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Diverse Regulation of Cardiac Expression of Relaxin Receptor by α_1- and β_1-Adrenoceptors

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Abstract

Purpose Relaxin, a new drug for heart failure therapy, exerts its cardiac actions through relaxin family peptide receptor 1 (RXFP1). Factors regulating RXFP1 expression remain unknown. We have investigated effects of activation of adrenoceptors (AR), an important modulator in the development and prognosis of heart failure, on expression of RXFP1 in rat cardiomyocytes and mouse left ventricles (LV).

Methods Expression of RXFP1 at mRNA (real-time PCR) and protein levels (immunoblotting) was measured in cardiomyocytes treated with α- and β-AR agonists or antagonists. RXFP1 expression was also determined in the LV of transgenic mouse strains with cardiac-restricted overexpression of α_{1A}-, α_{1B}- or β_2-AR. Specific inhibitors were used to explore signal pathways involved in α_1-AR mediated regulation of RXFP1 in cardiomyocytes.

Results In cultured cardiomyocytes, α_1-AR stimulation resulted in 2–3 fold increase in RXFP1 mRNA ($P<0.001$), which was blocked by specific inhibitors for protein kinase C (PKC) or mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK). Activation of β_1-, but not β_2-AR, significantly inhibited RXFP1 expression ($P<0.001$). Relative to respective wild-type controls, RXFP1 mRNA levels in the LV of mice overexpressing α_{1A}- or α_{1B}-AR were increased by 3- or 10-fold, respectively, but

unchanged in β_2-AR transgenic hearts. Upregulation by α_1-AR stimulation RXFP1 expression was confirmed at protein levels both in vitro and in vivo.

Conclusions Expression of RXFP1 was up-regulated by α_1-AR but suppressed by β-AR, mainly β_1-AR subtype, in cardiomyocytes. Future studies are warranted to characterize the functional significance of such regulation, especially in the setting of heart failure.

Keywords Relaxin family peptide receptor-1 (RXFP1) · Adrenoceptors · Cardiomyocyte

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Introduction

Rapid progress has been achieved in the last decade in our understanding of the pleiotropic actions of the peptide hormone relaxin in the cardiovascular system. Studies have examined the cardiovascular pharmacology, signaling mechanisms, and protective effects of relaxin in various pathological situations [1–3]. Findings both in vitro and in vivo have provided clear evidence of relaxin's cardiac protection against ischemia/reperfusion injury, oxidative stress-evoked cardiomyocyte apoptosis and cardiac hypertrophy [4–7]. While changes in circulating levels of endogenous relaxin in patients with cardiovascular diseases remain controversial [1], recent clinical trials in patients with acute heart failure have documented a beneficial effect of intravenous relaxin infusion with significant reduction in peripheral vascular resistance, alleviation of dyspnoea and an improved 180-day survival [8, 9].

Effects of relaxin are mediated by relaxin family peptide receptors (RXFP), leucine-rich repeat-containing G-protein coupled receptors [10]. RXFP1 is the major receptor subtype for relaxin with a high affinity [10, 11]. Cardiomyocytes express RXFP1 [12, 13], which mediates actions of relaxin through multiple pathways [1, 11]. Little is known about the

regulation of cardiac RXFP1 albeit such knowledge is important both in our understanding of the cardioprotective action of relaxin and in optimising its use in the clinic.

In addition to RXFP1, cardiomyocytes are also equipped with other G protein-coupled receptors. One of the most important classes is adrenoceptors, which are targets of the catecholamines. The importance of the sympathetic nervous activity via catecholamines in heart failure progression and mortality is well established [14]. Considering the recent success of the relaxin clinical trial for heart failure therapy [8], the routine use of β-adrenergic blockers [15] and a therapeutic potential of α-adrenergic agonists [16, 17], we studied the hypothesis that activation of α- and β-adrenergic receptors (ARs) regulates expression of RXFP1 in cardiomyocytes, both in vitro and in vivo. Part of the data was previously presented and published as an abstract in a conference preceding [18].

