RESEARCH PAPER

Effects of metoprolol therapy on cardiac gap junction remodelling and conduction in human chronic atrial fibrillation

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BACKGROUND AND PURPOSE

We investigated the influence of metoprolol on gap junction proteins connexin43 (Cx43) and connexin40 (Cx40) in atrial tissue from patients with/without atrial fibrillation (AF).

EXPERIMENTAL APPROACH

Left atrial tissue samples from 160 patients with AF or sinus rhythm (SR) with or without metoprolol (mean daily dose: 65.2 ± 9.1 mg·day⁻¹) were analysed for Cx43 and Cx40 by Western blot and immunohistology. Transverse and longitudinal conduction velocities were determined by 64 multi-electrode mapping.

KEY RESULTS

Both Cx43 and Cx40 expression were significantly increased in patients with AF versus SR. Cx43-expression in AF was significantly higher in patients receiving metoprolol, while Cx40 expression was unaffected by metoprolol treatment. In AF, the ratio of lateral/polar expression of Cx43 and Cx40 was enhanced due to increased expression at the sides of the cells (lateral) and a loss at the cell poles. This AF-induced increase in lateral/polar expression of Cx43, but not of Cx40, was significantly antagonized by metoprolol treatment. Functionally, in AF patients, transverse conduction velocity in atrial samples was significantly enhanced and this change was also significantly antagonized by metoprolol.

CONCLUSIONS AND IMPLICATIONS

AF induced enhanced lateral expression of Cx43 and Cx40 together with enhanced transverse conduction velocity in left atrial tissue. Alterations in localization of Cx43 and conduction changes were both antagonized by metoprolol, showing that pharmacological modulation of gap junction remodelling seems, in principle, possible. This finding may open new approaches to the development of anti-arrhythmic drugs.

Abbreviations

AF, atrial fibrillation; CHD, coronary heart disease; Cx40, connexin40; Cx43, connexin43; EF, ejection fraction; FITC, Fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; LM, length of the plasma membrane; MVD, mitral valve disease; PLM, length of the immunofluorescence-positive membrane; SR, sinus rhythm; TBS, Tris-buffered saline; V(L), longitudinal conduction velocity; V(T), transverse conduction velocity

Introduction

The prevalence of atrial fibrillation (AF) is about 1% in the total adult population (Fuster *et al.*, 2006) and increases with age. In people aged more than 65 years, about 5% and at more than 80 years, about 10% are affected (Go *et al.*, 2001; Miyasaka *et al.*, 2006). AF, thus, is the most common arrhythmia in humans (Go *et al.*, 2001). Concomitant cardiac diseases such as congestive heart failure, valvular disease, myocardial infarction or ischaemic heart disease also affect the prevalence of AF. Patients suffering from AF have both increased mortality, risk of stroke and morbidity (Wang *et al.*, 2003; Kirchhof *et al.*, 2009). The effect of AF on cardiovascular mortality rate is 2.5-fold higher in women than in men (Friberg *et al.*, 2004). Clinical experience shows that with prolonged duration of AF, it becomes increasingly difficult to convert patients to sinus rhythm (SR), and to maintain SR after successful conversion.

Chronic AF is accompanied by a remodelling process involving, among other factors, atrial fibrosis (Boldt *et al.*, 2004; Sakabe *et al.*, 2004), ionic channel modulation (Brundel *et al.*, 2002), changes in extracellular matrix with deposition of amyloid (Röcken *et al.*, 2002) and gap junctional remodelling (Polontchouk *et al.*, 2001; Kostin *et al.*, 2002). This cell structural remodelling was postulated as a second component of tachycardia-induced remodelling, distinct from the electrical remodelling (Tieleman and Crijns, 2000). This remodelling in AF results in the formation of an arrhythmogenic substrate stabilizing the AF, as summarized in the sentence 'Atrial fibrillation begets atrial fibrillation' (Wijffels *et al.*, 1995). It is still a matter of debate whether changes in gap junctions may lead to altered conduction properties. If so, another open question is whether this gap junction remodelling can be affected pharmacologically. Strategies targeting gap junction remodelling could open new approaches to the treatment of AF.

