gd$^{3+}$ 50 μmol/L decreased average AF duration vs baseline. *$P<0.05$. 

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**Figure 1.** Effect of atrial pressure on AF inducibility and AF duration in isolated rabbit heart during baseline. Bipolar electrogram recorded from right atrium. At low intra-atrial pressure (P) of 2.5 cm H$_2$O, no AF response evolved after cessation of burst pacing (1). With a stepwise increase in P, burst pacing induced AF episodes of progressively longer duration. At 12.5 cm H$_2$O, sustained AF was evoked.
Atrial enlargement is frequently observed in patients with AF. Atrial dilatation could develop as a result of the arrhythmia itself. Yet AF is also frequently associated with elevated intracavitary pressure and cardiac disorders that favor this condition. Numerous studies have demonstrated that the vulnerability to arrhythmias increases with myocardial stretch. For intact canine and rabbit atria, it has been shown that acute dilatation promotes tachyarrhythmic responses to burst pacing or single extrastimuli. Our study confirmed these findings. A fibrillatory response to electrical stimulation could be elicited only when atrial myocardium was preconditioned by stretch. Moreover, the degree of atrial dilatation influenced the duration of induced AF. The susceptibility to develop sustained AF increased with progressively higher pressure levels. The most widely accepted theory of the mechanism of AF suggests multiple wave fronts that wander through the atria and create continuous electrical activity. According to this hypothesis, progressive atrial dilatation must have changed atrial electrical properties in favor of the coexistence of multiple wavelets and their ability to continually encounter excitable tissue.

**Electrical Changes Underlying AF Vulnerability**

Acute stretch might contribute to arrhythmogenesis by several mechanisms. In isolated cardiac tissue, mechanical stretch decreased resting membrane potential and reduced action potential amplitude and upstroke velocity. In whole-heart preparations and in situ hearts, ventricular stretch decreased APD, thereby shortening myocardial refractoriness. For the intact atrium, conflicting data exist. In vivo studies investigated the influence of rather modest alterations in atrial pressure due to changes in paced AV intervals or infusion of plasma expander. Refractory periods were reported by 1 group to lengthen in response to an increase in atrial pressure, whereas others found either no effect on ERP or ERP shortening. Isolated heart preparations exposed to defined degrees of atrial stretch showed shortening...
of refractoriness with increased atrial pressure, paralleled by an increase in AF vulnerability.8–9 It has been postulated that ERP shortening implies shortening of excitation wavelength and thereby favors reentrant activity, which is generally recognized to entrap AF.10 The high correlation between ERP shortening and AF inducibility in the recent study by Ravelli and Allessie8 supports this hypothesis. Our data confirm previous results by demonstrating a decrease in ERP and increased AF with progressive atrial stretch. Yet we found the local atrial ERP response unaltered after application of Gd3+1, whereas the vulnerability to AF was significantly reduced. Overall shortening of ERP might therefore not fully explain the increased susceptibility to AF during stretch. Evidence has been provided that acute atrial dilatation increases the spatial dispersion of atrial refractoriness.25 The inhomogeneous structure of the atria can create regional differences in wall stress during elevated intra-atrial pressure. Nonuniform distribution of local atrial refractory periods due to heterogeneous wall stress could establish a basis for the initiation and maintenance of atrial reentry during stretch.25 SAC block by Gd3+ might interfere with local electrical properties dependent on the magnitude of regional wall stress and possibly reduce ERP dispersion. This has to be further elucidated.

The wavelength of the atrial impulse is also determined by myocardial conduction properties. To date, the effect of acute myocardial stretch on intra-atrial conduction time has not been systematically evaluated. Studies performed in ventricular preparations reported no effects on conduction velocity within a physiological range of dilatation, whereas stretch beyond the optimal fiber length decreased conduction velocity.21 Alterations in conduction time depending on atrial load could influence the susceptibility to atrial reentry. If SACs mediate stretch-dependent changes in atrial conduction time, SAC block by Gd3+ can be expected to mitigate them. In some of our experiments, the increased atrial irritability during stretch manifested in spontaneous onset of AF. A rise in atrial pressure elicited nonsustained runs of AF initiated by premature depolarizations. This is in accordance with previous observations in dog hearts that developed spontaneous atrial arrhythmias on atrial balloon dilatation.9 A possible explanation for premature depolarizations is the occurrence of afterdepolarizations. Sustained myocardial stretch induced afterdepolarizations in atrial tissue.14 Afterdepolarizations provoked by cesium chloride have recently been reported to account for onset of polymorphic atrial tachycardia degenerating into AF.26

