Cyclical stretch induces structural changes in atrial myocytes

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Abstract

Atrial fibrillation (AF) often occurs in the presence of an underlying disease. These underlying diseases cause atrial remodelling, which make the atria more susceptible to AF. Stretch is an important mediator in the remodelling process. The aim of this study was to develop an atrial cell culture model mimicking remodelling due to atrial pressure overload. Neonatal rat atrial cardiomyocytes (NRAM) were cultured and subjected to cyclical stretch on elastic membranes. Stretching with 1 Hz and 15% elongation for 30 min. resulted in increased expression of immediate early genes and phosphorylation of Erk and p38. A 24-hr stretch period resulted in hypertrophy-related changes including increased cell diameter, reinduction of the foetal gene program and cell death. No evidence of apoptosis was observed. Expression of atrial natriuretic peptide, brain natriuretic peptide and growth differentiation factor-15 was increased, and calcineurin signalling was activated. Expression of several potassium channels was decreased, suggesting electrical remodelling. Atrial stretch-induced change in skeletal \( \alpha \)-actin expression was inhibited by pravastatin, but not by eplerenone or losartan. Stretch of NRAM results in elevation of stress markers, changes related to hypertrophy and dedifferentiation, electrical remodelling and cell death. This model can contribute to investigating the mechanisms involved in the remodelling process caused by stretch and to the testing of pharmaceutical agents.

Keywords: Atria \( \cdot \) stretch \( \cdot \) remodelling \( \cdot \) cell culture

Introduction

Atrial fibrillation is the most common cardiac arrhythmia [1] and it often occurs because of an underlying disease, such as hypertension or heart failure [2]. Structural remodelling seen in patients with AF as well as in animal models includes atrial dilatation, cellular hypertrophy, dedifferentiation, fibrosis, apoptosis and myolysis [3–7]. Electrophysiological changes also occur and may contribute to the progressive nature of AF [4, 8]. The underlying disease is a major causal factor of atrial remodelling [5–7, 9–15]. The common factor in these underlying disease states is haemodynamic and pressure overload of the ventricles and the atria which causes stretching. However, it is difficult to determine in animal models whether the critical mechanism is mechanical stress or neurohormonal activation. In \( \text{in vitro} \), stretching of ventricular cardiomyocytes has been associated with hypertrophy, increased calcineurin activity, apoptosis and release of reactive oxygen species [16–18]. Stretching of atrial cells, however, is not well characterized. Atria from hypertensive animals show increased weight, fibrosis and inflammation [11, 13, 15] and long-term hypertension (4–5 years) results in enlarged atria, hypertrophy, fibrosis, dedifferentiation, cell death and myolysis, but without any sign of inflammation [5]. In \( \text{in vitro} \) experiments, static stretch leads to electrical remodelling, hypertrophy, increased calcineurin activity and extracellular matrix remodelling [19–21]. Static stretch, however, may not reflect the physiological situation.

In an effort to better simulate the clinical setting of atrial pressure overload, our objective was to develop an atrial cell culture model mimicking atrial remodelling due to atrial pressure overload.

Materials and methods

Ethics statement

All animal studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the University of Groningen (Approval ID: DEC 5495 and DEC 6002).

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Isolation and culture of neonatal rat atrial cardiomyocytes

Neonatal rat atrial cardiomyocytes (NRAM) were isolated and cells were cultured, as described previously [22]. Briefly, the hearts of 1- to 3-day-old Sprague Dawley rats were excised and transferred in a tube containing cold calcium- and bicarbonate-free Hank's solution (CBFHH) (137 mmol/l NaCl, 5.36 mmol/l KCl, 0.81 mmol/l MgSO₄, 5.55 mmol/l dextrose, 0.44 mmol/l KH₂PO₄, 0.34 mmol/l Na₂HPO₄ and 20 mmol/l HEPES, pH 7.4). Atria and ventricles were then separated and atrial cardiomyocytes were isolated by successive rounds of stirring and pipetting the tissue up and down in CBFHH supplemented with 1.5 mg/ml trypsin (BD Biosciences, Breda, the Netherlands) and 20 μg/ml DNase. To enrich the cardiomyocytes, the cell suspension was plated on regular polystyrene dishes for 45 min. and the cardiomyocytes were then seeded on flexible collagen-coated silicone rubber plates for 24 hrs. After washing twice with PBS, the cells were plated on the Flexercell-4000 system (Flexcell International Corporation, Hillsborough, NC, USA) and cultured for 24 hrs at 37°C under 5% CO₂ in MEM and supplemented with sodium bicarbonate for 5% CO₂, 25 mmol/l Hepes (Invitrogen Corporation, Breda, the Netherlands), 1.5 mmol/l vitamin B12, 5% FCS, 0.1 mmol/l BrdU and penicillin-streptomycin (50 U/ml and 50 mg/ml, respectively; Invitrogen Corporation). The cells were then cultured in the same medium without serum and BrdU, but supplemented with transferrin (10 μg/ml), insulin (10 μg/ml) and bovine serum albumin (BSA; 1 mg/ml) for 18–48 hrs before the experiments were carried out.

