Experimental studies of atrial fibrillation: a comparison of two pacing models

Gabriel Laurent,1,2 Gordon Moe,1,2* Xu dong Hu,1,2 Howard Leong-Poi,2,3 Kim A. Connelly,2,3,4 Petsy Pui-Sze So,5 Ramadeen A, Doumanovskaia,1,2 Konig A, Trogadis J, Courtman D, Strauss B, Dorian P. Experimental studies of atrial fibrillation: a comparison of two pacing models. Am J Physiol Heart Circ Physiol 294: H1206–H1215, 2008. First published January 4, 2008; doi:10.1152/ajpheart.00999.2007.—Rapid ventricular pacing (RVP) is a well-established animal model of atrial fibrillation (AF). However, this model is limited by a high mortality rate and severe heart failure. The purpose of our study was to assess a new canine model of inducible AF. We performed acute, short-term, simultaneous atrioventricular pacing (SAVP) and RVP (in random order) in 14 dogs for 30 s. SAVP produced more echocardiographic pulmonary venous flow reversal, a greater increase in mean pulmonary capillary wedge pressure, and a significantly greater decrease in left atrial emptying function (84.4 ± 38.6% vs. 23.7 ± 27.1%, P < 0.05) than RVP. Thirty dogs were randomized to three, longer-term, study groups: eight dogs in the control group (no pacing), eight dogs in the RVP group (2 wk at 240 beats/min followed by 3 wk at 220 beats/min), and fourteen dogs in the SAVP group (2 wk at 220 beats/min). SAVP induced less left ventricular dysfunction but more left atrial dysfunction than RVP. SAVP dogs had similar atrial effective refractory periods as RVP dogs but more heterogeneity in conduction and more AF inducibility (83% vs. 40%, P < 0.05) and maintenance (median 1,660 vs. 710 s, P < 0.05) than RVP dogs. SAVP induced more collagen turnover and was associated with a significantly greater increase in type III collagen in the atria compared with RVP dogs (1.5 vs. 4.8 ± 1.1 and 1.6 ± 0.7, respectively, P < 0.05 vs. 1.1 ± 0.7 in un paced control dogs). In conclusion, the SAVP model induced profound mechanical and substrate atrial remodeling and reproducible sustained AF. This new model is clinically relevant and may be useful for testing AF interventions.

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Experimental atrial arrhythmia; extracellular matrix; remodeling

ATRIAL FIBRILLATION (AF) is the most common sustained cardiac arrhythmia and frequently causes cardiovascular morbidity and mortality. Although AF can occur in patients with normal hearts (lone AF), the majority of patients with AF have coexisting structural heart disease, most commonly hypertension or heart failure (1). To better understand the relationship between the atrial substrate and AF vulnerability and to evaluate therapies for AF, multiple animal models of AF have been developed. They include sterile pericarditis, acute atrial ischemia, atrial volume overload, mitral regurgitation, pacemaker-induced atrial tachycardia, and ventricular pacing-induced heart failure (25).

One of the most commonly used models employs rapid atrial pacing (RAP) at 400 beats/min with control of the ventricular response (atrioventricular node ablation) (24). This model helps to understand the mechanisms underlying the tendency of paroxysmal AF to become persistent but does not relate closely to the most frequent clinical conditions associated with AF, in which the left atrium (LA) dilates and is fibrosed, exhibiting conduction slowing (14, 21). The other commonly used model is rapid ventricular pacing (RVP) to induce heart failure (240–280 beats/min for 3–8 wk), sometimes combined with RAP (RVP at 240 beats/min for 2 wk combined with additional RAP at 400 beats/min during the second week) (5). This model mimics heart failure–related AF associated with regional atrial conduction slowing due to increased collagen turnover and interstitial fibrosis (16). However, it also results in substantial interanimal variability in AF production and in overt congestive heart failure, occasionally leading to premature death (prior to completion of the protocol) (11).

An ideal AF model should be simple, clinically relevant (i.e., simulate human disease), and allow for reliable sustained AF production and thereby sensitivity to intervention (25).

Based on these considerations and inspired by observations made in humans that a very short ventriculoatrial interval (during reciprocating tachycardia) can cause a marked increase in atrial pressure by inducing atrial contractions during mitral valve closure (18, 20, 31), we developed a fast simultaneous atrioventricular pacing (SAVP) model. This new model was designed to produce hemodynamic consequences that have been shown to lead to atrial electrical and structural remodeling and AF vulnerability (15).

The purpose of this study was to compare the new SAVP dog model to a “standard” RVP model. We hypothesized that SAVP at a moderate rate of 220 beats/min would induce more atrial than ventricular mechanical remodeling and less overt congestive heart failure than the RVP model, yet more AF vulnerability.

