Expression of Angiotensin II Receptors in Human Left and Right Atrial Tissue in Atrial Fibrillation With and Without Underlying Mitral Valve Disease

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OBJECTIVES We postulated a change of angiotensin II receptor subtype expression in patients with lone atrial fibrillation (AF) and AF with underlying mitral valve disease (MVD) both compared with sinus rhythm (SR).

BACKGROUND Atrial fibrillation is a progressive disease associated with electrical and structural remodeling. Angiotensin II (ANGII) is involved in the process of myocardial remodeling. Actions of ANGII are mediated by ANGII receptor subtypes 1 and 2 (AT1 and AT2).

METHODS Left atrial (LA) and right atrial (RA) tissue samples were obtained from patients with AF or SR with or without underlying MVD. The AT1 and AT2 protein levels were measured by quantitative Western blotting techniques.

RESULTS The AT1 protein level in the LA was significantly increased in patients with AF (all forms) compared with SR (p < 0.05), whereas AT2 expression was not significantly altered. Comparison of the subgroups revealed a similar increase of AT1 in both paroxysmal AF and chronic AF with or without MVD. Additionally, investigations of ANGII receptor subtypes in the RA did not exhibit any significant changes either in AT1 or in AT2 in patients with AF versus SR. Underlying MVD did not significantly affect AT2 receptor subtype expression in LA.

CONCLUSIONS Atrial fibrillation is associated with an up-regulation of AT1 in LA, but not in RA, and did not appear to influence the AT2 expression in the atrium. Because we found an enhanced expression of AT1 in the LA, we conclude that AT1 might be involved in the pathogenesis of AF in the LA. (J Am Coll Cardiol 2003;42:1785–92) © 2003 by the American College of Cardiology Foundation

About 1% of the population is suffering from the most common cardiac arrhythmia, atrial fibrillation (AF). Its incidence increases with age and/or will be promoted by risk factors such as rheumatic and ischemic heart disease, hypertension, or congestive heart failure. However, AF may also occur in the absence of cardiovascular disease (lone AF) (1–3).

The pathophysiology of AF with electrical and structural remodeling of the atria has been described (e.g., electrical and structural remodeling might be responsible for an enhanced stability of AF) (4–9). Nakashima et al. (7) suggested that angiotensin II (ANGII) may be involved in the process of atrial electrical remodeling. The activity of ANGII is mediated by its receptor subtypes 1 and 2 (AT1 and AT2) (10). However, Goette et al. (11) found in right atrial (RA) tissue of patients with AF an up-regulation of AT2 and a down-regulation of AT1. The role of underlying cardiac disease was not investigated in that study. However, because fibrotic changes of the tissue are often observed in AF and in case of an involvement of ANGII, this would be mediated via AT1. The study of Goette et al. (11) is not easy to fit into the pathophysiology of AF but might indicate, as they suggest, a counter-regulation of AT1-induced fibrosis via AT2 up-regulation. However, these authors investigated RA tissue, while AF normally originates in and depends on the left atrium (LA). Sources of initiation and maintenance of AF are localized in the LA and AF propagates through both atria (12). Therefore, we chose human tissue samples from the LA and, for comparison, RA. Recent clinical findings favor a promoting role of AT1 in AF (13). Furthermore, previous studies suggest that an up-regulation of AT1 leads to increased extracellular matrix component production, cell growth, or vasoconstriction, whereas AT2 counter-regulates the effects of the AT1. The stimulation of AT2 promotes antiproliferative and antigrowth effects. Moreover, AT2 receptor stimulation attenuates the progression of myocardial fibrosis and advances antifibrotic processes (9,14). Because AF is associated with an increased amount of atrial fibrosis and AT1 has profibrotic effects, we suppose an up-regulation of AT1 and a down-regulation of AT2 in the LA in patients with AF.

