Chronic \(\beta_2\)-adrenergic receptor stimulation increases proliferation of human cardiac fibroblasts via an autocrine mechanism

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Abstract

**Objective:** The aim of this study was to determine whether chronic \(\beta\)-adrenergic receptor (\(\beta\)-AR) stimulation induces proliferation of human cardiac fibroblasts and to investigate the mechanism(s) involved. **Methods and results:** In vitro cultures of human cardiac fibroblasts were established from biopsies of right atrial appendage. RT-PCR analysis and pharmacological studies demonstrated that these cells express predominantly the \(\beta_2\)-AR subtype coupled to activation of adenylyl cyclase and p44/42 mitogen-activated protein kinase (MAPK). Proliferation was determined by cell counting over a 6-day period in medium containing 2.5% fetal calf serum (control) or supplemented with the non-selective \(\beta\)-AR agonist isoproterenol (ISO). ISO induced a concentration-dependent increase in cardiac fibroblast proliferation, which was maximal at 1 \(\mu\)mol/l. This increased proliferation was inhibited by the \(\beta_2\)-AR-selective antagonist2 ICI-118,551, but not the \(\beta_1\)-AR-selective antagonist atenolol. Direct activation of adenylyl cyclase alone (0.1–10 \(\mu\)mol/l forskolin) stimulated cyclic AMP production and MAPK activation, but did not induce cell proliferation. Since catecholamines are not considered to be ‘classical’ growth factors, we subsequently investigated whether \(\beta_2\)-AR stimulation results in secretion of growth factors that are able to stimulate proliferation in an autocrine manner. Conditioned medium obtained from cardiac fibroblasts treated with ISO for 48 h increased proliferation of parallel cultures of fibroblasts in the presence of the \(\beta_2\)-AR antagonist alprenolol. Heat-treatment of this conditioned medium fully prevented the increase in cell proliferation, indicating that the autocrine factor(s) are heat-sensitive proteins. **Conclusions:** Chronic \(\beta_2\)-AR stimulation increases proliferation of human cardiac fibroblasts via a mechanism involving increased secretion of heat-sensitive growth factors.

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**Keywords:** Adrenergic (ant)agonists; Cell culture/isolation; Growth factors; Heart failure; Remodeling

1. Introduction

In heart failure patients, myocardial sympathetic nervous activity is increased to compensate for loss of cardiac function [1,2]. However, prolonged increases in adrenergic drive are ultimately damaging to the heart. A number of large clinical trials have demonstrated a remarkable benefit of \(\beta\)-adrenergic receptor (\(\beta\)-AR) antagonists (\(\beta\)-blockers) in reducing the mortality associated with heart failure [3].

The mechanisms that underlie the detrimental effects of chronic \(\beta\)-AR stimulation in heart failure are therefore of immense interest and clinical relevance.

The adult heart consists of both myocytes and non-myocytes (fibroblasts, endothelial cells and smooth muscle cells). The vast majority of non-myocytes are cardiac fibroblasts, which account for up to two-thirds of the total cells in the heart. Cardiac fibroblasts are responsible for regulating the synthesis and degradation of extracellular matrix (ECM) components in the cardiac interstitium, thereby maintaining the structural integrity of the heart. In the adult, myocardial remodeling is regulated primarily by

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hormones and growth factors acting on cardiac fibroblasts, resulting in alteration of ECM metabolism and increased cardiac fibroblast proliferation [4,5].

To date, most studies on the adverse consequences of chronic adrenergic stimulation have focused on the effects of catecholamines on cardiac myocytes. Catecholamines are toxic to myocytes and cause cell death and/or hypertrophy [5–7], ultimately contributing to the loss of cardiac function associated with heart failure. A key benefit of β-AR blockade in heart failure patients appears to be at the level of adverse myocardial remodeling which is reduced, or even reversed, in patients receiving β-blockers [3]. Despite the key role of cardiac fibroblasts in regulating myocardial ECM metabolism, relatively little is known about the chronic effects of β-AR stimulation on cardiac fibroblast function.