METHODS

Thirty adult mongrel dogs were randomly assigned to three groups. Fourteen dogs were included in an acute temporary pacing study, including an echocardiographic Doppler analysis (n = 12) and a...
hemodynamic study (n = 4) while pacing the right ventricle (RVP) or both chambers simultaneously (SAVP) at 220 beats/min (in random order) for 30 s. These 14 dogs became part of a chronic pacing study and served as the SAVP group (220 beats/min for 2 wk). Eight other dogs were stimulated only in the right ventricle (RVP group) at 240 beats/min for 2 wk followed by pacing at 220 beats/min for an additional 3 wk. A third group of nonpaced normal dogs served as controls (Ctrl group, n = 8). The protocol was approved by the Animal Care Committee of St. Michael’s Hospital. The investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, Revised 1996).

Pacemaker Implantation

Dogs were premedicated with buprenorphine (0.10 mg/kg), acepromazine (0.10 mg/kg), and atropine (0.05 mg/kg) administered subcutaneously, anesthetized with pentobarbital sodium (30 mg/kg iv), additional doses of 4 mg/kg as needed), and maintained with 1–2% isoflurane. Respiration was maintained via an endotracheal tube and a mechanical ventilator. With aseptic surgical techniques, an incision was made in the lateral aspect of the neck to expose the right external jugular vein.

Under fluoroscopic guidance, two bipolar screw-in pacing leads (Tendril SDX, St. Jude Medical, St. Paul, MN) were fixed in the right atrial (RA) appendage and right ventricular apex of the SAVP group of dogs. The leads were then connected to a “Y connector” and a SSI pacemaker (model 5156 Verity ADx XL SR, St. Jude Medical) in a subcutaneous pocket in the neck. For the RVP group, a single active-fixation (screw-in lead) or passive-fixation lead (tined) (Medtronic, Minneapolis, MN) was placed at the apex of the right ventricle and connected to a VVI pacemaker unit (model 8084, Medtronic, Minneapolis, MN) under the same conditions.

Acute Temporary Pacing Study

In the first group of 14 dogs, leads were connected via temporary wires to a Programmer analyzer (Medtronic). Temporary RVP (220 beats/min) or SAVP at 220 beats/min in a 270 ms cycle length (CL), 1.0-ms pulse duration at two times threshold current (n = 4) while pacing the right ventricle (RVP) or both chambers simultaneously (SAVP) at 220 beats/min (in random order) for 30 s. These 14 dogs became part of a chronic pacing study and served as the SAVP group (220 beats/min for 2 wk). Eight other dogs were stimulated only in the right ventricle (RVP group) at 240 beats/min for 2 wk followed by pacing at 220 beats/min for an additional 3 wk. A third group of nonpaced normal dogs served as controls (Ctrl group, n = 8). The protocol was approved by the Animal Care Committee of St. Michael’s Hospital. The investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, Revised 1996).

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Hemodynamic study. A double-lumen 7.5-Fr Swan-Ganz catheter (Edwards Lifesciences LLC, Irvine, CA) was advanced via the external left jugular vein to the pulmonary artery for measurements of RA, pulmonary artery, and mean pulmonary capillary wedge pressures (PCWP) (simultaneously with the echocardiographic data acquisition at baseline and 30 s after the initiation of the two pacing modes). The catheter was connected to two pressure transducers with 0-mmHg pressure set at the level of the mid-RA. The PCWP was used to estimate LA pressure (10). After the study had been completed, the catheter was removed, and the pacemaker electrodes were reconnected via a Y-connector to the pacemaker.

Chronic Pacing Study

Dogs were then free of stimulation during the following week to allow for recovery from the surgery. In the SAVP group, pacemakers were programmed at 220 beats/min with 1.0-ms pulse duration at three times the highest threshold current of both chambers to capture the RA and right ventricle simultaneously. Pacemakers of the RVP group were programmed at 240 beats/min for the first 2 wk, and, for the remaining 3 wk, the rate was reduced to 220 beats/min. Clinical evaluations were conducted on a daily basis, and telemetry was repeated every 2 days to confirm continuing ventricular and atrial capture.

Transthoracic echocardiographic study. Two-dimensional trans-thoracic echocardiograms were performed (at baseline 1 wk after surgery and at the end of the pacing protocol) in normal sinus rhythm (15–20 min after the pacemaker had been turned off) on standing conscious dogs using a phased array transducer (S3, Philips Ultrasound, Bothell, WA). The two-dimensional left ventricular (LV) area was planimetered; LV diastolic areas (LVDAs) and LV systolic areas (LVSA) were taken as the maximum and minimum cavity dimensions, respectively, from two-chamber short-axis views. LV endocardial tracings were drawn to include the papillary muscles inside the outlines. The LV shortening fraction area was calculated as follows: 100 × (LVDAs − LVSA)/LVDA. LA diastolic areas (LADAs) and LA systolic areas (LASA) were obtained at the plane of aortic valve (parasternal short-axis view), incorporating the area defined by both the LA free wall and appendage. The LA emptying function was defined as the LA shortening fraction area as follows: 100 × (LADAs − LASA)/LADA.

