Mitochondrial respiration and microRNA expression in right and left atrium of patients with atrial fibrillation

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Mitochondrial respiration and microRNA expression in right and left atrium of patients with atrial fibrillation. Physiol Genomics 46: 505–511, 2014. First published May 13, 2014; doi:10.1152/physiolgenomics.00042.2014.—Atrial fibrillation (AF) is the most common cardiac arrhythmia with a potential to cause serious complications. Mitochondria play central roles in cardiomyocyte function and have been implicated in AF pathophysiology. MicroRNA (miR) are suggested to influence both mitochondrial function and the development of AF. Yet mitochondrial function and miR expression remain largely unexplored in human atrial tissue. This study aims to investigate mitochondrial function and miR expression in the right (RA) and left atria (LA) of patients with AF and sinus rhythm (SR). Myocardial tissue from the RA and LA appendages was investigated in 37 patients with AF (n = 21) or SR (n = 16) undergoing coronary artery bypass surgery and/or heart valve surgery. Mitochondrial respiration was measured in situ after tissue permeabilization by saponin. MiR expression was assessed by miR array and real-time quantitative reverse-transcription polymerase chain reaction. Maximal mitochondrial respiratory rate was increased in both RA and LA tissue of patients with AF vs. SR. Bialtral downregulation of miR-208a and upregulation of miR-106b, -144, and -451 were observed in AF vs. SR. In addition, miR-15b was upregulated in AF within RA only, and miR-106a, -18a, -18b, -19a, -19b, -23a, -25, -30a, -363, -486-5p, -590-5p, and -93 were upregulated in AF within LA only. These findings suggest that mitochondrial function and miR are involved in AF pathophysiology and should be areas of focus in the exploration for potential novel therapeutic targets.

METHODS

Patients. This study includes 37 patients with normal SR (n = 16) or AF (n = 21) undergoing isolated CABG surgery, mitral valve replacement, aortic valve replacement (AVR), or combined CABG/AVR surgery at St. Olav’s University Hospital, Trondheim, Norway. Applicable patients underwent concomitant peroperative ablation treatment for AF. The AF group consisted of patients with either paroxysmal AF (pAF, n = 11) or chronic AF (cAF, n = 10). Researchers were blinded to patient category during data collection and processing. All patients gave written informed consent prior to participation. The study was approved by the Regional Committee for Medical Research Ethics of Norway and conformed to the principles outlined in the Declaration of Helsinki. The study was registered at http://www.clinicaltrials.gov under identification number NCT01493128.

Study design. Standard procedures of the department were followed, including presurgical preparation, anesthesia, and surgical approach. Premedication was administered 1–3 h before surgery and included acetaminophen and morphine-scopolamine. Anesthetic procedures included intravenous use of thiopental, fentanyl, propofol, and cisatracurium, as well as inhalation of isoflurane during pulmonary ventilation. Cardiopulmonary bypass (CPB) was conducted with a membrane oxygenator at mild hypothermia of 32–34°C. In cases of

atrial fibrillation; atrium; myocardium; mitochondria; microRNA

ATRIAL FIBRILLATION (AF) CONSTITUTES the most common cardiac arrhythmia in clinical practice, the incidence of which is expected to increase over the next decades (7). Current treatment modalities of AF, such as antiarrhythmic and rate-regulating drugs have constrained effectiveness because they only influence a subset of ionic channels, and the success rate of ablation therapy is also limited in its long-term ability to prevent disturbance in myocardial electrophysiology (26). This may be attributed to the fact that none of the currently available treatment options target the underlying pathophysiological processes of the disease (26). Further understanding of cellular alterations in AF have been called for to ultimately improve management strategies for AF (25). While investigations of human myocardium are sparse in general, studies of left atrial (LA) tissue are particularly limited, as the right atrial (RA) appendage is generally more readily available for investigation.

Although AF-induced atrial remodeling involves processes closely linked to mitochondrial function, mitochondria have not been a major focus in research on AF. A recent study links preoperative mitochondrial function of the RA appendage and the risk for new-onset postoperative AF after coronary artery bypass graft (CABG) surgery in patients with metabolic syndrome (17). While mitochondria must keep up with a high demand for energy through oxidative phosphorylation, balancing its different tasks is important and focus must also remain on sustaining homeostasis of electrolytes and pH, as well as limiting production of reactive oxygen species (ROS) (9). MicroRNA (miR) are small RNA molecules that regulate mRNA and have recently been indicated to influence both mitochondrial function (12) and cardiac arrhythmia (14, 28).

