ARTICLE

Adenosine-Induced Atrial Fibrillation
Localized Reentrant Drivers in Lateral Right Atria due to Heterogeneous Expression of Adenosine A1 Receptors and GIRK4 Subunits in the Human Heart

BACKGROUND: Adenosine provokes atrial fibrillation (AF) with a higher activation frequency in right atria (RA) versus left atria (LA) in patients, but the underlying molecular and functional substrates are unclear. We tested the hypothesis that adenosine-induced AF is driven by localized reentry in RA areas with highest expression of adenosine A1 receptor and its downstream GIRK (G protein-coupled inwardly rectifying potassium channels) channels ($I_{K,Ado}$).

METHODS: We applied biatrial optical mapping and immunoblot mapping of various atrial regions to reveal the mechanism of adenosine-induced AF in explanted failing and nonfailing human hearts (n=37).

RESULTS: Optical mapping of coronary-perfused atria (n=24) revealed that adenosine perfusion (10–100 $\mu$mol/L) produced more significant shortening of action potential durations in RA (from 290±45 to 239±41 ms, 17.3±10.4%; P<0.01) than LA (from 307±24 to 286±23 ms, 6.7±6.6%; P<0.01). In 10 hearts, adenosine induced AF (317±116 s) that, when sustained (≥2 minutes), was primarily maintained by 1 to 2 localized reentrant drivers in lateral RA. Tertiapin (10–100 nmol/L), a selective GIRK channel blocker, counteracted adenosine-induced action potential duration shortening and prevented AF induction. Immunoblotting showed that the superior/middle lateral RA had significantly higher adenosine A1 receptor (2.7±1.7-fold; P<0.01) and GIRK4 (1.7±0.8-fold; P<0.05) protein expression than lateral/posterior LA.

CONCLUSIONS: This study revealed a 3-fold RA-to-LA adenosine A1 receptor protein expression gradient in the human heart, leading to significantly greater RA versus LA repolarization sensitivity in response to adenosine. Sustained adenosine-induced AF is maintained by reentrant drivers localized in lateral RA regions with the highest adenosine A1 receptor/GIRK4 expression. Selective atrial GIRK channel blockade may effectively treat AF during conditions with increased endogenous adenosine.

© 2016 American Heart Association, Inc.

Key Words: adenosine ◼ atrial fibrillation ◼ G protein-coupled inwardly rectifying potassium channels ◼ optical mapping ◼ receptor, adenosine A1 ◼ tertiapin

Correspondence to: Vadim V. Fedorov, PhD, Department of Physiology and Cell Biology, The Ohio State University Wexner Medical Center, 300 Hamilton Hall, 1645 Neil Ave, Columbus OH 43210-1218. E-mail vadim.fedorov@osumc.edu, fedorov.2@osu.edu

Sources of Funding, see page 497
Adenosine and Atrial Fibrillation

Clinical Perspective

What Is New?

• This study elucidates the molecular and functional mechanisms that may underlie adenosine-induced atrial fibrillation (AF) in the human heart. The integration of panoramic optical mapping and regional immunoblot allowed us to resolve that protein expression of the 2 main components of the adenosine signaling pathway (adenosine A1 receptor and GIRK4 [G protein-coupled inwardly rectifying potassium channels]) is 2 to 3 times higher in human right atria versus left atria, leading to greater right atrial versus left atrial action potential duration shortening in response to adenosine.

• We also found that adenosine induces AF sustained by localized reentrant drivers anchored to regions of both shortest action potential duration and highest adenosine A1 receptor expression in the right atrium.

What Are the Clinical Implications?

• This study suggests that, in the clinical setting, adenosine injection may unmask the location of right atrial AF drivers by selectively shortening action potential duration and increasing frequency in driver regions and aid in targeted ablation treatment.

• We also demonstrated that the selective GIRK channel blocker tertiapin successfully terminated and prevented AF, suggesting that the arrhythmogenic effect of adenosine in the human atria is mediated by activation of GIRK channels. Based on our results, specific blockade of the GIRK channels may offer a novel mechanism to prevent adenosine-mediated AF in patients.

Adenosine and supraventricular tachyarrhythmias. However, intravenous adenosine administration may provoke spontaneous or pacing-induced atrial fibrillation (AF) in up to 12% to 16% of patients at clinically relevant doses. Moreover, endogenous production of adenosine during metabolic stress conditions (e.g., ischemia and heart failure) has been suggested as a trigger of AF. In clinical and animal model studies, adenosine was shown to shorten atrial action potential duration (APD) and refractoriness that, in turn, could provoke AF. However, the specific functional and molecular mechanisms of adenosine-induced AF, specifically in the human heart, are yet to be resolved.

Several clinical studies directly link endogenous adenosine and AF by showing that AF occurrence after acute myocardial infarction and coronary artery bypass graft may be treated by blockade of adenosine receptors. At a cellular level, adenosine has been shown to induce atrial APD shortening mainly through activation of specific G protein–coupled adenosine A1 receptors (A1R) and the downstream outward potassium channel I K,Ado formed by GIRK1 (G protein-coupled inwardly rectifying potassium channel 1) and GIRK4 subunits. Based on these findings, selective inhibition of I K,Ado may prevent adenosine-induced proarrhythmic APD shortening and AF; however, this hypothesis has never been evaluated in human atria.

Moreover, 2 clinical studies have proposed that the magnitude of adenosine’s effect on repolarization/refractoriness is greater in the right atrium (RA) than in the left atrium (LA), indicating that a gradient of sensitivity might exist across the atria. However, the molecular mechanisms that cause the heterogeneous effect of adenosine across the human atria are unknown. A limited number of studies have explored GIRK1 and GIRK4 channel expression and function and acetylcholine-mediated effects (I K,ACh) in human hearts, but they were restricted to using right or left atrial appendage tissue, which are not typical arrhythmogenic regions for AF. At present, no data exist regarding expression, localization, and functional contribution of A1R and adenosine-mediated activation of GIRK channels (I K,Ado) to adenosine-induced AF across multiple regions of the human atria and particularly in the regions that are prone to AF drivers such as the posterior LA and lateral RA.

In the present study, we used high-resolution biatrial optical mapping of human atria combined with detailed regional molecular mapping of A1R and GIRK expression across 10 regions of the RA and LA to address the functional and molecular mechanisms of adenosine-induced AF. We specifically compared human RA and LA sensitivities to adenosine and evaluated the correlation of regional A1R and GIRK protein expression across the RA and LA with AF initiation. Our study reveals that expression of A1R and GIRK4 protein are higher in the human RA, specifically in the superior lateral region, that directly correlate with the source of AF maintenance by localized reentrant drivers. We further show that the selective GIRK channel blocker tertiapin successfully terminated and prevented AF, suggesting that the arrhythmogenic effect of adenosine in human atria is mediated by activation of GIRK channels. Based on our results, specific blockade of the GIRK channels may offer a novel mechanism to prevent adenosine-mediated AF in the human heart.

METHODS

An expanded Material and Methods can be found in the online-only data Supplement Material.

Patient Groups

Deidentified, coded human hearts (n=37) were obtained from The Ohio State University Cardiac Transplant Team and

August 9, 2016 487

Circulation. 2016;134:486–498. DOI: 10.1161/CIRCULATIONAHA.115.021165
LifeLine of Ohio Organ Procurement Organization. This study was approved by The Ohio State University Institutional Review Board. Human atrial tissue was used for optical mapping experiments (n=24) and immunoblotting analysis (n=18). Patient-specific data are presented in online-only data Supplement Tables I and II. Six-digit deidentified case numbers are presented in parentheses after heart number.

Optical Mapping of Coronary-Perfused Human Atrial Preparations

Human atrial preparations (n=24) were isolated and coronary perfused as previously described. The atrial preparations were immobilized with 10 μmol/L blebbistatin and stained with near-infrared dye di-4-ANBDQBS (10–40 μmol/L). Imaging was simultaneously conducted with 2 to 4 MiCAM Ultima-L CMOS cameras (SciMedia, Ltd.) from atrial epicardial (Figure 1) and endocardial (Figure 2) fields of view (330–940 mm² resolution, 100×100 pixels), sampled at 1000 frames/s.