Inhibitors were incubated with the cells for 30 min. prior to the stretch period. Concentrations used were: 1 μmol/l liso, 1 μmol/l of the selective Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN93, 1 μmol/l cyclosporin A (Merck, Darmstadt, Germany), 10 μmol/l eplerenone and 10 μmol/l pravastatin.

Application of mechanical stretching

Cardiomyocytes were stretched using the Flexercell-4000 system (Flexcell International Corporation), a modification of the system initially described by Banes et al. [23]. Cyclic stretching of 1 Hz and 15% elongation was applied for different durations ranging from 30 min. to 24 hrs. Control cardiomyocytes were cultured on identical Flexerwell plates and maintained under similar conditions, but without mechanical stretch. Following the stretch period, the cells were washed twice with PBS and used for protein analysis, immunofluorescence or RNA isolation.

Cell diameter

Cells were fixed in 4% paraformaldehyde for 5 min. on ice, permeabilized with ice-cold PBS + 0.3% Triton X100 for 5 min. and then washed with PBS. A solution containing 3% BSA, 2% normal goat serum and 0.1% tween in PBS was used to block and reduce the background. Antibodies were diluted in 1% BSA in PBS and incubated with the cells for 1 hr at room temperature. The primary antibody was a mouse monoclonal anti-α-actinin (clone EA-53) and the secondary antibody was a goat antimouse FITC (Santa Cruz Biotechnology, Heidelberg, Germany). Counterstaining with fluorescent rhodamine-phalloidin (Invitrogen Corporation) was used to differentiate non-cardiomyocytes. Coverslips were mounted using Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA). Four to eight pictures were taken and 5–10 cells/picture were used per experiment. Image processing software (Image J 1.43u, NIH, Bethesda, MD, USA) was used to determine Feret’s cell diameter. Cell diameter was expressed as a percentage of the diameter of control cells.

Real-time quantitative PCR

Total RNA was isolated using TRIzol reagent (Invitrogen Corporation) or Nucleosil II kit (Macherey-Nagel, Düren, Germany) and converted to cDNA by Quantitect Reverse Transcription (Qiagen, Venlo, the Netherlands). Gene expression was measured with Absolute QPCR SYBR Green ROX Mix (Abgene, Epsom, UK) in the presence of 7.5 ng cDNA and 200 nM forward and reverse primers. qRT-PCR was performed on the Biorad CFX384 (Bio-Rad, Veenendaal, the Netherlands). The initial denaturation and activation of the DNA polymerase (95°C for 3 min.) was followed by 35 cycles with denaturation for 15 sec. at 95°C and annealing and elongation for 30 sec. at 60°C followed by a melt curve. Gene expression levels were corrected for ribosomal protein, large, P0 (Rplp0) reference gene expression and values were expressed relative to the respective control group of each experiment. Primers used included: jun proto-oncogene (c-jun) forward gattatcagctaggacctg, c-jun reverse tattttgtcatcagttcg, FBjury eosinosa oncogene (c-fos) forward tcaagccgagaacagatc, c-fos reverse tgtgacctctgccgctga, myelocytomatosis oncogene (c-myc) forward ctatgcctgtcagagagac, c-myc reverse tttgctgtgctctttgat, early growth response gene 1 (egr-1) forward gcagcttgaccagagctgtc, egr-1 reverse ggtggattctggtgctga, atrial natriuretic peptide (ANP) forward attgcgcttctcttcag, ANP reverse ttacggccatctctcct, brain natriuretic peptide (BNP) forward aacatcctagcatcagag, BNP reverse ggtggccttcttcttgag, growth differentiation factor-15 (GDF15) forward ttgacacgcgctgtgctga, GDF15 reverse gttgacacgcgtctgctga, c-jun proto-oncogene (c-jun) forward gattatcagctaggacctg, c-jun reverse tattttgtcatcagttcg, FBjury eosinosa oncogene (c-fos) forward tcaagccgagaacagatc, c-fos reverse tgtgacctctgccgctga, myelocytomatosis oncogene (c-myc) forward ctatgcctgtcagagagac, c-myc reverse tttgctgtgctctttgat, early growth response gene 1 (egr-1) forward gcagcttgaccagagctgtc, egr-1 reverse ggtggattctggtgctga.
nitric oxide synthase (iNOS) forward gcaggaactgcgagagag, iNOS reverse gcagattggattgttcagag, endothelial nitric oxide synthase (eNOS) forward tctactaggttctgctatcc, eNOS reverse gcagccaaacaccaagaagtc, Riplp0 forward gtggctcagtcgctcactc and Riplp0 reverse gcacgcgcaathtgctagtt.

