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QUARTERLY FOCUS ISSUE: HEART RHYTHM DISORDERS

Altered Na^+ Currents in Atrial Fibrillation

Effects of Ranolazine on Arrhythmias and Contractility in Human Atrial Myocardium

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Objectives We investigated changes in Na^+ currents (I_{Na}) in permanent (or chronic) atrial fibrillation (AF) and the effects of I_{Na} inhibition using ranolazine (Ran) on arrhythmias and contractility in human atrial myocardium.

Background Electrical remodeling during AF is typically associated with alterations in Ca^{2+} and K^+ currents. It remains unclear whether I_{Na} is also altered.

Methods Right atrial appendages from patients with AF (n = 23) and in sinus rhythm (SR) (n = 79) were studied.

Results Patch-clamp experiments in isolated atrial myocytes showed significantly reduced peak I_{Na} density (~16%) in AF compared with SR, which was accompanied by a 26% lower expression of Nav1.5 ($p < 0.05$). In contrast, late I_{Na} was significantly increased in myocytes from AF atria by ~26%. Ran (10 μmol/l) decreased late I_{Na} by ~60% ($p < 0.05$) in myocytes from patients with AF but only by ~18% ($p < 0.05$) in myocytes from SR atria. Proarrhythmic activity was elicited in atrial trabeculae exposed to high $[Ca^{2+}]_o$ or isoprenaline, which was significantly reversed by Ran (by 83% and 100%, respectively). Increasing pacing rates from 0.5 to 3.0 Hz led to an increase in diastolic tension that could be significantly decreased by Ran in atria from SR and AF patients.

Conclusions Na^+ channels may contribute to arrhythmias and contractile remodeling in AF. Inhibition of I_{Na} with Ran had antiarrhythmic effects and improved diastolic function. Thus, inhibition of late I_{Na} may be a promising new treatment option for patients with atrial rhythm disturbances and diastolic dysfunction. (J Am Coll Cardiol 2010;55:2330–42) © 2010 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is the most common arrhythmia and is associated with substantial morbidity and mortality (1,2). Major determinants of electrical remodeling in AF include: 1) reduced action potential duration (APD); 2) decreased L-type Ca^{2+} current amplitude; and 3) altered K^+ currents (3,4). Whether electrical remodeling in permanent (or chronic) AF also affects Na^+ channels is unclear. Evidence comes from 2 nonclinical studies showing reduced peak Na^+ current (I_{Na}) densities in dog models of experimentally induced AF (5,6).

There is increasing recognition of the importance of a small persistent component of I_{Na} (late I_{Na}). Although the amplitude of this current is only ~1% of the peak I_{Na}, due to its persistent nature during the action potential (AP), it can contribute to the amount of Na^+ that enters the cell. The late I_{Na} integral seems to be significantly larger than the peak I_{Na} integral under pathological conditions (7). There is evidence of an increased late I_{Na} in ventricular myocytes of patients with heart failure and myocardial ischemia (8,9), which may contribute to the increased $[Na^+]_i$ observed (10–12). However, the role of late I_{Na} in atrial myocytes of AF patients is not known. Thus, our first objective was to determine whether AF is associated with altered Na^+ channel expression as well as changes in peak and late I_{Na} compared with sinus rhythm (SR).

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Ventricular proarrhythmia and negative inotropy are important limitations to the current drug therapies used in AF (13). Thus, the development of agents that preferentially modulate the function of atrial rather than ventricular

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ion channel currents is an attractive therapeutic strategy. Ranolazine (Ran) is an antianginal agent that preferentially inhibits late over peak I_{Na} in ventricular myocytes (14–16). A large clinical trial (MERLIN–TIMI 36 [Metabolic Efficiency With Ranolazine for Less Ischemia in Non–ST-Elevation Acute Coronary Syndromes–Thrombolysis In Myocardial Infarction 36]) revealed that Ran significantly reduces the incidence of supraventricular arrhythmias and new episodes of AF in patients with non–ST-segment elevation acute coronary syndrome (17). In canine perfused right atrial preparations, Burashnikov et al. (18) demonstrated differences in the inactivation characteristics of atrial versus ventricular Na^+ channels, and an atrial selective action of Ran to cause a use-dependent block of Na^+ channels and suppression of AF. Hence, our second objective was to determine the effects of Ran on peak and late I_{Na} of atrial myocytes from patients with AF and SR.

Last, the third objective was to determine the effects of Ran on contractile function as well as possible antiarrhythmic properties in human atrial trabeculae.

Abbreviations and Acronyms

AF = atrial fibrillation
ANOVA = analysis of variance
AP = action potential
APD = action potential duration
HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
I_{Na} = Na^+ current
Iso = isoprenaline
I_{Ti} = transient inward current
PAC = premature atrial contraction
Ran = ranolazine
SR = sinus rhythm

Methods

Tissue. Right atrial appendages were obtained from patients undergoing heart surgery who were in SR or permanent AF (Table 1). All procedures were in compliance with the ethical committee of Georg-August-University Göttingen.

Cell isolation. Pieces of atrial myocardium were transported to the laboratory in cardioplegic solution (mmol/l: NaCl 110, KCl 16, $MgCl_2$ 16, $NaHCO_3$ 16, $CaCl_2$ 1.2, glucose 11) immediately after excision. They were rinsed, cut into small pieces, and incubated (36°C) in a Ca^{2+}-free solution containing 1.4 mg/ml collagenase (Worthington type 2, 290 U/mg), 30 μg/ml proteinase (Sigma type XXIV, 9 U/mg), and (mmol/l): NaCl 88, sucrose 88, KCl 5.4, $NaHCO_3$ 4, NaH_2PO_4 0.3, $MgCl_2$ 1.1, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10, taurine 20, glucose 10, sodium pyruvate 5 (pH 7.4). After 45 min, the supernatant was discarded. The tissue was digested again in a collagenase solution until myocytes appeared. Solutions containing cells were centrifuged at 60 rpm (3 min). In the following steps, the same solution was used and aliquots were incubated (10 to 15 min). This procedure was repeated 4 to 5 times. Cells were stored for 1 h in medium containing (mmol/l): taurine 10, glutamic acid 70, KCl 25, KH_2PO_4 10, dextrose 22, ethylene glycol tetraacetic acid 0.5 (pH 7.4, KOH). Only elongated cells with cross striations and without granulation were used.

Patch-clamp experiments. Whole-cell voltage-clamp was used to measure I_{Na} (12). Microelectrodes (2 to 3 MΩ) were filled with (mmol/l): 40 CsCl, 80 Cs glutamate, 10 NaCl, 0.92 $MgCl_2$, 5 magnesium adenosine triphosphate, 0.3 lithium guanosine triphosphate, 10 HEPES, 0.03 niflumic acid, 0.02 nifedipine, 0.004 strophanthidin, 5 (1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid) (tetracesium salt), 1 5,5′-dibromo (1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid) (tetrapotassium salt), 1.49 $CaCl_2$ (free $[Ca^{2+}]_i$ = 100 nmol/l; pH 7.2, CsOH). The bath solution contained (mmol/l): 130 NaCl, 10 tetraethylammonium chloride, 4 CsCl, 1 $MgCl_2$, 10 glucose, 10 HEPES or 10 NaCl, 130 tetramethylammonium chloride, 4 CsCl, 1 MgCl2, 10 glucose, 10 HEPES (pH 7.4, NaOH). Note that the high $[Na^+]$ solution was used for late I_{Na} measurements (12,14). Myocytes were placed in a recording chamber mounted on the stage of a microscope. Fast capacitance was compensated in cell-attached configuration. Liquid junction potentials (3 to 6 mV) were corrected. Membrane capacitance and series resistance were compensated after rupture; access resistance was <10 MΩ. Recordings were started 5 min after rupture. Signals were filtered with 2.9- and 10-kHz Bessel filters and recorded with an EPC10 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany). Myocytes were held at