Materials and Methods

Animals

Animals were bred and housed under standard conditions at the AMREP (Alfred Medical Research and Education Precinct) Animal Service Centre. Experimental procedures were approved by a local Animal Ethics Committee in accordance with the National Health and Medical Research Council of Australia guideline for the use and care of animals.

Sprague–Dawley neonatal rats (0–3 day-old) were used for preparation of cardiomyocytes. In addition, we used three transgenic (Tg) stains of mice with cardiac-restricted overexpression, driven by the α-myosin heavy chain promoter, of subtypes of ARs for measurement of changes in cardiac RXFP1 expression. They were α_{1A}AR-Tg mice (A1A2 line, kindly provided by Professor Robert Graham, Victor Chang Cardiac Research Institute, Sydney) [19], constitutively active mutant (CAM) α_{1B}-AR-Tg mice (CAM21-α_{1B}AR-Tg line) [20], and β_2-AR-Tg mice (TG4-β_2AR-Tg line) [21] (both were kindly provided by Professor Robert Lefkowitz, Duke University, Durham). The extent of increase in receptor density was 66-fold for α_{1A}-AR, 3-fold for mutant α_{1B}-AR, and 195-fold for β_2-AR lines [19–21] relative to their respective non-transgenic (NTg) littermates. Both male and female Tg and NTg mice aged 4–8 months were used.

Cell Preparation and Treatment

Cardiomyocytes were prepared from the ventricle of neonatal rats and cultured as previously described [6]. The cell purity was over 98 %. Cardiomyocytes were subjected to 24 h treatment with α- or β-AR agonists, in the presence or absence of specific α_1- or β-antagonists or selective inhibitors of signalling molecules. Changes in RXFP1 expression were then determined by real-time PCR and Immunoblotting.

The following adrenergic agonists and antagonists were tested (in μM): isoproterenol (10, β-agonist), norepinephrine (10, α- and β-agonist), phenylephrine (5, mainly an α-agonist and relatively weak β-agonist activity at μM concentrations), methoxamine (100, α_1-agonist), phentolamine (1, α-antagonist), propranolol (1, β-antagonist, ICN), atenolol (1, β_1-antagonist), CGP20712A (0.1, β_1-antagonist), ICI-118551 (1, β_2-antagonist, ICN), dobutamine (10, β_1-agonist) and zinterol (1, β_2-agonist).

To explore signalling pathways involved in regulation of RXFP1 expression in cardiomyocytes, we tested specific protein kinase C (PKC) inhibitors (bisindolylmaleimide at 1 μM and Ro-32-0432 at 10 μM, Calbiochem) and mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) inhibitors (PD98050 at 30 μM and U0126 at 10 μM, Calbiochem). Effect of a PKC activator, phorbol-12-myristate-13-acetate (PMA, 0.1 μM), was also tested.

We selected concentrations of the antagonists/agonists or the inhibitors/activators according to the dose-testing results from our pilot experiments and from the literature that allow for full stimulation or blockade. The organic solvent used was DMSO with the final concentration <0.1 % (V/V), a concentration showing no effect on RXFP1 expression (data not shown). To prevent oxidation and biotransformation of the test agents, ascorbic acid at the final concentration of 100 μM was added into the culture media. Unless specified above, all chemicals were purchased from Sigma (St. Louis, MO).

Reverse Transcription and Real-Time PCR

Total RNAs was extracted from cardiomyocytes or the LVs using TRIzol reagent (Invitrogen). After deoxyribonuclease I (Promega, Madison, WI) treatment, 1 μg of total RNA was reverse-transcribed into cDNA using Supercript III (Invitrogen). Real-time quantitative PCR was performed on 50 ng cDNA using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and the primers rat RXFP1 5′-CAGGAAGTACCATGACCTCCA-3′ and antisense primer 5′-CTGACGGAGCGAATCTTATTG-3′; rat GAPDH 5′-ATGATTCTACCCACGGCAAG-3′ and antisense primer 5′-CTGGAAGATGGTGATGGGTT-3′; mouse RXFP1 5′-GAGGCAGAAACTTCCGAATG-3′ and antisense primer 5′-ATCTCGGCACAAACAATGC-3′; mouse GAPDH 5′-AGCTTGTCATCAACGGGAAG-3′ and antisense primer 5′-TTTGATGTTAGTGGGGTCTCG-3′. The primers used were verified for their target-specificity by preliminary PCR experiments prior to the quantification of RXFP1 mRNA. PCR reaction was performed using an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). Amplifications were carried out in a 96-well plate with