Atrial preparations (n=19) were sequentially imaged during perfusion by regular Tyrode solution (baseline), 10 μmol/L and 100 μmol/L adenosine (Sigma) followed by the selective GIRK channel blocker tertiapin (10–100 nmol/L; Tocris) or washout. In 5 preparations, 100 μmol/L adenosine was added after 100 μmol/L tertiapin perfusion. The time interval between drug applications was 20 to 30 minutes. All preparations were paced at a basic cycle length of 500 ms, and paced incrementally until the functional refractory period was reached or AF was induced. Burst pacing with a cycle length faster than functional refractory period was also used to induce AF.

Optical mapping data were analyzed using a customized Matlab program as previously described. Atrial activation patterns and 80% of repolarization (APD80) were analyzed during baseline, adenosine, and tertiapin perfusion. Activation frequency of RA and LA during AF was measured with dominant frequency (DF) analysis and discrete islands of highest DF were considered AF driver regions, which were limited to 2.5×2.5 cm² regions (Figures 3 through 5). Additionally, activation

**Figure 1.** Epicardial optical mapping of the intact human atria.

A, Schematic of the optical fields of view of the 4 epicardial cameras. B, Representative optical action potential from the lateral RA (location indicated with a circled 1 in D) of human heart #1 (case no. 947202). C, Average APD80 of RA versus LA at baseline, 10 μmol/L adenosine, 100 μmol/L adenosine and tertiapin of all biatrial preparations (n=11). Values are presented as mean±standard deviation. *P<0.05 versus baseline. #P<0.05 versus LA. D, Activation (top) and APD80 (bottom) maps of heart #1 (947202) at baseline, 10 μmol/L adenosine, 100 μmol/L adenosine, and 10 nmol/L tertiapin perfusion. Ado indicates adenosine; APD80, action potential duration with 80% repolarization; BB, Bachmann bundle; CS, coronary sinus; CT, crista terminalis; IAS, interatrial septum; IVC, inferior vena cava; LA, left atrium; LAA, left atrial appendages; LRA, lateral right atria; PLA, posterior left atria; RA, right atrium; RAA, right atrial appendages; SAN, sinoatrial node; and SVC, superior vena cava.
maps (Figures 3 through 5) were used to identify the mechanism of AF reentrant drivers as done previously. Here, AF drivers are defined as a localized source(s) of fastest electric activity visualized as reentrant circuits where 2 pivot points were mapped or as breakthrough pattern and incomplete reentry circuits where 1 pivot point was mapped. These drivers were temporally stable for >70% of AF duration if only 1 driver or >30% if 2 drivers. The temporal stability of the AF driver is estimated by the percentage of activation cycles with activation source origin within a driver region during an 8 s recording. Based on our previous transmural mapping study, we suggest that the incomplete reentry/breakthrough visualized in the present study by single-sided mapping is intramural reentry, and this pattern is referred to as incomplete reentry/breakthrough (online-only Data Supplement Figure I). Breakthroughs distributed within 1×1 cm² area of the driver region during an AF episode are defined as spatially unstable breakthroughs. Breakthroughs distributed between 1×1 cm² and 2.5×2.5 cm² areas of the driver region are defined as spatially unstable breakthroughs. Detailed methods for optical mapping and data analysis are provided in the online-only Data Supplement Material.

**Immunoblotting**

Fresh atrial tissue (n=13) was collected from different atrial locations (as shown in Figure 6) to study the A1R and GIRK1/4 protein expression. Detailed methods for protein isolation and immunoblotting are provided in the online-only Data Supplement Material.

**Statistical Analysis**

Data are presented as mean±standard deviation other than AF episode duration, which is presented as mean±standard error of the mean. P values of 0.05 or below were considered significant. Additional methods for statistical analysis are provided in the online-only Data Supplement Material.
Li et al

RESULTS
Adenosine Induces Heterogeneous APD Shortening

RA versus LA APD comparisons were obtained from biatrial epicardial (n=9) or endocardial (n=2) mapping experiments during 500 ms cycle length pacing. At baseline, average APD was similar in RA versus LA (281±49 versus 310±22 ms, P=0.10, n=11). Adenosine perfusion induced heterogeneous APD shortening, as shown in Figures 1 and 2. Maximal APD shortening occurred after 2 to 5 minutes of adenosine perfusion. APD shortening from baseline to adenosine 10 μmol/L perfusion was significantly greater in RA than LA (17.4±11.7% versus 6.3±5.5%, P=0.02, n=11). Adenosine 100 μmol/L perfusion did not significantly shorten APD further (Figure 1C). In all RA and LA preparations (n=23), adenosine perfusion (10–100 μmol/L) produced greater APD shortening in RA (from 290±45 to 239±41 ms, 17.3±10.4%, P<0.01, n=21) than LA (from 307±24 to 286±23 ms, 6.7±6.6%, P<0.01, n=13). The APD shortening induced by 10 and 100 μmol/L adenosine perfusion decreased the functional refractory period in lateral RA (n=19) from 232±60 ms (baseline) to 199±56 ms with 10 μmol/L adenosine (P<0.05 versus baseline) and 171±58 ms with 100 μmol/L adenosine (P<0.01 versus baseline). Simultaneous dual-sided mapping experiments showed that 100 μmol/L adenosine-induced APD shortening was similar in epicardial (17.2±7.8%) and endocardial (16.8±7.4%) atrial layers in both RA and LA. No significant difference in APD shortening in response to adenosine was detected between failing versus nonfailing groups (online-only Data Supplement Table III).

To confirm that the observed functional changes were attributable to adenosine-activated I_{Ado} current, 50 to 100 nmol/L tertiapin was added after adenosine perfusion (n=12). Tertiapin completely reversed the adenosine-induced APD shortening. Importantly, in 5 preparations tertiapin addition at baseline caused insignificant APD changes (from 310±40 ms at baseline to 302±35 ms, P=0.74). Figure 1C shows the average APD of different atrial regions (RA versus LA) at baseline, and during adenosine and tertiapin perfusion. Neither adenosine nor tertiapin had a significant effect on either RA or LA activation patterns (Figures 1 and 2).

Adenosine Induces Sustained AF with Localized Reentrant Drivers in Right Atria

At baseline, burst pacing at rates up to the functional refractory period induced nonsustained AF episodes (duration of 16±9 s) in only 3 intact atrial preparations. Adenosine significantly increased AF inducibility and duration; burst pacing induced AF in 5 preparations (duration of 241±143 s, P=0.03 versus baseline, 3/5 sustained AF) at 10 μmol/L adenosine and in 9 prepara-
tions (duration of 222±74 s, \(P=0.02\) versus baseline, 5/9 sustained AF) at 100 \(\mu\)mol/L adenosine. Addition of tertiapin (n=8) terminated adenosine-induced AF and prevented its reinduction (Figure 3A). After 15 minutes of tertiapin perfusion, only 1 nonsustained AF episode (30 s) was induced in 1 preparation where AF (88 s) was induced at baseline.

During adenosine-induced AF, the average DF in the RA was higher than LA (8.2±2.7 versus 4.8±1.2 Hz, \(P<0.01\)) and the maximal DF was also greater in RA than LA (9.6±3.1 versus 6.2±2.3 Hz, \(P<0.01\)). Figures 3 through 5 show that the maximal DF was localized to the region of shortest APD during adenosine perfusion (Figures 1 and 2). This was a consistent observation for all AF-inducible atrial preparations (n=10).