Table 1 Patient Characteristics

	SR	AF
n	79	23
Age, yrs	68 ± 1*	73 ± 1
Male	50 (63.3)	13 (60.8)
Surgery		
CABG	39 (49.4)	8 (34.8)
AVR	13 (16.5)	7 (30.4)
MVR	1 (1.3)	3 (13.0)
CABG + AVR	12 (15.3)	3 (13.0)
CABG + MVR	2 (2.5)	2 (8.7)
HTX	3 (3.8)	0 (0.0)
HOCM	9 (11.4)	0 (0.0)
Drug treatment		
Ca^{2+}-channel blocker	14 (17.7)	6 (26.1)
Beta-blocker	61 (77.2)	18 (78.3)
ACE inhibitor	46 (58.2)	14 (60.9)
Amiodarone	4 (5.1)	2 (8.7)
Diuretic	39 (49.4)	14 (60.9)
Catecholamines	2 (2.5)	0 (0.0)
LV function		
EF >45%	53 (67.1)	11 (55.0)
EF 35%–45%	18 (22.8)	7 (35.0)
EF <35%	8 (10.1)	2 (10.0)
LA diameter (mm)	42.6 ± 1*	51.3 ± 1

Values are n, mean ± SEM, or n (%). *p < 0.05 SR versus AF.

ACE = angiotensin-converting enzyme; AF = atrial fibrillation; AVR = aortic valve replacement; CABG = coronary artery bypass graft surgery; EF = ejection fraction; HOCM = hypertrophic obstructive cardiomyopathy; HTX = heart transplantation; LA = left atrial; LV = left ventricular; MVR = mitral valve replacement; SR = sinus rhythm.

−120 mV, and I_{Na} was elicited using depolarizing pulses to −30 mV. To measure peak I_{Na}, myocytes were held at −80 mV followed by a depolarizing step to −30 or −10 mV. Current-voltage (I-V) relationships were generated using a holding potential of −120 mV followed by steps from −80 to +30 mV (0.5 Hz). For the measurement of late I_{Na}, pulses were preceded by a 5-ms pre-pulse to +50 mV to optimize voltage control. Measured currents were normalized to the membrane capacitance. Late I_{Na} was measured and integrated from 50 to 250 ms of the beginning of the depolarizing pulse (room temperature).

Western blots. Atrial tissue was homogenized in Tris buffer containing (mmol/l): 20 Tris-HCl, 200 NaCl, 20 NaF, 1 Na_3VO_4, 1 dithiothreipol, 1% Triton X-100 (pH

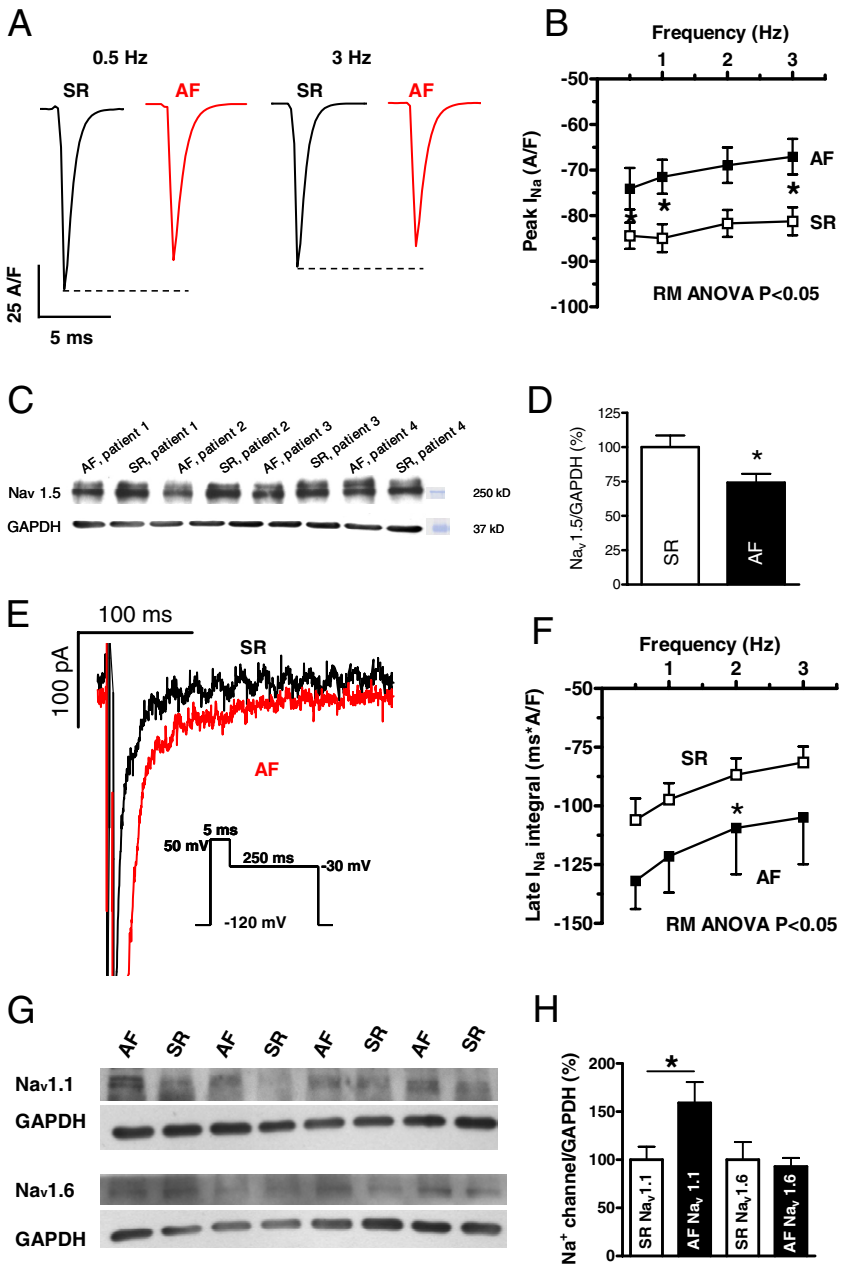

Figure 1 **Altered Na$^+$ Currents and Channel Expression in AF Versus SR**

(A) Original peak Na^+ current (I_{Na}) tracings. **(B)** Mean peak I_{Na} in atrial fibrillation (AF) (n = 18 cells from 9 hearts) versus sinus rhythm (SR) (n = 19 cells from 14 hearts; $p < 0.05$). **(C)** Western blots for Nav1.5. **(D)** Mean data for Nav1.5 in AF (n = 9) versus SR (n = 9). **(E)** Original late I_{Na} recordings. **(F)** Average late I_{Na} integral in AF versus SR (n = 13 cells from 9 hearts vs. 30 cells from 16 hearts, $p < 0.05$). **(G)** Western blots for Nav1.1, Nav1.6. **(H)** Mean data for Nav1.1 (AF, n = 6 vs. SR, n = 10), Nav1.6 (AF, n = 6 vs. SR, n = 5). *$p < 0.05$ versus SR. A/F = ampere/farrad; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; RM ANOVA = repeated-measure analysis of variance.