In our present study, we have focused on the role of connexin43 (Cx43) and connexin40 (Cx40), two gap junction proteins, in the remodelling process and on the question of whether changes in gap junction localization may be associated with changes in conduction. We previously found that α - and β -adrenoceptor stimulation up-regulated the expression of Cx43 (Salameh *et al.*, 2006, 2008; Rojas Gomez *et al.*, 2008) and mechanical stretch enhanced the expression of Cx43 and altered Cx43 localization (Salameh *et al.*, 2010a). Moreover, in a cell culture study, we have found enhanced Cx43 expression in cells treated with metoprolol (Salameh *et al.*, 2010b). Thus, we proposed the hypothesis that β -adrenoceptor antagonists may have an effect on connexin expression and/or on connexin localization. We have tested this hypothesis in a group of patients taking a β_1 -adrenoceptor antagonist (receptor nomenclature follows Alexander *et al.*, 2009), without intrinsic sympathomimetic activity. In Saxony, most of the patients taking β_1 -adrenoceptor antagonists have been prescribed metoprolol, so we decided to study tissue samples from patients on metoprolol.

Methods

All patients gave informed written consent and the study was approved by the institutional ethical committee. This inves-

tigation conformed to the principles outlined in the Declaration of Helsinki. We studied left atrial tissue from 160 patients with AF (duration > 1 year; type of AF: permanent or, in most cases, persisting) or without AF (i.e. in SR) and with or without metoprolol treatment (duration > 3 months). The tissue samples were obtained during cardiac surgery for mitral valve replacement or repair, for aortic valve replacement or for coronary bypass surgery. Metoprolol therapy was not discontinued. The patients enrolled in this study received either metoprolol [mean daily dose was 65.2 ± 9.1 mg; (minimum–maximum, 23.25–190 mg·day⁻¹); in most of the patients, metoprolol was given as a post-infarction prophylaxis for coronary heart disease, or for blood pressure control], or they did not take any other β -adrenoceptor antagonist. Atrial tissue from all patients was obtained from the left atrial free wall near the inter-atrial septum (about 5 to 10 mm from atriotomy) during cardiac surgery used for electrophysiological mapping (see below) and, thereafter, quickly frozen in liquid nitrogen and stored at -80°C until use, or processed for immunohistology. For patient characteristics, see Table 1.

Immunohistology

Formalin-fixed, paraffin-embedded atrial tissue sections (5 μm thick) were deparaffinized, blocked with 5% milk powder–Tris-buffered saline (TBS) solution and incubated with primary antibodies rabbit anti Cx43 (Sigma-Aldrich, Deisenhofen, Germany) or Cx40 (Chemicon/Millipore, Billerica, MA, USA) overnight at 4°C , followed by incubation with a secondary γ -chain-specific ALEXA488-labelled anti-rabbit antibody (A11008, Gibco/Invitrogen, Darmstadt, Germany). Specificity was controlled by omitting the primary antibodies.

For suppression of the autofluorescence of the connective tissue in the atria, we incubated the slides after immunostaining with 0.5% Chicago blue in TBS (pH 7.6, 10 min), washed again with TBS for 5 min and finally covered with immunofluorescence mounting medium (Dako Cytomation, Glostrup, Denmark). Fluorescence pictures were taken for fluorescein isothiocyanate (FITC) fluorescence and for red fluorescence and then combined according to the protocol reported by Kwak *et al.* (2001).