In the regions of maximal DF during sustained AF (duration ≥2 minutes), 1 or 2 localized drivers were identified in the pectinate muscle network of the lateral RA (online-only Data Supplement Figures II and III). Localized AF drivers were seen as complete reentry circuits in 3 hearts (Figures 4 and 5) and incomplete reentry circuits/breakthrough in 7 hearts (Figure 3 and online-only Data Supplement Figure I). Specific characteristics of AF drivers are given in Table. The size of the complete reentry circuits and the average conduction velocity around the driver circuit were measured as shown in online-only Data Supplement Figures III and IV. The average size of complete reentry circuits was 18.5±8.4 \(\times\) 6.7±2.5 mm\(^2\). Complete reentrant circuits and stable breakthroughs were observed during 8 episodes of adenosine-induced AF in 6 hearts. In these 6 hearts, the APD80 of the AF driver regions (n=8) was shorter than that of the driver regions (n=4) with unstable breakthroughs in the other 4 hearts (177±37 versus 235±15 ms, \(P<0.05\)). Additionally, the APD80 of the AF driver regions in 8 sustained AF episodes was shorter than the driver regions in 5 non-sustained AF episodes (181±40 versus 225±24 ms, \(P<0.05\), online-only Data Supplement Figure VA).

Figure 4A. Activation map of reentrant driver region during sustained adenosine-induced AF. Top left, Magnified activation map of AF reentrant driver. Bottom left, Optical action potentials (OAPs 1–6) from regions across RA and LA; numbers correspond to location from Top left. Top right, Optical action potentials (OAPs 1–4) showing stable reentry sustaining AF. A, Top left, Magnified activation map of reentrant driver region during sustained adenosine-induced AF. Top right, Magnified activation map of AF reentrant driver. Bottom left, Optical action potentials (OAPs 1–6) from regions across RA and LA; numbers correspond to location from Top left. Bottom right, OAPs 1 to 4 showing stable reentry sustaining AF. B, Dominant frequency (DF) and frequency power map during this sustained AF shows greater DF and frequency power in the driver location of the RA Ado indicates adenosine; AF, atrial fibrillation; BB, Bachmann bundle; CS, coronary sinus; CT, crista terminalis; IAS, interatrial septum; Inf, inferior; IVC, inferior vena cava; LA, left atrium; LAA, left atrial appendages; LLA, lateral left atria; LRA, lateral right atria; Mid, middle; MV, mitral valve; OAP, optical action potential; PLA, posterior left atria; RAA, right atrial appendages; SAN, sinoatrial node; Sup, superior; SVC, superior vena cava; and TV, tricuspid valve.

Figure 5. Magnified activation map of reentrant driver region during sustained adenosine-induced AF. A, Top left, Magnified activation map of AF reentrant driver. Bottom left, Optical action potentials (OAPs 1–6) from regions across RA and LA; numbers correspond to location from Top left. Top right, Optical action potentials (OAPs 1–4) showing stable reentry sustaining AF. B, Dominant frequency (DF) and frequency power map during this sustained AF shows greater DF and frequency power in the driver location of the RA Ado indicates adenosine; AF, atrial fibrillation; BB, Bachmann bundle; CS, coronary sinus; CT, crista terminalis; IAS, interatrial septum; Inf, inferior; IVC, inferior vena cava; LA, left atrium; LAA, left atrial appendages; LLA, lateral left atria; LRA, lateral right atria; Mid, middle; MV, mitral valve; OAP, optical action potential; PLA, posterior left atria; RAA, right atrial appendages; SAN, sinoatrial node; Sup, superior; SVC, superior vena cava; and TV, tricuspid valve.

DF had a strong inverse correlation with APD in the driver region (\(r^2=0.76, \ P<0.01\), online-only Data Supplement Figure VB).
A1R and GIRK4 Protein Expression Is Higher in Right versus Left Atria

Adenosine A1R and the 2 main cardiac subunits of the I_{K,Ado} channel protein (GIRK1 and GIRK4) were analyzed in 10 different regions across the atria. Figure 7A shows that A1R and GIRK4 proteins were expressed most highly in the superior lateral RA tissue isolated from the region of maximum APD shortening (Figure 2B) and the reentrant AF driver location (Figure 4). Figure 7B shows immunoblotting results from nonmapped donor heart #30, which also exhibited higher A1R and GIRK4 protein expression in the superior-middle lateral RA versus other atrial regions. However, GIRK1 protein expression was similar in lateral RA versus LA, a consistent observation in other hearts. Figure 7C summarizes A1R and GIRK4 protein expression in 6 representative atrial regions from both failing and nonfailing hearts (n=14). Molecular mapping revealed significantly higher A1R (2.7±1.7-fold; P<0.01) and GIRK4 (1.7±0.8-fold; P<0.05) protein expression in the superior-middle lateral RA versus other atrial regions. However, GIRK1 protein expression was similar in lateral RA versus LA, a consistent observation in other hearts. Figure 7C summarizes A1R and GIRK4 protein expression in 6 representative atrial regions from both failing and nonfailing hearts (n=14).
were compared across diseases of heart failure and AF; however, the protein expression ratios were not significantly different between groups (online-only Data Supplement Figure VI).

**DISCUSSION**

In this study, integration of optical mapping and regional immunoblot analysis allowed us to resolve the functional and molecular mechanisms underlying adenosine-induced AF in the ex vivo human atria. The major findings of the study are: expression of A1R and GIRK4 protein is heterogeneous across the human atria and is highest in the superior lateral region of the right atria; adenosine induced heterogeneous APD shortening and increased the incidence of AF; during adenosine-induced AF, DF was higher in the RA than LA, and regions of highest DF correspond to regions of highest A1R and GIRK protein expression in the RA; the direct correlation of AF driver location to location of both shortest APD and highest A1R and GIRK4 protein expression in the lateral RA reveals the mechanism of adenosine-induced AF.

**Right and Left Atrial Differences of Adenosine Sensitivity Predisposes AF Drivers to the Right Atria**

In our human atria ex vivo optical mapping experiments, atrial repolarization times analyzed during 500 ms pacing at baseline in RA (290±45 ms) and LA (307±24 ms) were within the range of clinical monophasic action potential measurements of RA (209–351 ms) and LA (202–339 ms) in patients with and without AF history.18,30 Tebbenjohanns et al6 previously reported that intravenous bolus of 6 mg and 12 mg of adenosine induced 19% and 27% APD shortening at 500 ms pacing in RA, which is similar to our ex vivo findings (Figures 1 and 2).

Our observation of an RA-to-LA gradient of adenosine-induced APD shortening in the human heart is supported by clinical studies of adenosine’s effect on AF driver preference in RA and LA,17,18,31 because DF values can represent

---

**Table. Characteristics of AF Episode in Human Heart**

<table>
<thead>
<tr>
<th>Heart No.</th>
<th>Condition</th>
<th>Duration (s)</th>
<th>RA</th>
<th>LA</th>
<th>DF (Hz)</th>
<th>Driver Region</th>
<th>DF (Hz)</th>
<th>CL (ms)</th>
<th>Stability</th>
<th>Spatial</th>
<th>Visualization Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Baseline</td>
<td>88</td>
<td>5.3</td>
<td>4.8</td>
<td>RA sup</td>
<td>7.2</td>
<td>141</td>
<td>24</td>
<td>38</td>
<td>Unstable</td>
<td>BT</td>
</tr>
<tr>
<td>5</td>
<td>Baseline</td>
<td>40</td>
<td>5.8</td>
<td>4.2</td>
<td>RA sup</td>
<td>6.6</td>
<td>148</td>
<td>10</td>
<td>79</td>
<td>Stable</td>
<td>BT/reentry</td>
</tr>
<tr>
<td>6</td>
<td>Baseline</td>
<td>29</td>
<td>3.9</td>
<td>4.6</td>
<td>(1) RA sup</td>
<td>6.1</td>
<td>172</td>
<td>19</td>
<td>55</td>
<td>Unstable</td>
<td>BT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2) PLA</td>
<td>5.9</td>
<td>182</td>
<td>12</td>
<td>69</td>
<td>Unstable</td>
<td>BT</td>
</tr>
</tbody>
</table>

