Key roles of endothelin-1 and p38 MAPK in the regulation of atrial stretch response

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ATRIAL FIBRILLATION (AF) has a ~2% prevalence in the general population and is associated with increases in morbidity and mortality (13). Hypertension and left ventricular hypertrophy often lead to pathologic remodeling of the heart and diastolic dysfunction resulting in left atrial enlargement, which is an important step in progression for AF. AF may also result from atrial hypertrophy from the heart, arising from conditions such as pulmonary hypertension, chronic obstructive pulmonary disease, and congenital heart disease. These diseases alter the structure of atrial tissue, thereby increasing the risk of AF. Atrial fibrosis is a common finding in patients with AF (8). Fibrosis may result from pathologies such as cardiac dysfunction, mitral valvular disease, and myocardial ischemia, and atrial fibroblast function has actually been the focus of studies investigating the mechanisms of AF. Cardiac fibroblasts are not only responsible for the synthesis of major extracellular matrix proteins but also secrete a number of factors that affect the structure and function of adjacent cardiomyocytes. Studies indicate that fibroblasts isolated from atrial tissue respond more robustly to growth factors than fibroblasts isolated from ventricular tissue (7). Atrial fibroblast also express much higher levels of myofibroblast marker α-smooth muscle actin than ventricular fibroblasts, suggesting a highly secretory function for the atrial fibroblasts (7).

The central mediator of atrial fibrosis is ANG II (8). ANG II is produced by local cardiomyocytes in response to cardiomyocyte stress and exerts its effects on surrounding cells. Exposure of cardiac fibroblasts to ANG II induces fibroblast function and protein synthesis, and mice overexpressing angiotensin converting enzyme (ACE) show marked atrial dilatation with focal fibrosis and AF (5, 49). AF is actually associated with increased levels of ACE; and activation of ANG II-regulated intracellular signaling pathways and ACE inhibitors have been found beneficial in reducing the frequency of AF (40). In addition, AF is also associated with increased atrial mRNA levels of endothelin-1 (ET-1) (31). In the left ventricle, ET-1 augments left ventricular contractile function and activates hypertrophic signaling pathways (43, 44). In fact, the signaling mechanisms regulating hypertrophic response in ventricular cardiomyocytes have been studied extensively, but signaling mechanisms involved in stress response in atrial myocardium are not well understood. There is some evidence, however, suggesting that signaling mechanisms activated by cardiomyocyte stretch or by neurohumoral agonists may differ between the atrial and ventricular cardiomyocytes (18, 19, 29).

Cardiomyocyte stretch induces cardiac gene expression via activation of ion channels and activation of paracrine/autocrine mechanisms. A number of signaling pathways have been implicated in the regulation of mechanical load-induced response in ventricular cardiomyocytes, including mitogen-activated protein kinase (MAPK) pathways. MAPKs are divided into three subclasses; extracellular signal-regulated protein kinase (ERK) pathway, JNK pathway, and p38 MAPK pathway. All three MAPK pathways have been shown to be inducible by a variety of stimuli, such as myocyte stretch, ET-1, ANG II, and other G protein-coupled receptor agonists (for review, see Ref. 16). Importantly, key regulators of cardiac gene expression, nuclear factor of activated T cells, GATA-4, and activator protein-1 (AP-1), have been implicated as targets of MAPKs (16, 39).
The aim of the current study was to dissect intracellular signaling mechanisms activated by stretching of isolated rat atria and to study the possible involvement of paracrine or autocrine mechanisms in stretch-induced hypertrophic response. We show that atrial stretch activates p38 MAPK and ERK and transcription factors Elk-1, GATA-4, and AP-1. We also demonstrate that stretch-induced expression of several genes associated with cardiomyocyte hypertrophy is regulated by p38 MAPK. We also find evidence for activation of local ET-1 system and involvement of ET-1 in the regulation of stretch response in isolated rat atria.

MATERIALS AND METHODS

Materials. SB203580 and PD98059 were from Calbiochem (Darmstadt, Germany). Bosentan was generously provided by Actelson (Allschwil, Switzerland). All antibodies used were from Cell Signaling Technology (Hitchin, Hertfordshire, UK).