The slides were investigated at $1000\times$ magnification using a commercial image analysis system (Zeiss Axiovision, Zeiss, Jena, Germany) and a Zeiss Axioskop fluorescence microscope (Zeiss). For evaluation, only cells that were cut longitudinally were analysed. Each cell was divided into four areas as described previously (Polontchouk *et al.*, 2001). The longitudinal cell axis was determined and divided into four sections of equal length, giving four areas: the left and right cell poles and two mid areas. We measured the length of the plasma membrane (LM) of each section and the length of the immunofluorescence-positive membrane (PLM) of the section and calculated the ratio between positively stained membrane length and membrane length $\text{PLM}_{\text{section}}/\text{LM}_{\text{section}}$. Immunohistological evaluation was carried out without knowledge of the treatments and cross-checked by an independent person.

Twenty-four human (six per group) specimens were investigated by immunostaining. Four slide preparations per specimen were obtained and about 15 cells per slide were analysed.

Table 1

Patient characteristics

Parameter	SR	SR + metoprolol	AF	AF + metoprolol	P
N	23	20	37	80	
Age (years)	62.2 ± 2.4	58.7 ± 1.9	54.3 ± 0.1	57.1 ± 1.4	n.s.
Gender (female) (%)	35	25	34	36	n.s.
Aortic valve disease (%)	35	40	10	8.3	n.s.
MVD (%) (regurgitation)	64	40	59	49	n.s.
Mitral valve stenosis (%)	12	10	18	6	n.s.
CHD (%)	18	45	16	21	n.s.
Left atrial diameter (mm)	47.5 ± 1.7	42.8 ± 1.5	49.1 ± 1.1	47.2 ± 1.1	<0.05
EF (%)	58.4 ± 3.9	56.9 ± 2.5	59 ± 1.6	58.8 ± 1.5	n.s.
Metoprolol (%)	0	100	0	100	<0.05
ACE inhibitors/AT ₁ blockers (%)	41	60	35	50	n.s.
Digitalis (%)	17	5	46	15	
Anti-arrhythmics (%)	6	10	40	23	
Diabetes mellitus (%)	29	20	16	10	n.s.
Hypertension (%)	35	45	44	56	n.s.

n.s., not significant. MVD, mitral valve disease; CHD, coronary heart disease; EF, ejection fraction; ACE, angiotensin converting enzyme; AT₁, angiotensin AT₁ receptor.

Western blot analysis

Frozen atrial tissue was homogenized in HEPES buffer with 0.5 mg·mL⁻¹ leupeptin, 10 µg·mL⁻¹ aprotinin and 1 mM PMSF (Boehringer, Mannheim, Germany). For electrophoresis, 50 µg of total protein from homogenized total tissue was separated in a SDS-polyacrylamide gel and blotted onto PVDF membrane (Roth, Karlsruhe, Germany). Rabbit-anti-human CX40, rabbit-anti-human CX43 (Alpha Diagnostics, San Antonio, TX, USA) and goat-anti-human-collagen-I-antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used as primary antibodies. As a reference, we used glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) detected by mouse-anti-human GAPDH (Hytect, Turku, Finland). After washing, membranes were incubated with secondary antibodies using goat-anti-rabbit IgG (Dianova, Hamburg, Germany) for connexin signals, rabbit-anti-goat-IgG for collagen-I signals (Sigma, Deisenhofen, Germany) and rabbit-anti-mouse IgG for GAPDH signals (Sigma), both conjugated with horseradish peroxidase and subsequently developed with Super Signal Reagent (Pierce, Rockford, IL).

Densitometric analysis

Immunoblots were exposed to X-ray film (Eastman Kodak Co., Rochester, NY, USA), developed and analysed by ONE-Dscan 1.0 Software (Scanalytics, Los Angeles, CA, USA). The relative amount of connexin 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 connexin/GAPDH from each patient was used to calculate possible differences in connexin expression between the

patients. If not stated differently, total connexin (phosphorylated and non-phosphorylated) was used for calculation.