15 µl reaction volumes and 40 amplification cycles (94 °C, 15 s; 55 °C, 30 s; 72 °C, 34 s). Experiments were carried out in duplicates, and the mRNA expression was taken as the mean of three to four separate cardiomyocytes preparations or 6–8 mice per animal group. The expression of RXFP1 was normalized to GAPDH expression. Fold changes relative to controls were determined using the $^{\Delta\Delta}$Ct method.

Immunoblotting

Expression levels of RXFP1 were also measured by Immunoblotting in cardiomyocytes and the LVs from Tg mice and their respective NTg littermates. Proteins enriched with membrane fractions were extracted as we previously described with minor modifications [6, 22], cells or myocardium were lysed in a SDS buffer (0.125 M Tris-Cl, 15 % glycerine, 4 % SDS), homogenised and sonicated. Lysates were then centrifuged at 13,000 rpm for 15 min and supernatant was collected for determination of protein concentration (Piece BCA Protein Assay Kit). 50–100 µg lysate protein was subjected to 7.5 % SDS-PAGE gel electrophoresis. A rabbit polyclonal antibody against RXFP1 (IMGENEX, IMG-5906, 1:2,500) and a mouse monoclonal antibody against β-actin (abcam, ab8227, 1:2,500) were respectively used for detection of RXFP1 and β-actin (as a loading control). Following incubation with the secondary antibody, donkey anti-rabbit IgG-HRP (Santa Cruz, sc2313, 1:3,000) and donkey anti-mouse IgG-HRP (Santa Cruz, sc2314, 1:3,000), immunoreactive bands were visualized with the ECL reagent (Millipore) and quantified by densitometry (Quantity One, Biorad). The expression of RXFP1, normalized to respective β-actin expression, was

taken as the mean of three to four separate cardiomyocytes experiments or 6–8 mice per group.

Statistical Analysis

Triplicates per group were used in cell experiments and 6–8 mice per group in animal experiments. Each experiment was repeated independently three to four times and data are presented as mean ± SEM. Statistical comparisons among multiple groups were performed using one-way ANOVA with Newman-Keuls's test as post-hoc test, if $P<0.05$. Student's t-test was used for comparison between two groups. Software used was GraphPad Prism (version 5; GraphPad Software Inc. Say Diago, CA). $P<0.05$ was considered as statistically significant.

Results

α_1-AR Activation Up-Regulated RXFP1 Expression in Cardiomyocytes

Treatment with α_1-agonists phenylephrine or methoxamine for a period of 24 h increased RXFP1 gene expression by approximately 1.8- or 2.7-fold, respectively ($P<0.001$, Fig. 1a and b), effect that was prevented by the α-antagonist phentolamine ($P<0.01$, Fig. 1a). Phenylephrine is mainly an α_1-AR agonist, it however weakly activates β-AR at µM concentrations. Combination of phenylephrine and the β-antagonist propranolol further increased RXFP1 expression by about