Isolated perfused atrial preparations. The experimental model used in this study was the isolated rat atrial appendix of male Sprague-Dawley rats weighing 290–400 g (25). The rats were decapitated, and the heart from each rat was rapidly removed and placed in oxygenated (~10°C) buffer solution [in mM: 113.8 NaCl, 28.6 NaHCO3, 2.5 CaCl2, 5.0 HEPES, 1.1 MgCl2, 1.2 KH2PO4, 4.7 L-glutamic acid, 5.0 taurine, 5.0 creatine, 5.0 succinic acid, 5.0 glucose, and insulin (0.06 nM); pH 7.4], which was also used at 37°C for superfusion of the atrium. The left atrial auricle was attached to one of the four ends of a cross-branch polyethylene adapter, and the tissue was placed in a constant temperature (37°C) organ bath. A tube was attached to the opposite end of the cross-branch adapter, and another tube with a smaller diameter was inserted inside to carry the perfusate inflow into the lumen of the atrium. For the outflow, another tube was attached to one of the cross branches of the adapter. The same outflow tube was used to control the intra-atrial pressure by adjusting the height of the end of the tube. The four cross branch of the cross-branch adapter was connected to a pressure transducer (model TCB 100; Millar Instruments) to record the pressure in the lumen of the atrium. Inflow and outflow (2 ml/min) to both the atrial lumen and the organ bath at a constant temperature were controlled by a peristaltic pump (model 7553–85; Cole-Parmer Instrument). To induce atrial stretch, the intra-atrial pressure inside the lumen was elevated from 2 to 6 mmHg at the 30-min time point. Approval for experiments was granted by the Animal Use and Care Committee of the University of Oulu. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Radioimmunoassay. Aliquots of the guanidine thiocyanate homogenates used for RNA extraction (see below) were diluted with radioimmunoassay buffer (at least 100-fold dilution). Atrial natriuretic peptide (ANP) was measured by radioimmunoassay as described previously (48). B-type natriuretic peptide (BNP) was measured from the same extract utilizing an antisera raised in goat against rat BNP22–42. Synthetic Tyr-rat BNP22–42 was used for radioiodination and rat BNP1–45 as calibrator. The BNP antisem does not cross-react with C-type natriuretic peptide, apelin, adrenomedullin (AM), ET-1, or ANG II (<0.01%). Furthermore, unlike the commonly used commercial rat BNP antisem (Peninsula RIN9080), it does not show any detectable cross-reactivity with ANP (<0.01%), making it suitable also for samples with high levels of ANP, such as atria. The sensitivity of both the ANP and BNP assays was 1 fmol per tube, respectively. The within- and between-assay coefficients of variation were <10% and 15%, respectively, in both assays.

Immunoblot analysis. After the perfusion, the atrial preparation was frozen in liquid nitrogen and stored at ~70°C. The tissue was next homogenized into a buffer that consisted of 20 mM Tris (pH 7.5), 10 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, supplemented with 1 mM β-glycerophosphate, 2 mM dithiothreitol, 1 mM Na3VO4, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 2 μg/ml pepstatin, 2 mM benzamidine, 1 mM PMFS, and 50 mM NaF. The samples were cleared of the connective tissue by centrifugation for 1 min at 3,000 g. To extract the nuclear proteins, 0.6% NP-40 was added, the samples were further lysed with sonication, and supernatant was removed after centrifugation. The pellet was resuspended into a buffer consisting of 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA supplemented with 1 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM Na3VO4, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin, 2 mM benzamidine, 1 mM PMFS, 50 mM NaF, 3 μg/ml N-tosyl-L-phenylalanine chloromethyl ketone, and 3 μg/ml N-p-tosyl-L-lysine chloromethyl ketone. For Western blot analysis, samples were matched for protein concentration (20–50 μg), loaded on SDS-PAGE and transferred to nitrocellulose filters. The membranes were blocked in 5% nonfat milk and incubated with primary antibodies overnight at 4°C, and the amount of protein was detected by enhanced chemiluminescence.