This study aims to investigate mitochondrial function and miR expression in the RA and LA of patients with AF and sinus rhythm (SR).
peroperative ablation, tissue samples were obtained prior to ablation. St. Thomas’ (Martindale Pharmaceuticals, Brentwood, UK) crystallloid or blood cardioplegia was administered for myocardial protection approximately every 20 min. Heart rhythm was monitored pre- and peroperatively by electrocardiography.

**Tissue samples.** Tissue was obtained from the RA appendage during venous cannulation, whereas tissue from the LA appendage was obtained during CPB. One part of each sample was transferred to a preservation solution and used for mitochondrial respiratory assessment, and another was immediately snap-frozen in liquid nitrogen and kept at −80°C for miR analyses.

**Mitochondrial respiration in situ.** Assessment of mitochondrial respiratory rates was performed in situ as previously described and reviewed by others (11, 19). Connective tissue was removed by fine dissection under a microscope, and the remaining myocardium transformed from dense tissue to a fine net with precise forceps, while continuously maintained at 4°C. The sample was submerged in a storage solution with an added 50 μg/ml saponin for 30 min at 4°C under continuous shaking to permeabilize cell membranes while leaving mitochondrial membranes intact. Next, the sample was placed into pure storage solution and shaken for ≥10 min to remove any residue of saponin, followed by another rinse cycle of 10 min in respiration solution. The permeabilized myocardial tissue was placed into 3 ml of respiration solution inside a water-jacketed respiration chamber, where a Clark-type microcathode oxygen electrode (Strathkelvin Instruments, Glasgow, UK) with a fluorinated electrolyte propylene membrane was used for respiration measurement. The storage solution contained 2.77 mM CaK2EGTA, 7.23 mM K2EGTA (100 nM free Ca2+), 6.56 mM MgCl2 (1 mM free Mg2+), 20 mM taurine, 0.5 mM dithiothreitol (DTT), and 20 mM imidazole 50 mM potassium-methanesulfonate (CH3KO3S), 5.7 mM Na2ATP, 15 mM phosphocreatine (pH 7.1 at 22°C). The respiration solution contained 2.77 mM CaK2EGTA, 7.23 mM K2EGTA (100 nM free Ca2+), 1.38 mM MgCl2 (1 mM free Mg2+), 20 mM taurine, 0.5 mM DTT, and 20 mM imidazole (pH 7.1 at 22°C), 90 mM potassium-methanesulfonate (CH3KO3S), 10 mM sodium-methanesulfonate (CH3SO3Na), 3 mM K2HPO4, 10 mM glutamate, 4 mM malate, and 2 mg/ml bovine serum albumin. Basal respiratory rate (V0) was assessed with glutamate and malate as substrates for Complex I of the electron transport chain in the absence of adenine diphosphate (ADP), followed by the addition of a subsaturating amount of 0.1 mM ADP, registering VADP. The addition of 20 mM creatine allowed measurement of Vcreatine. Supplementation of a saturating amount of 2 mM ADP preceded assessment of the maximal respiratory rate of the electron transport chain complex I through IV with glutamate and malate as substrates (Vmax). We added 10 mM succinate, supplementing complex II of the respiratory chain (Vsuccinate), after which addition of complex I inhibitor amytal (1 mM amobarbital) allowed assessment of complex II (Vamytal). Furthermore, the supplement of ascorbate (0.5 mM) and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD, 0.5 mM) induced complex IV activity (VascorbateTMPD). At last, respiratory rates were assessed with irreversible inhibition of cytochrome c oxidase (complex IV), accomplished by addition of azide (4 mM) (Vazide). Tissue samples were dried in a heat centrifuge at 60°C for 75 min before being weighed. Respiratory rates are given as micromoles O2·min−1·g dw−1. The ratio of Vmax/V0, referred to as the acceptor control ratio (VADP/Vmax), the effect of creatine on respiration is given as percent decrease in respiratory rate (ΔRR Cr). Vazide/Vmax estimates excess respiration of the cytochrome oxidase complex. The apparent constant of Michaelis for ADP was estimated both in the absence [Km(ADP−C)] and in the presence of creatine [Km(ADP−C)] (19). miR expression. All samples with sufficient amount of tissue underwent miR analyses: RA SR n = 12, RA AF n = 20, LA SR n = 8, LA AF n = 20. All miR analyses including RNA isolation, miR array and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) were performed at Exiqon Services, Vedbaek, Denmark. Tissue samples were transported from St. Olav’s Hospital, Norway, to Exiqon Services, Denmark, on dry ice transportation medium. Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) was used to control RNA quality. Samples were labeled for miR array using Exiqon’s miRCURY LNA microRNA Hi-Power Labeling Kit, Hy3/Hy5 and hybridized on the miRCURY LNA microRNA Array 6th gen (Exiqon) containing capture probes targeting all human miRs registered in miBASE 16.0. A Tecan HS4800 hybridization station (Tecan) was used for hybridization. Array slides were scanned on the Agilent G2565BA Microarray Scanner System (Agilent Technologies), and the image analysis was carried out with the ImaGene 9 (miRCURY LNA microRNA Array Analysis Software, Exiqon). The quantified signals were background corrected and then normalized with the global Lowess (Locally Weighted Scatterplot Smoothing) regression algorithm. The miRmiRNA LUNA Universal RT miRNA PCR kit and mix custom panel was used for miR RT-qPCR designed to detect miR-1, -100, -106a, -106b, -125b, -133a, -133b, 138-1*, -142-5p, -144, -146a, -155, -15b, -17, -18, -18a, -18b, -18c, -191, -193a-3p, -199a-5p, -19a, -19b, -208a, -208b, -21, -22a, -23a, -25, -26a, -26b, -29b, 30a, 30b, -32a, -32c, -363, -451, -486-5p, -590-5p, -600, -92a, -92b, -93. Amplification was performed with LightCycler 480 Real-Time PCR System (Roche). The amplification curves were analysed with Roche LC software, both for determination of crossing point (Cp) and for melting curve analysis. The amplification efficiency was calculated with algorithms similar to the LinReg software. The average of five normalization assays detected in all samples was used for normalization. miR expression from RT-qPCR are presented as normalized crossing point (dCp), calculated by subtracting the Cp of the specific miR from the average Cp of normalization miRs.