The aim of the present study was to quantify the AT1 and AT2 expression in human LA and RA. We compared patients with sinus rhythm (SR) and AF (paroxysmal atrial fibrillation [PAF] and chronic atrial fibrillation [CAF]) with or without underlying mitral valve disease (MVD).
**METHODS**

**Patients.** The study groups consisted of patients undergoing cardiac surgery for lone AF (lone AF group, n = 40; PAF, n = 19; CAF, n = 21), for mitral valve repair or replacement in conjunction with intraoperative radiofrequency ablation of AF (MVD + AF group, n = 35; PAF, n = 6; CAF, n = 29) and patients undergoing cardiac surgery for coronary artery bypass grafting, aortic valve replacement, or valve repair (Table 1) (SR group, n = 15; n = 8 with coronary artery bypass grafting or aortic valve replacement as control group for the lone AF group; n = 7 with an underlying MVD). Patients in the control group were matched to the AF groups according to age, LA size, and left ventricular (LV) function. Patients were included in the study only if they had preserved LV function and a LA size of ≤45 mm as assessed by echocardiography for the lone AF group. The surgical procedure and the concept of intraoperative ablation of AF have been described in detail previously (18). All patients gave informed consent. The institutional ethical committee approved the study. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Atrial tissue from all patients was obtained from the LA free wall near the interatrial septum (about 5 to 5 mm from atriotomy) or RA free wall near the auricle, both during cardiac surgery, quick-freeze in liquid nitrogen, and stored at −80°C until use.

**Western blot analysis.** Frozen atrial tissue was homogenized in N-(2Hydroxymethyl)piperazine-N’-(2ethanesulonicacid) buffer with 0.5 mg/ml leupetin, 10 μg/ml aprotinin, and 1 mM phenylmethylsolfonic acid (Boehringer, Mannheim, Germany). For electrophoresis, 20 μg of total protein from homogenized total tissue was separated in a sodium dodecyl sulfate-polyacrylamide gel and blotted onto polyvenylinendifloride membrane (Roth, Karlsruhe, Germany). Rabbit–anti-human AT1 and rabbit–anti-AF

### Table 1. Patient Characteristics

<table>
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<tr>
<th></th>
<th>Lone AF (Left Atrium)</th>
<th>MVD + AF (Left Atrium)</th>
<th>Control Group</th>
<th>AF (Right Atrium)</th>
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<tr>
<td>n (male/female)</td>
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<td>PAF (male/female)</td>
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<td>4 (4/0)</td>
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<td>CAF (male/female)</td>
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<td>29 (14/15)</td>
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<td>1 (0/1)</td>
<td>—</td>
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<td>MVR (1)</td>
<td>CABG (2)</td>
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<td></td>
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<td>AVR (2)</td>
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<td></td>
<td></td>
<td></td>
<td>AVR + CABG (2)</td>
<td>AVR + CABG + AAR (1)</td>
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<td></td>
<td></td>
<td></td>
<td>59 ± 7</td>
<td>68 ± 7</td>
<td>68 ± 3</td>
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<td>Age (yrs) (total)</td>
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<td>65 ± 8</td>
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<td>LVEF (%) (total)</td>
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<td>Left atrium (mm) (total)</td>
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<td>Duration of AF (months)</td>
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<td>LED (total)</td>
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<td>6 ± 17%</td>
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<td>Spironolactone (total)</td>
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<td>(PAC/CAF)</td>
<td>(0/3)</td>
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AAR = aortic ascendence replacement; ACE = angiotensin-converting enzyme; AF = atrial fibrillation; AVR = aortic valve replacement; CABG = coronary artery bypass grafting; CAF = chronic atrial fibrillation; IRAAF = intraoperative radiofrequency ablation of atrial fibrillation; LVEF = left ventricular ejection fraction; MVD = mitral valve disease; MVR = mitral valve repair or replacement; n.a. = not available; PAF = paroxysmal atrial fibrillation.