EMSA. Nuclear extracts from myocytes were prepared as described previously (36, 41). Protein concentration from each sample was determined by using Bradford assay (4) (Bio-Rad Laboratories). Double-stranded oligonucleotides used for EMSA were as follows: GATA (5′-TGTGTCGTGATATAACCGAGTAGCTGACC-3′), AP-1 (5′-GGACTTTCGAGCTTCACCTTATTTGCATAAGCGATTGA-3′), octamer-1 (5′-AGCTTTCGAGCTTCACCTTATTTGCATAACCGAGTAGCTGACC-3′), or octamer-1 (5′-TCGAGCTTTCGAGCTTCACCTTATTTGCATAACCGAGTAGCTGACC-3′). Probes were sticky end-labeled with [α-32P]dCTP by Klenow enzyme. For each reaction mixture (20 μl) 6–12 μg of nuclear protein and 2 μg of poly(dI-dC) was used in a buffer containing 10 mM HEPES (pH 7.9), 1 mM MgCl2, 50 mM KCl, 1 mM DTT, 1 mM EDTA, 10% glycerol, 0.025% NP-40, 0.25 mM PMFS, and 1 μg/ml of each leupeptin, pepstatin, and aprotinin. Protein phosphatase inhibitors NaF (50 mM) and Na3VO4 (1 mM) were also added to the mixture. Reaction mixtures were incubated with a labeled probe for 20 min followed by non-denaturing
gel electrophoresis on 5% polyacrylamide gel. Subsequently, gels were dried and exposed in a PhosphorImager screen and analyzed with Molecular Imager FX Pro MultiImager System (Bio-Rad Laboratories).

mRNA analysis. Northern blotting and quantitative RT-PCR analysis using TaqMan chemistry were performed as described (29, 30). See Table 1 for sequences of the forward and reverse primers and probes used for real-time PCR analysis (Table 1). Expression levels were normalized to 18S mRNA in each sample.

Statistics. Differences between data groups were evaluated for significance using a Student’s t-test of unpaired data or one-way ANOVA and followed by Bonferroni’s post test. Area under the curve analysis was used to evaluate the significance for changes in contractility. Results are expressed as means ± SE.

RESULTS

Activation of ERKs and p38 by atrial stretch. Adult rat heart was dissected, and the left atrial auricle was attached to the end of a perfusion tube consisting of two lumens. After 30-min perfusion, the auricle was stretched by raising the end of the buffer outflow tube and therefore increasing the pressure inside the auricle. The stretch-induced p38 phosphorylation was increased at 5 min and peaking at 10–15 min (Fig. 1A). After 30-min stretch, the p38 phosphorylation had returned close to basal level. Stretch also induced a time-dependent upregulation in ERK phosphorylation that peaked at 10 min (Fig. 1B). Immunoblot analysis with anti-nonphospho-specific antibodies for p38 and ERK showed that comparable amounts of samples were quantitated in these experiments.

Inhibition of p38 MAPK enhances cardiac contractility in isolated atria. Stretching of the atrial preparate induced a rapid increase in the developed pressure that was identical between the groups during the first 20 s from the onset of the stretch (Fig. 2A). This was followed with a slower increase in con-
traction force, which accounted for the bulk of the total increase in the force. Inhibition of p38 MAPK with SB203580 (5 μM) resulted in a significant augmentation of this slower phase (Fig. 2A). Concomitantly, the maximum + dP/dtmax was significantly higher in the SB203580 treated group (Fig. 2B). Pharmacological attenuation of ERK signaling had no effect on the development of contraction force (data not shown).

**Effect of stretch on cardiac transcription factors.** We next studied possible activation of cardiac transcription factors in isolated rat atria in response to increased pressure. We found that atrial stretch induced a significant increase in the DNA binding activity of Elk-1, GATA-4, and AP-1 (Fig. 3, A–D). Atrial stretch had no effect on DNA binding activity of nuclear factor of activated T cells or serum response factor (data not shown). To investigate the role of p38 MAPK on stretch-induced activation of cardiac transcription factors, we treated the cells with specific p38 inhibitor SB203580. Administration of SB203580 (5 μM) reduced the stretch-induced DNA binding activity of Elk-1 and GATA-4, but had no effect on AP-1 (Fig. 3, A–D). Inhibition of ERK by PD98059 modestly decreased Elk-1 binding activity, but had no effect on GATA-4 or AP-1 (data not shown).