7.4), and complete protease inhibitor cocktail (Roche Diagnostics, Grenzach-Wyhlen, Germany). Protein concentration was determined by bicinchoninic acid assay (Pierce Biotechnology, Rockford, Illinois). Denatured cell lysates and tissue homogenates (30 min, 37°C, 2% β-mercaptoethanol) were subjected to Western blotting (7.5% sodium dodecylsulfate-polyacrylamide gel) using cardiac-specific anti-Nav1.5 (1:500), neuronal anti-Nav1.1, anti-Nav1.6 (1:400, Alomone Labs, Jerusalem, Israel), anti-glyceraldehyde-3-phosphate dehydrogenase (1:20,000, Biotrend Chemikalien, Köln, Germany) as primary, and a horseradish peroxidase-conjugated donkey anti-rabbit and sheep anti-mouse immunoglobulin G (1:10,000, Amersham Biosciences, Freiburg, Germany) as secondary antibody. Chemiluminescent detection was performed with SuperSignal West Pico Substrate (Pierce Biotechnology).

Preparation of right atrial trabeculae. Thin right atrial trabeculae were microdissected (19) in a cardioprotective solution (mmol/l): Na^+ 152, K^+ 3.6, Cl^- 135, HCO_3^- 25, Mg^{2+} 0.6, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, Ca^{2+} 2.5, glucose 11.2, 2,3-butanedione monoxime oxygenated (95% O_2, 5% CO_2). Trabeculae were mounted in an organ chamber and connected to a force transducer. Trabeculae were superfused with solution (mmol/l: $NaCl_2$ 116, KCl 5, NaH_2PO_4 2, $MgCl_2$ 1.2, Na_2SO_4 1.2, $NaHCO_3$ 20, $CaCl_2$ 0.25, glucose 10) that was oxygenated (95% O_2, 5% CO_2, 37°C) and stimulated at 1 Hz (voltage 25% above threshold, pulse width of 5 ms). Ca^{2+} was added stepwise every 2 min until the final concentration of 1.25 mmol/l was reached. After an equilibration period (45 min), the trabeculae were gradually stretched until maximum steady-state twitch force was achieved.

Drug solutions and experimental protocol. Ran ([+]N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)-propyl)-1-piperazine acetamide dihydrochloride]) was freshly dissolved in 10% HCl solution. In trabeculae experiments, the Ran concentration used was 10 μmol/l because it is within the range of therapeutic plasma levels (<10 μmol/l) and inhibitory concentration of 50% values for inhibition of late I_{Na} (6 to 15 μmol/l), which does not significantly inhibit I_{Ca}, $I_{Na/Ca}$, or I_{Ks} (15). A force-frequency relationship was obtained by increasing stimulation rates from 0.5 to 3.0 and back to 1 Hz. To measure sarcoplasmic reticulum characteristics, post-rest behavior was assessed by measuring force after rest intervals of 10 and 30 s at 1 Hz (20). Premature atrial contractions (PACs) were induced using high Ca^{2+} (5 mmol/l) or isoprenaline (Iso) (30 nmol/l). To induce Na^+ overload and diastolic dysfunction, we used 0.25 μmol/l ouabain (21).

Data analysis and statistics. Force values were normalized to cross-sectional areas of each trabeculae (width × thickness × π/4) and expressed as mN/mm^2. All data are expressed as mean ± SEM. Student *t* test, 2-way repeated-measures analysis of variance (ANOVA) with Holm-Sidak tests, 2-way ANOVA, or the Fisher exact test was used to test for significance where appropriate and as indicated in the figures ($p < 0.05$).

Table 2 Membrane Capacitances

Figure	Membrane Capacitance (pF)
1B	AF: 114 ± 5 vs. SR: 105 ± 5
1F	AF: 105 ± 12 vs. SR: 88 ± 5
2B	Vehicle/Ran 10 μmol/l: 90 ± 7 vs. Ran 20 μmol/l: 95 ± 19
2C	Vehicle: 78 ± 5 vs. Ran: 73 ± 10
2D	Vehicle: 105 ± 13 vs. Ran: 86 ± 6
2E	Vehicle: 114 ± 11 vs. Ran: 124 ± 11
3B	Vehicle: 89 ± 9 vs. Ran: 97 ± 10
3C	Vehicle: 114 ± 11 vs. Ran: 117 ± 12

No statistical significance could be obtained.
Abbreviations as in Tables 1 and 2.

Results

I_{Na} in AF versus SR. Peak and late I_{Na} were measured in atrial myocytes. The magnitude of peak I_{Na} over a broad range of stimulation frequencies was significantly smaller between both groups (2-way repeated-measures ANOVA) of atrial myocytes from patients with AF versus SR (Figs. 1A and 1B). Mean peak I_{Na} at 1 Hz was −71.5 ± 3.7 for AF versus −84.9 ± 3.1 A/F for SR ($p < 0.05$, Holm-Sidak). This ~16% smaller peak I_{Na} was accompanied by a reduced expression of the cardiac Na^+-channel isoform Nav1.5 by ~26% in AF (74 ± 6%) versus SR (100 ± 8%) ($p < 0.05$) (Figs. 1C and 1D).

In marked contrast, late I_{Na} (Figs. 1E and 1F) was significantly greater (~26% at 2 Hz, $p < 0.05$, Holm-Sidak) between both groups of myocytes from AF versus SR patients (2-way repeated-measures ANOVA) with a similar difference over the wide range of frequencies studied (Fig. 1F) ($p < 0.05$). Membrane capacitances of myocytes in both groups were not different for each dataset used (Table 2). Because late I_{Na} may also be due to altered expression of other Na^+-channel isoforms, 2 neuronal Na^+-channel isoforms were studied. An increase in Nav1.1 (by 59 ± 22%, $p < 0.05$) but no change in Nav1.6 protein expression (−7 ± 9%) in AF versus SR was observed (Figs. 1G and 1H). In summary, divergent regulation of peak versus late I_{Na} was found in AF versus SR patients.

Inhibition of I_{Na}. Paired experiments were performed with Ran to determine its effect on peak and late I_{Na}. Ran significantly inhibited peak I_{Na} in atrial myocytes from SR patients (Figs. 2A and 2B). To perform experiments at more physiological holding potentials, myocytes were held at −80 mV followed by a depolarization step to −30 mV (high $[Na^+]_o$) or −10 mV (low $[Na^+]_o$); at high $[Na^+]_o$ peak, I_{Na} was reduced by 15.0 ± 3.6% at 1 Hz and 22.2 ± 6.3% at 3 Hz ($p < 0.05$, n = 5 cells from 3 hearts each; data not shown) showing no difference to our observations at more negative holding potential (Figs. 2A and 2B). Experiments