**Statistical analysis.** Statistics were performed with SPSS 21.0 for Mac (IBM SPSS Statistics, Chicago, IL). Unpaired Student’s t-test was used for between-group comparison for continuous variables. A two-tailed P value < 0.05 was considered significant. Pearson’s χ2 and Fisher’s exact test were used for categorical data. Hochberg-Bonferroni correction was applied to analyses of miR expression. Graphics were produced by the use of GraphPad Prism 5 (GraphPad Software, San Diego, CA).

**RESULTS**

Clinical data are presented in Table 1. Patient characteristics were similar in AF vs. SR, except for an increased number of patients with mitral valve disease in patients with AF (76% in AF vs. 44% in SR). Preoperative transthoracic echocardiographic evaluation of LA diameter was documented for 25 patients (68%) (12 in SR and 13 in AF), revealing a tendency of LA dilatation (defined as LA diameter ≥41 mm) in AF, though not statistically significant (17% in SR vs. 54% in AF, P = 0.10). Among patients with a history of AF, 55% were in AF on electrocardiography (ECG) immediately preoperatively (of which 10 were diagnosed with cAF and 1 with pAF).

**Mitochondrial respiration.** AF was associated with increased maximal mitochondrial respiration rates (Vmax) in the myocardium of both RA (Fig. 1A) and LA (Fig. 1B) compared with patients with SR.

Within RA, significantly higher mitochondrial respiration rates were observed in AF samples for VADP (AF 4.2 ± 1.8 vs. SR 2.8 ± 1.4, P = 0.02), Vmax (AF 9.8 ± 3.4 vs. SR 7.1 ± 2.6, P = 0.01), and Vsuccinate (AF 12.3 ± 4.3 vs. SR 8.2 ± 3.2, P = 0.01).

Within LA, respiratory rates were significantly elevated in AF compared with SR with regards to Vcreatine (AF 7.7 ± 3.0
Table 1. Patient characteristics and perioperative parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SR (n = 16)</th>
<th>AF (n = 21)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Female sex, n (%)</td>
<td>4 (25)</td>
<td>5 (24)</td>
<td>0.62</td>
</tr>
<tr>
<td>Age, μ ± SD</td>
<td>71 ± 8</td>
<td>70 ± 8</td>
<td>0.84</td>
</tr>
<tr>
<td>Isolated CABG surgery, n (%)</td>
<td>10 (63)</td>
<td>10 (48)</td>
<td>0.29</td>
</tr>
<tr>
<td>Isolated AVR surgery, n (%)</td>
<td>3 (19)</td>
<td>4 (19)</td>
<td>0.66</td>
</tr>
<tr>
<td>Combined CABG/AVR surgery, n (%)</td>
<td>2 (13)</td>
<td>5 (24)</td>
<td>0.67</td>
</tr>
<tr>
<td>Isolated MVR surgery, n (%)</td>
<td>1 (6)</td>
<td>2 (10)</td>
<td>0.60</td>
</tr>
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No significant differences were observed between AF vs. SR in ACR, ADP sensitivity, creatine-induced respiration rate, excess respiration of the cytochrome oxidase complex, appKm(ADP–Cr), or appKm(ADP+Cr) in either RA (Table 2) or LA (Table 3).