**Effect of MAPK inhibition on markers of cardiac hypertrophy and contractility in isolated rat atria.** Stretching of the atrial prepare for 90 min induced an increase in expression levels of c-fos, a known growth-promoting oncogene (Fig. 4A). Inhibition of p38 by SB203580 (5 μM) resulted in a significant decrease in both basal and stretch-induced c-fos gene expression (Fig. 4A). β-Myosin heavy chain (β-MHC) mRNA levels were elevated by 73% in response to increased stretch (Fig. 4B). Inhibition of p38 by SB203580 (5 μM) resulted in modest but significant decrease in the stretch-induced increase in β-MHC mRNA levels. Interestingly, p38 inhibition resulted in a slight, but nonsignificant augmentation of β-MHC mRNA levels in nonstretched preparates (Fig. 4B). Skeletal muscle α-actin (skαA) mRNA levels were also increased by atrial stretch, but not affected by p38 inhibition with SB203580 (data not shown). An increase in atrial stretch was also sufficient to induce BNP gene expression by 1.9-fold (Fig. 4C). Inhibition of p38 with SB203580 (5 μM) resulted in a significant de-

![Fig. 3. Stretch-induced activation of cardiac transcription factors in isolated rat atria. Atrial preparate was perfused for 30 min without stretch and next stretched (at 6 mmHg) for 15 min. Nuclear protein was extracted as described in MATERIALS AND METHODS, and equal amounts of protein were subjected to gel mobility shift assays and analyzed for DNA binding activity of (A) Elk-1, (B) GATA-4, and activator protein-1 (AP-1) (C). SB203580 (5 μM) was added to the perfusion buffer when appropriate. Representative bands are shown in D. Data are expressed as means ± SE, n = 3–5. *P < 0.05 compared with vehicle; #P < 0.05 compared with vehicle + stretch.](http://ajpregu.physiology.org/)
crease in both basal and stretch-induced BNP gene expression. Induction of the BNP gene expression in the stretched atria was accompanied by a 2.9-fold increase in perfusate ir-BNP levels, which was attenuated by 40% with the inhibition of p38 (not shown). A 90-min stretch was not sufficient to induce ANP gene expression, but ANP secretion was rapidly induced in response to stretch (Fig. 4D). Inhibition of p38 with SB203580 (5 \( \mu \)M) significantly attenuated the stretch-induced ANP secretion (Fig. 4D). Administration of ERK inhibitor PD98059 (5 \( \mu \)M) had no effect on ANP and BNP gene expression or secretion in the stretched atria (data not shown).

**Effect of atrial stretch on autocrine/paracrine factors.** To dissect the possible autocrine or paracrine mechanisms involved in stretch-induced activation of cellular signaling mechanisms in isolated atria, we studied regulation of a number of potential effectors known to regulate myocardial function. We first determined the effect of atrial stretch on ET-1 signaling. A 90-min stretch of atrial preparate significantly increased ET-1 gene expression (Fig. 5A). In contrast, atrial stretch resulted in a significant decrease in endothelin converting enzyme-1 (ECE-1) mRNA levels (Fig. 5B). Inhibition of p38 by SB203580 (5 \( \mu \)M) had no effect on ET-1 gene expression, but ECE-1 mRNA levels were restored to basal levels (Fig. 5, A and B). Atrial stretch also resulted in a 50% decrease in AM gene expression (Fig. 5C). In parallel, AM receptor gene expression was reduced by 60% (Fig. 5D). We then analyzed apelin mRNA levels, which was not affected by stretching of the isolated atria for 90 min (data not shown). In contrast, expression of apelin receptor was significantly attenuated in response to atrial stretch (Fig. 5E). Administration of p38 MAPK inhibitor SB203580 (5 \( \mu \)M) had no significant effect on AM, AM receptor, apelin, or apelin receptor mRNA levels (Fig. 5, C–E).