Electrophysiological investigation

If specimens of atrial tissue were large enough to allow functional investigation (7 × 7 to 15 × 15 mm), these samples from SR or AF patients were submitted to electrophysiological mapping analysis using 64 unipolar electrodes being attached on the tissues surface. Tissue was superfused (10 mL·min⁻¹) with Tyrode solution [Na⁺ 161.44, K⁺ 5.36, Ca⁺⁺ 1.8, Mg⁺⁺ 1.05, Cl⁻ 147.86, HCO₃⁻ 23.8, PO₄²⁻ 0.42 and glucose 11.1 mmol·L⁻¹, equilibrated with 95% O₂ and 5% CO₂ (pH 7.4)], stimulated at a site within the electrode matrix with 1 ms square pulses of double threshold at a rate of 1 Hz. The surface temperature of the atrial tissue was 37°C. The specimen were connected to the mapping system HAL4 (temporal resolution: 20 kHz per channel) as described previously (Dhein *et al.*, 1999). A total of 64 AgCl electrodes were cast in a polyester plate (8 × 8 orthogonal matrices with 1 mm inter-electrode distance), which was carefully attached to the tissues surface, thus mapping an area of 7 × 7 mm.

For evaluation of the mapping data, the activation time points at each electrode were determined as $t(dU/dt \text{ min})$ as described previously (Dhein *et al.*, 1999). From the activation times, we generated 0.25 ms isochrones and analysed longitudinal and transverse conduction velocity. Longitudinal axis was determined by histological analysis of the fibre orientation as described previously (Dhein *et al.*, 1999).

Statistical analysis

All data are given as mean ± SEM. Statistical evaluation of the patient data was performed by using multi-factorial ANOVA

Figure 1

A. Expression of atrial Cx43/GAPDH in patients with SR and patients with AF with and without β -blocker (metoprolol) treatment (BB) (data given as mean $\pm$ SEM). * $P < 0.05$, significant difference between AF and SR. # $P < 0.05$, significant effects of β -blockade. The number of patients for each column is indicated in brackets in the column. B. Original Western blot of Cx43 and GAPDH in patients with SR and patients with AF with and without metoprolol treatment (BB).

followed by *post hoc*-Mann-Whitney *U*-test. Values of $P < 0.05$ were considered statistically significant. As described above, all data are specified as ratios (connexin/GAPDH) and did therefore not require any units.

Results

The patient characteristics are given in Table 1. Regarding the expression of the cardiac gap junction protein Cx43, we found that in patients suffering from AF, Cx43 expression was significantly higher than in patients with SR ($P = 0.001$) (Figure 1A, B). Gender had no effect on Cx43 expression ($P = 0.94$). In both groups of patients, SR and AF, the Cx43 expression was significantly higher if patients were on metoprolol therapy ($P = 0.027$) (Figure 1A). Other drugs – in this study population – had no significant effect on Cx43 expression

Figure 2

A. Expression of atrial Cx40/GAPDH in SR and AF patients with and without metoprolol treatment (BB). * $P < 0.05$, significant difference between AF and SR. The slight effects of β -blocker treatment on Cx40 expression did not reach significance. The number of patients for each column is indicated in brackets in the column. B. Original Western blot of Cx40 and GAPDH in patients with SR and patients with AF with and without metoprolol treatment (BB).

(digitalis: $P = 0.565$; calcium antagonists: $P = 0.562$; angiotensin converting enzyme (ACE) inhibitors/ angiotensin AT₁ receptor blockers: $P = 0.08$).

Regarding Cx40, we also found significantly enhanced expression in AF ($P = 0.029$) (Figure 2A, B). However, in contrast to Cx43, Cx40 expression was not significantly ($P = 0.131$) affected by metoprolol treatment (Figure 2A). There was no gender-dependent effect on Cx40.