An analysis of subgroups pAF vs. cAF was judged inappropriate due to the number of patients included. However, a tentative examination revealed similar maximal mitochondrial respiration rates in both subgroups (RA Vmax: SR 7.1 ± 2.6, pAF 9.6 ± 3.8, cAF 9.9 ± 3.2, and LA Vmax: SR 7.5 ± 2.1, pAF 10.2 ± 3.5, cAF 10.1 ± 3.4).

miR. Within RA, expression of miR-106b, miR-144, miR-15b, and miR-451 was significantly increased in AF compared with SR, whereas miR-208a was significantly downregulated in AF vs. SR (Fig. 2A).

The largest number of differences in miR between the experimental groups was found in LA samples (Fig. 2B); significantly elevated expression levels were observed for miR-106a, -106b, miR-144, miR-18a, miR-18b, miR-19a, miR-19b, miR-23a, miR-25, miR-30a, miR-363, miR-451, miR-486-5p, miR-590-5p and miR-93, whereas there was significantly reduced expression of miR-208a in AF vs. SR.

**DISCUSSION**

This is to our knowledge the first study to demonstrate that AF is associated with increased maximal mitochondrial respiratory rates of both RA and LA myocardium compared with patients with normal SR. This is interesting both because it supports the hypothesis that mitochondria play a role in AF pathophysiology and because it provides evidence that AF-associated alterations involve both atria. Most previous human studies investigating underlying mechanisms of AF have included the RA appendage only (1, 10, 16, 22), whereas investigations including the LA appendage have mainly been re...

![Fig. 1. Mitochondrial respiration rates in patients with normal sinus rhythm (SR) vs. patients with atrial fibrillation (AF) within the right atrium (RA) (A) and left atrium (LA) (B) given as means ± SD in μmol O2·min⁻¹·g dry weight⁻¹ (dw) of myocardial tissue. RA SR n = 16, RA AF n = 21, LA SR n = 10, LA AF n = 21. V0, basal respiration rate in the presence of glutamate and malate (substrates for complex I in the electron transport chain) without adenosine diphosphate (ADP); VADP, respiration rate with subsaturating amount of ADP; Vcreatine measurement after addition of creatine; Vmax, maximal respiration rate with saturating amount ADP and glutamate and malate as substrates; Vsuccinate, respiration rate with succinate supplementing complex II; Vamytal, respiration rate while inhibiting complex I with amytal; VascorbateTMPD, following addition of ascorbate (0.5 mM) and N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD, 0.5 mM) which induces respiration in complex IV; Vazide, respiration after irreversible inhibition of complex IV by azide. Error bars ± SE, *P < 0.05.](http://physiolgenomics.physiology.org/Downloadedfromhttp://physiolgenomics.physiology.org/
Mitochondrial respiratory parameters of the right atrium

<table>
<thead>
<tr>
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<th>SR, μ ± SD</th>
<th>AF, μ ± SD</th>
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<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>(V_{\text{amylal/V}_{\text{max}}})</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.28</td>
</tr>
<tr>
<td>(V_{\text{ADP/V}_{\text{max}}})</td>
<td>0.4 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.92</td>
</tr>
<tr>
<td>ACR</td>
<td>4.9 ± 3.9</td>
<td>5.0 ± 1.9</td>
<td>0.95</td>
</tr>
<tr>
<td>(K_m^{(\text{ADP}/\text{Cr})})</td>
<td>194 ± 149</td>
<td>158 ± 101</td>
<td>0.41</td>
</tr>
<tr>
<td>(K_m^{(\text{ADP}+\text{Cr})})</td>
<td>101 ± 123</td>
<td>65 ± 39</td>
<td>0.37</td>
</tr>
<tr>
<td>↑ RR Cr</td>
<td>73 ± 57</td>
<td>63 ± 35</td>
<td>0.52</td>
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\(\text{n} \), Number of patients; \(V_{\text{amylal/V}_{\text{max}}}\), quantification of excess respiration of the cytochrome oxidase complex; \(V_{\text{ADP/V}_{\text{max}}}\), ADP sensitivity ratio; ACR, acceptor control ratio; \(K_m^{(\text{ADP}/\text{Cr})}\), apparent Michaelis-Menten constant for ADP (μM) in the absence of creatine; \(K_m^{(\text{ADP}+\text{Cr})}\), apparent Michaelis-Menten constant for ADP (μM) in the presence of creatine; ↑ RR Cr, increase in respiration rate after addition of creatine.