We therefore sought to determine whether an additional mechanism underlying the detrimental effects of chronic β-AR stimulation in heart failure patients is that β-AR stimulation increases proliferation of human cardiac fibroblasts, thus contributing to adverse myocardial remodeling.

2. Methods

2.1. Materials

All cell culture reagents were purchased from Invitrogen (Paisley, UK), except fetal calf serum (FCS) that was from Helena Biosciences (Sunderland, UK). Unless stated otherwise, all additional chemicals were obtained from Sigma–Aldrich (Poole, Dorset, UK).

2.2. Culture of human cardiac fibroblasts

Biopsies of right atrial appendage were taken from patients without left ventricular dysfunction undergoing coronary artery bypass surgery. Local ethical committee approval and informed patient consent were obtained. Heart tissue was minced and digested by incubation with 800U/ml collagenase type II solution (Worthington Biochemical Corporation, Lakewood, NJ, USA) in Dulbecco’s modified Eagle medium (DMEM) containing 0.05% bovine serum albumin (BSA) for 4 h at 37°C. Cells were pelleted by centrifugation, washed with DMEM/BSA, and resuspended in full growth medium (DMEM supplemented with 10% FCS, 2 mmol/l -glutamine, 100 µg/ml penicillin G, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin). Cells were plated into cell culture flasks for 30 min to allow fibroblasts to adhere. Following removal of non-adherent cells, fibroblasts were cultured to confluence in fresh full growth medium in a humidified atmosphere of 5% CO2 in air at 37°C, and subsequently passaged by trypsinization. Experiments were performed on early passage cells (2–5) from up to 15 different patients.

2.3. Immunocytochemistry

Fibroblasts grown on glass coverslips were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 prior to incubation with goat anti-vimentin primary antibody (Chemicon International, Harrow, UK) at 4°C overnight. After incubation with FITC-conjugated anti-goat secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at room temperature for 4 h in the dark, coverslips were mounted and cells visualised using a Zeiss LSM-510 confocal microscope.

2.4. Determination of β-AR subtype expression using RT-PCR

Confluent 75-cm2 flasks of cardiac fibroblasts were incubated in serum-free medium (SFM) for 48 h prior to RNA extraction using the SV Total RNA Isolation System (Promega, Southampton, UK). Control human genomic DNA was extracted from whole blood using the PureGene Genomic DNA Purification Kit (Gentra Systems, Minneapolis, MN, USA), according to the manufacturer’s instructions. RT-PCR was performed on 50 ng RNA or DNA using the Access RT-PCR System (Promega), according to the manufacturer’s instructions. PCR primers were synthesized by Invitrogen with the following sequences: 5’-ATGCACTGGTGCGGGCGAGAGC-3’ (β1-AR forward), 5’-AAGGCCAGCACGAGCCGCGG-3’ (β2-AR reverse), 5’-AAGCCATGCGCGGAGACAC-3’ (β2-AR forward), 5’-ATGATCACCGGG-3’ (β2-AR reverse). The RT-PCR conditions were 48°C (45 min) followed by 94°C (2 min) and then 40 cycles of 94°C (30 s), 66°C (1 min) and 72°C (2 min) with a final extension phase of 72°C (7 min). PCR products were resolved by 1.5% agarose gel electrophoresis and visualized under UV illumination following ethidium bromide staining. The predicted sizes of the PCR products were 434, 414 and 240 bp for β1-AR, β2-AR and GAPDH, respectively.

2.5. Measurement of intracellular cAMP production

Fibroblasts were incubated in SFM for 24 h and then loaded with [3H]adenine before measuring intracellular cAMP accumulation over a 12-min period in the presence of 0.5 mmol/l IBMX, as we have described previously [8].