Immunohistological analysis (see Figure 3 for quantitative data and Figure 4 for original immunohistology images) revealed that in SR patients (see Figure 4A), staining for Cx43 (green) was more or less confined to the cell poles (Figure 4A) in a regular pattern, while at the side of the cells (lateral localisation), there was only low expression. In AF (Figure 4B), however, the pattern became more irregular, and Cx43 was also expressed at the sides of the cells (Figure 4B), while its polar accumulation was less pronounced. In AF patients receiving metoprolol (Figure 4C), the AF-induced alterations were less prominent, and more Cx43 was seen at the cell poles (Figure 4C) and less at the sides (as compared with AF).

Regarding quantitative analysis of polar Cx43 expression (ratio between Cx43 positively stained membrane length and membrane length $PLM_{section}/LM_{section}$ at the cell poles), we found a significant decrease in polar Cx43 in AF patients

Figure 3

Subcellular localization of atrial Cx43. A. Expression of Cx43 at the cell pole, expressed as the percentage of the polar cell length staining positive for Cx43. B. Expression of Cx43 at the lateral cell side, expressed as the percentage of the polar cell length staining positive for Cx43. C. Ratio of lateral/polar Cx40 expression. Data are means $\pm$ SEM of 6 patients per group. * $P < 0.05$, significant difference between AF and SR. # $P < 0.05$, significant effects of β -blockade.

(Figure 3A). Interestingly, we found that, in AF, the polar expression was significantly higher in patients receiving metoprolol therapy (Figure 3A). In contrast to the cell pole, Cx43 expression at the side of the cells (PLM_{section}/LM_{section} ratio in the mid segments) was significantly enhanced in AF (Figure 3B). This increase in lateral Cx43 was slightly diminished in AF-patients on metoprolol therapy.

Because, in terms of biophysics, one important factor determining how much current is flowing either in longitudinal or in transverse directions is the ratio of lateral and polar gap junction conductances (Seidel *et al.*, 2010), we determined from the data already collected, the lateral/polar Cx43 ratio. This ratio was significantly enhanced in AF samples, compared with SR samples (Figure 3C) and such changes in the lateral/polar ratio was significantly antagonized by metoprolol treatment (Figure 3C).

Regarding Cx40 distribution, there were no significant changes in Cx40 expression at the cell poles (Figure 5A), while we also found significantly enhanced expression of Cx40 at the sides of the cells in AF (Figure 5B). The lateral/polar Cx40 expression ratio was also significantly enhanced in AF (Figure 5C). In contrast to Cx43, the AF-induced changes in Cx40 localization were not affected by metoprolol treatment (Figure 5).

Taken together, these data showed that AF led to enhanced expression and lateralisation of Cx43 and Cx40, and that in patients receiving metoprolol, the ratio of polar/lateral Cx43 expression was shifted towards a higher polar and less lateral expression.

Regarding the question whether this altered polar/lateral Cx ratio may be accompanied by functional electrophysiological changes, we investigated atrial tissue from SR and AF patients, stimulated this tissue electrically and registered epicardial potentials at 64 sites (8×8 electrodes). In each specimen, we investigated ≥ 5 pulses and tested two sites for stimulation. From these data, we determined transverse conduction velocity [V(T)] and longitudinal conduction velocity [V(L)].

This mapping of atrial specimens revealed that atrial tissue from patients with normal sinus rhythm exhibited higher longitudinal [V(L)] than transverse propagation velocity [V(T)] (Figures 6 and 7). Original isochrones from SR (Figure 6A) or AF patients (Figure 6B) demonstrate that the stimulated response (shown as the rectangle) propagates in an elliptical pattern around the indicated stimulated site in SR (Figure 6A) with a higher propagation velocity in the direction of the fibre axis (macroscopic fibre axis direction is indicated by the light yellow arrow) than transverse to it, while in AF, the propagation pattern was less elliptical and more uniform in both directions, so that in AF, longitudinal and transverse velocities were less different (Figure 6B). This increase in transverse conduction velocity was attenuated in AF patients receiving metoprolol (Figure 6C).