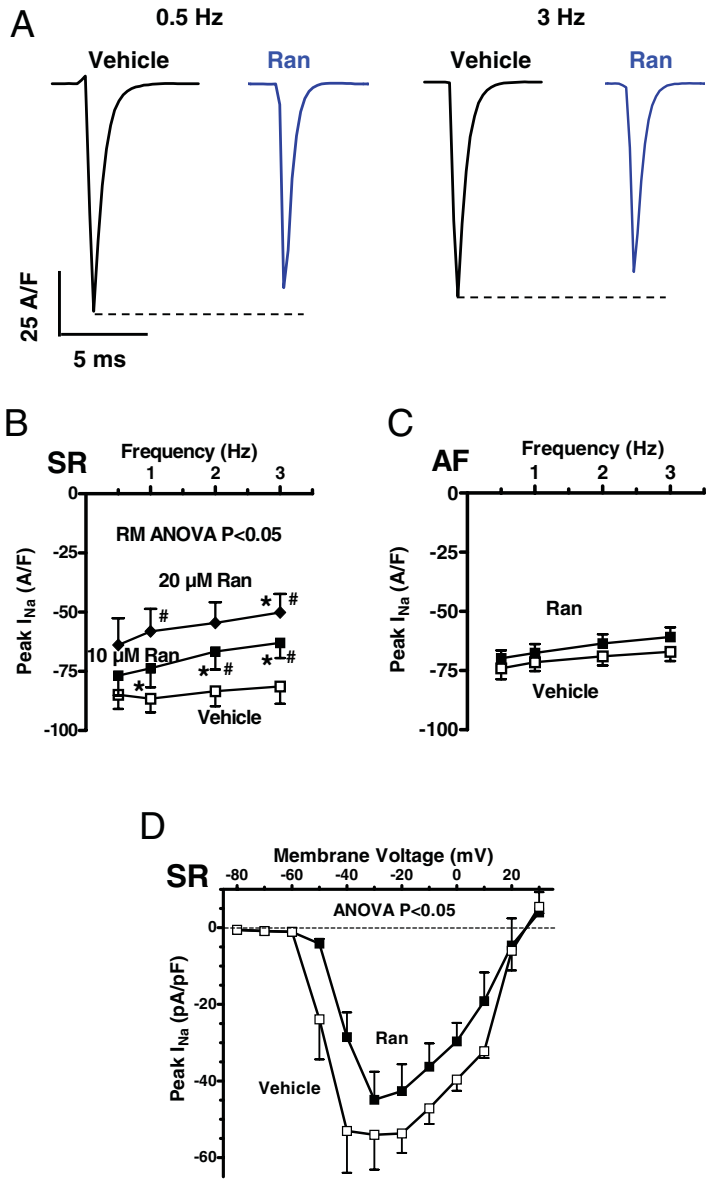

Figure 2 **Ranolazine and Peak I_{Na}**

(A) Original tracings in SR myocytes. **(B)** Mean peak I_{Na} in SR myocytes in the presence of 10 μmol/l (n = 7 cells from 5 hearts each, $p < 0.05$) and 20 μmol/l Ran (n = 6 cells from 3 hearts, $p < 0.05$). **(C)** Mean values in AF myocytes (ranolazine [Ran] vs. vehicle, n = 12 cells from 6 hearts vs. 18 cells from 9 hearts, p = NS). **(D)** Current-voltage curves in SR myocytes in the presence of 10 μmol/l Ran (n = 7 cells from 5 hearts each, $p < 0.05$). *$p < 0.05$ versus vehicle. #$p < 0.05$ versus baseline. Abbreviations as in Figure 1.

in low $[Na^+]_o$ similarly showed a significant inhibition of peak I_{Na} by Ran (1 Hz: −24 ± 2 for Ran vs. −34 ± 8 A/F for vehicle; 3 Hz: −21 ± 2 for Ran vs. −36 ± 8 ampere/farrad (A/F) for vehicle, $p < 0.05$; data not shown). In contrast, in myocytes from patients with AF, Ran reduced peak I_{Na} density only slightly, without statistical significance (Fig. 2C). In contrast, I-V relationships (Fig. 2D) in SR myocytes illustrate a typical I-V relationship in which Ran again reduced peak I_{Na} density ($p < 0.05$).

Late I_{Na} was found to be dramatically reduced by Ran in AF myocytes ($p < 0.05$) (Figs. 3A and 3C) in contrast to a much smaller effect in SR myocytes (Fig. 3B) ($p < 0.05$).

Ran inhibits PACs. To determine whether Ran exhibits antiarrhythmic effects, experiments were carried out in isolated trabeculae. Mean data (Fig. 4A) show that increasing $[Ca^{2+}]_o$ induced PACs in 32% (7 of 22) of vehicle-treated trabeculae, whereas only in 9% (2 of 21) of atrial trabeculae pre-treated with Ran. Original charts (Figs. 4C and 4D) show that application of Ran effectively suppresses

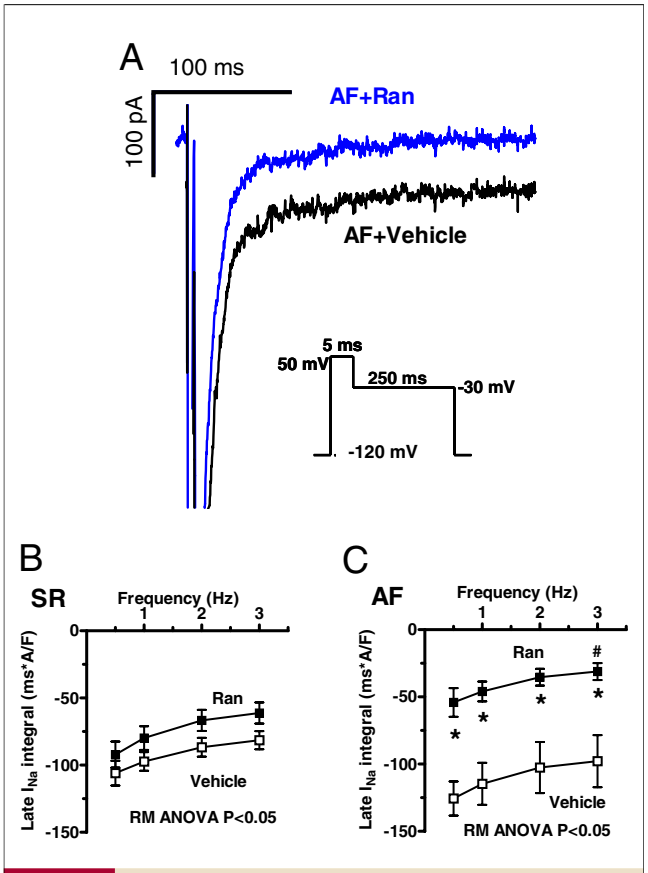

Figure 3 Ranolazine Inhibits Late I_{Na}

(A) Original recordings in an AF myocyte. **(B)** Mean late I_{Na} in SR myocytes (Ran vs. vehicle, n = 9 cells from 8 hearts vs. 30 cells from 16 hearts, $p < 0.05$). **(C)** Mean late I_{Na} in AF myocytes (Ran vs. vehicle, n = 10 cells from 5 hearts vs. 13 cells from 9 hearts, $p < 0.05$). *$p < 0.05$ versus vehicle. #$p < 0.05$ versus baseline. Abbreviations as in Figure 1.

PACs. Ran was found to suppress PACs in 5 of 6 trabeculae ($p < 0.05$), which corresponds to an 83% success rate for terminating PACs (Fig. 4B). In contrast, in vehicle-treated trabeculae, no PAC could be terminated (0 out of 6).

The incidence of Iso-induced PACs in vehicle- and Ran-treated trabeculae was 38% versus 14% (Fig. 5A). Ran was effective in converting PACs to a regular rhythm (7 of 7, $p < 0.05$) (Fig. 5B). Original tracings show that a PAC was abolished by Ran (Figs. 5C and 5D). In trabeculae from patients with AF (Figs. 5E and 5F), Ran was also effective in preventing (Fig. 5E) and terminating PACs (Fig. 5F). These data show that Ran also has antiarrhythmic properties in AF trabeculae.

Ran reduces atrial twitch amplitude concentration dependently. Increasing concentrations of Ran (6, 10, 15 μmol/l) reduced twitch amplitude ($p < 0.05$) in SR and AF trabeculae (Fig. 6). This effect was reversible on washout of the drug (Fig. 6B) (mean twitch amplitude in vehicle- and Ran-treated trabeculae was 3.1 ± 0.5 mN/mm^2 vs. 3.7 ± 0.6 mN/mm^2). Because Ran improves diastolic function in human ventricular failing myocardium (16), we investigated atrial contractile function during increasing stimulation frequencies.