2.6. Measurement of MAPK activation

Fibroblasts were incubated in SFM for 24 h before incubation with appropriate drugs for 5 min at 37°C in SFM. Mitogen-activated protein kinase (MAPK) activation was determined by immunoblotting whole cell homogenates with a phospho-specific p44/42 MAPK antibody.
Cardiac fibroblasts were seeded into 24-well plates (10,000 cells per well) in 1 ml of full growth medium. After incubation overnight, cells were growth-arrested in SFM for 48 h. SFM was then replaced with 1 ml of minimal growth medium (medium containing 2.5% FCS) containing the appropriate drugs. This time point was designated ‘Day 0’. Medium and drugs were replaced every 2 days throughout the experiment and cell number was determined in duplicate by trypsinizing and counting viable cells using Trypan Blue and a hemocytometer. In order to demonstrate that the responses to isoproterenol (ISO) were not attributable to non-specific passage effects, experiments were performed on cells from different passages. Similar proliferative responses to ISO were observed using cells from passages 2–5 inclusive (data not shown).

2.8. Preparation of conditioned medium

In order to investigate whether β-AR stimulation could induce cell proliferation in an autocrine manner, we prepared conditioned medium from fibroblasts exposed to β-AR stimulation, then studied its effects on cell proliferation in the following way. ‘Feeder’ flasks were prepared by seeding fibroblasts into 25-cm² flasks (100,000 cells per flask) in 10 ml of full growth medium. After incubation overnight, growth medium was replaced with SFM for 48 h. SFM was then exchanged for 10 ml of minimal growth medium to produce control conditioned medium (C-CM), or minimal growth medium supplemented with 1 µmol/l ISO to produce ISO-CM. After 48 h, CM was collected and centrifuged to remove cellular debris. After addition of alprenolol (1 µmol/l), with or without FCS (2.5%), this CM was added every 2 days to parallel cultures of fibroblasts previously prepared for proliferation assays, as described above. For heat inactivation experiments, an aliquot of CM was heated to 80°C for 20 min, then cooled to below 37°C before addition of FCS and alprenolol. For dilution experiments, C-CM and ISO-CM were diluted 1:5 or 1:10 in SFM before addition of FCS. The feeder flasks were replenished with fresh minimal growth medium (with or without ISO) every 2 days throughout the experiment for the continued production of CM.

2.9. Data analysis

Data analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). All data are expressed as mean values±S.E.M. EC₅₀ and IC₅₀ values were determined using a non-linear regression curve-fitting program. Proliferation curves were compared by calculating areas under curves and performing one-way analysis of variance (ANOVA) and two-tailed paired t-tests. All other data were analysed as appropriate using two-tailed paired t-tests. P<0.05 was considered statistically significant.

3. Results

3.1. Human cardiac fibroblasts express the β₂-AR subtype coupled to cAMP production and MAPK activation

Immunocytochemical staining with anti-vimentin antibody confirmed that >98% of cells cultured from biopsies of human right atrial appendage were fibroblasts (Fig. 1). Analysis of mRNA expression using RT-PCR revealed that these cells express the β₂-AR, but not the β₁-AR (Fig. 2, lane 2). As a positive control, we detected expression of both β-AR subtypes in a human genomic DNA sample (Fig. 2, lane 1). No PCR products were detected in the

Fig. 1. Immunofluorescent detection of vimentin in human cardiac fibroblasts obtained from right atrial appendage. Cells were incubated with anti-vimentin antibody and FITC-conjugated secondary antibody. (A) Phase-contrast image of cultured cells. (B) Immunofluorescent image of the same field of cells. (C) Phase-contrast image overlaid with the immunofluorescent image. Scale bar represents 200 µm.
fibroblast RNA samples in the absence of reverse transcriptase (Fig. 2, lane 3), confirming that the samples were not contaminated with genomic DNA. RNA integrity was confirmed by amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fig. 2).

The function of the receptors was demonstrated by performing cAMP assays with the non-selective β-AR agonist epinephrine, and the more β₁-AR-selective agonist norepinephrine (Fig. 3A). Both agonists stimulated cAMP accumulation in a concentration-dependent manner, with epinephrine (EC₅₀=2.4±0.2 μmol/l) being more potent than norepinephrine (EC₅₀=12.7±2.4 μmol/l) (P = 0.008).