Accordingly, quantitative evaluation revealed that in AF patients in particular transverse velocity was altered. In comparison to SR samples, V(T) was significantly enhanced in AF ($P = 0.01$) (Figure 7B). This AF-induced increase in V(T) was significantly attenuated in atrial tissue from patients receiving metoprolol ($P = 0.01$). V(L), however, was not significantly higher in AF nor was it modified by metoprolol (Figure 7A).

Figure 4

Original immunohistology for Cx43 (green) in human atrium in SR (A; arrows point to examples of polar Cx43 accentuation), AF (without β -blocker; B; arrows point to examples of Cx43 expression at the lateral cell borders) and AF with β -blocker (metoprolol) treatment (BB) (C; arrows point to examples of polar Cx43 accentuation). All specimens were counterstained with Chicago Blue for visualization of connective tissue (light orange).

To rule out the possibility that metoprolol may have affected AF-induced fibrosis, we also assessed collagen-I expression in the tissue samples. Collagen-I/GAPDH was significantly enhanced in AF [AF (all cases): 192 ± 21 vs. SR: 79 ± 38 ; $P < 0.05$]. However, metoprolol treatment did not affect AF-induced collagen expression (AF without metoprolol: 208 ± 46 vs. AF with metoprolol: 186 ± 24 , n.s.).

Discussion

The data obtained from human atria showed an up-regulation of both Cx43 and Cx40 in AF together with an increased expression of both connexins at the sides of the cell (laterally) and increased transverse conduction velocity. Moreover, we found that in both SR and AF patients, metoprolol treatment resulted in a higher expression of Cx43 and in a reduction of the AF-induced increase in lateral/polar Cx43 expression ratio together with a reduction of the transverse velocity. Thus, this is – to the best of our knowledge – the first description that the disturbance of the intracellular distribution pattern of Cx43 and Cx40 in AF is accompanied by enhanced transverse conduction velocity, and that enhanced V(T) and Cx43 remodelling are – at least partially – normalized in AF patients receiving metoprolol.

As regards the changes in the intracellular distribution pattern, it is known from previous studies (Polontchouk *et al.*, 2001; Kostin *et al.*, 2002; Li *et al.*, 2004) that besides many other effects, AF leads to increased expression of Cx43 at the lateral side of the cells (i.e. lateralization) and to heterogeneities (i.e. areas with low or almost no expression of connexins) (Dupont *et al.*, 2001). It has been assumed, that gap junctional heterogeneity or lateralization may contribute to conduction abnormalities (Brundel *et al.*, 2002; Nattel *et al.*, 2007), which might predispose to multiple wavelet re-entrant arrhythmia, although it remains still a matter of debate whether these lateral channels are functional (Duffy and Wit, 2008; Burstein *et al.*, 2009), which was until now only observed in a rat model of rapid pacing (Polontchouk *et al.*, 2001).

Thus, an important question is whether Cx43 lateralization might have a functional correlate. The mapping analysis revealed that indeed in AF, transverse conduction velocity was enhanced, and that this was significantly counteracted by metoprolol treatment in good accordance with the immunohistological reduction of the AF-induced increase in lateral/polar Cx43 ratio: In a first simplifying approximation, the activated cell can be considered as a current divider with two ohmic resistors in parallel: the polar and the lateral membranes. According to Ohm's law, the current flowing from this cell to neighbouring cells will be split between the resistors in inverse proportion to their impedances. Thus, it is the proportion of these lateral and polar impedances (and not the absolute impedance), which in conjunction with the current sinks in the lateral and transverse direction (i.e. beyond the considered cell) is essential for the current split ratio (Seidel *et al.*, 2010). Following this theory, we determined the ratio of lateral/polar Cx43 and Cx40 expression. The present results show that in AF, this ratio is markedly enhanced (Cx43: Figure 3C; Cx40: Figure 5C), and – on a functional level – the mapping experiments showed that this is accompanied by enhanced transverse velocity (Figure 7B), which would be in favour of the assumption that the lateral gap junctions are functional. This is further supported by the finding that reduction of the AF-induced increase in lateral/polar Cx43 expression ratio by metoprolol (Figure 3C) is accompanied by a reduction in transverse velocity (Figure 7B). However, the finding that metoprolol treatment only affected Cx43, but not Cx40, lateral/polar ratio might also help to explain why this treatment clinically is of only partial efficacy in the treatment of AF.