Electrophysiological study. After the complete pacing protocol had been achieved (2 wk of SAVP or 5 wk of RVP), premedicated dogs were anesthetized with intravenous propofol (10 mg/ml, 2.5–3.5 mg/kg) and maintained under isoflurane (1–2%). The pacemaker was turned off 30 min before anesthesia. Animals were then intubated and ventilated using a Harvard respirator (Harvard Apparatus, Holliston, MA). Body temperature was maintained (37°C) with a circulating water system (humidifier) and heating carpet. A median sternotomy was performed in all 27 surviving dogs (8 Ctrl, 14 SAVP, and 5 RVP dogs), and 5 bipolar stainless steel epicardial electrodes were sewn at the same atrial sites for every dog as follows: high and low RA, LA appendages, and the posterior wall of the LA. In 20 dogs (5 Ctrl, 12 SAVP, and 3 RVP dogs), an additional “clock-face electrode” (16 peripheral unipolar electrodes, all equidistant from each other and from a central bipolar electrode, radius 7.5 mm; Fig. 1) was sutured to the epicardium of the posterior wall of the LA. This
electrode allowed the calculation of the atrial conduction velocity independent of orientation, between the atrioventricular groove and inferior PV ostium. Intracardiac electrograms were recorded in the bipolar mode at a filter setting of 30–300 Hz (bipolar) and in the unipolar mode (0.05–300 Hz) and were stored digitally on a custom acquisition system (AQUI 2, Cartesian Labs, Toronto, ON, Canada).

Atrial effective refractory periods (AERPs) were measured at each of the five sites (high and low RA, RA and LA appendages, and the posterior wall of the LA) at twice the stimulation threshold as follows: to achieve a steady state, we paced, continuously at each site, for 30 s at 400 and 200 ms CL, before measuring AERPs at that CL. An atrial extrastimulus was then introduced after every eighth drive beat to avoid any pauses to alter the heart rate sequence. This way, the first extrastimulus that captured the atrium occurred after >30 s of uninterrupted pacing. The first extrastimulus coupling interval was set at 120 ms (for 400 ms CL), 100 ms (for 300 ms CL), or 80 ms (for 200 ms CL). The coupling interval was increased by 10 ms, after every eighth beat, until the extrastimulus resulted in atrial capture. The coupling interval was then reduced by 10 ms and increased in 2-ms steps until the extrastimulus captured the atria again. AERP was defined as the longest S1S2 coupling interval that failed to result in atrial capture. Atrial conduction was measured after a stable 30-s period at twice the diastolic threshold and two CLs of stimulation (400 and 200 ms CL). Global conduction times were defined as the time delay between the stimulus artefact at the pacing site and local activation at each of four unipolar recording sites (maximum negative peak of the change in voltage over time) and averaged to calculate global conduction. Each conduction time value corresponded to an average of five consecutive beats and was calculated for each of the five pacing sites.

Using the clock-face electrode, local atrial conduction properties at the posterior wall of the LA were expressed as follows: the average conduction velocity, conduction time heterogeneity (corresponding to the difference between the shortest (fastest) and the longest (slowest) conduction times) and conduction delay were calculated for each of the five pacing sites.

![Fig. 2. Representative pulmonary venous Doppler tracings in one dog at baseline (A), 30 s after simultaneous atrioventricular pacing (SAVP; B), and 30 s after rapid ventricular pacing (RVP; C). At baseline in sinus rhythm, the Doppler pulmonary venous flow profile showed positive waves during systole (S) and early diastole (D) and a small negative atrial flow reversal (AFR) wave. After the initiation of SAVP, there were giant systolic flow reversal (SFR) waves after each contraction. After the initiation of RVP, retrograde P waves (arrows) appeared in a 2:1 ventriculoatrial conduction relationship. Corresponding to systolic P waves, there were prominent SFRs.](image)

Fig. 3. Representative pulmonary venous Doppler tracings in one dog at baseline (A), 30 s after SAVP (B), and 30 s after RVP (C). At baseline in sinus rhythm, the Doppler pulmonary venous flow profile showed positive waves during systole and early diastole and a small negative AFR wave. After the initiation of SAVP, there were giant SFR waves after each contraction. After the initiation of RVP, P waves (arrows) appeared randomly because of a complete ventriculoatrial dissociation. When P waves appeared during systole, prominent SFRs were observed; when P waves appeared during diastole, there were small AFRs followed by forward systolic and early diastolic pulmonary venous flows.
atrial conduction time], and the conduction anisotropic index. Based on prior descriptions of anisotropic conduction properties in canine atria, we derived this new index, which is the ratio of the fastest conduction velocity divided by the slowest conduction velocity (19). Local partial or complete atrial block in this area was also recorded when 30 s of burst pacing at 150 ms CL was applied.