**Unsustained adenosine-induced AF**

| 3         | Ado 10    | 62           | 4.8| 3.5| RA inf  | 6.0           | 144     | 48      | 61        | Unstable | BT                |
| 3         | Ado 100   | 63           | 4.1| 3.4| RA inf  | 5.3           | 204     | 43      | 68        | Unstable | BT                |
| 13        | Ado 100   | 106          | 6.4| N/A| RA mid  | 7.8           | 121     | 33      | 64        | Unstable | BT                |
| 18        | Ado 100   | 11           | 7.4| N/A| (1) RA sup | 8.3         | 128     | 20      | 81        | Stable   | BT/reentry       |
|           |           |              |    |    | N/A     | (2) RA mid  | 8.3     | 122     | 10        | 41       | Stable BT/reentry |
| 19        | Ado 100   | 42           | 7.4| N/A| RA inf  | 8.1           | 117     | 31      | 72        | Unstable | BT                |

**Sustained adenosine-induced AF**

| 4         | Ado 10    | 347          | 6.7| 5.3| RA sup  | 7.2           | 135     | 28      | 100       | Stable   | BT/reentry       |
| 6         | Ado 10    | 1173         | 11.6| 6.5| RA sup  | 13.9          | 81      | 18      | 70        | Unstable | BT                |
| 10        | Ado 10    | 335          | 10.2| 4.1| RA sup  | 13.6          | 74      | 4       | 100       | Stable   | Reentry          |
| 1         | Ado 100   | 120          | 8.8| 5.5| RA inf  | 11.6          | 86      | 7       | 100       | Stable   | BT/reentry       |
| 2         | Ado 100   | 145          | 8.5| 4.9| RA mid  | 9.4           | 106     | 9       | 100       | Stable   | Reentry          |
| 4         | Ado 100   | 425          | 6.8| 4.5| RA sup  | 7.5           | 138     | 27      | 100       | Stable   | BT/reentry       |
| 5         | Ado 100   | 471          | 13.2| 6.8| (1) RA sup | 13.8        | 71      | 6       | 74        | Stable   | Reentry          |
|           |           |              |    |    | (2) RA inf | 13.6        | 72      | 6       | 33        | Stable   | Reentry          |
| 10        | Ado 100   | 616          | 11.2| 3.7| RA sup  | 12.8          | 78      | 4       | 100       | Stable   | Reentry          |

Ado 10/100 indicates adenosine 10 µmol/L/100 µmol/L; AF, atrial fibrillation; APD, action potential duration; Avg, average; BT, breakthrough; CL, cycle length; DF, dominant frequency; LA, left atria; N/A, not available; PLA, posterior left atria; RA sup/mid/inf, right atria superior/middle/inferior; and SD, standard deviation.
the local functional refractoriness of the tissue.\textsuperscript{17} We observed higher DF in lateral RA versus LA (9.6±3.1 versus 6.2±2.3 Hz) during adenosine-induced AF (Figures 3 through 5). In a similar pattern, the clinical study by Botteron et al\textsuperscript{17} showed that a bolus of 12 mg adenosine during paroxysmal or pacing-induced AF dramatically increased DF more in the lateral RA (6.4±0.7 to 12.2±1.9 Hz) than LA (6.1±0.6 to 8.7±1.2 Hz). Importantly, the high RA DF values (9.6±3.1 Hz) in our study are in the range of DF values reported by this (12.2±1.9 Hz)\textsuperscript{17} and other clinical AF studies.\textsuperscript{32,33}

Moreover, adenosine triphosphate injection also was shown to augment this RA-to-LA DF gradient by predominantly increasing the DF in the superior RA versus LA (10.7±0.7 versus 7.9±1.8 Hz).\textsuperscript{34} Atienza et al\textsuperscript{31} reported that adenosine increased DF in RA more than LA in persistent AF patients. These clinical observations\textsuperscript{6,7,17,18,31} of a greater DF increase in the superior lateral RA versus LA suggest a more pronounced adenosine-induced atrial repolarization and refractoriness in RA versus LA. These in vivo findings are supported by our ex vivo observations that the highest DF during adenosine-induced AF was always located in the shortest APD region in lateral RA (Figures 3 through 5, online-only Data Supplement Figure V).

**Figure 6. Distribution patterns of AF drivers and APD across the human atria.**

A, Locations and visualizations of AF drivers in 10 AF-inducible hearts shown on epicardial and endocardial atrial schematics. Heart number is distinguished by color, and drivers from the same heart are labeled with the corresponding color. A complete reentry pattern is denoted by a complete ellipsoidal arrow; partial reentry pattern is denoted by a dashed ellipsoidal arrow. Circled numbers 1 to 6 indicate the regions where APD80 was measured in B. B, Average APD80 of different atrial regions in AF-inducible and AF-noninducible groups at baseline and adenosine perfusion. C and D, Minimal APD and APD dispersion in the RA of AF-inducible and AF-noninducible preparations at baseline and adenosine perfusion. E, APD shortening in AF driver and nondriver regions. Values are presented in mean±standard deviation. *P<0.05 versus baseline. #P<0.05 versus AF-noninducible group. Ado indicates adenosine; AF, atrial fibrillation; APD, action potential duration; BB, Bachmann bundle; CS, coronary sinus; CT, crista terminalis; IAS, interatrial septum; Inf, inferior; IVC, inferior vena cava; LAA, left atrial appendages; LLA, lateral left atria; LRA, lateral right atria; Mid, middle; MV, mitral valve; OAP, optical action potential; PLA, posterior left atria; RA, right atrium; RAA, right atrial appendages; SAN, sinoatrial node; Sup, superior; SVC, superior vena cava; and TV, tricuspid valve.
Mechanism of Adenosine-Induced AF

The direct effect of adenosine on cardiomyocytes is activation of the outward potassium (K+) current, $I_{\text{K,Ado}}$, via activation of the G protein–coupled A1R.1 $I_{\text{K,Ado}}$ channels also are regulated by the neurotransmitter acetylcholine (ACh) via M2 muscarinic receptors, referred to as $I_{\text{K,ACh}}$.15,16 Augmented (endogenous or external) adenosine, and vagal nerve stimulation, as well, readily promotes AF induction and maintenance because of accelerated atrial repolarization and shortened atrial refractoriness as a consequence of the activation of the GIRK current ($I_{\text{GIRK}}$).17,20

Clinical studies31,34 have suggested the presence of AF reentrant drivers localized in the RA during adenosine infusion, but the direct mechanism sustaining AF was not shown. In this study, we have located areas of highest DF in the lateral RA during adenosine-induced AF and resolved in these regions that localized reentrant drivers may sustain AF (figures 3 through 5). We observed that sustained adenosine-induced AF may be driven by 1 to 2 localized reentry circuits, but during nonsustained AF, only unstable reentries/breakthroughs were observed in the areas of highest DF. These observations are in agreement with Schuessler et al35,36 canine ACh-induced AF studies that demonstrated ACh dose-dependently decreases refractory period and produces unstable AF until a critical level of the refractory period, when AF becomes sustained and driven by a localized source.

Our recent dual-sided endo-epicardial optical mapping study of ex vivo human atria integrated with 3-dimensional gadolinium-enhanced MRI revealed that pinacidil-induced sustained AF was driven by spatially and temporally stable intramural reentry, and that targeted ablation of these reentrant tracks terminated AF.26 The detailed 3-dimensional structural analysis in that study26 showed pectinate muscles and areas of disorganized myofiber orientation between the endo- and epicardium create microanatomic tracks that stabilize reentrant AF drivers. Online-only Data Supplement Figure II shows the endocardial pectinate muscle anatomy that may harbor a reentrant track that was only partially seen from the subepicardial mapping. Online-only Data Supplement Figure III shows the full subendocardial microanatomic track revealed by 3-dimensional micro-CT. Importantly, these microana-
tomic tracks often involve both the subendocardial and subepicardial myocardial layers. The simultaneous du-
al-sided electrode mapping in the canine ACh-induced AF model also revealed that the reentrant pathways could be outside the epicardial or endocardial plane, and the reentry activation could appear in a stable breakthrough pattern on the single atrial surface. Thus, it is not surprising that single-surface mapping may have only visualized part of the localized intramu-
rual reentry circuit during sustained AF, and instead revealed 3 main patterns of how intramural drivers can be visualized: (1) complete reentry circuits with 2 pivoting points; (2) spatially stable breakthrough with incomplete reentry circuits where 1 pivoting point was mapped; and (3) spatially unstable beat-to-beat variable breakthroughs (Table, Figure 6, Online-only Data Supplement Figure I). Therefore, once again, this study suggests that, to disclose the accurate AF mechanism, a simultaneous endo-epicardial and panoramic optical-mapping approach must be applied, which is currently impossible in the whole intact human atria.