In order to confirm the relative contribution of β-AR subtypes to cAMP production, we studied the effects of selective β-AR antagonists on cAMP accumulation stimulated by the non-selective β-AR agonist ISO. One μmol/l ISO induced an increase in cAMP production that was inhibited in a concentration-dependent manner by the non-selective β-AR antagonist alprenolol (CN Biosciences, Nottingham, UK) and the β₁-AR-selective antagonist ICI-118,551 (Tocris, Bristol, UK), with IC₅₀ values of 6.1±2.5 and 6.3±1.0 nmol/l, respectively (Fig. 3B). The β₁-AR selective antagonist atenolol (CN Biosciences) did not inhibit cAMP production at concentrations up to 100 nmol/l (Fig. 3B).

ISO also stimulated activation of p44/42 MAPK in cultured human cardiac fibroblasts in a concentration-dependent manner (EC₅₀=2.5±0.8 nmol/l), as determined by immunoblotting whole cell homogenates with a phospho-specific MAPK antibody (Fig. 4A). ISO-induced MAPK activation was inhibited by co-treatment with the β₁-AR-selective antagonist ICI-118,551, but not the β₁-AR selective antagonist atenolol (Fig. 4B).

3.2. β₁-AR stimulation increases human cardiac fibroblast proliferation

To determine whether β₁-AR stimulation could increase cell proliferation we exposed fibroblasts to different concentrations of ISO in minimal growth medium (2.5% FCS) for up to 6 days and measured changes in cell numbers (Fig. 5A). Fibroblasts grown in minimal growth medium alone (control) underwent a low level of proliferation that resulted in a 50.5±19.8% increase in cell number over a 6-day period. No further increase in proliferation was observed when fibroblasts were treated with 0.1 nmol/l ISO over the same period (P = 0.952). However, cardiac fibroblasts incubated in the presence of 10 nmol/l or 1
ISO activates MAPK in human cardiac fibroblasts via stimulation of the β₁-AR. p44/42 MAPK activation was determined by immunoblotting whole cell homogenates with a phospho-MAPK antibody. MAPK phosphorylation was quantified by densitometric analysis of autoradiographs. (A) Cells were incubated with increasing concentrations of ISO for 5 min. Upper blot: MAPK activation (phospho-MAPK antibody). Lower blot: MAPK expression (MAPK antibody). Data are expressed as the mean percentage ±S.E.M. of the maximum response (n=5). (B) Cells were incubated in the absence (control, C) or presence of 1 μmol/l ISO for 5 min, with or without 100 nmol/l atenolol (ATE) or 1 μmol/l ICI-118,551 (ICI), before measuring MAPK activation. Data are expressed as the mean percentage ±S.E.M of the response to ISO (n=4).

μmol/l ISO showed an enhanced rate of proliferation (P=0.108, P=0.029, respectively) (Fig. 5A). This resulted in an increase in cell number of 67.6±15.7 and 109.7±15.3%, respectively, after 6 days. In comparison, full growth medium (10% FCS) induced a 193.2±52.6% increase in cell number compared with control after 6 days (n=5; data not shown). The effects of ISO on cell proliferation were confirmed to be mediated via the β₁-AR, as the proliferative response was inhibited by ICI-118,551, but not atenolol (Fig. 5B).

Many physiological responses to β-AR stimulation are mediated via activation of adenyl cyclase [10]. We therefore determined the effects of forskolin, a direct activator of adenyl cyclase, on human cardiac fibroblast proliferation. Although forskolin stimulated cAMP production (Fig. 6A) and MAPK activation (Fig. 6B) in a concentration-dependent manner, it failed to stimulate fibroblast proliferation (Fig. 6C).