Mitochondrial respiratory parameters of the left atrium

<table>
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<th>SR, μ ± SD</th>
<th>AF, μ ± SD</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>(V_{\text{amylal/V}_{\text{max}}})</td>
<td>1.3 ± 0.5</td>
<td>1.0 ± 0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>(V_{\text{ADP/V}_{\text{max}}})</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>0.69</td>
</tr>
<tr>
<td>ACR</td>
<td>4.2 ± 2.4</td>
<td>4.5 ± 1.7</td>
<td>0.74</td>
</tr>
<tr>
<td>(K_m^{(\text{ADP}/\text{Cr})})</td>
<td>210 ± 168</td>
<td>141 ± 77</td>
<td>0.27</td>
</tr>
<tr>
<td>(K_m^{(\text{ADP}+\text{Cr})})</td>
<td>76 ± 80</td>
<td>43 ± 34</td>
<td>0.27</td>
</tr>
<tr>
<td>↑ RR Cr</td>
<td>65 ± 50</td>
<td>69 ± 33</td>
<td>0.81</td>
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\(\text{n} \), Number of patients; \(V_{\text{amylal/V}_{\text{max}}}\), quantification of excess respiration of the cytochrome oxidase complex; \(V_{\text{ADP/V}_{\text{max}}}\), ADP sensitivity ratio; ACR, acceptor control ratio; \(K_m^{(\text{ADP}/\text{Cr})}\), apparent Michaelis-Menten constant for ADP (μM) in the absence of creatine; \(K_m^{(\text{ADP}+\text{Cr})}\), apparent Michaelis-Menten constant for ADP (μM) in the presence of creatine; ↑ RR Cr, increase in respiration rate after addition of creatine.
hindlimb ischemia in mice overexpressing miR-93 (8). One may hypothesize that these features would represent an advantage in rapidly contracting atria during AF. It has recently been demonstrated that miR-18b may inhibit the process of transforming growth factor-β-induced differentiation into smooth muscle cells (13), and miR-486-5p has been linked to accumulation of superoxide anion, induction of DNA damage, and reduced cell proliferation, contributing to a senescent phenotype in human fibroblasts (6). While downregulation of miR-590-5p increased atrial collagen production and fibrosis, upregulation of miR-590-5p decreased the fibrotic response in a canine model of nicotine-induced AF (24).

miR expression profiles have recently been investigated in the RA appendages of patients with mitral stenosis with and without AF (30) and in RA and LA appendages from patients with AF and valvular heart disease (4). The described miR expression profiles diverge between the two studies. miR-208a was downregulated and miR-18b was upregulated in RA of patients with AF relative to that of healthy controls, in accordance with our observations (30). However, RA miR-18a and -19b were downregulated in AF as opposed to in our study (30). None of the miRs assessed in our study were investigated with PCR in the study of patients with valvular heart disease and AF, precluding detailed comparison (4). The inconsistency of results may reflect both that the current understanding of miR expression in patients with AF is at a preliminary stage and that the population of patients with AF is heterogeneous.

Study implications and limitations. Although we intended to obtain comparable patient groups, there is a possibility of interference between AF-induced alterations vs. alterations due to comorbidities. Investigations of structural and functional alterations in myocardial tissue of “lone” AF in human subjects are sparse; however, studies of goats and dogs have demonstrated AF-specific alterations in the absence of significant comorbidity (2, 18).

The atrial appendage was used for investigation because it constitutes the most accessible and nontraumatic location for sampling human myocardial tissue. Investigations of structural changes in a goat model of experimental AF indicates that alterations of the atrial appendages also applies to other parts of the atrial myocardium (2), but this has not been verified in human myocardium.

While assessment of mitochondrial respiratory capacity in situ has several advantages (11), the method does not identify whether the difference in mitochondrial respiratory capacity is due to qualitative or quantitative changes of the mitochondria. An investigation allowing quantification of the number of mitochondria in the atrial tissue would have provided valuable additional information but was precluded in the current study due to limited amount of tissue.

Direct measurement of ROS production as well as investigations of mitochondrial morphology would have added valuable information but was precluded due to limited tissue availability.

Existing data on the roles of miRs in the human heart and AF are sparse and mainly associative in nature. Further research is needed to establish causal relations.
Conclusion

AF is associated with increased maximal mitochondrial respiration rates and altered miR expression in RA and LA compared with patients with SR. These results suggest that mitochondrial function and miR are involved in AF pathophysiology and should be areas of focus in the exploration for potential novel therapeutic targets.

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