**Abbreviations and Acronyms**

- **AF** = atrial fibrillation
- **ANGII** = angiotensin II
- **ANOVA** = analysis of variance
- **AT1** = angiotensin II receptor type 1
- **AT2** = angiotensin II receptor type 2
- **CAF** = chronic atrial fibrillation
- **CHF** = congestive heart failure
- **GAPDH** = glyceraldehyde-3-phosphate dehydrogenase
- **LV** = left ventricular
- **LA** = left atrial/atrium
- **MVD** = mitral valve disease
- **PAF** = paroxysmal atrial fibrillation
- **RA** = right atrial/atrium
- **SR** = sinus rhythm
- **AAR** = aortic valve replacement
- **ACE** = angiotensin-converting enzyme
- **AF** = atrial fibrillation
- **AVR** = aortic valve replacement
- **CABG** = coronary artery bypass grafting
- **CAF** = chronic atrial fibrillation
- **IRAAR** = intraoperative radiofrequency ablation of atrial fibrillation
- **LVEF** = left ventricular ejection fraction
- **MVD** = mitral valve disease
- **MVR** = mitral valve repair or replacement
- **n.a.** = not available
- **PAF** = paroxysmal atrial fibrillation
- **LA** = left atrial/atrium
- **LV** = left ventricular
- **CHF** = congestive heart failure
- **AT1** = angiotensin II receptor type 1
- **AT2** = angiotensin II receptor type 2
- **ANOVA** = analysis of variance
- **ANGII** = angiotensin II
human AT$_2$ (Santa Cruz Biotechnology, Santa Cruz, California) were used as primary antibodies. As reference, we used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) detected by mouse–anti-human GAPDH (Hytest, Turku, Finland). After washing, membranes were incubated with secondary antibodies using goat–anti-rabbit IgG (Dianova, Hamburg, Germany) for ANGII receptors and rabbit–anti-mouse IgG for GAPDH signals (Sigma, Deisenhofen, Germany), both conjugated with horseradish peroxidase and subsequently developed with Super Signal Reagent (Pierce, Rockford, Illinois).

**Immunohistology.** Formalin fixed, paraffin-embedded atrial tissue sections (5-μm thick) were incubated with primary antibodies, as specified in the preceding text, against human AT$_1$ and AT$_2$ receptor (dilution 1:50). Goat–anti-rabbit IgG conjugated with horseradish peroxidase (1:500) (Dianova) was used as a secondary antibody. After washing, atrial tissue sections were incubated with biotinylated tyramid (1:50) (Perkin Elmer, Boston, Massachusetts), and subsequently incubated with streptavidin–horseradish peroxidase (1:100) (Perkin Elmer). Finally, the sections were developed using 3-amino-9-ethylcarbazolates substrate for peroxidase. The specificity of the detected signals was controlled by omitting the primary antibodies.

**Densitometric analysis.** Immunoblots were exposed to X-ray film (Eastman Kodak Co., Rochester, New York), developed, and analyzed by ONE-Dscan 1.0 Software (Scanalytics, Los Angeles, California). The relative amount of ANGII receptors in each sample was investigated by comparison of gray scale value of target proteins with the gray scale signal of GAPDH. The GAPDH value was used as an adjusting factor to assure that the same amount of cellular proteins in each patient was determined. The ratio of ANGII receptors/GAPDH from each patient was used as the adjusting factor to assure that the same amount of cellular proteins in each patient was determined. The ratio of ANGII receptors/GAPDH from each patient was used to calculate possible differences in ANGII receptors synthesis between the patients.

**Statistics.** All data are represented as mean ± standard error of mean. Statistical evaluation was performed by using Mann-Whitney test (two groups) or one-way analysis of variance (ANOVA) with subsequent post-hoc Tukey–HSD (for three or more groups). Values of p < 0.05 were considered statistically significant. As described in the preceding text, all data are specified as ratios (ANGII receptors/GAPDH) and, therefore, did not require any units.

**RESULTS**

**Patients.** Clinical characteristics of the patient population are summarized in Table 1.

**LA.** Patients in the lone AF group were significantly younger compared with all SR patients (p < 0.05). Furthermore, both patients in the lone AF group and in the SR group were significantly younger than patients in the MVD + AF group (p < 0.05). The LA sizes in both the lone AF and the SR group were significantly lower than in the MVD + AF group (p < 0.05), while there was no difference between lone AF and SR. There were no significant differences in the LV ejection fraction between lone AF, MVD + AF, and SR groups.

**RA.** There were no significant differences, except patients in SR had a slightly lower LA size than patients in AF (p = 0.055), while RA size was normal in both groups.

**Histological results.** Immunohistochemical staining for AT$_1$ and AT$_2$ receptors of LA tissue of patients in SR compared with patients in AF showed clear differences; there was a higher presence of AT$_1$ in patients in AF (all forms) (Fig. 1B) compared with patients in SR (Fig. 1A). Expression of AT$_2$ was unaltered in patients in AF (Fig. 1D) compared with patients in SR (Fig. 1C). After detecting clear histological differences in the LA tissue between patients in SR and patients in AF, the differences were quantified by Western blot techniques.