3.3. β-AR stimulation increases cardiac fibroblast proliferation via an autocrine mechanism

Although ISO increased cardiac fibroblast proliferation over a 6-day period, the effect was not apparent until 3–4 days after the initial exposure to ISO (Fig. 5A). This delayed proliferative response suggested that the mechanism by which ISO enhanced proliferation was indirect and possibly due to secretion of growth factors that subsequently increased proliferation in an autocrine manner. To test this hypothesis, we prepared conditioned media by treating cardiac fibroblasts with either minimal growth medium alone (C-CM) or supplemented with 1 μmol/l ISO (ISO-CM) for 48 h. We then investigated whether these CM could increase proliferation in parallel cultures of fibroblasts. We had previously observed that the ISO-induced increase in cell proliferation was dependent on the presence of FCS (data not shown). We therefore supplemented both C-CM and ISO-CM with fresh 2.5% FCS to replace any serum-derived factors that may have been depleted by the fibroblasts in the feeder flasks during the 2-day production of CM. Alprenolol (1 μmol/l) was also added to the CM to negate any growth-promoting effects of ISO-CM that were due to residual ISO.

As shown in Fig. 7A (columns 3 and 4), treatment of cardiac fibroblasts with ISO-CM for 6 days increased the cell number by 60±5% over that observed with C-CM. This compared with a 72±15% increase induced by ISO in control cells (Fig. 7A, columns 1 and 2). C-CM increased cell number by 60±18% over untreated control cells (Fig. 7A, columns 1 and 3) due to the additional 2.5% FCS.
This effect was abolished if the additional 2.5% FCS was omitted (Fig. 7A, column 5). Furthermore, the ability of ISO-CM to increase cell proliferation was also prevented in the absence of fresh 2.5% FCS (Fig. 7A, column 6). Heating the CM to 80°C prior to addition of 2.5% FCS and alprenolol completely

Fig. 5. ISO enhances human cardiac fibroblast proliferation via stimulation of the β₂-AR. (A) Cells were exposed to minimal growth medium alone (C) or supplemented with 0.1 nmol/l (●), 10 nmol/l (▲) or 1 μmol/l (▼) ISO. Data are expressed as the mean percentage ±S.E.M. of cell number observed on Day 0 (n=4). P<0.001 (ANOVA). (B) Cells were exposed to minimal growth medium alone (control, C) or supplemented with 1 μmol/l ISO for 4 days in the presence or absence of 100 nmol/l atenolol (ATE) or 1 μmol/l ICI-118,551 (ICI). Data are expressed as the mean percentage ±S.E.M. of control cell number on Day 4 (n=4).

Fig. 6. Forskolin increases intracellular cAMP levels and activates MAPK, but does not stimulate proliferation of human cardiac fibroblasts. (A) cAMP accumulation in response to increasing concentrations of forskolin. Data, from which basal cAMP levels have been subtracted, are expressed as the mean percentage ±S.E.M. of the response to 1 μmol/l ISO. (B) MAPK activation in response to forskolin was determined by immunoblotting whole cell homogenates with a phospho-MAPK antibody. MAPK phosphorylation was quantified by densitometric analysis of autoradiographs. Data are expressed as the mean percentage ±S.E.M. of the response to 100 nmol/l FSK (n=4). (C) Cell proliferation in response to forskolin. Cells were exposed to minimal growth medium alone (control, C), or supplemented with 1 μmol/l ISO or forskolin at the indicated concentrations for 6 days. Data are expressed as the mean percentage ±S.E.M. of control cell number on Day 6 (n=3). P values represent comparisons between control and drug-treated groups.
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b-AR subtype that is expressed by human cardiac fibroblasts. Firstly, RT-PCR demonstrated that these cells express the β2-AR, but not the β1-AR (Fig. 2). Secondly, the preferential stimulation of cAMP production by epinephrine compared with norepinephrine (Fig. 3A) is indicative of a β2-AR-mediated response. Thirdly, the ability of both alprenolol and ICI-118,551, but not atenolol, to abolish ISO-induced cAMP production (Fig. 3B) and MAPK activation (Fig. 4B) further supports a major role for the β2-AR in these cells.

