

# Endothelin system and atrial fibrillation post-cardiac surgery

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Received: 19 November 2007 / Accepted: 8 January 2008 / Published online: 9 February 2008  
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## Abstract

**Objective** We investigated the relation between the endothelin system and atrial fibrillation.

**Background** Endothelin has been implicated in the pathophysiology of atrial fibrillation, but the exact role of A- and B-receptors is unknown.

**Methods** We obtained right atrial biopsies from patients in sinus rhythm and preserved left ventricular function, undergoing off-pump coronary artery bypass grafting. The expression of endothelin, A- and B-receptors was measured using real time reverse-transcribed polymerase chain reaction.

**Results** We studied 52 patients (45 male, mean age  $66 \pm 1$  years, mean ejection fraction  $52 \pm 1\%$ ). During a 5-day post-operative period, persistent atrial fibrillation occurred in 15 patients (28.8%). Endothelin mRNA expression was comparable in patients who subsequently developed atrial fibrillation and in those maintaining sinus rhythm. However, the former group displayed down-regulation of endothelin

A- (by approximately 60%,  $p=0.0059$ ) and of B-receptors (by approximately 40%,  $p=0.0084$ ). The decreased endothelin A-receptor expression could predict atrial fibrillation occurrence (Wilks  $\lambda=0.86$ ,  $F=6.16$ ,  $p=0.017$ ).

**Conclusion** Decreased endothelin A- and B-receptor expression is associated with atrial fibrillation after bypass surgery.

**Keywords** Endothelin · Atrial fibrillation · Bypass surgery

## 1 Introduction

Atrial fibrillation (AF) represents the most frequent arrhythmia encountered in clinical practice, accounting for about one third of hospitalizations for arrhythmia [1]. As a result of its prevalence and its negative impact on morbidity and mortality, AF is one of the most vexing disease entities in cardiovascular medicine [1]. A number of diseases are associated with this arrhythmia, such as congestive heart failure, hypertension, valvular heart disease and coronary artery disease [2].

The mechanisms involved in the genesis of AF are complex and several aspects remain obscure. There are indications that endothelin-1 (ET-1) participates in the pathophysiology of AF [3, 4]. These indications stem from observations of elevated plasma concentrations in patients with AF and concomitant heart disease, such as congestive cardiac failure [3] and mitral valve disease [4]. The vasoconstrictive 21-amino-acid peptide ET-1 increases intracellular calcium concentration and induces cell growth, acting via two G-protein-coupled receptors [5]. Amidst a plethora of predisposing clinical conditions, AF is frequent after cardiac surgery and is associated with significant morbidity, prolonged hospital stay and increased costs [6]. Despite

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current pharmacological therapies and operative techniques, the incidence of the arrhythmia remains high, occurring in approximately one-third of such patients [6]. ET-1 plasma levels are increased as a result of cardiac surgery and have been implicated in the genesis of atrial tachyarrhythmias [7]. However, the precise pathophysiologic role of the endothelin system in post-operative AF is unclear.

The present study investigated the mRNA expression of endothelin-A-receptors (ETA-Rs) and endothelin-B-receptors (ETB-Rs) in right atrial appendages of patients undergoing cardiac surgery and its potential association with the incidence of post-operative AF.

## 2 Methods

Patients in sinus rhythm, with preserved left ventricular function and without significant valvular heart disease were included. The study complies with the principles outlined in the declaration of Helsinki. The Institutional Ethics' Committee approved the protocol and all patients gave written informed consent.

### 2.1 Patient selection

Consecutive patients below 85 years of age, scheduled for elective coronary artery bypass grafting for stable angina pectoris were screened. All patients had a 12-lead electrocardiogram, transthoracic echocardiography, coronary angiography, as well as hematological and biochemical examinations as part of their clinical assessment. Apart from beta-blockade, no patient was on any antiarrhythmic medication, including amiodarone, and none had received hormone replacement therapy. Aspirin was discontinued at least 10 days prior to the scheduled operation. Patients in sinus rhythm without previously documented episodes of AF and without recent infection, malignancies, autoimmune or inflammatory diseases, renal failure, or hepatic failure were considered eligible for the study. Patients were excluded in the presence of the following additional criteria: acute or recent (<2 months) myocardial infarction, unstable angina, heart failure, left ventricular ejection fraction below 45% (measured by left ventricular angiography), systolic pulmonary artery pressure above 50 mmHg (measured by Doppler echocardiography), left atrial diameter greater than 55 mm and plasma creatinine above 1.8 mg/dl. Thyroid function tests were performed prior to study entry and patients with thyroid dysfunction (including sub-clinical hyperthyroidism, defined as thyroid stimulating hormone below 0.05 ng/ml) were also excluded.