Western blotting

Proteins from whole cell lysates were isolated using radioimmuno-preparation assay buffer [0.5% sodium deoxycholate, 0.1% sodium dodecysulfate (SDS) and 1% Igepal ca-630 in TBS] supplemented with a protease inhibitor cocktail (Roche Diagnostics Corp., Indianapolis, IN, USA), a phosphatase inhibitor cocktail, PMSF (1 mmol/l; Roche Diagnostics Corp.) and sodium vanadate (15 mmol/l). SDS sample buffer was added and samples were denatured by heat at 99°C for 5 min. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel, transferred to PVDF membranes (Bio-Rad) and blocked with 5% milk in 0.1% tween in TBS (TBST). Blots were incubated overnight with antibodies against either phospho-Erk (E-4), troponin T-C (C-19) (both Santa Cruz Biotechnology), troponin I-C (MF4; Fitzgerald Industries International, Acton, MA, USA), phospho-Akt (Ser473) (D9E), phospho-p38 (Thr180/Tyr182; both Cell Signaling Technology, Danvers, MA, USA) or microtubule-associated protein light chain 3 (LC3; MBL, Nakaku Nagoya, Japan) diluted in either 5% milk or BSA in TBST. For loading control, membranes were reprobed with α-tubulin or GAPDH antibodies (Fitzgerald Industries International). For loading phosphorylated proteins controls, the membranes were stripped (stripping buffer containing 2% SDS, 100 mmol/l β-mercaptoethanol and 62.5 mmol/l Tris buffer) for 30 min. at 55°C, washed with TBST and incubated with the corresponding antibody detecting the total protein amount, i.e. Erk 1/2 (MK1; Santa Cruz Biotechnology) and Akt (C67E7) and p38 (both Cell Signaling Technology). Signals were detected by ECL and quantified by densitometry (Syngene, Cambridge, UK). Protein levels were expressed relative to the respective control group of each experiment.

Reagents and chemicals

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands).

Statistics

Data are expressed as mean values ± S.E.M. Comparisons between groups were done using one-way ANOVA with post-hoc Dunnett correction. For Figure 5b, a Bonferonni correction was used. Comparison of two groups was done using a two-tailed Student’s t-test. All analyses were carried out using PASW (Version 18, SPSS Inc., Chicago, IL, USA). P values of <0.05 were considered statistically significant.

Results

Immediate early genes

Stretch for 30 min. increased the mRNA expression of the immediate early genes c-fos by 647%, c-jun by 212%, c-myc by 56% and early growth response gene 1 (egr-1) by 465% (Fig. 1a). In addition, phosphorylation levels of Erk and p38 were increased 68% and 101% respectively (Fig. 1b-d).

Hypertrophy and dedifferentiation

Stretch for 24 hrs increased the cell diameter by 10% (P < 0.05, Fig. 2a and b) along with troponin T and troponin I expression by 51% (P < 0.05) and 42% (P < 0.05), respectively (Fig. 2c and d) suggesting hypertrophy-related increase in contractile protein expression. To investigate the dedifferentiation that often accompanies pathological hypertrophy, mRNA expression of skeletal α-actin, α-MHC and β-MHC were investigated. After 24 hrs of stretch, the β/α-MHC ratio and skeletal α-actin expression were increased by 41% and 76% respectively (Fig. 2e and f).

Stress markers

Stretch of NRAM caused a biphasic release of ANP (Fig. 3a), significantly different from control at 30 min., 1 hr, 24 and 48 hrs (increased by 43, 53, 44 and 102% respectively). Furthermore, 24 hrs of stretch increased mRNA levels of ANP by 47% (Fig. 3b). The biphasic
response, with increased ANP mRNA expression, implies immediate release of ANP upon stretch, subsequent increased ANP production and a second phase of release of ANP. After 3 hrs of stretch, BNP mRNA expression levels were increased by 99% (Fig. 3c). Levels remained 70% increased after 6 and 24 hrs of stretch and the expression level of GDF15 was increased 77% after 24 hrs (Fig. 3d).