Molecular Substrates of Right versus Left Atrial Adenosine Sensitivity: Heterogeneous A1R and GIRK4 Distribution

Using isolated cardiomyocytes from human atrial appendages, Voigt et al reported that GIRK1 and GIRK4 proteins were higher in RA versus LA, and that car-
bachol-activated $i_{K,ACH}$ was 70% larger in RA versus LA in patients in sinus rhythm. However, no data have been reported on A1R distribution across the human atria. For the first time, we performed molecular mapping of A1R and GIRK1/4 proteins from multiple regions across the entire atria and directly correlated protein expression with functional mapping data. We found that, although GIRK1 protein expression level is similar in both human atria, A1R and GIRK4 protein are more highly expressed in the RA than LA (Figure 6), which is supported by clini-
cal and our ex vivo functional data on RA versus LA adenosine sensitivity. Importantly, the correlation of expression of these proteins with regions of shortest APD and AF driver locations in superior/middle lateral RA during adenosine perfusion suggests that A1R and GIRK4 expression may contribute to the molecular sub-
strate for human AF.

A1R/GIRK4 Remodeling in Heart Failure

Heart failure causes structural and molecular remodeling in the atria, and increased incidence of AF, as well. Plasma adenosine levels increase progressively with the severity of chronic heart failure. Recently, we reported that, in the canine chronic heart failure model, upregulation of A1R and GIRK4 expression in the RA is correlated with APD shortening and high occurrence of adenosine-induced AF, suggesting that an increased sensitivity of failing atria to adenosine may be a risk factor for AF in heart failure. In the current study, we found A1R and GIRK4 protein expression gradients between lateral RA and LA also are highly present in hearts with a history of heart failure and AF (Figure 7B and online-only Data Supplement Figure VI), which, in combination with the elevated levels of endogenous adenosine reported in patients with heart failure, may initiate AF with an RA-to-LA DF gradient. The variety of disease history within each group may limit the ability to show the effects of independent disease remodeling on protein expression, which requires further investigation.

Limitations

In this study, we used ex vivo atrial preparations that are unbiased to the compounding influence of the autonomic nervous system, which could have partially modulated the response to adenosine in vivo. The small sample size of hearts with a variety of diseases limits our ability to fully account for and make specific suggestions regarding A1R and GIRK expressions, and AF susceptibility/inducibility for disease factors such as heart fail-
ure, hypertension, coronary heart disease, and diabetes mellitus, as well. Moreover, incomplete atria (paucity of pulmonary vein and intraseptal regions) received from transplanted failing hearts limited our statistical analysis of AF inducibility between failing versus nonfailing hearts. Although the mechanism of adenosine-induced AF in ex-
planted human hearts also may play an important role in human AF in vivo, its significance in patients with persist-
ent AF relative to other factors is still unclear. Because we used primarily single-sided epicardial or endocardial mapping, we could not visualize complete reentry activation during AF in some preparations. In addition, because we focused on estimating the antiarrhythmic efficacy of tertiapin, we did not perform targeted ablation of any of the driver areas to determine whether it would eliminate the AF. However, our previous studies support the as-
sumption that ablation of these reentrant drivers would result in termination or significant alteration of AF. Even though all AF drivers were localized in the lateral RA, we cannot exclude the possibility that adenosine-provoked AF can be maintained by a LA driver in some patient categories, because many factors can be involved in the induction and maintenance of AF.

Potential Implications and Future Directions

For the first time, our study reveals the potential molecular and functional mechanisms that may underlie adenosine-induced AF events in the human heart. Mechanistically, the high expression of the 2 main components (A1R and GIRK4 channels) of the adenosine signaling pathway, particularly
in the superior lateral RA, correlates with the localization of reentrant drivers. Our results also indicate that all AF drivers were localized in the shortest APD regions, which suggests that, in the clinical setting, adenosine injection may be a useful tool for unmasking the location of RA AF drivers and improving targeted ablation treatment. Interestingly, tertiapin reversed the APD shortening caused by adenosine and prevented AF induction, confirming the potential utility of selective GIRK channel blockers in the treatment of AF in humans. The mechanism of why A1R expression is at least 2-fold higher in the RA than LA in the human heart requires further evaluations.

CONCLUSIONS

We demonstrated for the first time in the human heart that a near 3-fold greater RA-to-LA A1R protein expression leads to significantly greater APD shortening in the RA versus the LA in response to adenosine.

Highest A1R and GIRK4 expression is present in the pectinate muscle region of the superior and middle lateral RA and is correlated with the areas of greatest adenosine-induced repolarization shortening. Adenosine primarily induces AF sustained by localized reentrant drivers in the superior/middle lateral RA, where shortest APD and highest A1R expression are observed. The selective GIRK channel blocker tertiapin successfully prevents and terminates adenosine-induced AF, suggesting that selective blockade of cardiac GIRK channels is a potential treatment for adenosine-mediated AF.

ACKNOWLEDGMENTS

We thank the Lifeline of Ohio Organ Procurement Organization and the Division of Cardiac Surgery at The Ohio State University Wexner Medical Center for providing the explanted hearts. The human heart program repository is supported by the Davis Heart and Lung Research Institute. We thank Benjamin Canan and Eric Schultz for their help with tissue processing.

SOURCES OF FUNDING

This work was supported primarily by NIH HL115580 (to Dr Fedorov), and, in part, by CR Webb Fund in Cardiovascular Research and The Ohio State University Heart and Vascular Center Trifit Challenge Discovery Fund (to Dr Fedorov), AHA Grant-in-Aid (to Dr Fedorov), the NIH HL1113084 (to Dr Janssen), HL084583, HL083422, HL114383 (to Dr Mohler), HL114940 (to Dr Biesiadecki), and HL111314 (to Dr Van Wagoner).

DISCLOSURES

None.

AFFILIATIONS

From Department of Physiology & Cell Biology, The Ohio State University Wexner Medical Center, Columbus (N.L., T.A.C., B.J.H., L.V.S., A. Kalyanasundaram, S.O.Z., A.G., P.J.M., P.M.L.J., B.J.B., V.V.F.); Davis Heart & Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus (N.L., T.A.C., B.J.H., L.V.S., A. Kalyanasundaram, A. Kilic, P.J.M., P.M.L.J., B.J.B., J.D.H., R.W., V.V.F.); Auckland Bioengineering Institute, The University of Auckland, New Zealand (J.Z.); Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus (A.G., A. Kilic, P.J.M., P.M.L.J., J.D.H., R.W.); Department of Molecular Cardiology, Cleveland Clinic, OH (D.R.V.W.); and Department of Surgery, Division of Cardiac Surgery, Wexner Medical Center, The Ohio State University, Columbus (A. Kilic, J.D.H., R.W.).

FOOTNOTES

Received December 31, 2015; accepted June 2, 2016.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.115.021165/-/DC1.

Circulation is available at http://circ.ahajournals.org.