Fifty-two consecutive patients were finally enrolled in the study. Their characteristics are presented in Table 1 and their pharmacological treatment is shown in Table 2.

### 2.2 Tissue sampling and mRNA analysis

All patients underwent coronary artery bypass grafting without the use of extracorporeal circulation. After exposure of the heart and prior to the grafting procedure, a right atrial appendage biopsy was obtained. The specimens were immediately frozen in liquid nitrogen and stored in  $-80^{\circ}\text{C}$  until analyzed.

Total cellular RNA was isolated using the Qiagen RNeasy Mini Reagent Set (Qiagen, Germany), according to the manufacturer's recommendations. Preparation and handling of RNA were performed in a laminar flow hood, under RNase free conditions. The concentration and purity of RNA were determined by spectrophotometry at 260 and 280 nm and the isolated RNA was stored at  $-80^{\circ}\text{C}$  until further manipulations. Reverse transcription of RNA was carried out with the SuperScript III Platinum Two-Step qRT-PCR kit (Invitrogen, CA, USA), according to the manufacturer's instructions, using 1  $\mu\text{g}$  of total RNA as template.

### 2.3 Real time RT-PCR

A real-time RT-PCR assay was developed for the quantification of each gene of interest. The primers and probes were designed using the Primer Premier software. Real time RT-PCR was performed in the LightCycler Instrument (Roche Applied Science, Germany) in a total volume of 10  $\mu\text{l}$  per glass capillary. For each reaction, 1  $\mu\text{l}$  of cDNA was placed in a 9- $\mu\text{l}$  reaction mixture, containing the following: 0.1  $\mu\text{l}$  (5 U/ $\mu\text{l}$ ) of a temperature-released Taq DNA polymerase (Platinum DNA Polymerase, Invitrogen, CA, USA), 1  $\mu\text{l}$  of 10x PCR buffer, 1.0  $\mu\text{l}$  (50 mM) of  $\text{MgCl}_2$ , 0.2  $\mu\text{l}$  (10 mM) of deoxynucleotide triphosphates (Invitrogen, CA, USA), 0.15  $\mu\text{l}$  (10  $\mu\text{g}/\mu\text{l}$ ) of bovine serum albumin (Sigma-Aldrich Corporation, MO, USA), 0.5  $\mu\text{l}$  (3  $\mu\text{M}$ ) of the primers, 1  $\mu\text{l}$  (3  $\mu\text{M}$ ) of the probe, and 5.05  $\mu\text{l}$  of diethylpyrocarbonate-treated  $\text{H}_2\text{O}$ . The cycling protocol for ETA-Rs consisted of an initial 5-min denaturation step at  $95^{\circ}\text{C}$  for activation of the DNA polymerase, followed by 45 cycles of denaturation at  $95^{\circ}\text{C}$  for 10 s, annealing at  $60^{\circ}\text{C}$  for 15 s, and extension at  $65^{\circ}\text{C}$  for 20 s. The cycling protocol for ETB-Rs was identical, with the exception of the extension being performed at  $72^{\circ}\text{C}$  for 15 s. The glyceraldehyde-3-phosphate dehydrogenase gene was used for normalization and quantification, as described previously [8].

The evaluation of the assay and the quantification of gene expression levels were performed with calibrators prepared and quantified as described previously [9]. For each gene, a calibration curve was generated from serial dilutions ranging from 106 to 102 copies/ $\mu\text{l}$  of the target of interest. All calibration curves showed linearity over the entire quantification range, with correlation coefficients over 0.99.

**Table 1** Patient characteristics

	No atrial fibrillation (n=37) <sup>a</sup>	Atrial fibrillation (n=15) <sup>a</sup>	p value <sup>b</sup>
Weight	83 (69, 87)	86 (70, 94)	0.54
Height	170 (165, 173)	172 (165, 178)	0.28
Age	66 (59, 73)	65 (59, 74)	0.94
Male sex, n (%)	31 (84)	14 (93)	0.36
Diabetes, n (%)	24 (65)	8 (53)	0.44
Insulin dependent diabetes, n (%)	5 (14)	1 (7)	0.48
Hypertension, n (%)	23 (62)	11(73)	0.44
LM, n (%)	7 (19)	2 (13)	0.63
LAD, n (%)	33 (89)	12 (80)	0.24
LCX, n (%)	21(57)	11 (73)	0.27
RCA, n (%)	23 (62)	12 (80)	0.21
Total cholesterol	201 (187, 221)	212 (195, 229)	0.27
Triglycerides	155 (133, 180)	165 (149, 185)	0.37
HDL cholesterol	38 (34, 41)	38 (37, 42)	0.46

HDL: high density lipoprotein, LAD: left anterior descending coronary artery, LCX: left circumflex coronary artery, LM: left main coronary artery, RCA: right coronary artery

<sup>a</sup> Continuous variables are presented as median (25th, 75th percentiles) and categorical variables as number of patients (percent value).  
<sup>b</sup> No significant differences were found.