AF induction. Bursts of atrial pacing (10 V, 10-ms pulse duration at 10 Hz for 10 s) were applied 10 times at each of the 5 sites. An AF episode was defined as an irregular supraventricular arrhythmia with A-A intervals (distance between two consecutive atrial electrograms) of <150 ms and lasting >1 min. The tendency to develop and maintain AF in this model was defined in two different ways as follows.

First, the ability to induce AF was defined as the percentage of dogs with J) at least one AF episode during AERP measurements (induced by a single extrastimulus) and 2) the percentage of burst attempts leading to AF episodes (percentage of attempts leading to AF).

Second, the ability to maintain AF was defined as J) the median AF duration per dog (expressed as median and 25th-75th percentile) and 2) the percentage of dogs with at least one AF episode lasting >10 min. AF episodes lasting >15 min were cardioverted using an external biphasic defibrillator (LIFEPAK12, Medtronic).

AF CL measurements. Mean AF CL corresponded to the mean value of 30 consecutive atrial electrogram intervals recorded after the first 60 s of the longest AF episode observed in each dog.

Gelatinase Activity Assays

Gelatin zymography (23) was done on tissue samples from LA and RA appendages of all dogs. Clear gelatinolytic zones were quantified by densitometric analysis using Molecular Analyst software (Bio-RAD Laboratories, Hercules, CA) and were expressed as a percentage of the value in Ctrl dogs.

Histology

Transmural tissue samples from RA and LA appendages of 6 Ctrl, 5 RVP, and 5 SAVP dogs were fixed in 10% neutral buffered formalin. Tissue was processed, embedded in paraffin, and sectioned in 4- to 5-μm-thick sections. RA and LA appendage cross sections were stained with picrosirius red to visualize collagen. The intensity range and within regions of interest (tissue inner folds) were counted. Connective tissue was expressed as a percentage of the reference tissue area, representing the fractional amount of total collagen.

Collagen and TGF-β1 mRNA Quantification Using Quantitative RT-PCR

Frozen transmural tissue samples (LA appendages) from five CTRL, five RVP, and five SAVP dogs were collected. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. cDNA was synthesized from DNase-treated total RNA samples by reverse transcription with a High-Capacity cDNA Archive Kit (Applied Biosystems Group, Foster City, CA) according to the manufacturer’s protocol. PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems Group). The increase in fluorescence of the SYBR green dye was monitored using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems Group). Relative mRNA levels in each sample were normalized to 18S content in the sample and expressed as fold change with respect to a housekeeping gene. Cycle parameters were 55°C × 5 min and 95°C × 10 min and then 40 cycles of 95°C × 15 s and 60°C × 60 s. Nucleotide sequences of the primers and probes were as follows: collagen I, forward 5′-GTG TGT ACA GAA CGG CCT CA-3′ and reverse 5′-TGG TGG GGG TGA AGA TAC-3′; and TGF-β, forward 5′-ATA GGT GCT TTT AGG GAC GAA-3′ and reverse 5′-AGT CCT TCA CCA GGA GC-3′; and TGF-β, forward 5′-CAA GGA TCT GTG CGT GAA GTG GA-3′ and reverse 5′-CCA GGA CCT TGC ACT GCG TGT-3′.

Data Analysis

Echocardiographic and electrophysiological measurements obtained during this study were analyzed using one-way ANOVA with repeated measures for comparisons between groups. If parameters were different, a Tukey’s post hoc analysis was done to compare each group to another. Proportions of dogs with inducible AF were com-

Table 1. Acute temporary pacing study

<table>
<thead>
<tr>
<th></th>
<th>Mean Values</th>
<th>Percent Changes</th>
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<td>Baseline</td>
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<td>RVP</td>
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<tr>
<td>Echocardiographic parameters</td>
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<tr>
<td>LASA, cm²</td>
<td>6.2±1.0</td>
<td>7.0±0.6</td>
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<td>LADA, cm²</td>
<td>9.6±1.4</td>
<td>10.9±1.3</td>
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<tr>
<td>LAFAS, %</td>
<td>39.7±7.4</td>
<td>36.2±5.4</td>
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<tr>
<td>Doppler parameters</td>
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<tr>
<td>Peak velocity of SFR, cm/s</td>
<td>12.5±4.1</td>
<td>24.3±7.5</td>
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<tr>
<td>Velocity time interval of SFR, cm/s</td>
<td>1.1±0.6</td>
<td>1.0±1.3</td>
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Values are means ± SD; n = 14 dogs/group. Echocardiographic Doppler changes from baseline are shown. RVP, rapid ventricular pacing (220 beats/min for 30 s); SAVP, simultaneous atrioventricular pacing (220 beats/min for 30 s); LA, left atrial; LASA, LA systolic area; LADA, LA diastolic area; LAFAS, LA fractional area shortening; SFR, systolic flow reversal. *P < 0.05, SAVP vs. RVP percent changes.