Fig. 1 Activation of α_1-adrenoceptors (AR) upregulated expression of relaxin family peptide receptor-1 (RXFP1) in cardiomyocytes. **a** Upregulation of RXFP1 mRNA by α_1-adrenergic agonists, phenylephrine (PE, 5 µM) and methoxamine (Met, 100 µM), was abolished by α-antagonist phentolamine (Phent, 0.1 µM). SFM: serum-free media. **b** Upregulation of RXFP1 mRNA by the non-selective adrenergic agonists norepinephrine (NE, 10 µM) and PE, could be revealed by simultaneous blockade of β-AR using propranolol (Pro, 1 µM). **c** Immunoblotting showed upregulation of RXFP1 proteins by Met. **$P<0.01$, ***$P<0.001$
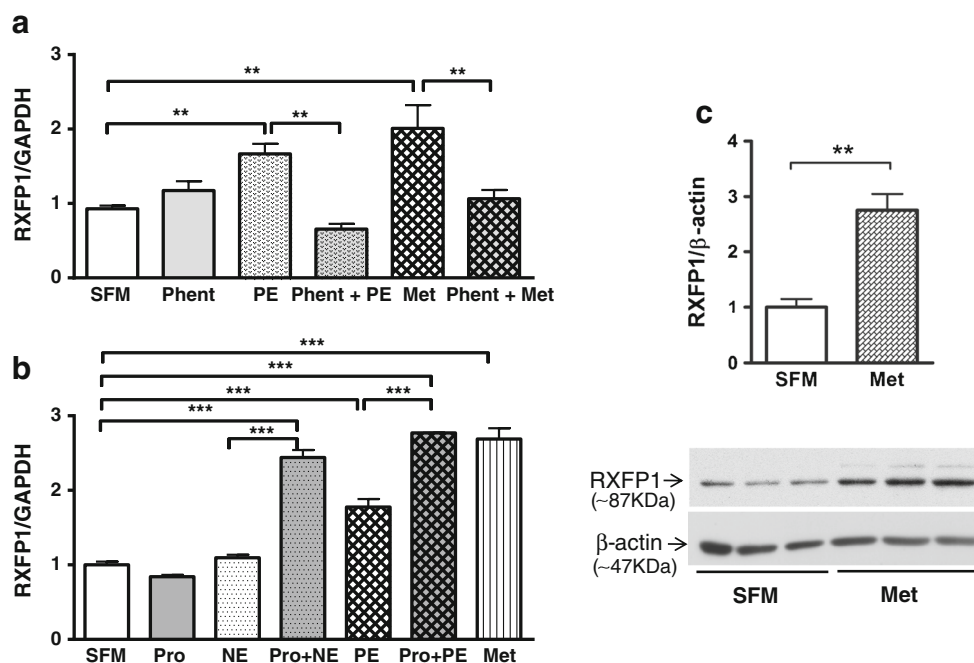
30 % ($P<0.001$) relative to that by phenylephrine alone (Fig. 1b). Importantly, whilst treatment with norepinephrine, a non-selective and naturally occurring α- and β-agonist, showed no effect on RXFP1 expression, simultaneous blockade of β-AR with propranolol resulted in a 2.6-fold increase ($P<0.001$) in RXFP1 expression, indicating an inhibitory action of β-AR on the α-AR mediated up-regulation of RXFP1 in cardiomyocytes (Fig. 1b).