Cell death

Three different forms of cell death exist: apoptosis, cell death with autophagy and necrosis. TUNEL staining showed 6.1% TUNEL-positive cells in control cells compared with 6.2% following stretch (Fig. 4a). Western blotting for LC3-II, a marker of autophagosomes (a double-membrane structure which contains elements of its own cytoplasm mediating autophagy), revealed no difference between control cells and stretched cells (Fig. 4d and e). Although no sign of cell death was shown by TUNEL staining or LC3-II Western blotting, a 78% increase (Fig. 4b) in the amount of cells in the medium was observed after 24 hrs of stretch. As a positive control for cell death, staurosporine was used; staurosporine increased the amount of cells in the medium 170% (Fig. 4b). Trypan blue staining was used to confirm that the cells released in the medium were indeed dead cells. The majority of the cells (95%) stained blue, showing that these cells did not have an intact cell membrane. In addition, LDH levels were threefold increased in medium from stretched cells (Fig. 4c). Also, a trend towards increased phosphorylation of Akt, a protein involved in cellular survival pathways, was observed (Fig. 4d and f).

Electrical remodelling

Figure 5 shows the effects of 24 hrs of stretch on genes related to electrical remodelling. Stretch for 24 hrs had no effect on expression of Scn5a which contributes to the sodium current (INa), expression of LTCC which contributes to the L-type calcium current (ICaL) or expression of the sodium/calcium exchanger (Ncx1; Fig. 5). Stretch reduced expression levels of Kcnd3 to 30% of control levels. Kcnd3 encodes Kv4.3 and contributes to the cardiac transient outward potassium current (Ito). Also expression of Kcnq1, Kcnj2 and Kcnj3 were reduced to 63, 63 and 75% of control levels, respectively. Kcnq1 encodes Kv7.1 and mediates slow delayed rectifying K+ (IKs), Kcnj2 codes for Kir2.1 and mediates the inward rectifier current (IK1) and Kcnj3 codes for Kir3.1 and mediates acetylcholine-activated inward rectifier K+ channel (IKACh). Interestingly, also the expression of small conductance Ca2+-activated K+ channel 1 (SK1) was reduced.

Mechanisms

Stretch for 24 hrs increased the expression of Rcan1 by 124% (Fig. 6a) suggesting the involvement of calcineurin signalling. Furthermore, treatment with 1 μM cyclosporine A was associated with a trend towards attenuation of the stretch-induced increase in the expression of skeletal α-actin (Fig. 6b). This suggests activation of calcineurin signalling upon stretching and involvement in the stretch-induced response.
We could not demonstrate the involvement of CaMKII in the stretch-activated response as no increase in expression of CaMKII, the major CaMKII isoform in the heart, was observed after 24 hrs of stretch (Fig. 6c) and inhibition of CaMKII with 1 uM KN93 did not alter the expression level of skeletal $\alpha$-actin (Fig. 6b).

Interventions

Treatment with losartan had no effect on the gene expression of skeletal $\alpha$-actin (Fig. 6d) which suggests that this response is not mediated via ligand activation of the angiotensin II type 1 receptor. However, the expression of the angiotensin II receptor type 1a was decreased (Fig. 6c). Incubation with eplerenone had no effect on stretch-induced gene expression, but pravastatin reduced stretch-induced expression of skeletal $\alpha$-actin (Fig. 6e). No effect of stretch on expression of Rac1 was observed (Fig. 6f), but the expression of eNOS and iNOS was reduced (Fig. 6f).

Discussion

This study shows that cyclic stretch of NRAM results in (1) activation of immediate early genes, distinct signalling pathways, changes related to hypertrophy and dedifferentiation, elevation of stress markers, electrical remodelling and cell death; (2) activation of calcineurin
signalling, but not of CaMKII signalling; (3) inhibition of skeletal α-actin expression with pravastatin and (4) the demonstration that BNP in atrial cells is a stronger stress marker than ANP in neonatal atrial cardiomyocytes in vitro.