**ANGII receptor expression in LA/RA.** Western blot analysis in the LA revealed an increase of AT$_1$ in patients with AF (all AF) compared with patients in SR (all SR) control group. The protein expression of AT$_1$ (1.46 ± 0.13; n = 74) was significantly (about two-fold) increased compared with the SR control group (0.60 ± 0.10; n = 14; p < 0.001). In contrast, in the LA, the protein expression of AT$_2$ exhibited no significant changes under influence of AF (1.45 ± 0.11; n = 74) compared with the SR control group (1.71 ± 0.27; n = 13) (Figs. 2 and 3A). The ratio of AT$_1$/AT$_2$ was significantly enhanced (about five-fold; p < 0.0001) in patients in AF (1.41 ± 0.15; n = 74) compared with patients in SR (0.32 ± 0.06; n = 14) (Fig. 3A).

Analysis of AT$_1$ and AT$_2$ expression in the RA tissue revealed no significant changes between patients in SR (n = 5) and AF (n = 5) (Fig. 3B). However, there was a slight, but not significant, increase in AT$_2$ expression in the RA (p = 0.09).

**Influence of MVD on ANGII receptor expression in the LA.** Next we analyzed the influence of MVD on the AT$_1$ and AT$_2$ expression. Comparing all patients without MVD (SR and lone AF) with patients with underlying MVD (SR + MVD and all MVD + AF), we could not detect any significant changes between both groups either in AT$_1$ (1.41 ± 0.17; n = 48 vs. 1.33 ± 0.18; n = 41) or in AT$_2$ (1.53 ± 0.14; n = 45 vs. 1.44 ± 0.15; n = 42). Furthermore, the ratio AT$_1$/AT$_2$ did not exhibit any significant changes between patients with (1.30 ± 0.19; n = 42) and without underlying MVD (1.21 ± 0.18; n = 48) (Fig. 4A).

**Differences between SR, lone AF, and MVD + AF in LA.** Using the ANOVA, significant changes in AT$_1$ expression among SR, lone AF, and MVD + AF were detected (p = 0.02). Patients in lone AF exhibited an increase in AT$_1$ expression (1.48 ± 0.18; n = 39; p < 0.05) compared with the SR control group (0.60 ± 0.10; n = 14). Likewise, there was also a considerable increase in patients with MVD + AF (1.43 ± 0.17; n = 35; p < 0.05) in comparison with the SR control group (Fig. 4B). In
contrast, there were no significant changes in AT_2 receptor expression (Fig. 4C). Exemplary Western blots are shown in Figure 2.

Considering the ratio AT_1/AT_2, there were significant changes comparing SR versus lone AF and MVD + AF using ANOVA (p < 0.001). The change of the ratio AT_1/AT_2 in the lone AF group (1.41 ± 0.21; n = 39; p < 0.05) was almost as high as in the MVD + AF group (1.41 ± 0.22; n = 35; p < 0.05) (vs. SR [0.32 ± 0.07; n = 14]), (Fig. 4D).

Influence of PAF and CAF in LA. To investigate the influence of PAF and CAF, the AT_2 expression in patients with lone PAF and lone CAF, MVD + PAF, and MVD + CAF, and patients in SR were compared.

Using ANOVA we could not detect any significant changes between PAF and CAF either in AT_1 expression or in AT_2 expression (Fig. 5).

The ratio AT_1/AT_2, however, was significantly changed in both lone PAF (1.63 ± 0.35; n = 19; p < 0.001) and lone CAF (1.22 ± 0.21; n = 21; p < 0.01) versus SR control group (0.18 ± 0.03; n = 8). Other differences among the subgroups did not reach the level of statistical significance (Fig. 5C).

DISCUSSION

The present study demonstrates a clear relationship between AT_1 up-regulation and AF in the LA. We were able to find an increase of about 100% in AT_1 expression in LA tissue of patients in AF (both lone AF and MVD + AF) compared with patients in SR. Interestingly, AF does not appear to be associated with the regulation of the AT_2 in human LA. Thus, our data seem to confirm an association between AF and (AT_1-mediated) actions of ANGII in both lone AF and MVD + AF.