Role of SACs

The presence of SACs capable of generating both inward and outward currents might explain how stretch influences cardiac electrophysiology and increases vulnerability to arrhythmia and cardiac automaticity. Various types of SACs have been identified in cardiac myocytes of different species, including rabbits and humans.11–13 The most commonly reported type of SAC nonselectively passes Ca2+ as well as monovalent Na+ and K+ ions, whereas other SACs are K+ or Cl− selective.11,12 Reversal potentials of SACs range between 10 and −70 mV.11 Opening of SACs that do not show fatigue during sustained stretch may account for repolarizing currents during electrical systole that shorten APD and refractoriness. During electrical diastole, SAC opening could establish depolarizing currents that lead to afterdepolarizations. Intra- cellular Ca2+ overload due to SAC opening may also account for abbreviation of the action potential and production of afterdepolarizations.27 An increase in [Ca2+], evolves when heart cells are stretched.28

Effect of SAC Blockade

In single myocardial cells, Gd3+ has proved to be a potent blocker of SACs that carry monovalent and divalent cations.11 Gd3+ has also been shown to reduce [Ca2+], during stretch.28 To date, only a few studies have focused on the effect of SAC blockade in multicellular preparations. Gd3+ suppressed stretch-induced depolarizations in isolated canine ventricles when brief stretch was applied during electrical diastole.6 The role of SACs during sustained stretch has been studied in the working rat heart. Occurrence of ventricular premature beats due to increased left ventricular pressure was reduced by streptomycin, another compound known to block SACs.29 Afterdepolarizations produced by constant stretch in atrial tissue were suppressed by Gd3+.14 SAC blockade by Gd3+ was also reported to inhibit the release of atrial natriuretic peptide,17 a substance that might contribute to action potential shortening during atrial stretch.30

Our study provides initial evidence that the propensity to AF during acute stretch can be modulated pharmacologically by a SAC-blocking agent. Gd3+ impeded electrical initiation of AF, hampered maintenance of burst-induced AF, and suppressed the generation of spontaneous AF during stretch. Although interference with myocardial propagation and/or repolarization properties during stretch appears to be crucial for the reduced ability to sustain AF, the absence of spontaneous AF after Gd3+ may be explained by suppression of afterdepolarizations and triggered activity. Gadolinium, a potent blocker of SACs, is a nonspecific ion channel blocker. In single myocardial cells, voltage-clamp studies provided evidence for block of L-type Ca2+ channels with Gd3+ concentrations as low as 10 μmol/L.15 Block of Ikr31 and sodium currents12 required up to 100 μmol/L Gd3+.

Inhibitory concentrations have been shown to vary between isolated cells and intact tissue or among species.14 We cannot exclude the possibility that part of the effect of Gd3+ on the inducibility of AF during stretch was mediated by its calcium, potassium, and sodium channel blocking effects. Yet calcium channel block, perhaps the most prominent nonspecific Gd3+ effect, did not affect the vulnerability to AF in our study. This finding is in accordance with the failure of specific calcium channel blockers to suppress stretch-induced afterdepolarizations in isolated atrial tissue14 and arrhythmogenic responses in ventricular myocardium,6 whereas Gd3+ achieved suppression in these systems. If the effect of Gd3+ on AF vulnerability had been mediated by block of Ikr, the ERP would have been expected to increase during Gd3+ application. The lack of ERP prolongation suggests that Ikr block is not responsible for the effects we have observed with Gd3+. This evidence links the inhibitory effect of Gd3+ to a different mode of action, namely to its SAC-blocking properties.
Limitations
Manifestation of AF in this rabbit heart model cannot be translated directly into the clinical manifestation of AF in patients. The present study evaluated AF of short duration (>2 seconds) compared with long-lasting AF episodes that can be observed in patients. Definitions of AF duration in this model were based on the low tendency of the rabbit atrium to fibrillate. This was emphasized by the inability to induce AF in the undilated atrium and the prompt termination of stretch-facilitated AF by return of atrial pressure to normal levels. The small size of rabbit atria does not provide sufficient tissue mass to entartain multivavelet reentrant activity. Unlike in the larger human atria, perpetuation of AF has to be promoted by interventions that shorten wavelength by 10% or increase atrial surface area. Therefore, we assumed that even brief episodes of AF in rabbit atria can reflect a considerable increase in AF vulnerability.

Conclusions
Our study showed that the SAC blocker Gd3+ modulates the electrophysiologic properties of the intact rabbit atrium during acute dilatation. A decrease in the stretch-induced vulnerability to AF was consistent with the concept that the facilitation of AF may be mediated by SACs. Thus, SAC block with such agents as Gd3+ may represent a novel antiarrhythmic approach to diminish the proarrhythmic effect of acute atrial stretch on AF.

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