REFERENCES

11. Franceschi F, Dehoro JC, Giorgi R, By Y, Monserrat C, Condo J, Ibrahim Z, Saadjian A, Gueiru T. Peripheral plasma adenosine re-


Adenosine-Induced Atrial Fibrillation: Localized Reentrant Drivers in Lateral Right Atria due to Heterogeneous Expression of Adenosine A1 Receptors and GIRK4 Subunits in the Human Heart

_Circulation_. 2016;134:486-498; originally published online July 26, 2016; doi: 10.1161/CIRCULATIONAHA.115.021165

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/134/6/486

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2016/07/26/CIRCULATIONAHA.115.021165.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Adenosine-Induced Atrial Fibrillation: Localized Reentrant Drivers in Lateral Right Atria due to Heterogeneous Expression of Adenosine A1 Receptors and GIRK4 Subunits in the Human Heart

Li et al.: Adenosine and Atrial Fibrillation

Authors: Ning Li, MD, PhD1,2; Thomas A. Csepe, BS1,2; Brian J. Hansen, BS1,2; Lidiya V. Sul1,2; Anuradha Kalyanasundaram, PhD1,2; Stanislav O. Zakharkin, PhD1; Jichao Zhao, PhD3; Avirup Guha, MD1,4; David R. Van Wagoner, PhD5; Ahmet Kilic, MD2,4,6; Peter J Mohler, PhD1,2,4; Paul ML Janssen, PhD1,2,4; Brandon Biesiadecki, PhD1,2; John D Hummel, MD2,4,6; Raul Weiss, MD2,4,6; Vadim V. Fedorov, PhD1,2

Affiliations: 1- Department of Physiology & Cell Biology, The Ohio State University Wexner Medical Center, Columbus, OH, USA
2- Davis Heart & Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA
3- Auckland Bioengineering Institute, The University of Auckland, Auckland, New Zealand
4- Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, USA
5- Department of Molecular Cardiology, Cleveland Clinic, Cleveland Ohio, USA
6- Department of Surgery, Division of Cardiac Surgery, Wexner Medical Center, The Ohio State University, Columbus, OH.

Address for correspondence: Vadim V. Fedorov, PhD

Department of Physiology and Cell Biology, The Ohio State University Wexner Medical Center
300 Hamilton Hall, 1645 Neil Avenue, Columbus OH 43210-1218
e-mails: vadim.fedorov@osumc.edu, fedorov.2@osu.edu
Supplemental Methods

Optical mapping of coronary-perfused atrial preparations

Explanted human hearts were obtained from The Ohio State University Cardiac Transplant Team and LifeLine of Ohio in accordance with The Ohio State University Institutional Review Board. Human hearts (n=37) were obtained in the operating room at the time of cross-clamp and immediately preserved with ice-cold cardioplegic solution and stored at 4°C during transport and dissection. Hearts were transported to the experimental lab within 15 minutes and coronary-perfused with oxygenated cardioplegic solution at 4°C to prevent any potential tissue degradation\(^1\)\(^-\)\(^3\). Human atrial tissue was utilized for optical mapping experiments (n=24, Supplemental Table I) and/or multi-regional immunoblotting analysis (n=18, Supplemental Table II).

Human atrial preparations (n=24) were isolated and coronary-perfused as previously described\(^4\). In nine of the atrial preparations, bi-atrial optical mapping\(^2\) of the whole atria from epicardium (epi) was performed (Figure 1 and Figure 5). In the remaining 15 hearts, the pulmonary veins region, including part of the interatrial septum, was kept by the surgical team for cardiac and/or lung transplantation. As such, endocardial (endo) or epi optical mapping was performed on preparations containing both lateral LA and RA (n=2, Figure 2) or only RA (n=6)\(^1\).

\(^4\). Simultaneous dual-sided optical mapping\(^1\) was utilized on isolated LA (n=2) and RA (n=5) to examine the APD response of sub-epi tissue vs. sub-endo tissue to adenosine. All mapped preparations excluded regions of poor coronary perfusion/ischemia.

After 40-70 minutes of washout with oxygenated Tyrode’s solution and warming to 37°C to ensure tissue recovery and stabilization, the human atrial preparations were immobilized by perfusion with 10μM blebbistatin and stained with near-infrared dye di-4-ANBDQBS (10-40μM)\(^5\).
Imaging was simultaneously conducted with two (n=19) to four (n=3) MiCAM Ultima-L CMOS cameras (SciMedia, Ltd., CA USA) from atrial epi and/or endo fields of view (330-940µm² resolution, 100×100 pixels), sampled at 1000 frames/s. The fluorescent signals were amplified, digitized, and visualized during the experiments. The preparations were instrumented with two customized bipolar pacing electrodes placed on the RA or LA epi or endo surface. Electrical activity was continuously recorded from a 2mm bipolar sensing catheter (7Fr, 8mm tip, Biosense Webster, CA) placed on the atrial epi or endo surface, and a far-field pseudo atrial ECG was recorded by two Ag–AgCl plaque electrodes (9-mm diameter).

Following motion suppression with 10µM blebbistatin and staining with near-infrared dye di-4-ANBDQBS (10-40µM), preparations were equilibrated for 20–30 min before imaging. Atrial preparations (n=19) were sequentially imaged during perfusion by regular Tyrode’s solution (baseline), 10µM and/or 100µM adenosine (Sigma MO, USA) followed by the selective GIRK channel blocker tertiapin (10-100nM) (Tocris Bristol, UK) or washout. In five of the preparations, 100µM adenosine was added after 100nM tertiapin perfusion. The time interval between drug applications was 20-30 minutes. In all whole atrial preparations, sinus rhythm was recovered prior to pacing protocol. All preparations were paced at a basic cycle length (CL) of 500ms, and paced incrementally until the functional refractory period was reached or AF was induced. This restitution pacing protocol was repeated after drug application. Additionally, burst pacing with a CL faster than the functional refractory period was used to induce AF.

All optical mapping data were analyzed by a customized Matlab program as previously described. Activation maps and conduction velocity were constructed from activation times, which were determined from maximum upstroke of OAPs (dV/dt max) for each channel. Atrial activation patterns and 80% of repolarization (APD80) were analyzed at baseline and during adenosine, and tertiapin perfusion. APD changes were compared using recordings taken at 2-5
minutes after adenosine perfusion and 15-20 minutes after tertiapin perfusion, when the maximum drug effects were reached. Activation frequency of RA and LA during AF was measured with dominant frequency (DF) analysis and discrete islands of highest DF were considered AF driver regions, which were limited to 2.5x2.5cm² regions (Figure 3). Additionally, activation maps (Figures 3-5) and movies were used to identify the mechanism of AF reentrant drivers. Here, AF drivers are defined as a localized source(s) of fastest electrical activity visualized as reentrant circuits where two pivot points were mapped or breakthrough pattern and incomplete reentry circuits where one pivot point was mapped that were temporally stable for >70% of the AF duration if only one driver was seen or >30% if two drivers were seen. The temporal stability of the AF driver is estimated by the percentage of activation cycles with activation source origin within a driver region during 8 seconds recording. Based on our previous transmural mapping study, we suggest that the incomplete reentry or stable breakthrough visualized in the present study by single-sided mapping is intramural reentry, and this pattern is referred to as incomplete reentry/breakthrough (Supplemental Figure I). Breakthroughs distributed within 1x1 cm² area of the driver region during an AF episode are defined as spatially stable breakthroughs. Breakthroughs distributed between 1x1cm² and 2.5x2.5cm² area of the driver region are defined as spatially unstable breakthroughs from one single driver.