Effects of Ran on contractile function during increasing frequencies. At all stimulation rates, a slight negative inotropic effect of Ran was observed (Fig. 7A). Ran reduces diastolic tension in SR trabeculae ($p < 0.05$) (Fig. 7B). Relaxation parameters were also assessed, but Ran only caused a small acceleration of relaxation time at 2 and 3 Hz (Table 3).

In line with this, sarcoplasmic reticulum function was not altered in atrial trabeculae treated with Ran as assessed by post-rest twitches (Figs. 7C and 7D).

Interestingly, we found that AF trabeculae (Fig. 7D) showed a slight positive force-frequency relationship. However, AF trabeculae generated twitches with lower amplitude compared with SR. The beneficial effect of Ran on diastolic tension was similar in atrial trabeculae from patients with AF (Fig. 7E) compared with SR (Fig. 7B).

Effects of Ca^{2+} and Iso on diastolic performance. The small negative inotropic effect of Ran was still present at high [Ca^{2+}] (Fig. 8A). Ran slightly reduced the increases in diastolic tension at higher [Ca^{2+}] (Fig. 8B). In Iso-treated trabeculae, force amplitude was significantly reduced by Ran at low Iso concentrations (Fig. 8C). Although force amplitude did not differ between Ran- and vehicle-treated trabeculae in the presence of high Iso concentrations, diastolic tension was significantly reduced by Ran (Fig. 8D). To induce Ca^{2+} overload and severe diastolic dysfunction by different means, ouabain was added to the bath solution (Fig. 8E). Ran significantly delayed the time to contracture ($p < 0.05$) (Fig. 8F). Taken together, the negative inotropic effect of Ran was associated with beneficial effects on diastolic performance under different "stress" conditions (i.e., increasing stimulation frequencies, Iso, or ouabain).

Discussion

The results show that permanent AF is associated with altered expression and function of Na^+ channels. Although Nav1.5 expression and peak I_{Na} density were significantly reduced in AF compared with SR, late I_{Na} was significantly greater as well as Nav1.1 expression. Ran reduced peak and late I_{Na} in SR, but it preferentially blocked late over peak I_{Na} in AF. Ran reduced Ca^{2+}- and Iso-induced PACs and caused a concentration-dependent and reversible negative inotropic effect associated with an improved diastolic tension at: 1) higher stimulation frequencies; 2) high [Ca^{2+}]$_o$; and 3) in the presence of Iso. Moreover, ouabain-induced diastolic contracture was attenuated by Ran.

Altered Na^+-channel expression and function in AF. AF is associated with changes in atrial function and structure (21–24) and electrical remodeling (3,4). Na^+ channels play a crucial role in cardiac excitation-contraction coupling by initiating the AP (25,26). The present study provides evidence that expression of Nav1.5 and peak I_{Na} density is

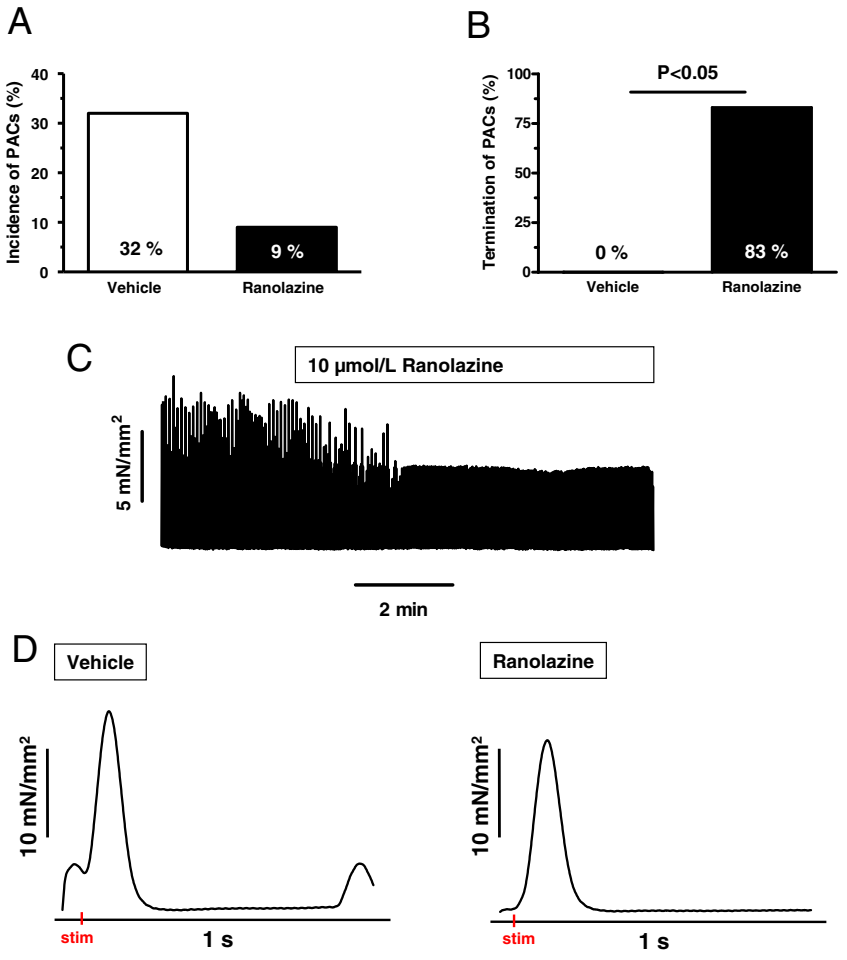

Figure 4 Ran Exhibits Antiarrhythmic Effects on Ca^{2+}-Induced PACs in SR Trabeculae

(A) Mean incidence of premature atrial contractions (PACs). **(B)** PACs could be terminated by Ran (5 of 6 trabeculae, $p < 0.05$). **(C)** Original recording of PACs. **(D)** Original tracings showing a PAC suppressed by Ran. **Red marks** indicate stimulus (stim). Abbreviations as in Figures 1 and 2.

decreased in the atrial myocardium of patients with AF. It is plausible that the decreased peak I_{Na} may be partly due to the down-regulation of Nav1.5 expression. Similar findings were reported by Yue et al. (27) showing a reduction of Na^+-channel α-subunit protein and mRNA expression in atrial myocardium of dogs with AF. This observation is in keeping with their previous findings of significantly less I_{Na} density in the same model (5), which has been confirmed in another AF dog model (6). Bosch et al. (28) reported that neither current density nor the I_{Na} voltage dependence was altered in human AF, although there was a trend toward a ~10% reduction in I_{Na} density. However, our 16% reduction in peak I_{Na} density is consistent with the decreased Na^+ channel protein expression of 26% and is in line with the results in dog AF models (5,27). It should be noted that we also recognize the difficulty in accurately measuring peak I_{Na} but have taken all precautions to correctly assess peak I_{Na}, as also shown previously (12,16).

In contrast, we found evidence that late I_{Na} is significantly increased in AF. To our knowledge, this is the first report of late I_{Na} in AF. There is increasing evidence that late I_{Na} plays a role in a number of cardiac diseases (16,29–36). Several possible explanations for the increase in late I_{Na} exist. One hypothesis is that the elevated Nav1.1 expression contributes to late I_{Na}. In line with this, Xi et al. (37) showed in a rat hypertrophy model that Nav1.1 and Nav1.6 contribute to elevated late I_{Na}. Another hypothesis is that calmodulin-dependent protein kinase II increases late I_{Na}. Calmodulin-dependent protein kinase II is found to be increased in AF (38) and known to regulate late I_{Na} (12,26). Alternatively, oxidative stress (39) may contribute to altered late I_{Na} (12,35).