### 2.4 Statistical analysis

All data are presented as mean±standard error of the mean, unless stated otherwise. Categorical variables were compared with chi square after Yates' correction. Since continuous variables were not normally distributed, the non-parametric Mann–Whitney *U* test was used for comparisons. Values of ET-1 receptor expression were dichotomized above and below the median value and sensitivity, specificity, positive and negative predictive values were calculated. Receiver operating characteristic (ROC) curves were constructed as a plot of true-positive rates and false positive rates.

Discriminant function analysis (with sigma-restricted parameterization) was used to determine the predictive value of gene expression and AF occurrence. Statistical significance was defined at a two-sided *p* value below 0.05. Data were analyzed with the Statistica software (version 7.0, StatSoft Inc., OK, USA).

## 3 Results

### 3.1 Patient population

The study cohort consisted of 52 patients (45 male, with a mean age of 66±1 years, ranging from 51 to 83 years).

**Table 2** Pharmacological treatment

	No atrial fibrillation (n=37)	Atrial fibrillation (n=15)	p value <sup>a</sup>
Beta-adrenergic blockers, n (%)	29 (78)	10 (67)	0.48
Statins, n (%)	30 (81)	12 (80)	0.76
Clopidogrel, n (%)	10 (27)	2 (13)	0.48
Angiotensin converting enzyme inhibitors, n (%)	22 (59)	7 (46)	0.59
Angiotensin receptor Blockers, n (%)	4 (11)	2 (13)	0.82
Nitrates, n (%)	30 (81)	12 (80)	0.76

<sup>a</sup> No significant differences were found.

Mean ejection fraction was 52±1%. During a 5-day post-operative period, 15 patients (28.8%) developed persistent AF, requiring pharmacological treatment with amiodarone (in 10 patients) or electrical cardioversion (in 5 patients). The patient characteristics and pharmacological treatment were comparable in the two groups, as shown in Tables 1 and 2, respectively. No serum electrolyte abnormalities were detected in any patient and none required temporary pacing.

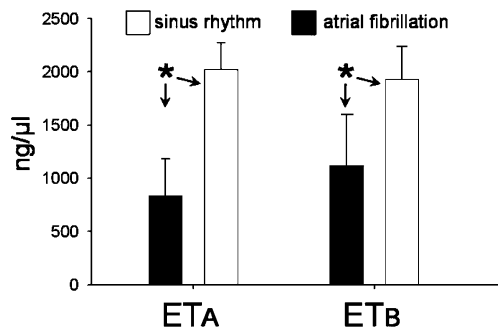
### 3.2 Endothelin mRNA expression

Atrial tissue ET-1 mRNA expression was slightly and non-significantly higher in patients who subsequently developed AF (8,088±4,039 ng/μl), compared to those remaining in sinus rhythm (5,911±1,237 ng/μl, two-sided *p*=0.20).

### 3.3 Endothelin receptor (A and B) mRNA expression

ETA-Rs were significantly (two-sided *p*=0.0059) lower in patients who subsequently developed AF, compared to those who did not. Similarly, ETB-Rs were significantly (two-sided *p*=0.0084) lower in patients who subsequently developed AF, compared to those remaining in sinus rhythm. Values are depicted in Fig. 1.

The sensitivity and specificity of ETA-R mRNA expression to predict AF occurrence were 0.90 and 0.64, respec-



**Fig. 1** Endothelin A- and B-receptor expression. Note the significantly lower expression of A- and B-receptors in patients who developed post-operative atrial fibrillation (black bars), compared to those maintaining sinus rhythm (white bars). *ETA* endothelin A-receptors, *ETB* endothelin B-receptors; asterisk denotes significant differences

tively. Positive and negative predictive values were 0.45 and 0.95, respectively. The sensitivity and specificity of ETB-R mRNA expression to predict AF occurrence were 0.83 and 0.62, respectively. Positive and negative predictive values were 0.45 and 0.90, respectively. For ETA-R expression, the area under the ROC curve was 0.78 (with 95% confidence intervals 0.62 to 0.89). For ETB-R expression, the area under the ROC curve was 0.75 (with 95% confidence intervals 0.60 to 0.86) (Fig. 2).