Table 2. Acute temporary pacing study

<table>
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<tr>
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<th>Mean Values</th>
<th>Percent Changes</th>
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<tr>
<td></td>
<td>RVP</td>
<td>SAVP</td>
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<tr>
<td></td>
<td>RVP</td>
<td>SAVP</td>
</tr>
<tr>
<td>RA pressure</td>
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</tr>
<tr>
<td>Peak mmHg</td>
<td>13.5±3.5</td>
<td>12.8±2.9</td>
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<tr>
<td>Mean mmHg</td>
<td>10.5±2.7</td>
<td>9.8±1.9</td>
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<td>PCWP</td>
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<tr>
<td>Peak mmHg</td>
<td>14.5±3.2</td>
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<tr>
<td>Mean mmHg</td>
<td>11.8±1.9</td>
<td>14.6±2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 4 dogs/group. Mean values are values at baseline; percent changes are changes after 30 s of rapid pacing. RA, right atrial; PCWP, pulmonary capillary wedge pressure. *P < 0.05, SAVP vs. RVP percent changes.
pared using a \( \chi^2 \)-test. A Kruskal-Wallis test was used to compare nonnormally distributed variables between groups. Continuous variables were expressed as means ± SD or medians (25th-75th percentile) and compared using the unpaired Student’s \( t \)-test. The \( \chi^2 \)-test was used for parametric tests on proportions. A value of \( P < 0.05 \) was considered significant.

RESULTS

Acute Temporary Pacing Study

Changes in transoesophageal echocardiographic Doppler parameters. At baseline, in sinus rhythm, Doppler PV flows profile usually showed positive waves during ventricular systole and early diastole along with inconsistent small atrial flow reversal (AFR) waves during atrial systole (Figs. 2A and 3A). In contrast to sinus rhythm, there were significantly greater PV flow reversal waves (velocity time integral and peak velocity) with different patterns after SAVP compared with RVP (Table 1). SAVP was characterized by giant PV systolic flow reversal (SFR) with a 1:1 ventriculoatrial association (Figs. 2B and 3B), whereas during RVP, a variable magnitude lower PV SFR was observed, depending on the ventriculoatrial timing (Figs. 2C and 3C). After 30 s of rapid pacing, the LA dilated and its function decreased (Table 1). The mean LA diastolic surface was similarly increased after SAVP and RVP, whereas the LA systolic surface was more enlarged after SAVP than RVP, corresponding to a significantly greater decrease in LA emptying function (LA fractional area shortening) in RVP dogs (54.9 ± 10.2%) than SAVP dogs (25.7 ± 17.9%; \( P < 0.05 \)). In contrast, SAVP induced a greater increase in mean and peak PCWP than RVP (Fig. 4).

Chronic Pacing Study

Changes in transthoracic echocardiographic parameters. Echocardiographic parameters at baseline were similar between the three groups (Table 3). After 2 wk of stimulation for SAVP dogs and 5 wk for RVP dogs, we observed a greater decrease in LV systolic function (LV fractional area shortening) in RVP dogs (54.9 ± 10.2%) than SAVP dogs (25.7 ± 17.9%; \( P < 0.05 \)). In contrast, SAVP dogs had a greater decrease in LA emptying function (LA fractional area shortening; -45.4 ± 13.3%) than RVP dogs (25.9 ± 30.4%, \( P < 0.05 \); Table 4).

Table 4. Changes in echocardiographic parameters after completion of the chronic pacing study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RVP</th>
<th>SAVP</th>
<th>Percent Changes</th>
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<tbody>
<tr>
<td>LASA, %</td>
<td>12.7 ± 1.6</td>
<td>12.9 ± 2.3</td>
<td>77.1 ± 41.4</td>
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<tr>
<td>LADA, %</td>
<td>15.4 ± 2.1</td>
<td>14.8 ± 2.8</td>
<td>63.5 ± 37.7</td>
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<tr>
<td>LAFAS, %</td>
<td>17.3 ± 7.7</td>
<td>12.1 ± 4.9</td>
<td>-25.9 ± 30.4</td>
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<tr>
<td>LVDA, cm²</td>
<td>15.5 ± 2.4</td>
<td>13.9 ± 3.3</td>
<td>156.2 ± 52.8</td>
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<tr>
<td>LVSA, cm²</td>
<td>20.3 ± 2.9</td>
<td>18.8 ± 3.9</td>
<td>37.8 ± 26.6</td>
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<tr>
<td>LVFAS, %</td>
<td>23.7 ± 5.4</td>
<td>26.2 ± 7.4</td>
<td>-54.9 ± 10.2</td>
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Values are means ± SD and are expressed as the percent change in the respective parameter from the baseline prepacing value; \( n = 8 \) RVP and 14 SAVP dogs. SAVP dogs had a lesser decrease in LVFAS and a greater decrease in LAVAS than RVP dogs. *\( P < 0.05 \), SAVP vs. RVP percent changes.
Table 5. AF inducibility