**Immunobloting**

In thirteen hearts, fresh atrial tissue was collected and flash-frozen in liquid nitrogen during heart dissection from different atrial locations in order to study the A1R and GIRK1/4 protein expression. The location of the collected tissue included LA and RA appendages, lateral LA, posterior LA/inferior pulmonary veins, interatrial septum, crista terminalis, superior, middle and inferior lateral RA (pectinate muscle regions), and RA base (vestibule) as shown in Figure 6. In another five hearts, the atrial tissue was collected from the tip of both appendages and the edge
of LA and RA lateral wall before optical mapping to confirm the direct correlation of functional and molecular data. Protein isolation and immunoblotting were performed by methods previously described\(^3\). Primary antibodies against A1R (1:500; Abcam), GIRK1 (1:500; Alomone, Israel), GIRK4 (1:500; Alomone, Israel) and GAPDH (1:10000, Sigma) were used to quantify corresponding proteins in atrial tissue homogenates\(^7\). Cy5 conjugated goat anti-rabbit (1:2000, Jackson) was used as secondary antibody. The specific bands were detected on a Typhoon 9410 imager (GE Healthcare) and quantified by densitometry analysis (ImageQuant, GE Healthcare). Based on previous publications\(^7,\ 9,\ 10\), specific bands at the expected molecular weights for A1R (~37kDa), GIRK4 (~50kDa) and GIRK1 (~65kDa) proteins were detected. A1R, GIRK1, and GIRK4 protein expression was normalized to GAPDH.

**Ex vivo Micro-Computed Tomographic Imaging**

We conducted detailed 3D structural analysis on the functionally mapped human atria, with emphasis on atrial structures that harbor localized drivers, using iodine-enhanced Micro-Computed Tomographic Imaging (micro-CT) for high-resolution imaging of atrial anatomy\(^11\). After functional mapping, the human atrial tissue was formalin fixed for 24 hours, then washed out with PBS and incubated at 4°C in 25% Lugol iodine solution for 6 days\(^12\). Whole atria or specific AF driver locations were imaged by a micro PET-CT (Inveon, Siemens) scanner to acquire a resolution of 20x20x20 µm\(^3\) with a 2x4x4 cm\(^3\) field of view (AF driver area). Structure-tensor analysis was used to characterize atrial fibers from the 3D atrial volume, as iodine preferentially accumulates within the muscular fibers rather than in connective tissues. **Supplemental Figure II** shows results from our experiment in which we scanned the main AF driver region from Heart #10 and revealed complex myofiber structures that play a critical role in anchoring reentrant arrhythmias. These data show our ability to quantitatively measure and analyze 3D atrial anatomy and fiber orientations together with complex AF activation/conduction patterns within the human atria.
Statistical Analysis

Data are presented as mean ± SD other than AF episode duration, which is presented as mean ± SEM. Comparison of measurements within each heart (LA vs. RA and between treatment conditions), was done using PROC MIXED in SAS 9.4 (SAS institute, Cary, NC) with group or treatment as a fixed factor and heart ID as a random factor. Pairwise comparisons were done with Tukey’s adjustment, which is commonly used and has been recommended for Circulation papers13. Comparisons of inducible vs. non-inducible and failing vs. non-failing hearts were done in R 3.2.3 using an independent groups two-sided t-test or non-parametric Wilcoxon test based on whether normality assumptions were met according to Anderson-Darling test. P-values of 0.05 or below were considered significant.
## Supplemental Tables

### Supplemental Table I. Human heart information for optical mapping experiments

<table>
<thead>
<tr>
<th>Heart No.</th>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>AF</th>
<th>HF</th>
<th>Main Diagnoses</th>
<th>Device</th>
<th>H.W. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>947202</td>
<td>34</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Head Trauma, Drug Abuse</td>
<td>None</td>
<td>446</td>
</tr>
<tr>
<td>2</td>
<td>442404</td>
<td>69</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH, DM, HTN</td>
<td>None</td>
<td>659</td>
</tr>
<tr>
<td>3</td>
<td>257102</td>
<td>43</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH, COPD</td>
<td>None</td>
<td>329</td>
</tr>
<tr>
<td>4</td>
<td>266541</td>
<td>57</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>ICB/ICH, DM</td>
<td>None</td>
<td>635</td>
</tr>
<tr>
<td>5</td>
<td>402879</td>
<td>54</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>ICB/ICH, HTN</td>
<td>None</td>
<td>474</td>
</tr>
<tr>
<td>6</td>
<td>984478</td>
<td>54</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>ICB/ICH, HTN</td>
<td>None</td>
<td>348</td>
</tr>
<tr>
<td>7</td>
<td>481041</td>
<td>41</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH, HTN</td>
<td>None</td>
<td>455</td>
</tr>
<tr>
<td>8</td>
<td>118258</td>
<td>38</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH, HTN</td>
<td>None</td>
<td>575</td>
</tr>
<tr>
<td>9</td>
<td>474083</td>
<td>41</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>CVA, HTN</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>522421</td>
<td>56</td>
<td>F</td>
<td>-</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant), Stroke, VT</td>
<td>ICD</td>
<td>470</td>
</tr>
<tr>
<td>11</td>
<td>240603</td>
<td>51</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>Blunt Head Trauma, HTN</td>
<td>None</td>
<td>320</td>
</tr>
<tr>
<td>12</td>
<td>685884</td>
<td>36</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Cardiac Arrest, HTN</td>
<td>None</td>
<td>415</td>
</tr>
<tr>
<td>13</td>
<td>749693</td>
<td>65</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH, HTN</td>
<td>None</td>
<td>643</td>
</tr>
<tr>
<td>14</td>
<td>450564</td>
<td>30</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant), Atrial Flutter</td>
<td>ICD</td>
<td>482</td>
</tr>
<tr>
<td>15</td>
<td>479062</td>
<td>50</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Ischemic HF (Transplant), VT</td>
<td>ICD</td>
<td>636</td>
</tr>
<tr>
<td>16</td>
<td>537114</td>
<td>48</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant)</td>
<td>ICD,</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LVAD</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>323104</td>
<td>63</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant), HTN</td>
<td>CRT</td>
<td>543</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>328163</td>
<td>63</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Ischemic HF (Transplant)</td>
<td>ICD</td>
<td>506</td>
</tr>
<tr>
<td>19</td>
<td>728878</td>
<td>40</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant)</td>
<td>LVAD, PM</td>
<td>747</td>
</tr>
<tr>
<td>20</td>
<td>380071</td>
<td>43</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>Respiratory Arrest, CAD, HTN, DM</td>
<td>None</td>
<td>603</td>
</tr>
<tr>
<td>21</td>
<td>645444</td>
<td>47</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Ischemic HF (Transplant)</td>
<td>LVAD</td>
<td>411</td>
</tr>
<tr>
<td>22</td>
<td>963542</td>
<td>60</td>
<td>F</td>
<td>-</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant)</td>
<td>ICD</td>
<td>329</td>
</tr>
<tr>
<td>23</td>
<td>422358</td>
<td>52</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Ischemic HF (Transplant)</td>
<td>LVAD</td>
<td>498</td>
</tr>
<tr>
<td>24</td>
<td>514489</td>
<td>42</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>Cardiac Arrest</td>
<td>None</td>
<td>327</td>
</tr>
</tbody>
</table>

Abbreviations: AF = Atrial fibrillation; CAD = Coronary artery disease; CRT = Cardiac resynchronization therapy; CVA/ICH = Cardiovascular attack/Intracranial hemorrhage; DM = Diabetes mellitus; HF = Heart failure; HTN = Hypertension; H.W. = Heart weight; ICD = Implantable cardiac defibrillator; LVAD = Left ventricular assist device; PM = Pacemaker; VT = Ventricular tachycardia.
Supplemental Table II. Human heart information for molecular mapping experiments