**ET-1 system is central for stretch response in rat atria.** We next compared the expression levels of members of ET-1 signaling system between the atrial and ventricular myocar-

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**Fig. 4.** p38 regulates stretch-induced expression of hypertrophic markers in isolated rat atria. Atrial preparate was subjected to stretch for 90 min with either vehicle or p38 inhibitor SB203580 (5 \( \mu \)M). Total RNA was extracted and analyzed by Northern blotting. Shown is densitometric analysis of c-fos (A), \( \beta \)-myosin heavy chain (\( \beta \)-MHC) (B), or B-type natriuretic peptide (BNP) (C) mRNA levels as ratio to 18S mRNA levels. Data is expressed as fold vs. control. D: analysis of atrial natriuretic peptide (ANP) secretion from atrial preparate. IR, immunoreactive. Shown are vehicle (○), vehicle + stretch (●), and SB203580 + stretch (■). Data are expressed as means ± SE from 4 separate experiments. *\( P < 0.05 \) compared with vehicle; **\( P < 0.01 \) compared with vehicle; #\( P < 0.05 \) compared with vehicle + stretch.
We found that the expression level of the ETA receptor was increased to almost fourfold in left atrial tissue compared with left ventricular tissue (Fig. 6A). mRNA levels of the ETB receptor were too low for reliable quantification. There was no difference in ET-1 gene expression in atrial myocardium compared with ventricular myocardium, whereas ECE-1 mRNA levels were significantly higher in left atria compared with left ventricle (Fig. 6, B and C). ANG II is known to play a central role in pathologic remodeling of both atrial and ventricular myocardium (8, 40). We found that gene expression levels of ANG II receptor type-1 (AT1) or type-2 (AT2) did not differ between atrial and ventricular myocardium (Fig. 6, D and E). Finally, we investigated the functional role of ET-1 and ANG II in regulation of transcription factors in isolated rat atria. Atrial stretch substantially increased GATA-4 DNA binding activity, which was significantly attenuated by administration of bosentan (1 μM) (Fig. 6F). In contrast, administration of CV-11974 (10 nM) had no effect on stretch-induced

Fig. 5. Effect of atrial stretch and p38 inhibition on autocrine/paracrine factors. Atrial preparate was subjected to stretch for 90 min with either vehicle or p38 inhibitor SB203580 (5 μM). Total RNA was extracted and analyzed with RT-PCR for expression of endothelin-1 (ET-1) (A), endothelin converting enzyme-1 (ECE-1) (B), adrenomedullin (AM) (C), AM receptor (AMR) (D), and apelin receptor (E). Values represent the means ± SE from 4 separate experiments. *P < 0.05 compared with vehicle; #P < 0.05 compared with vehicle + stretch.
activation of GATA-4 (Fig. 6G). Stretch-induced activation of Elk-1 or AP-1 were not affected by administration of bosentan or CV-11974 (data not shown).

DISCUSSION

We sought to investigate the mechanisms regulating the activation of signaling pathways and gene expression in response to stretch in atrial myocardium. Previously, ANG II has been shown to exert a positive inotropic effect in human atria but not in the ventricle (18). In addition, there is evidence from in vivo and in vitro studies suggesting that the stretch-induced early activation of BNP gene expression in rat atrial myocytes, but not in the ventricular myocytes, is mediated via an ET-1 dependent mechanism (29, 47). In cardiac myocytes, ET-1 acts...
through binding to ETₐ receptor on cell surface leading to activation of multiple intracellular signaling pathways, such as Ras, protein kinase C, MAPK cascades, and calcineurin and an increase in the levels of intracellular calcium (33, 43). Equally, hemodynamic stress has been shown to induce activation of MAPK cascades and their downstream effectors in cardiac myocytes (16). In the present study, atrial stretch provoked a rapid phosphorylation of both ERK and p38. Atrial stretch also induced activation of Elk-1, a known substrate for MAPKs, as well as transcription factors GATA-4 and AP-1. The stretch-induced activation of Elk-1 was regulated by p38 and, to a lesser extent, also by ERK. However, only p38 inhibition affected the stretch-induced activation of GATA-4 in isolated rat atria. Studies with neonatal rat ventricular cardiomyocytes in various in vitro models suggest that both ERK and p38 MAPK are involved in regulation of Elk-1 as well as GATA-4 (10, 23, 26, 37, 38).