#### 3.4 Discriminant analysis

Atrial mRNA expression of ET-1 (Wilks  $\lambda=0.98$ ,  $F=0.46$ ,  $p=0.49$ ) and ETB-Rs (Wilks  $\lambda=0.95$ ,  $F=2.05$ ,  $p=0.15$ ) were not significant predictors of AF occurrence. In contrast, ETA-R mRNA expression (Wilks  $\lambda=0.86$ ,  $F=6.16$ ,  $p=0.017$ ) could predict AF occurrence.

## 4 Discussion

### 4.1 Main findings and comparison with previous studies

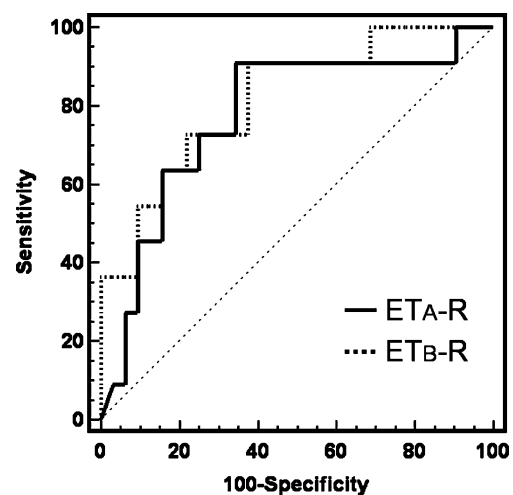
AF is the most common type of arrhythmia and is frequently observed post-cardiac surgery [1, 2, 6, 7]. The present study examined a cohort of 52 consecutive patients undergoing off-pump coronary artery bypass grafting, in the absence of heart failure, valvular heart disease or other predisposing conditions. In our series, post-operative AF occurred in approximately 30% of patients, a percentage comparable to previous reports in similar patients [6]. Down-regulation of both, ETA-R and ETB-R mRNA expression was found at the time of surgery in patients who developed AF in the immediate post-operative period.

Very few studies have previously investigated the endothelin system in human AF post-cardiac surgery; Brundel et al. [10] performed right atrial biopsies in a

heterogeneous group of 72 patients undergoing cardiac surgery. Half of these patients had valvular heart disease, approximately 25% had chronic AF and 8% had paroxysmal AF. Compared to control patients in sinus rhythm, this study [10] reported reduced protein amounts of ETA-Rs and ETB-Rs in patients with persistent AF with and without underlying valve disease. Taken together, our results and those by Brundel et al. [10] indicate a possible pathophysiologic link between AF and alterations in the endothelin system of the atria.

### 4.2 Endothelin-1 and atrial fibrillation

The potent electrophysiologic effects of ET-1 have been demonstrated long ago [11]. These effects differ in various cardiac sites: ET-1 induces prolongation of the action potential duration in ventricular myocytes and shortening in atrial myocytes [11], without affecting sinus node function [12]. Moreover, increased ET-1 plasma levels have been reported in patients with AF, independent of the underlying cardiac disease [3, 13]. Based on these observations, the hypothesis that ET-1 participates in the genesis of AF has been previously put forward [14]. ET-1 has been implicated in atrial structural remodeling, in cardiomyocyte hypertrophy [15], and in the genesis and perpetuation of AF [15, 16]. In right atrial trabeculae isolated from patients with cardiac disease, ET-1 has been shown to induce arrhythmic contractions [17], but the pathophysiologic link between AF and alterations in the endothelin system is unclear. Myocyte calcium overload is a crucial early participant in the atrial electrophysiologic remodeling process [18] and ET-1 has been reported to increase intracellular calcium in the atrial myocyte [5, 19].



**Fig. 2** Receiver operating characteristic curves of endothelin-1 receptors and post-operative atrial fibrillation. The area under the curve was 0.78 for endothelin-1 A-receptor (ETA-R) expression and 0.75 for endothelin-1 B-receptor (ETB-R) expression

This action is mediated by L-type calcium channels [5] and by the inositol 1,4,5-trisphosphate pathway [19].