<table>
<thead>
<tr>
<th>Heart No.</th>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>AF</th>
<th>HF</th>
<th>Main Diagnoses</th>
<th>Device</th>
<th>H.W. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>947202</td>
<td>34</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Head Trauma, Drug Abuse</td>
<td>None</td>
<td>446</td>
</tr>
<tr>
<td>2</td>
<td>442404</td>
<td>69</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH, DM, HTN</td>
<td>None</td>
<td>659</td>
</tr>
<tr>
<td>10</td>
<td>522421</td>
<td>56</td>
<td>F</td>
<td>-</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant), Stroke, VT</td>
<td>ICD</td>
<td>470</td>
</tr>
<tr>
<td>12</td>
<td>685884</td>
<td>36</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Cardiac Arrest, HTN, Drug/Alcohol Abuse</td>
<td>None</td>
<td>415</td>
</tr>
<tr>
<td>18</td>
<td>328163</td>
<td>63</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>Ischemic HF (Transplant)</td>
<td>ICD</td>
<td>506</td>
</tr>
<tr>
<td>25</td>
<td>911614</td>
<td>65</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>Non-Ischemic HF (Transplant), AF</td>
<td>CRT, LVAD</td>
<td>716</td>
</tr>
<tr>
<td>26</td>
<td>774694</td>
<td>50</td>
<td>F</td>
<td>-</td>
<td>+</td>
<td>Ischemic HF (Transplant), CAD</td>
<td>None</td>
<td>486</td>
</tr>
<tr>
<td>27</td>
<td>674541</td>
<td>64</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>Stroke, CAD, HF, DM, HTN, AF</td>
<td>ICD, LVAD</td>
<td>599</td>
</tr>
<tr>
<td>28</td>
<td>600245</td>
<td>51</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>CVA/ICH</td>
<td>None</td>
<td>507</td>
</tr>
<tr>
<td>29</td>
<td>809108</td>
<td>60</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Cardiac Arrest, HTN,DM</td>
<td>None</td>
<td>842</td>
</tr>
<tr>
<td>30</td>
<td>147381</td>
<td>58</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>Blunt Injury, CAD, HTN, AF</td>
<td>None</td>
<td>512</td>
</tr>
<tr>
<td>31</td>
<td>768159</td>
<td>44</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Cardiac Arrest, DM, VF</td>
<td>None</td>
<td>279</td>
</tr>
<tr>
<td>32</td>
<td>380071</td>
<td>43</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>Respiratory Arrest, HTN, DM, COPD, CAD, MI</td>
<td>None</td>
<td>603</td>
</tr>
<tr>
<td>33</td>
<td>712301</td>
<td>67</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Blunt Injury, HTN</td>
<td>None</td>
<td>527</td>
</tr>
<tr>
<td>34</td>
<td>845013</td>
<td>26</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Cardiac Arrest, VSD, VF, VT</td>
<td>PM</td>
<td>497</td>
</tr>
<tr>
<td>35</td>
<td>724569</td>
<td>64</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>Non Ischemic HF(Transplant), AF, VT, HTN</td>
<td>ICD</td>
<td>636</td>
</tr>
<tr>
<td>36</td>
<td>971258</td>
<td>57</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>Ischemic HF(Transplant), CAD, LVAD, ICD</td>
<td>LVAD, ICD</td>
<td>619</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MI, AF</td>
<td></td>
<td>- Blunt Injury, Drug/Alcohol abuse,</td>
<td>None</td>
<td>584</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>37</td>
<td>574165</td>
<td>62</td>
<td>M</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>AF</td>
</tr>
</tbody>
</table>

Abbreviations: VF = Ventricular fibrillation; VSD = Ventricular septal defect. Other abbreviations as seen in Supplemental Table I. * denotes that heart was used for both optical and molecular mapping.
Supplemental Table III. Adenosine effect on right atrial APD in failing vs non-failing hearts

<table>
<thead>
<tr>
<th></th>
<th>Average APD (ms)</th>
<th>Minimum APD (ms)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Ado10</td>
<td>Ado100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(%) APD shortening</td>
<td>(%) APD shortening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>295±38</td>
<td>241±43</td>
<td>244±42</td>
<td>288±36</td>
</tr>
<tr>
<td>(n=8)</td>
<td>(14.3%)</td>
<td>(16.8%)</td>
<td></td>
<td>(17.1%)</td>
</tr>
<tr>
<td></td>
<td>Ado10</td>
<td>Ado100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>237±47</td>
<td>227±55</td>
<td>237±47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(16.9%)</td>
<td>(16.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>287±50</td>
<td>249±32</td>
<td>218±38</td>
<td>279±48</td>
</tr>
<tr>
<td>(n=13)</td>
<td>(14.7%)</td>
<td>(18.4%)</td>
<td></td>
<td>(16.9%)</td>
</tr>
<tr>
<td></td>
<td>Ado10</td>
<td>Ado100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>207±37</td>
<td>236±32</td>
<td>207±37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(20.9%)</td>
<td>(16.9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Ado10 = Adenosine 10µM; Ado100 = Adenosine 100µM; APD = Action potential duration 80%; HF = Heart failure; NF = Non-failing
Supplemental Figures and Figure Legends

Supplemental Figure I.

**Different Types of Intramural Reentrant Driver Visualization by Single-Surface Mapping**

Based on our dual-sided optical mapping study, different types of intramural reentrant driver visualization by single-surface mapping exist: 1) complete reentry circuits, 2) incomplete reentry circuits and spatially stable breakthrough, and 3) spatially unstable breakthrough. Abbreviations: Epi - epicardium; Endo - endocardium; IVC - inferior vena cava; LRA - lateral right atria; PLA - posterior left atria; SVC – superior vena cava.
Supplemental Figure II. Spatially and temporally stable incomplete reentry circuits/breakthrough driving adenosine-induced AF in human Heart #1 (947202) from Figure 3.

A, Dominant frequency (DF) map during 100μM adenosine-induced AF. B, Activation map of the driver region in the lateral right atria (LRA). The colored dots indicate the mapped breakthrough locations for beats 1-5 shown in panel D. Dashed arrow shows the projected reentry circuit of the AF driver. C, The anatomy of the projected AF driver reentrant track along pectinate muscles. D, Activation maps of the AF driver region from 5 consecutive beats during adenosine-induced AF. E, Optical action potentials (OAPs) from the AF driver region. Abbreviations as in Supplemental Figure I; AF - atrial fibrillation; CT- crista terminalis; DF - dominant frequency; IAS- intra-atrial septum.
Supplemental Figure III. Spatially and temporally stable reentry driving adenosine-induced AF in human Heart #10 (522421) from Figure 4.

A, Activation map during 10μM adenosine-induced AF. Arrow shows the reentry circuit of the AF driver. B, Micro-CT revealed sub-endocardial structure of the microanatomic AF driver track in the superior lateral right atria (LRA). C, Optical action potentials on the reentry track from the location indicated by colored numbers in panel A and B. D, Activation maps showing stability of reentrant AF driver during 5 consecutive beats. Abbreviations as in Supplemental Figure I.
Supplemental Figure IV. Conduction velocity of the reentrant AF driver in Heart #10 (522421) from Figure 4.

Activation maps showing conduction velocity of the AF reentrant circuit in Heart #10 (522421) for five consecutive beats. Small black numbers and arrows indicate local conduction velocity and direction. White arrow indicates reentrant track shown in Supplemental Figure III. Grey oval indicates region of slow conduction at center of reentrant track. Numbers at bottom right of activation maps show the average conduction velocity and standard deviation along the reentrant track. Abbreviations as in Supplemental Figure I; CV - conduction velocity.
Supplemental Figure V. APD analysis in driver regions during adenosine-induced AF.

**A** APD of Driver Regions in AF Inducible Hearts

- Stable Driver (n=8)
- Unstable Driver (n=4)
- Sustained AF (n=9)
- Unsustained AF (n=3)

**B** Correlation of Highest DF vs APD in Driver Regions

- $r^2 = 0.76$, $p < 0.01$, n=14

A, The APD of stable driver regions is shorter than APD in unstable driver regions and the APD in driver regions of sustained AF is shorter than that of unsustainable AF. See Table 1 for driver characteristics. B, The correlation of highest DF vs APD in driver regions.
Supplemental Figure VI. Right to left atrial expression ratio of A1R and GIRK4 proteins.

A, Immunoblot of A1R and GIRK4 protein in LA and RA from failing vs non-failing and non-AF hearts. B, AF vs non-AF and non-failing hearts. GAPDH normalized band density is shown in mean±SD and normalized to the value of the lateral left atria. See Figure 7 for more details.
Supplemental References