p38 MAPK and atrial phenotype. We next examined how inhibition of p38 MAPK affects the phenotype provoked by atrial stretch. We found that stretch-induced increase in cardiac contractility in isolated rat atria was enhanced by inhibition of p38 MAPK. There is already convincing data indicating a negative role for the p38 MAPK mediating the inotropic effect in ventricular cardiomyocytes both in vivo and in vitro (27, 28, 44) (for review, see Ref. 22). Interestingly, we discovered that stretch-induced decrease in ECE-1 mRNA levels was restored by p38 MAPK inhibition in isolated atria, which, with unaffected ET-1 production, would lead to further enhancement of ET-1-induced contractility. However, this does not explain the acute effect that p38 inhibition had on contractile function in our study.

Hemodynamic load-induced activation of the intracellular signaling pathways is known to result in increased activity of several nuclear effectors leading to transcriptional activation of a pattern of genes including structural proteins, such as skα1 and β-MHC, and the natriuretic peptides ANP and BNP (39). Previously, Elk-1 together with serum response factor (SRF) has been shown to regulate c-fos gene expression (1). Minimal c-fos promoter with a point mutation at the p62TCF/Elk-1 binding site of the serum response element (SRE) fails to respond to pressure overload (1). Nearly identical Elk-1 and p62TCF share the same DNA sequence requirements for DNA binding (17). In addition to synergistic mechanism with SRF, ETS domain transcription factors have also been shown to act without adjacent SRE in the promoter to regulate cardiac gene expression (14). In the present study, atrial stretch provoked an increase in c-fos, β-MHC, and BNP mRNA levels. Inhibition of p38 MAPK decreased the stretch-induced Elk-1 activity and was sufficient to suppress both the basal and stretch-induced increase in c-fos and BNP mRNA levels, and stretch-induced increase in β-MHC mRNA levels. Interestingly, p38 inhibition had no effect on either basal or stretch-induced skα1 mRNA levels, which could be attributable to the fact that the SRE-1 in the skα1 promoter lacks the flanking ETS-domain sequence (42).

Autocrine/paracrine mechanisms in rat atria. We next investigated the activation of autocrine/paracrine mechanisms in the isolated atria in response to stretch. AM is a vasorelaxant peptide with favorable hemodynamic effects in vivo. Prior studies suggest that AM exerts potent inotropic effect in the atria as well as in the ventricle (2, 45). We found that atrial stretch led to a significant decrease in both AM and AM receptor mRNA levels, suggesting a substantial downregulation of local AM system. Similarly, we found that apelin signaling was suppressed in the isolated atria in response to mechanical stretch. Apelin is a potent stimulator of cardiac contractility that exerts its effects via apelin receptor (APJ orphan receptor) in the heart (46). It is actually plausible that the observed downregulation of local AM and apelin systems in response to atrial stretch may oppose the stretch-induced increase in atrial contractility. ET-1, the principal peptide of the ET family, is strongly expressed in the vascular endothelium and also in cardiac myocytes in vivo (50). ET-1 is initially synthesized as an inactive prepropeptide that is activated by posttranslational processing to produce the mature peptide. The final step in this proteolytic processing is catalyzed by ECE-1. ET-1 activates a number of downstream signaling cascades through binding to two distinct G protein-coupled receptors, ET₁ and ET₂, to exert their biological effects (21). In the heart, ET-1 acts as a chronotropic and inotropic agent, vasoconstricting arteries and inducing myocardial cell hypertrophy (11, 24). Mixed ET₁/ET₂ receptor antagonist bosentan is in clinical use for the treatment of pulmonary artery hypertension. In patients, bosentan treatment not only induces vasodilatation and decreases pulmonary vascular resistance, but also lowers the mean right atrial pressure (3, 9). We found that the mechanical stretch induced an increase in ET-1 gene expression, which suggests that the effects of atrial stretch were, in
part, mediated by ET-1. In contrast, the ECE-1 gene expression was inversely regulated, which could counter the effects of increased ET-1 expression. ET-1 mRNA and peptide levels are elevated in failing human myocardium, and ET-1 mRNA levels are also elevated in right atrial samples from patients with valvular diseases (6, 34, 35). Interestingly, patients with end-stage heart failure and failing atrial myocardium were previously found to have increased ECE-1 gene expression levels in the myocardium (34), but there are no previous reports of the effect of acute pressure overload on ECE-1 mRNA levels. Our data here suggests that pharmacologic inhibition of p38 MAPK in stretched adult atria may be sufficient to restore the reduced ECE-1 mRNA levels, thereby enhancing the processing of ET-1 to a mature peptide.