Thus, we have reasons to believe that at least partially the increased lateral/polar ratio of Cx43 seems to be functional. However, it should be mentioned that this analysis is only possible if the specimen are large enough, and that other factors like fibrosis also contribute to the changes in activation pattern, as shown in a dog model of congestive heart failure-associated AF (Burstein *et al.*, 2009). Our own data also showed that AF enhanced collagen-I expression, but that this was not affected by metoprolol.

Figure 5

Subcellular localization of atrial Cx40 given as expression of Cx40 at the cell pole (A), at the lateral cell side (B) and as the lateral/polar ratio (C). For details, see legend to Figure 3 and text. Data are means $\pm$ SEM of $n = 6$ patients per group. * $P < 0.05$, significant difference between AF and SR. The slight effects of β -blockade (BB) on Cx40 localization did not reach the level of significance.

Regarding the expression of Cx43 in AF in humans, there is some controversy whether Cx43 is up-regulated as seen by Yan *et al.* (2004), Wetzel *et al.* (2005) and Dhein *et al.* (2008) or not (Polontchouk *et al.*, 2001; Li *et al.*, 2004), or may even be down-regulated in AF as was described by others (Kostin *et al.*, 2002 (right atrium); Luo *et al.*, 2007). The discrepancies between the observed levels of expression among these studies may be related to the various patient populations with varying percentages of patients suffering from mitral valve disease (MVD) or coronary heart disease (CHD) as the reason for the cardiac surgery, so that a sample can be obtained, and the question whether right or left atrial tissue has been investigated, and whether this was from the free wall or from the appendage. In a recent study, it was reported that Cx43 is increased in AF if there is no or only mild heart failure but is decreased in presence of severe heart failure (Hui *et al.*, 2008). In patients suffering from CHD undergoing bypass surgery, Cx43 was not up-regulated in AF (Polontchouk *et al.*, 2001), while in MVD-positive AF patients, Cx43 is up-regulated (Wetzel *et al.*, 2005). Accordingly, in our present study, nearly 90% of all patients suffered from MVD, and ventricular function was normal with ejection factor values of about 62%. Thus, an up-regulation in Cx43 in AF seems to be in agreement with earlier results, taking the concomitant factors into account. However, according to the results of our study, we would infer that increased expression of connexins *per se* is not the prime factor stabilizing AF, but rather that the altered distribution with enhanced expression at the lateral cell borders favours or stabilizes AF.

Regarding Cx40, the finding of enhanced Cx40 expression and lateralization in AF in our patients is in good agreement with some other studies (Dupont *et al.*, 2001; Polontchouk *et al.*, 2001; Yan *et al.*, 2004; Wetzel *et al.*, 2005), although Gaborit *et al.* (2005) observed reduced Cx40 expression in valvular heart disease-associated AF. However, in contrast to our study, these authors investigated a group of patients consisting of four patients with MVD and seven with aortic stenosis (ratio mitral: aortic: 0.57), while in our patients, this ratio was 3.7; that is, in our population, the proportion of MVD was much higher (see Table 1). Nevertheless, this supports the view of Gaborit *et al.* (2005) that underlying heart disease is an important factor modulating gap junction remodelling. While Cx40 exhibited similar, although attenuated, changes in AF as did Cx43, changes in Cx40 were not affected by metoprolol treatment. This, however, is in good agreement with cell culture data showing that only Cx43, but not Cx40, is regulated by β -adrenoceptor stimulation (Salameh *et al.*, 2009). Similarly, other stimuli such as endothelin or angiotensin also only affected Cx43, but not Cx40 (Polontchouk *et al.*, 2002), so that these data again support the view that Cx43 and Cx40 are differentially regulated.