<table>
<thead>
<tr>
<th></th>
<th>Ctrl</th>
<th>RVP</th>
<th>SAVP</th>
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</thead>
<tbody>
<tr>
<td>Percentage of AERP induced-AF</td>
<td>0.0*</td>
<td>0.0†</td>
<td>30.0</td>
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<tr>
<td>Percentage of burst attempts leading to AF</td>
<td>1.0±1.7*</td>
<td>8.2±4.1†</td>
<td>34.4±14.2</td>
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<tr>
<td>Median AF duration, s</td>
<td>0 (0-0)*</td>
<td>710 (160-1,180)†</td>
<td>1,687 (1467-1,800)</td>
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<td>Percentage of dogs with AF &gt;10 min</td>
<td>0.0*</td>
<td>40.0†</td>
<td>83.3</td>
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Data are presented as percentages with or without SD and as medians (25th–75th percentile); *n = 8 Ctrl dogs, 5 RVP dogs, and 14 SAVP dogs. AF, atrial fibrillation; percentage of AERP induced-AF, AF induced by a single extrastimulus during refractory period measurements; percentage of burst attempts leading to AF, percentage of bursts attempts leading to AF episodes lasting >1 min; percentage of dogs with AF >10 min, percentage of dogs presenting at least one AF episode lasting >10 min. SAVP dogs had more AF inducibility and AF maintenance than RVP and Ctrl dogs. *P < 0.05 between Ctrl and SAVP dogs; †P < 0.05 between RVP and SAVP dogs.

Clinical data. Six of the fourteen SAVP dogs developed clinical ascites (confirmed during the final surgery), whereas eight SAVP dogs had mild pericardial effusion. None of the dogs showed signs of pulmonary edema. All RVP dogs developed pulmonary edema symptoms (crackles and coughing) from moderate (n = 6) to severe (n = 2); half of the RVP dogs had ascites and pericardial effusions. All SAVP dogs completed the entire planned experiment, whereas three of the eight RVP dogs died prematurely (37.5%) before or during the final end study.

Electrophysiological study. SAVP dogs were significantly more likely to have AF induced by atrial extrastimuli (during AERP measurements) than RVP dogs (Table 5). SAVP dogs more often had successful burst attempts (leading to AF episodes) than RVP and Ctrl dogs. Median AF durations and proportions lasting >10 min (needing cardioversion) were significantly greater in the SAVP group than in the RVP group. Mean AF CL was not different between SAVP and RVP dogs (128.7 ± 17.3 vs. 136.1 ± 15.2 ms, respectively, P = not significant). Wave duration, QRS duration, and QT interval at 400 ms CL were similarly prolonged in both pacing groups (RVP and SAVP dogs) compared with the Ctrl group (Table 6). Mean heart rate and blood pressure were not different between the two paced groups.

All three groups had similar mean AERPs (Fig. 5A); both pacing groups had similarly prolonged mean global atrial conduction times compared with Ctrl dogs at 200 and 400 ms CL (Fig. 5B). Both pacing modes induced a similar reduction in conduction velocity measured at the posterior wall of the LA (clock-face electrode) when pacing at 400 and 200 ms CL (Fig. 5C). However, five SAVP dogs (41.6%) developed intra-atrial block at 150 ms CL stimulation, whereas none of the three RVP dogs did. SAVP dogs also had greater conduction heterogeneity than Ctrl dogs (23.3 ± 3.5 vs. 17.6 ± 2.9 ms, respectively, P < 0.05) and a lower conduction anisotropic index (2.35 ± 0.61 vs. 3.05 ± 0.53, respectively, P < 0.05), indicating a relatively greater slowing in the direction of the fastest conduction velocity. We observed no significant differences between SAVP and RVP dogs with regard to conduction time heterogeneity (23.3 ± 3.5 vs. 20.5 ± 3.0 ms) and anisotropic index (2.35 ± 0.61 vs. 2.50 ± 0.56).

Metalloprotease Activity

Direct observation of the gels, as well as the corresponding densitometry, showed an increase in the pro-matrix metalloprotease (MMP)-9 and active MMP-9 activity in the LA of SAVP dogs compared with RVP dogs (Fig. 6).

Histopathological Analysis

Histopathological analyses using picrosirius red staining of selective midmyocardium layers in the LA revealed significantly more total collagen area fractions in SAVP and RVP dogs compared with Ctrl dogs (17.6 ± 4.8% and 15.0 ± 4.2% vs. 8.7 ± 2.5%, P < 0.05, respectively). There were no differences between SAVP and RVP dogs. Representative histological sections from each group are shown in Fig. 7.

TGF-β1 and Collagen Isoform Analysis

Assessment of LA collagen type I and III mRNA demonstrated significant increases in both the RVP (10.5 ± 5.8 and 4.8 ± 1.6, respectively) and SAVP (15.4 ± 8.6 and 6.9 ± 1.5, respectively) groups compared with the Ctrl group (1.1 ± 0.5 and 1.1 ± 0.7, respectively). Furthermore, SAVP was associated with a significant increase in collagen type III compared with RVP dogs (P < 0.05). The profibrotic cytokine TGF-β1 was increased in both RVP (1.81 ± 1.41) and SAVP models (1.30 ± 0.27) compared with Ctrl dogs (1.05 ± 0.34). There were no differences between RVP and SAVP dogs.