Activation of immediate early genes is an early response to a wide variety of growth factors, cytokines as well as injury. Previous studies in isolated rat atria showed that stretch increased the expression of c-fos, c-myc and egr-1, but not of c-jun [25, 26] which is in agreement with our study. In ventricular cells, activation of immediate early genes has also been demonstrated in response to stretch [27, 28]. Kerkela et al. showed a rapid increase in phosphorylated p38 and Erk due to stretch which is in agreement with our data showing that 30 min. of stretch increased phosphorylation levels of p38 and Erk [26]. Similar findings have also been reported in stretched ventricular cardiomyocytes [17, 29].

In atrial cardiomyocytes subjected to stretch, hypertrophy was suggested via an increase in protein to DNA ratio and ANP mRNA levels [19, 20]. We also observed an increase in cell length, tropo- 
nin expression and the expression levels of ANP, which is often used as a marker of hypertrophy. In animal models of hypertension, enlarged atria have also been observed [5, 11, 13, 15, 30–32], as well as atrial dedifferentiation, in models of hypertension, mitral valve disease and atrial fibrillation [5, 32, 33] which is in agreement with the increased levels of gene expression markers of dedifferentiation we observed in our study. An important aspect of dedifferentiation is myolysis, which has been described in cells subjected to tachypacing and in patients with AF [34], but has not been described in association with stretch. Reduced expression of troponins was found in AF patients, which correlated with the amount of myolysis [35]. We, however, did not observe any reduction in troponin protein levels which suggests that stretch for 24 hrs is a model in which remodelling takes place without the loss of contractile elements.

GDF15 is a potential new biomarker and a 25-fold increased expression has been observed in stretched ventricular myocytes [36]. In our experimental model, we only observed a 1.8-fold increase in expression. This discrepancy may be due, at least in part, to the different stretch protocols used (cyclic versus static).

Cell death has not been described in stretched atrial cardiomyocytes. In hypertensive sheep, atrial apoptosis has been demonstrated [5], but in a dog model of mitral regurgitation, no sign of necrosis was found [31]. We observed an increase in cell death which probably did not occur via apoptosis because there was no increase in TUNEL-positive cells. In addition, no protective effect of the autophagosome was found (unchanged protein levels of LC3-II).

Electrical remodelling has been described extensively related to AF [37, 38]. In cells, Yang et al. showed that rapid electrical stimulation of HL-1 cells induced electrical remodelling, including shortening of action potential duration [39]. In patients with hypertension, electrical remodelling has also been described [9]. More recently, also SK1 and SK2 were recognized to play a role in electrical remodelling. Saygili et al. found a reduced I_h and a reduced expression of Kv4.2, in addition, an increased I_Ks and an increased expression of Kir2.1 and Kir2.3 was observed [19]. These findings are partially in agreement with ours; we observed a reduced expression of Kv4.3 and also a reduced expression of Kir2.1.

Fig. 3 Stretching increases mRNA expression of stress specific markers and induces release of ANP. (A) ANP release into the medium (n = 3–9). (B) ANP expression (n = 8–21). (C) BNP expression (n = 8–20). (D) GDF15 expression (n = 8–19). Data are expressed relative to control. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with unstretched control. ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; GDF15: growth differentiation factor-15; Rplp0: ribosomal protein, large, P0.
Many changes in calcium handling and Ca\textsuperscript{2+}-dependent mechanisms are related to atrial remodelling [41]. Static atrial stretch has been shown to increase calcineurin activity and Rcan1 expression [20]. Furthermore, cyclosporine A, a calcineurin inhibitor, reduced stretch-induced changes in extracellular matrix remodelling in atrial cardiomyocytes [21]. Our observations are in agreement with these previous studies. We observed a stretch-induced increased Rcan1 mRNA expression, and cyclosporine A tended to attenuate the increase in skeletal \(\alpha\)-actin expression. In AF, down-regulation of the expression of the L-type Ca\textsuperscript{2+}-channel has been observed [42]. Similarly, reduced atrial L-type Ca\textsuperscript{2+}-current has been demonstrated in patients with mitral valve disease or reduced ejection fraction [43]. However, we did not observe any reduction in L-type Ca\textsuperscript{2+}-channel expression after 24 hrs of stretch.

In an atrial cardiomyocytes model of static stretch, losartan attenuated the stretch-induced hypertrophy and electrical and extracellular matrix remodelling [19, 21]. In our experiments, losartan had no effect on stretch-induced increase in skeletal \(\alpha\)-actin expression. We did, however, observe a down-regulation of the angiotensin II type 1 receptor which might be protective. An important difference between these studies and ours is the type of stretch used. In our experimental model, we used a similar elongation, but a cyclical stretch pattern which, as opposed to static stretch, mimics a more physiological condition. Furthermore, we could not show protective effects of eplerenone.