Using radioligand-binding studies, it has been previously observed that the β2-AR accounts for 30–40% of total β-AR expression in human atrium and 20–30% in human ventricle, with the remainder being attributed to the β1-AR [10]. However, the expression of β-AR subtypes by cardiac myocytes and fibroblasts may well differ. Functional evidence exists for the co-expression of β-AR and β1-AR subtypes in human cardiac myocytes [11,12]. However, our present observations in human, and those previously in rat [13–15], support the conclusion that cultured cardiac fibroblasts express predominantly the β2-AR subtype.

The present study provides the first report of a proliferative effect of catecholamines on human cardiac fibroblasts. Previous animal studies have shown that catecholamines stimulate DNA synthesis via the β-AR in adult rabbit and neonatal rat cardiac fibroblasts [13,16–18]. However, the identity of the β-AR subtypes involved, and whether increased DNA synthesis translated into increased cell number, were not investigated in any of these studies. Our data take these interesting observations an important stage further and clearly demonstrate that chronic β-AR stimulation increases proliferation of human cardiac fibroblasts, as measured by increases in cell number. ISO did not induce proliferation in SFM, indicating that ISO stimulates cardiac fibroblast proliferation via an autocrine mechanism.

Fig. 7. ISO stimulates cardiac fibroblast proliferation via an autocrine mechanism. (A) Cells were exposed to minimal growth medium alone (control, C), minimal growth medium supplemented with 1 μmol/l ISO (ISO), control conditioned medium (C-CM) or 1 μmol/l ISO-conditioned medium (ISO-CM) for 6 days. CM was supplemented with alprenolol (1 μmol/l), with or without fresh 2.5% FCS as indicated. For heat inactivation experiments, CM was heated to 80 °C, cooled and alprenolol and 2.5% FCS added. Data are expressed as the mean percentage ±S.E.M. of control cell number on Day 6 (n=3–6). (B) For dilution experiments, C-CM and ISO-CM were either undiluted or diluted 1:5 or 1:10 before addition of FCS. Cells were exposed to each medium for 6 days before counting cells. Data are expressed as the mean percentage ±S.E.M. of the cell number observed in response to undiluted C-CM (n=4).

4. Discussion

The present study is the first to identify the predominant β-AR subtype that is expressed by human cardiac fibroblasts. Firstly, RT-PCR demonstrated that these cells express the β2-AR, but not the β1-AR (Fig. 2). Secondly, the preferential stimulation of cAMP production by epinephrine compared with norepinephrine (Fig. 3A) is indicative of a β2-AR-mediated response. Thirdly, the ability of both alprenolol and ICI-118,551, but not atenolol, to abolish ISO-induced cAMP production (Fig. 3B) and MAPK activation (Fig. 4B) further supports a major role for the β2-AR in these cells.

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The mechanism of β2-AR-induced cardiac fibroblast proliferation in man differs from that reported for rat. A recent study demonstrated that adult rat cardiac fibroblasts proliferate in response to catecholamines via stimulation of the β2-AR [19]. However, in these rat cells, direct stimulation of intracellular cAMP production by forskolin (0.1–20 μmol/l) also enhanced cell proliferation to a similar level to that observed with ISO, without activating MAPK. This directly contrasts with our finding in human cardiac fibroblasts, where a range of concentrations of forskolin (1 nmol/l–10 μmol/l) did not increase cell proliferation even though intracellular cAMP levels were elevated and MAPK was activated (Fig. 6).