DISCUSSION

The aim of this study was to compare the well-known RVP model to a new SAVP model in terms of acute echocardiographic Doppler and hemodynamic changes and chronic structural atrial remodeling and AF inducibility.

Compared with RVP, SAVP for 30 s induced 1) more SFR in the upper PV and, consequently, a greater increase in PCWP; and 2) a greater increase in the LASA and, consequently, greater decrease in the LA emptying function.

Compared with RVP for 5 wk, SAVP for 2 wk induced 1) relatively more mechanical remodeling in the LA and less mechanical remodeling in the LV; 2) fewer pulmonary edema symptoms and no premature deaths; 3) more reliable AF inducibility and maintenance; 4) more heterogeneity in con-
study has shown that this phenomenon can be observed during ventricular stimulation at 200 beats/min if associated with a 1:1 ventriculoatrial conduction (31) and generates PV SFR. In our study, RVP at 220 beats/min was associated with either a 2:1 ventriculoatrial conduction or a complete ventriculoatrial dissociation responsible for less SFR in the upper PV and, consequently, less increase in PCWP than SAVP. This may also explain the observed greater increase in LASA and, consequently, the greater decrease in the LA emptying function during SAVP for 30 s.

Chronic Consequences of SAVP Versus RVP

Mechanical remodeling. We have shown in this study that pacing both chambers simultaneously for 2 wk created more atrial than ventricular dysfunction, as opposed to RVP, which is known to be mostly associated with LV dysfunction (26). In RVP models, the resulting dilation of the LV is responsible for mitral annulus enlargement and mitral regurgitation (28). The severe decrease in LV function we observed in our RVP dogs (−54.9 ± 10.2%) was accompanied by heart failure symptoms (pulmonary edema in 100% of the dogs) and premature mortality in 37.5% of the dogs. Most previous studies have not mentioned any premature death in chronic RVP dogs; however, in a recent publication (11), two of seven dogs died during anesthesia induction (28%).

AF inducibility and maintenance. The SAVP model leads to more easily induced and more frequently sustained AF episodes than the RVP model. The observation that SAVP dogs had a greater degree of LA systolic dysfunction and longer episodes of AF is consistent with prior observations that a decrease in LA fractional area shortening is correlated with AF duration in dogs (29) and suggests that structural changes associated with decreases in LA function are an important determinant of the propensity to AF.

**Acute Consequences of SAVP Versus RVP**

Inappropriate LA contraction during mitral valve closure corresponds to the LA “cannon A” wave phenomenon, which is associated with an acute increase in LA pressure and dimension (8). A recent transesophageal echocardiographic dog
In addition, whereas none of the RVP dogs developed AF induced by a single premature extrastimulus, half of the SAVP dogs did. This observation suggests that the SAVP model is clinically relevant with respect to AF induced by premature atrial contractions in heart failure patients prone to arrhythmia (13). In prior animal studies, extrastimulus-induced AF has been mostly described in RAP models (55.7%) as opposed to combined (RVP at 240 beats/min for 2 wk combined with an additional RAP at 400 beats/min during the second week) and RVP models (16.2% and 6.3%, respectively) (5, 12). Heterogeneity of refractoriness has been described as the primary determinant of AF induction by extrastimuli (33); however, premature beats can also initiate AF by leading to intra-atrial block in areas of slow conduction (observed in 5 SAVP dogs and no RVP dogs), which degenerates into multiple electrical wavefronts (35).

**Electrophysiological substrate for AF.** Both pacing models resulted in similar surface ECG changes (prolonged P wave and QRS duration), corresponding to global slowing of conduction, which have been shown to be correlated to the percentage of fibrosis (12). Paced dogs also had similar QT prolongation; this observation has been described as the consequence of the downregulation of repolarizing K+ currents induced by heart failure (27).

There were no differences in refractory periods between the three groups of dogs.

In vitro analyses of ion currents have shown that the minor changes in AERP observed in RVP dogs are due to inhibition of depolarizing currents being offset by the inhibition of repolarizing currents (22).

Both pacing models induced similar global and local (at the posterior wall of the LA) conduction slowing at 200 ms CL. However, intra-atrial blocks were only observed in SAVP dogs when they were paced at 150 ms CL. More heterogeneity of conduction is consistent with previous experiments that have suggested that structural, not ionic, remodeling was the primary contributor to AF maintenance in experimental congestive heart failure (5).