An increase in late I_{Na} causes $[Na^+]_i$ to rise, which leads to cellular Ca^{2+} overload via the reverse-mode Na^+/Ca^{2+} exchanger (16) causing contractile dysfunction and electrical instability (29). Increased diastolic $[Ca^{2+}]$ would increase

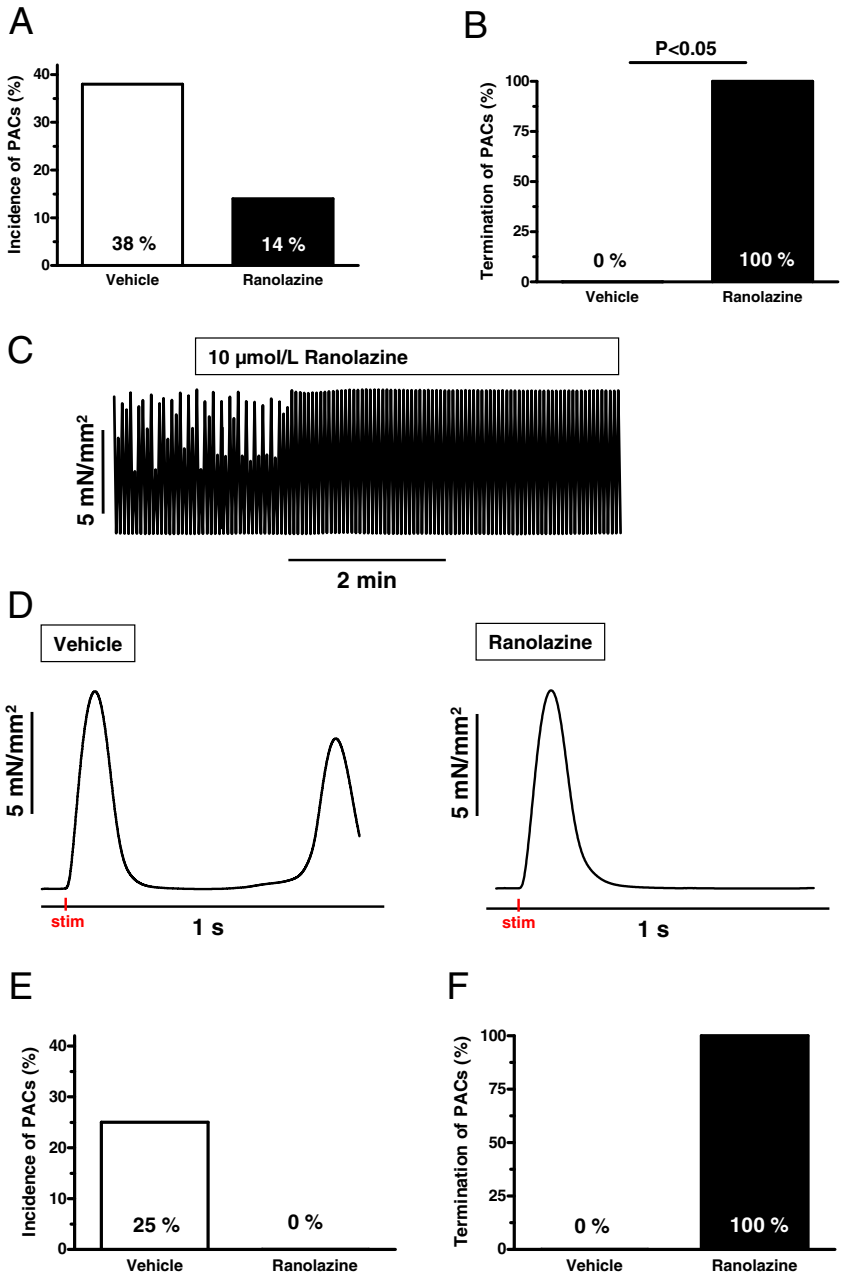

Figure 5 **Ran Exhibits Antiarrhythmic Effects on Iso-Induced PACs**

(A) Mean PACs in SR trabeculae. **(B)** PACs could be terminated by Ran (7 of 7, $p < 0.05$). **(C)** Representative tracing. **(D)** Twitch with Iso-induced PACs antagonized by Ran. Red marks indicate stimulus (stim). **(E)** Ran similarly exhibits antiarrhythmic effects in AF trabeculae: mean PACs (vehicle 2 of 8, Ran 0 of 12). **(F)** PACs could be terminated by Ran (2 of 2). Iso = isoprenaline; other abbreviations as in Figures 1, 2, and 4.

the open probability of the sarcoplasmic reticulum Ca^{2+}-release channel (ryanodine receptor [RyR]), augmenting spontaneous Ca^{2+}-release events. By removing Ca^{2+} from the cytosol, the Na^+/Ca^{2+} exchanger generates a transient inward current (I_{Ti}), giving rise to delayed afterdepolarizations and possibly to the PACs observed. A recent study in guinea-pig atrial myocytes demonstrated that increased late I_{Na} induced I_{Ti}, delayed after depolarizations, and sustained triggered activity (40). Typically, APD and effective refractory periods have been shown to be reduced in AF (41), thus promoting re-entry contributing to AF.

Ran differentially inhibits I_{Na}. In the present study, we found that peak I_{Na} is highly sensitive to inhibition of Ran in SR myocytes, but not in AF. On the contrary, the inhibition of late I_{Na} was minimal in SR but marked in AF. The finding in SR is surprising in view of the results of

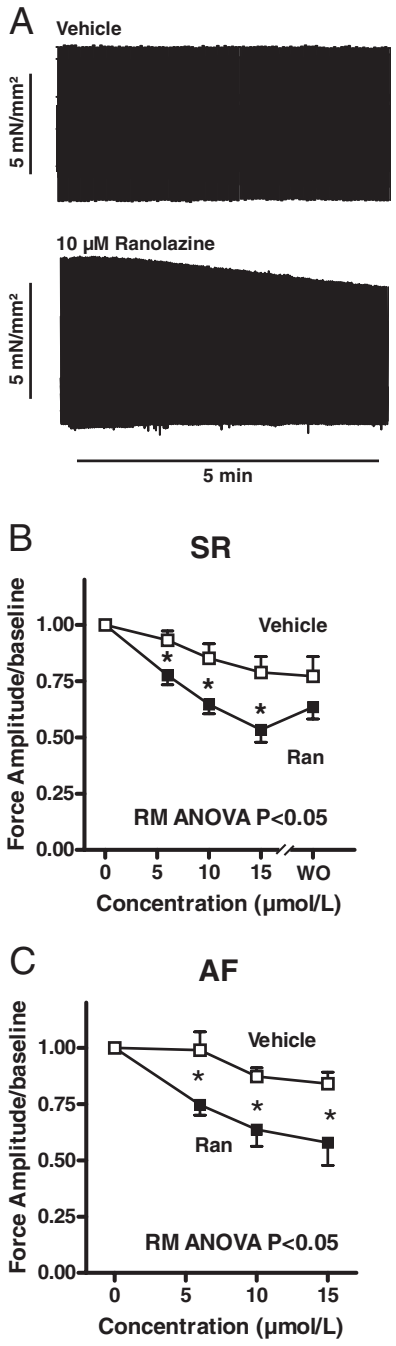

Figure 6 **Concentration-Dependent Effect of Ran on Force Amplitude**

(A) Original recordings in SR trabeculae. **(B)** Mean data showing a negative inotropic effect (vehicle vs. Ran n = 12 cells from 7 hearts vs. 12 cells from 10 hearts, $p < 0.05$) reversible on washout ($p = 0.2$ vehicle vs. Ran). **(C)** Mean data for AF trabeculae (vehicle vs. Ran n = 5 cells from 3 hearts vs. 6 cells from 3 hearts, $p < 0.05$). *$p < 0.05$ versus vehicle. Abbreviations as in Figures 1 and 2.