Our results with forskolin indicate that cAMP generation, together with subsequent MAPK activation, do not provide a sufficient stimulus for proliferation of human cardiac fibroblasts. Indeed, at higher concentrations of FSK there was a trend towards reduced proliferation (Fig. 6C),
although this was not statistically significant (control versus 10 μmol/l FSK, P = 0.099). In rat cardiac fibroblasts, a number of previous studies have demonstrated a growth-inhibitory effect of cAMP [20–22], although a stimulatory effect has also been reported [19]. The differential effects of ISO and FSK on the increase in cell proliferation may be due to differences in the size and duration of the cAMP and MAPK signals generated by these two agents. Alternatively, additional signal transduction pathways may be responsible for the proliferative effect of ISO. For example, the phosphatidylinositol-3-kinase pathway is activated following β2-AR stimulation in rat cardiac fibroblasts [23], and was shown very recently to be necessary for β-AR-mediated DNA synthesis in adult rat cardiac fibroblasts [24]. The phosphatidylinositol-3-kinase pathway is also required for platelet-derived growth factor (PDGF)-induced DNA synthesis in human cardiac fibroblasts [25].

The proliferative effect of ISO on human cardiac fibroblasts that we observed was not apparent until 3–4 days after the initial exposure to ISO (Fig. 5A). This is clearly different from some other reports in animal cardiac fibroblasts where marked DNA synthesis was observed within 24 h of β-AR stimulation [16–18] and significant cell proliferation within 48 h [19]. However, one report in keeping with our findings in human cells showed that DNA synthesis was not apparent until 72 h after initial exposure to ISO in neonatal rat cardiac fibroblasts [13]. Another recent study reported that treatment with norepinephrine for <48 h inhibited rat cardiac fibroblast cell proliferation, but longer treatments (48–96 h) resulted in increased cell proliferation [22].

The delayed proliferative response we observed suggested that the stimulatory effect of ISO on cell growth was indirect. We hypothesized that this was due to increased secretion of one or more factors capable of inducing cell proliferation in an autocrine manner. We therefore collected conditioned medium from cardiac fibroblasts treated with ISO for 48 h (ISO-CM) and showed that it caused a significant increase in fibroblast proliferation in parallel cultures in the presence of the β-AR antagonist alprenolol (Fig. 7A). Heating the ISO-CM to 80°C completely abolished the proliferative response, suggesting that the secreted autocrine factor is a bioactive protein or peptide, rather than a chemical mediator.

Cardiac fibroblasts have the ability to secrete a variety of different growth factors including PDGF, basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α, angiotensin II and endothelin-1 [26–29], all of which can induce cardiac fibroblast growth [4]. Importantly, catecholamines have been shown to increase synthesis of angiotensinogen (a precursor of angiotensin II) and TGF-β in neonatal rat cardiac fibroblasts [13,30,31]. ISO has also been shown to induce angiotensinogen, TGF-β, PDGF and bFGF mRNA production in cultured rat vascular smooth muscle cells [32].

In the present study, we report for the first time that the predominant β-AR subtype expressed by human cardiac fibroblasts is the β2-AR. Stimulation of the β2-AR resulted in cAMP production, MAPK activation and cell proliferation. Our current observations differ from those reported in animal cardiac fibroblasts in that β2-AR-induced proliferation in the human cells occurred via an indirect mechanism. We demonstrate here that this increase in proliferation was due to enhanced secretion of one or more heat-sensitive autocrine factors. Cardiac fibroblasts are known to secrete a variety of different growth factors and we are currently determining the precise identity of the autocrine factor(s) involved using neutralizing antibodies and specific receptor antagonists.

A number of large clinical trials have demonstrated that β-AR antagonists improve cardiac performance and significantly reduce mortality in heart failure patients [3,33]. Investigation of the underlying mechanisms has focused mainly on the role of β-ARs in cardiac myocytes [6,7]. The effects of chronic β-AR stimulation on cardiac fibroblast behaviour have received less attention and have been almost exclusively studied in animal models. The relative merits of β1-selective blockade versus non-selective β-blockade in the treatment of heart failure is the subject of current debate [3,34]. Our observations that human cardiac fibroblasts express the β2-AR, and that chronic β2-AR stimulation increases fibroblast proliferation, offer a further rationale for potential differential effects of commonly used β-blockers according to their selectivity.

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