#### 4.3 Atrial endothelin-1 expression and down-regulation of endothelin receptors

In the present study, we found only slightly (and non-significantly) higher tissue mRNA expression of ET-1 in patients who subsequently developed post-operative AF, compared to those maintaining sinus rhythm. This is in accordance with the findings of Brundel et al. [10], who reported increased tissue mRNA expression of pre-pro-ET-1 in patients with chronic AF and concomitant underlying valve disease, but unchanged levels in AF patients without valvular heart disease. Consequently, our data, as well as those by Brundel et al. [10] do not support the notion that chronically elevated local atrial tissue ET-1 may induce receptor down-regulation. This notion was based on findings in hypertensive rats, in which both receptors were down-regulated, being almost absent in the spontaneously hypertensive rat model [20]. Thus, the mechanisms of down-regulation of ETA-Rs and ETB-Rs remain speculative and merit further study.

In our patient population, a significant down-regulation (by approximately 60%) of ETA-R mRNA expression was found at the time of surgery in patients who subsequently developed AF, compared to patients remaining in sinus rhythm. ETB-R mRNA expression was also significantly down-regulated, but to a lesser extent (by approximately 40%). The lower significance of ETB-R down-regulation in AF occurrence was further underscored by discriminant analysis, which revealed that only ETA-R expression could predict AF occurrence. Although such analysis was not performed in the study by Brundel et al. [10], protein expression of ETA-Rs and ETB-Rs was evenly reduced in AF patients, when compared to patients in sinus rhythm. However, the wide heterogeneity of the patient population included in this study [10] precludes firm conclusions. Interestingly, the data by Burrell et al. [17] suggest that ETA-Rs and ETB-Rs have differing pathophysiological significance in the genesis of atrial arrhythmias. In this study [17], the arrhythmogenic effects of ET-1 in human atria were prevented by a dual ETA-R/ETB-R antagonist, but not by a selective ETA-R antagonist.

The widely held view on the arrhythmogenic properties of ET-1 has been challenged by data on the electrophysiologic effects of ET-1 on the atrial [17] and ventricular myocardium [21]. In the setting of myocardial ischemia, the findings of Sharif et al. [21] suggested a dual (both pro- and antiarrhythmic) action of ET-1. In their [21] experiments, endogenously released ET-1, acting via both receptors, produced a pro-arrhythmic effect, while low dose ET-1, administered exogenously, was antiarrhythmic. Our results,

in conjunction with previous findings [10, 17], may allow the generation of hypotheses on the underlying electrophysiological mechanisms of atrial fibrillation. It may be postulated that, under certain circumstances, ET-1 exerts antiarrhythmic actions in the atria, an effect lost after a decrease in ETA-Rs. This hypothesis is further supported by previous findings in isolated human atrial cells, where isoproterenol prolonged the action potential duration and produced arrhythmic depolarizations; both effects were prevented by ET-1, independently of beta-blocker treatment [22]. Thus, the antiarrhythmic potential of ET-1 in human atria in the setting of hypercatecholaminemia, such as post-surgery, may explain our findings.

An interesting alternative hypothesis was put forward by Boyden [14], raising the possibility that the electrophysiologic properties of ET-1 in human atria are mediated neither by ETA-Rs nor by ETB-Rs. This assumption is based on the fact that, despite the reported down-regulation of both receptors [10], functional data are absent. In particular, there are no solid data available to indicate a pathophysiologic link between the function of the remaining receptors and abnormal calcium handling, leading to the initiation of atrial fibrillation. Moreover, there is evidence to suggest that the positive inotropic effects of ET-1 in human right atrial trabeculae may be mediated neither by ETA-Rs nor by ETB-Rs [17]. Clearly, further research is warranted to elucidate the pathophysiologic significance of ET-1 and its receptors in the genesis of AF.

#### 4.4 Conclusions

Post-operative atrial fibrillation is frequently observed in patients undergoing cardiac surgery. These patients do not have substantial increases in atrial tissue ET-1 expression, but they display significant down-regulation mainly of ETA-Rs, but also of ETB-Rs. Thus, alterations in atrial ET-1 system may be important in the genesis of AF post-cardiac surgery, but the exact pathophysiologic mechanisms remain unclear. These conclusions should be extrapolated with caution in other groups of patients.

**Acknowledgements** Paraskevi Lioni, BSc, offered invaluable help as a research coordinator. We are grateful to our patients for their participation in the study.

**Funding** This work was supported by two grants from the Hellenic Ministry of Development, General Council for Research and Technology (1st Project: PENED Prosmeno-7034-05/21/2001 and 2nd Project: Operational Program for Competitiveness, Axis 4-Measure 4.5, EPAN 2002, Hercules).

**Disclosures** Conflict of Interest: None declared.

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