Previous studies reported about the relationship of actions of ANGII and (other) cellular components that are involved in electrical and structural remodeling in AF such as, for example, connexin 43. Shyu et al. (19) and Polontchouk et al. (20) observed an increase of connexin 43 mediated by activation of AT_1 in cultured rat cardiomyocytes. Additionally, Emdad et al. (21) described an important role of AT_1 in gap-junctional remodeling. Moreover, Elvan et al. (22) observed an increased expression and changed distribution of connexin 43 in dogs with CAF, which became reduced by intraoperative radiofrequency ablation, whereas in a similar goat model of persistent AF, the expression of connexin 43 remained unchanged (23,24). These studies might indirectly indicate a possible role of

Figure 1. Immunostaining of angiotensin II (ANGII) receptors. Exemplary pictures of immunostaining of ANGII subtypes in human left atrial tissue. (A, B) Antibodies against human AT_1 (red-stained pixels) in sinus rhythm (SR) (A) and lone chronic atrial fibrillation (CAF) (B) were used for immunostaining in paraffin-embedded tissue sections. (C, D) Antibodies against human AT_2 (red-stained pixels) were used for immunostaining of paraffin-embedded tissue sections of patients with SR (C) and lone CAF (D).

Figure 2. Exemplary Western blots for human AT_1 (A) and AT_2 (B) expression in atrial fibrillation (AF). (A) A specific band at 52 kDa for AT_1 is detected in left atrial tissue of patients in sinus rhythm (SR), lone chronic atrial fibrillation (CAF), and patients with CAF and underlying mitral valve disease (MVD). (B) Western blots for AT_2 with a specific band at 52 kDa were performed for patients with SR, lone CAF, and MVD + CAF. Monoclonal antibodies for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with a specific band at 36 kDa were used as reference.
AT1 and angiotensin in the remodeling process in AF. Attributes of structural remodeling such as fibroblast and myocyte cell growth or the promotion of extracellular matrix component (e.g., collagen type 1, fibronectin) synthesis could be mediated by AT1 (14,25–27). AT1, as a promotor of extracellular matrix component synthesis, and the knowledge of an enhanced fibrosis in tissue with AF might indicate a possible role of AT1 in structural remodeling in AF (3,17,28).

However, Goette et al. (11) showed an up-regulation of AT2 and a down-regulation of AT1 in the human RA in 19 patients with AF versus 11 patients with SR. We also found some slight up-regulation of AT2 in the RA. The difference in the changes in the LA with enhanced AT1 and unchanged AT2 might be caused by the fact that the RA is not the source of genesis of AF, but the LA (29). The increase in AT1 (in LA) might be related to increased fibrosis in AF, because it is known that ANGII mediates its growth-promoting effects via AT1 (3,16,28). Both Goette et al. (11) and we interpret the increase in AT2 in the RA in AF (which did not reach significant levels in our study probably due to the smaller number of RA) as a counter-regulation against ANGII-AT1-induced fibrosis in AF.

Because AF pathophysiologically depends on the LA, it seems important to investigate LA (29). In fact, from our data we believe that ANGII is associated with AF and that changes in the expression of AT1 might contribute to the remodeling process and stabilization of AF with time. This is supported by a recent clinical trial, showing that additional treatment with the AT1 antagonist irbesartan in combination with amiodarone reduced the rate of recurrence of AF more than treatment with amiodarone alone (13).

Furthermore, a previous study indicated that ANGII plays an important role in congestive heart failure (CHF)-induced atrial enlargement with atrial remodeling and atrial fibrosis, which can make the atrium more susceptible to AF (26). In this study, the action of ANGII was blocked with the angiotensin-converting enzyme inhibitor enalapril, resulting in a significantly reduced atrial fibrosis during CHF-induced atrial enlargement. In a similar dog model, Li et al. (30) investigated CHF-induced atrial remodeling (CHF by tachypacing) and found in the LA increased AT1 and AT2 expression as well as increased fibrosis in CHF, which induced susceptibility to pacing-induced AF. Atrial fibrosis-increased AT1 (and, in parts, AT2) expression could be inhibited by enalapril, indicating a pathophysiologic role for ANGII in CHF-induced atrial remodeling. However, we did not observe an effect of MVD on AT1 expression, which might be related to the different models (tachypacing-induced CHF with atrial enlargement in dogs vs. MVD in humans). On the other hand, the papers by Shi et al. (26) and Li et al. (30) also indicate in principle a role for AT1 in a process leading to enhanced atrial susceptibility for AF in LA tissue, which seems to be in accordance with our findings.