**Extracellular matrix remodeling.** In this study, both models were associated with an increase in collagen turnover compared with Ctrl dogs. However, whereas the total collagen area fraction was similarly increased in both pacing models, SAVP dogs had more MMP-9 activity than RVP dogs. Total matrix collagen content is a function of both synthesis and degradation, and degraded products of matrix proteins serve as a stimulus for collagen synthesis; this, in turn, may result in increased deposition and altered structure of the extracellular matrix. Assessment of collagen types demonstrated significantly increased collagen I and III mRNA in both SAVP and RVP animals. Furthermore, SAVP dogs demonstrated a greater increase in collagen III, likely as a result of the increased hemodynamic stress demonstrated in this group. Increased collagen I concentrations in the human LA occurs even in lone AF, whereas the mechanical stress induced by mitral valve diseases causes further changes, especially in collagen III (3). Whereas the relationship of increased collagen III mRNA to MMP-9 and AF vulnerability has not been clearly elucidated, it is clear that alterations in local atrial conduction properties associated with interstitial fibrosis have been described to play an important role in stabilizing the reentry that causes AF in failing hearts (7).

TGF-β1, a potent prosclerotic cytokine, has been implicated in the pathogenesis of an arrhythmogenic substrate. In the present study, both models were associated with a similar increase in mRNA expression of TGF-β1 compared with unpaced Ctrl dogs, which was associated with increased fibrillar collagen deposition. Verheule et al. (32) demonstrated that increased atrial TGF-β1 expression in mice was associated with increased collagen deposition, conduction heterogeneity, and atrial vulnerability. However, the differences demonstrated between RVP and SAVP dogs suggest that the roles of TGF-β1 and MMPs in cardiac remodeling are not completely elucidated and that other cytokines may be involved in the pathogenesis of atrial fibrosis and atrial arrhythmogenesis.

In patients with dilated cardiomyopathy, atrial extracellular matrix remodeling (downregulation of tissue inhibitor of metalloproteinase-2 and upregulation of MMP-2) is associated with increased susceptibility to AF, because the heterogeneity of fiber thickening and disarray may facilitate local intra-atrial conduction block (4).
Sympathetic activation in patients with heart failure can also influence extracellular matrix turnover via MMP induction (9). Mechanical stretch and strain induced by hemodynamic load in the atria may contribute to the transcriptional regulation of MMPs, and this factor may have played a role in this study since SAVP dogs had a greater decline in LA emptying function than RVP dogs (2).

In summary, we have shown that acute SAVP produced more pronounced changes in hemodynamics in the LA than RVP. After 2 wk of SAVP, we observed more atrial than ventricular dysfunction and more abnormal collagen deposition compared with RVP for 5 wk. We infer that the main difference between the SAVP and RVP models is that, in the SAVP model, atrial dysfunction preceded LV dysfunction and therefore produced earlier atrial remodeling. This allowed us to stop pacing before severe LV failure occurred, yet yielded more inducible AF than after 5 wk of RVP. Changes in atrial mechanical remodeling are only part of the explanation as to how the two models are different in terms of AF inducibility. Changes in collagen turnover and the consequent shift in collagen isoforms are possibly involved, but differences in gap junction distribution and function and/or differential alteration of the atrial bundle architecture might also be involved.

Limitations

Control dogs were not sham-operated dogs, because this study was mainly designed to compare the two pacing models. There are several differences between this study and previous studies with respect to atrial conduction analysis. First, we did not measure the overall conduction velocity, but the mean atrial conduction time was calculated between each of the five epicardial electrodes. The distance between the electrodes may be another variable, as it was not taken into account, as we tried to place it at the exact same location for every dog. Second, the only true conduction velocity calculated (taking into account the interelectrode distance) was at the posterior wall of the LA. In humans, this area is of interest for its AF vulnerability, because of the muscular fiber disarray responsible for complex fractionated atrial electrograms, which have been reported as ablative targets for the treatment of AF (29). Mapping the LA with the clock-face electrode (only 16 unipolar electrodes) is also a limitation. However, the clock-face electrode allows electrophysiological analysis in a 360° equidistant area from a central bipolar pacing electrode at different stimulation CLs. It was possible to accurately measure conduction velocity, conduction time heterogeneity, and the conduction anisotropic index, which reflect the conduction status of the small area covered by the electrode. By placing the clock-face electrode at the posterior wall of the LA, we wanted to analyze more specifically an area that is known to display changes in conduction associated with AF vulnerability.

Conclusions

Pacing the RA and right ventricle simultaneously induces more mechanical and structural atrial remodeling than pacing only the right ventricle. More LA dysfunction and abnormal collagen deposition may explain why this new model yields more reliable induction of AF than the RVP model. This model may be clinically relevant and may be useful for testing AF interventions.

ACKNOWLEDGMENTS

The authors thank Suzan O’Donnell for statistical help and Marta Gadacz for editorial assistance with the manuscript.

GRANTS

This work was supported by The Heart and Stroke Foundation of Canada. G. Laurent was supported by the “Fédération Française de Cardiologie.” K. A. Connelly is supported by a Tailored Advanced Collaborative Training in Cardiovascular Sciences (TACTICS) scholarship (Canada) and by Neil Hamilton Fairley Scholarship 440712 from the National Health and Medical Research Council.

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