Finally, we investigated the significance of ET-1 signaling system in atrial tissue compared with ventricular tissue. We found that mRNA levels of ETA receptor and ECE-1 were significantly higher in atrial tissue compared with ventricular tissue, while there was no difference in the of AT1 or AT2 receptor mRNA levels. These results are in agreement with previous data from radioligand binding assays, which also suggest that the proportion of ETA and ETB receptors is higher in atrial compared with ventricular myocardium (32). Finally, we analyzed the functional difference between the ET-1 and ANG II signaling systems in isolated rat atria. In the isolated left ventricle, the stretch-induced binding activity of both GATA-4 and AP-1 are suppressed by administration of ET-1 inhibitor bosentan and, importantly, also by AT1 antagonist CV-11974 (15). We found that administration of AT1 receptor antagonist CV-11974 had no effect on transcription factors studied in isolated left atria. In contrast, the stretch-induced GATA-4 DNA binding activity was substantially reduced by administration of mixed ETA/B receptor antagonist bosentan. Our findings suggest that ET-1 plays a central role in the regulation of atrial stretch response. In addition, members of the ET-1 signaling system are expressed in higher levels in atrial compared with ventricular myocardium, which may be relevant in cardiovascular conditions that increase the atrial load.

Perspectives and Significance

Studies investigating the signaling mechanisms activated by stretch and involved in pathologic processes in the ventricular myocardium have been in the focus of interest for the past several years. It is evident, however, that maladaptation of atrial tissue plays a role in pathogenesis of certain diseases, such as rhythm disorders. Normal atrial tissue has higher fibroblast density than the ventricular tissue, and the atrial fibroblast proliferation rate in response to growth factors is greater than that of ventricular fibroblasts (7). This would imply that the autocrine/paracrine mechanisms activated by wall stretch may play a more central role in atrial compared with ventricular tissue. Cardiac fibroblasts respond to growth factors, such as ET-1, and are actually an important source of ET-1 in the heart. There is evidence, in fact, that hypertrophic response to ANG II in isolated cardiomyocytes is actually mediated by local production of ET-1 (20). In the present study, we show that p38 MAPK and the ET-1 system are key mediators in the stretch-induced hypertrophic response in isolated rat atrium. We find that p38 MAPK regulates stretch-induced activation of cardiac transcription factors and, similar to findings in ventricular cardiomyocytes, augments the stretch-induced atrial contractile function. Our studies also identify GATA-4 as a promising target for stretch-induced ET-1/p38 MAPK signaling. A recent study by Glenn et al. (12) found that GATA-4 is actually an important mediator of ET-1 production in cultured cardiac fibroblasts. Our findings with stretched isolated rat atrium containing both cardiomyocytes and fibroblasts suggest, however, that inhibition of p38 and consequent depression of GATA-4 DNA binding are not sufficient to attenuate ET-1 gene expression. The importance for understanding the role of ET-1 in atrial myocardium is emphasized by the fact that there are a number of novel compounds in development targeting ET-1 signaling (24). Our data suggests that the effect of endothelin antagonists on the development of atrial hypertrophy, a known risk factor for AF, should be further investigated.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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