Regarding the underlying mechanism, stimulation of β -adrenoceptors is known to up-regulate Cx43, but not Cx40 (Salameh *et al.*, 2006, 2009), and stretch up-regulates Cx43 (Salameh *et al.*, 2010a), so that it seems paradoxical that β -adrenoceptor antagonists like metoprolol also up-regulate Cx43. Are there possible explanations? First, there are indirect effects: ischaemia leads to down-regulation of Cx43 (Kaprielian *et al.*, 1998), and metoprolol may reduce oxygen consumption and thus alleviate ischaemic events. Another interesting possible explanation comes from a cell culture

Figure 6

Isochrones (0.25 ms) of electrical activation mapped at 64 electrodes (inter-electrode distance: 1 mm; temporal resolution: 20 kHz per channel) in atrial tissue samples from an SR patient (A), a patient suffering from chronic AF (B) and from an AF patient receiving metoprolol treatment (AF + BB, C). An action potential was elicited in the tissue with a rectangular pulse (indicated) at the site within the electrode matrix and propagated in longitudinal and transverse directions as shown by the arrows. The main longitudinal fibre orientation is indicated by a light yellow arrow.

Figure 7

V(L) (A) and V(T) (B) in atrial tissue samples from patients with SR (n = 6) and patients suffering from AF without (n = 5) or with treatment using the β -blocker metoprolol (AF + BB, n = 6). All data are given as means $\pm$ SEM. * P < 0.05, significant difference between AF and SR. # P < 0.05, significant effects of β -blockade.

study that showed that β -adrenoceptor-independent effects may also contribute, since in that study the R-isomer of metoprolol also increased Cx43 expression, similar to S-metoprolol or the racemate (Salameh *et al.*, 2010b). Another highly interesting aspect comes from the recent

finding by Smyth *et al.* (2010) demonstrating the role of oxidant stress in connexin trafficking. Taking into account that β -blockers can have antioxidant properties (Gomes *et al.*, 2006), which typically are not stereospecific, an antioxidant effect might – at least in parts – also be involved.

Why may the findings of this study be of interest? Regarding the question whether connexin changes are rather an epiphenomenon of AF as suggested by a study in dogs suffering from heart failure-associated AF (Burststein *et al.*, 2009) or whether they might contribute to conduction changes, our data in patients comparing SR and AF suggest that the changes in lateral/polar connexin expression may contribute to changes in propagation of the electrical activation and thus may contribute to the stabilization of AF by enhanced V(T). Taken together with the considerations above, changes in connexins are likely to be contributing factors in AF but probably not the only factor. Besides this pathophysiological aspect, our findings may also open a new perspective on treatment of AF, as reversal of the gap junction remodelling could perhaps normalize the propagation pattern. The metoprolol data show – to the best of our knowledge – for the first time that AF-induced gap junction remodelling can be influenced pharmacologically. Admittedly, metoprolol is not among the most clinically effective drug for AF treatment. However, as the data show, metoprolol only affected Cx43 remodelling but not Cx40 remodelling. Both connexins are involved in AF remodelling and it would be most interesting to find other drugs, which affect both connexins. The present study shows that it is, in principle, possible to reverse gap junction remodelling by drug treatment *in vivo*.

Limitations of this study

It should clearly be stated that our data refer to the special selection of patients as indicated in Table 1. That means that the finding of Cx43 lateralization and changes in anisotropy and its improvement by metoprolol therapy is limited to patients suffering from AF associated with MVD (mainly mitral valve regurgitation) in the absence of heart failure. Thus, these data cannot be uncritically transferred to other pathophysiologicals of AF (lone AF, etc.). Moreover, the statement that other drugs (digitalis, calcium antagonists, ACE inhibitors) had no influence is limited to this study population, as the number of patients receiving these drugs was too small for a generalized statement.

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Conflict of interest

None.

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