Other authors found that immediate early gene, late genes, and growth factors (e.g., transforming growth factor beta-1 gene) were completely blocked by an AT1 receptor antagonist and not by AT2 receptor antagonist (31). Otsuka et al. (32) showed that increased collagen I and aortic hydroxyproline concentration were prevented by inhibition of AT1 and not by inhibition of AT2. Accordingly, these data and our results suggest that AF is associated with an overexpression of AT1 simultaneously with an increase in fibrosis in cardiac tissue. Furthermore, AT2 did not appear to be involved in this remodeling process in AF. However, according to Goette et al. (11), it might be that in certain circumstances there is counter-regulation of AT1-induced process by up-regulation of AT2 in AF.

In fact, the up-regulation of AT1, as shown in our study may influence the enhanced synthesis of fibrotic factors
(e.g., collagen type 1 and fibronectin) and increased connexin 43 expression. Accordingly, actions of ANGII, mediated by AT1, might play a role in structural and electrical remodeling in AF. The finding that AT1 was only upregulated in LA may indicate a pathophysiologic role for ANGII in AF.

Conforming with previous studies, we could detect a larger LA diameter in patients with MVD + AF compared with patients with SR and patients with lone AF (Table 1) (33,34). However, patients with underlying MVD (MVD + AF and MVD + SR) did not exhibit any significant changes either in AT1 or in AT2 expression compared with patients without underlying MVD (lone AF and SR). Furthermore, lone AF and MVD + AF exhibited a similar increase in AT1 expression compared with SR. The AT2 expression among SR, lone AF, and MVD + AF remained unchanged. Additionally, both SR and MVD + SR exhibited similar low AT1 expression compared with lone PAF, lone CAF, MVD + PAF, or MVD + CAF (Fig. 4A). Thus, we assume that an up-regulation of AT1 was not associated with MVD. Supporting our suggestion, Schotten et al. (28) observed that the differences in extracellular matrix synthesis between patients in SR compared with those in AF were similar to patients with MVD.

It is known that systematic differences between PAF and CAF exist. Thus, patients with CAF exhibit a shorter cycle length and a higher degree of disorganized activity compared with patients with PAF (35,36). However, we could not confirm a systematic progressive up-regulation of AT1 or down-regulation of AT2 from PAF to CAF either in the lone AF group or in MVD + AF group. This is supported by other studies, which also could not find differences between PAF and CAF in human AF of different causes (e.g., AF with MVD, coronary artery disease, and lone AF) regarding angiotensin or other pathophysiologic factors such as endothelin (11,37).

Figure 4. Influence of mitral valve disease (MVD) on angiotensin II receptor expression. (A) Expression of AT1 and AT2 and ratio AT1/AT2 in left atrial tissue of patients with MVD (sinus rhythm [SR] and atrial fibrillation [AF], left columns) and without MVD (SR and AF, right columns). (B) AT1 and (C) AT2 expression and ratio of AT1/AT2 (D) in left atrial tissue of patients in SR, lone AF, and AF with underlying MVD. GAPDH = glyceraldehyde-3-phosphate dehydrogenase.
To the best of our knowledge, this is the first study that determined the regulation of AT_1 and AT_2 in the human LA in patients suffering from AF. Furthermore, we considered the separate subgroups, both PAF and CAF, with or without an underlying MVD.

In conclusion, AF is associated with an up-regulation of AT_1 in human LA (but not RA) tissue and did not influence the expression of AT_2. However, there was no systematic difference in ANGII receptor subtype expression between PAF and CAF. Mitral valve disease in combination with AF has no influence on AT_1 expression and was not associated with a change in AT_2 expression. Because AF normally originates from LA, our finding that AT_1 expression was only enhanced in LA, but not in RA, tissue can only cause speculation that this may indicate a possible pathophysiologic role for ANGII and AT_1 in AF.

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