studies in ventricular myocytes showing higher selectivity of Ran to inhibit late I_{Na} over peak I_{Na} (31). Burashnikov et al. (18) reported on the atrial versus ventricular selectivity to inhibit peak I_{Na} but without quantifying the effect of Ran on late I_{Na} in atrial myocytes. Because Ran is known to act as an inactivated state blocker (31), a more depolarized resting membrane potential (18), a less steep repolarization phase in atria, and shorter diastolic intervals at rapid rates may account for the effect of Ran on peak I_{Na} (42). Consistent with this, our results show that at increased stimulation frequencies, Ran caused a greater inhibition of peak I_{Na}. However, other studies also suggested that Ran preferentially binds to open versus inactivated Na^+ channels (43,44).

The preferential inhibition of Ran on peak I_{Na} in SR versus AF myocytes may be attributed to a rightward shift of the steady-state inactivation curve of peak I_{Na} in AF (28). This may decrease the percentage of inactivated Na^+ channels and increase the fraction of resting Na^+ channels, reducing binding and promoting unbinding of Ran. Importantly, the smaller inhibition of peak I_{Na} by Ran in AF is desirable because the drug would be expected to cause less slowing of conduction velocity.

Which patients might clinically benefit from Ran? Inhibition of late I_{Na} would be expected to further shorten APD in AF. However, there are reports of APD prolongation leading to polymorphic atrial tachycardia that degenerates into AF (45). Similarly, APD prolongation has been reported in atria of patients with heart failure (46), dilated atria (47), atrial tachyarrhythmias having long QT syndromes 1 and 2 (48), and an Na^+ channel mutation responsible for long QT syndrome 3 and familial AF (49). In this latter study, flecainide shortened QT interval and terminated AF.

What may be the effect of Ran in most other AF patients with shortened APD? Of note, Burashnikov et al. (18) showed that Ran does not shorten APD but rather even slightly prolongs APD in dog atrial myocytes. This may have been due to the fact that Ran also inhibits I_{Kr} (14). Most importantly, these authors could suppress experimentally induced AF. Therefore, we believe that there may be also beneficial effects of Ran independent of APD (e.g., by decreasing Na^+ and Ca^{2+} overload).

Ran terminates Iso- and Ca^{2+}-induced PACs. Ran was found to prevent and suppress Ca^{2+}- and Iso-induced PACs. This effect may be explained by 2 mechanisms: first, the inhibition of Na^+ channels, I_{Kr}, and, to a lesser extent, late I_{Ca} (14); and second, Ran inhibits late I_{Na}, which should reduce intracellular $[Na^+]$ and consequently cytosolic $[Ca^{2+}]$ via the Na^+/Ca^{2+} exchanger. This would result in reduced sarcoplasmic reticulum Ca^{2+} load and reduced RyR open probability, rendering spontaneous sarcoplasmic reticulum Ca^{2+} release and I_{TI} less likely. Both Iso and increased $[Ca^{2+}]_o$ have the opposite effect; they increase sarcoplasmic reticulum Ca^{2+} load and spontaneous Ca^{2+} release and thereby the likelihood of I_{TI}. The antiarrhythmic effects of Ran observed are consistent with abolished early afterdepolarizations, delayed afterdepolarizations, and triggered activity induced

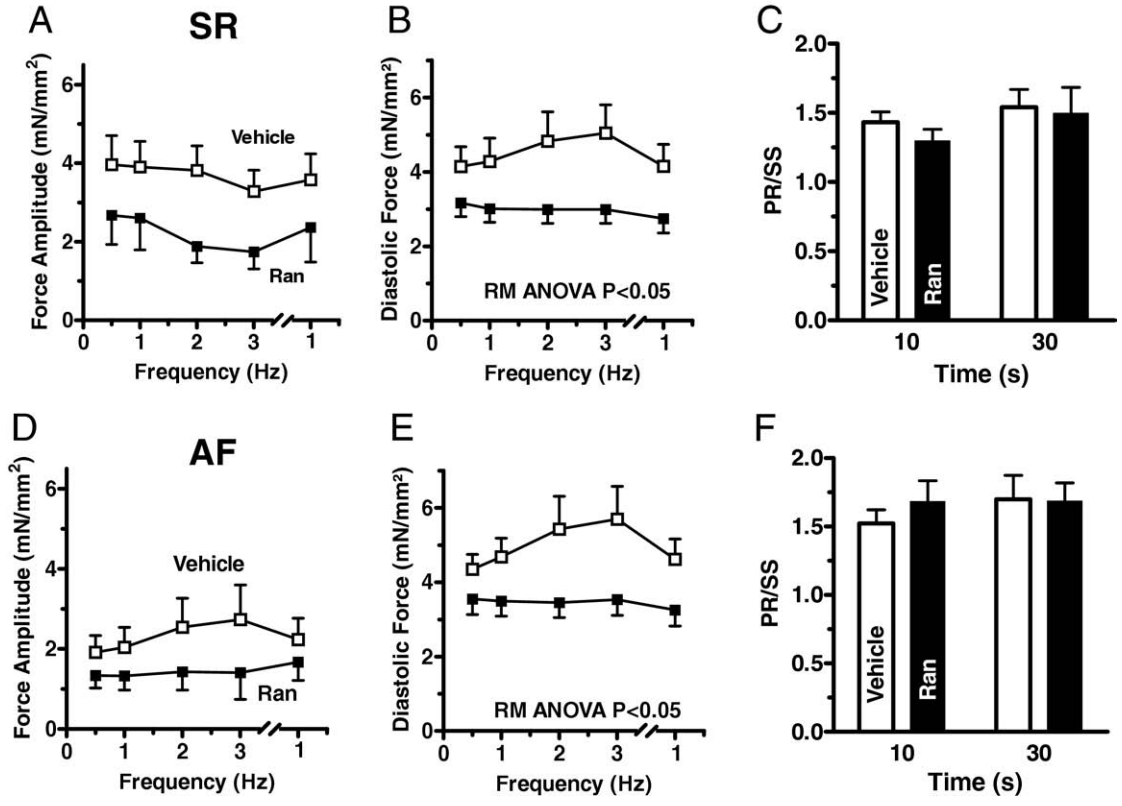

Figure 7 **Effect of Ran on Contractile Behavior During Increasing Stimulation Rates**

(A) Mean force amplitude for SR (n = 24 cells from 20 hearts vs. 23 cells from 21 hearts). **(B)** Average diastolic force. **(C)** Mean values of post-rest twitches (PR) normalized steady-state (SS) contraction. **(D)** Mean force amplitude of AF trabeculae (n = 11 cells from 8 hearts each). **(E)** Average diastolic force. **(F)** Mean values of post-rest twitches (n = 7 cells from 5 hearts vs. 9 cells from 6 hearts). Abbreviations as in Figures 1 and 2.

by late I_{Na} (40). In a small study, Ran was reported to maintain SR in patients with resistant AF (50). The inhibitory effects of Ran on late I_{Na} and I_{Kr} are likely to play an important role in the antiarrhythmic properties in the atrium.