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none, but pravastatin prevented the stretch-induced increase in skeletal α-actin expression. This suggests a role for reactive oxygen species which has not been described in atrial cells. However, in ventricular myocytes, stretch has been shown to induce the release of reactive oxygen species and these reactive oxygen species mediated the effect of stretch [17, 44]. Rac1 is a small GTPase that regulates the activity of NADPH oxidase, producing superoxide. Increased expression of Rac1 has been implicated in AF [45]. However, we did not observe changes in the expression of Rac1, but we did observe reduced eNOS and iNOS expression.
As expected, ANP expression, a key factor in cardiac stress, was increased upon stretch. We observed a comparable small increase in ANP mRNA levels as previously described in atrial cardiomyocytes [19, 20]. Increased expression of BNP has also been found following atrial stretch [26]. In human atrial strips, BNP is also induced by ischaemia; this is independent of mechanical stress [46]. In our experiments, changes in BNP mRNA levels were more pronounced than changes in ANP expression levels. Our findings confirm that atrial cardiomyocytes are a source of BNP and show, in vitro, that stretch induced BNP expression. Interestingly, our results suggest that during stretch, BNP is a better stress marker than ANP in neonatal atrial cardiomyocytes in vitro.

In summary, we have demonstrated that cyclical stretch of atrial cardiomyocytes results in activation of immediate early genes and distinct signalling pathways including phosphorylation of Erk and p38, changes related to hypertrophy and dedifferentiation, elevation of stress markers such as ANP, BNP and GDF15, changes related to electrical remodelling and cell death (Fig. 6g). Previous studies demonstrated that static stretch results in hypertrophy [19, 20], increased MMP2 and MMP9 activity [21] and changes in potassium currents and gene expression suggesting electrical remodelling [19, 20]. We observed the involvement of calcineurin, but not of CaMKII signalling. Incubation with losartan or eplerenone was without effect, but pravastatin reduced skeletal α-actin expression upon stretch. The role of calcineurin [20, 21] and angiotensin II-dependent signalling pathways has been suggested in previous studies [19, 21]. Importantly, our results were obtained using cyclical stretch, a context reflecting more adequately the physiological condition where the pressure in the atrium increases during each heart cycle.

In ventricular cardiomyocytes, more data are available on the effects of in vitro stretch [16-18, 27, 28, 36, 47]. In ventricular cardiomyocytes another method of stretch, using glass microspheres to induce mechanical load, did not induce hypertrophy, but did induce arrhythmias and caused the release of angiogenic factors [48]. Results obtained using ventricular cardiomyocytes cannot be directly extrapolated because atrial and ventricular cardiomyocytes are anatomically and functionally different and express genes to a different extent [49, 50]. In a microarray performed by Barth et al., differences in gene expression were shown between human atria and ventricles [51]. In the atria, genes associated with fibrosis, apoptosis and neurohormonal activation were more highly expressed whereas, in the ventricles, genes associated with the contractile function were more abundant [51].

Limitations

We used cyclical stretch as a model of atrial overload caused by disease states that create a substrate for AF. It is not known whether the mechanisms described in our experimental model could be extrapolated to humans. We tested a limited number of mechanisms and signalling pathways that are often activated in ventricular disease as well as mechanisms that have been described in atrial fibrillation. It is likely that other signalling pathways are also activated, but this warrants further investigation. In vitro, stretch of atrial and ventricular cardiomyocytes can be dissected. In vivo, however, atrial fibrillation results in a high ventricular rate which has been shown to be an important causal factor of atrial remodelling [6, 10].

Conclusions

The present stretch model resulted in changes mimicking the situation caused by underlying disease. Our model used cyclical stretch of the atrial cardiomyocytes and provides useful data on different aspects of remodelling. This model can be used to investigate mechanisms involved in the remodelling process as well as to assess the effect of new pharmaceutical agents on the remodelling process.

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Conceived and designed the experiments: ADJ, AM, RDB, WVG, IVG. Performed the experiments: ADJ, AM, SOM, RDB, WVG, IVG. Wrote the manuscript: ADJ, AM, SOM. Reviewed the data: ADJ, AM. The study is funded by ICIN (Anne Margreet De Jong) and unrestricted grants to the University Medical Center Groningen, Department of Cardiology from Medtronic and Biotronik.

Conflict of interest

The authors confirm that there are no conflicts of interest.
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