Effects of Ran on atrial contractility. Ran reduced atrial contractility. Several antiarrhythmic agents depress ventricular contractility. This is an undesirable effect, especially in patients with reduced left ventricular function. In contrast, Ran exerts no negative inotropic effect on the left ventricular myocardium (16,51). Regardless, the difference between ventricular and atrial myocardial responsiveness to Ran might be explained by the distinct electrophysiological properties of both tissues.

Ran reduced the increase in diastolic tension associated with fast stimulation rates in SR and AF trabeculae and also during stress conditions. This suggests that Na^+ overload and hence Ca^{2+} overload may contribute to diastolic dysfunction in atrial trabeculae, even if others found preserved atrial relaxation and unimpaired diastolic function in AF (52). Because many

Table 3 **Twitch Parameters of Trabeculae From Patients in Sinus Rhythm**

	Frequency (Hz)			
	0.5	1	2	3
TTP Ran, ms	143 ± 7	151 ± 10	140 ± 8	135 ± 9
TTP vehicle, ms	142 ± 8	147 ± 12	148 ± 14	131 ± 5
$RT_{50\%}$ Ran, ms	69 ± 4	75 ± 10	67 ± 3	63 ± 3
$RT_{50\%}$ vehicle, ms	67 ± 4	70 ± 5	71 ± 6	71 ± 6
$RT_{90\%}$ Ran, ms	175 ± 13	175 ± 14	153 ± 11	143 ± 8
$RT_{90\%}$ vehicle, ms	168 ± 15	164 ± 11	162 ± 13	148 ± 7

Ran = ranolazine; $RT_{50\%}$ = time to 50% relaxation; $RT_{90\%}$ = time to 90% relaxation; TTP = time to maximum force peak.

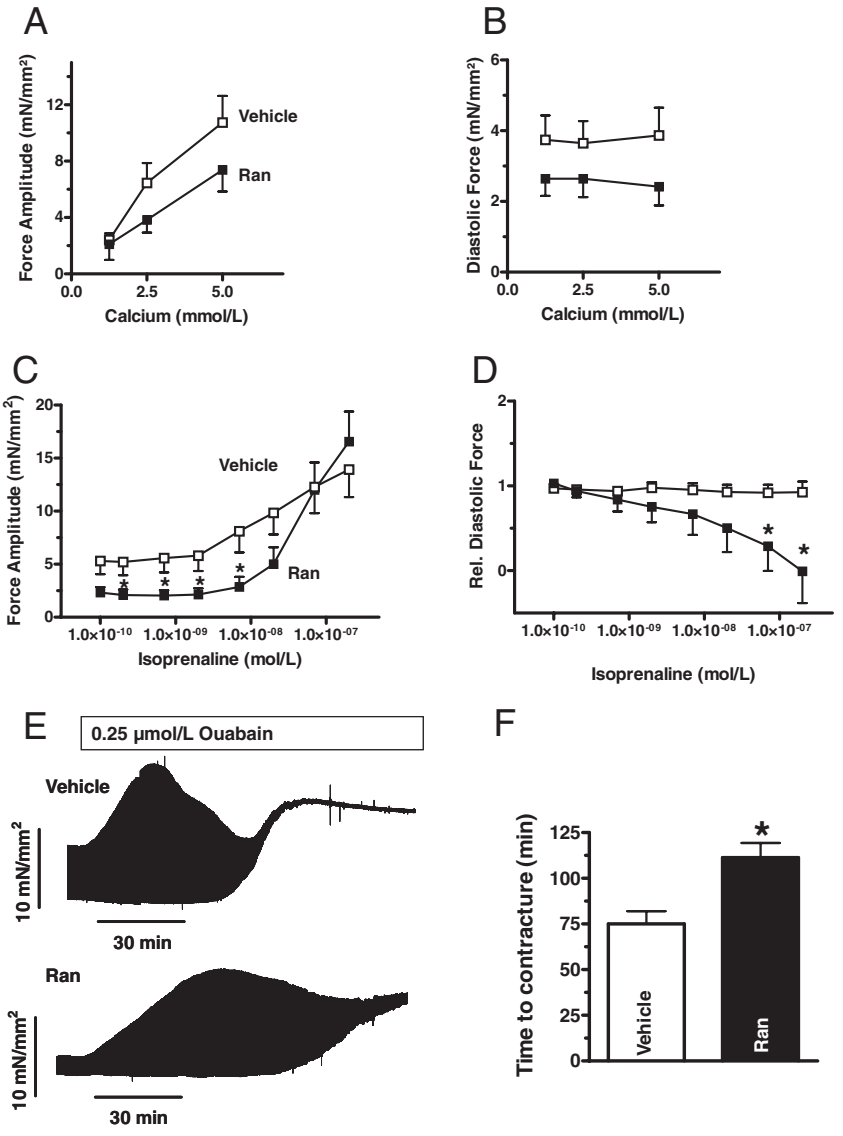

Figure 8 Contractile Behavior in the Presence of Ran During Increasing Concentrations of Ca^{2+}, Iso, or Ouabain

(A) Mean data of a Ca^{2+} concentration-response curve (vehicle vs. Ran, n = 18 cells from 16 hearts vs. 17 cells from 15 hearts). **(B)** Diastolic force. **(C)** Average force during increasing concentrations of Iso (vehicle vs. Ran, n = 11 cells from 5 hearts vs. 13 cells from 8 hearts). **(D)** Diastolic force. **(E)** Representative tracings of ouabain-induced diastolic dysfunction of trabeculae. **(F)** Mean values of time to contracture (vehicle vs. Ran, n = 5 cells from 4 hearts vs. 6 cells from 4 hearts, $p < 0.05$). *$p < 0.05$ versus vehicle. Rel. = relative; other abbreviations as in Figure 2.

features of atrial remodeling could be a consequence of load-dependent signaling pathways, this observation may be of interest. The reduction of load due to the decrease in diastolic tension may slow or even reverse atrial remodeling. Decreased diastolic tension could also reduce sarcoplasmic reticulum Ca^{2+} load and the likelihood of I_{TI} due to changes in AP morphology (53).

Conclusions

Recent studies revealed a potential role for Na^+ channels in the pathogenesis of AF (6,7). The results of the present study show that alterations of Na^+-channel expression and function occur in the atrial myocardium of patients with AF. This may represent an additional mechanism for AF or may simply be part of a well-known constellation of ion channel dysregulation that leads to changes in AP morphology and contractility (53). Ran restores the physiological relationship between peak and late I_{Na} and consequently suppresses known proarrhythmogenic mechanisms in vitro. This points to a potentially new therapeutic benefit of this drug with fewer side effects on the ventricular myocardium of patients with atrial rhythm disorders. The effect of Ran to reduce diastolic tension provides a rationale for further studies in animal models of diastolic dysfunction.

One major limitation is that only right atrial appendages were used instead of more relevant left atrial tissue. It is generally accepted that right atrial appendages do not significantly contribute to the initiation or perpetuation of AF. Therefore, one should not extrapolate our findings for AF in general. It is unclear whether the cellular proarrhythmic mechanism described here is applicable to the clinical situation whereby a left atrial re-entry mechanism is a key mechanism underlying AF. The fact that Ran improves diastolic tension and also suppresses arrhythmogenic events in AF and SR trabeculae questions whether late I_{Na} inhibition solely contributes to these beneficial effects. Nevertheless, these novel findings should trigger further studies.

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Key Words: antiarrhythmia agents ■ atrial fibrillation ■ diastolic function ■ sodium channels.