In agreement with upregulated mRNA expression of RXFP1 by α-AR agonists, the protein expression levels were approximately 3-fold higher in cardiomyocytes treated with methoxamine versus untreated cells ($P<0.01$, Fig. 1c).
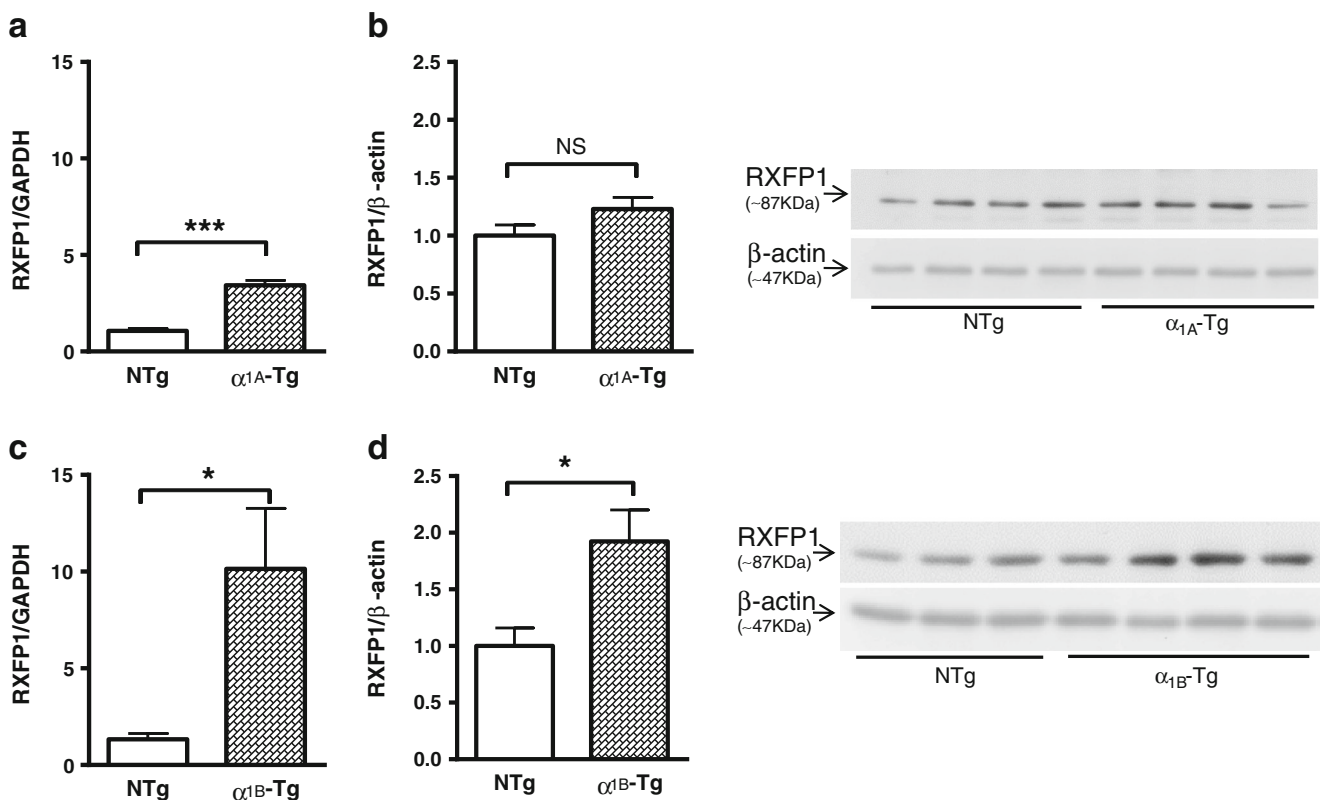
RXFP1 Expression in Transgenic Mouse Hearts

Compared to respective Ntg littermates, mRNA levels of RXFP1 increased by 3.2-fold ($P<0.001$) and 9.8-fold ($P<0.05$), respectively, in the LV of Tg mice with cardiomyocyte-restricted overexpression of α_{1A}- or CAM α_{1B}-AR (Fig. 2a and c). Immunoblotting revealed a 2-fold increase ($P<0.01$) in the RXFP1 at protein level in the CAM α_{1B}-Tg mouse hearts (Fig. 2d). No significance difference in RXFP protein expression, however, was observed in α_{1A}-Tg hearts (Fig. 2b).

### β-AR Activation Down-Regulated RXFP1 Expression in Cardiomyocytes

Following the observation in cardiomyocytes that co-treatment with norepinephrine and propranolol resulted in an increased RXFP1 expression (Fig. 1b), we further examined the subtypes of β-AR involved and whether β-AR activation alone would actually suppress RXFP1 expression. Cardiomyocytes were treated with norepinephrine in the presence of either the β_1-antagonist CGP20712A and atenolol or the β_2-antagonist ICI-118551. Treatment with CGP20712A, atenolol, or ICI-118551 alone had no effect on RXFP1 expression. Norepinephrine alone or the combination of norepinephrine and ICI-118551 did not alter RXFP1 expression (Fig. 3a). In contrast, combination of norepinephrine and CGP20712A or norepinephrine and atenolol upregulated RXFP1 expression by 1.5 to 2-fold (both $P<0.05$, Fig. 3a), indicating that β_1-AR attenuated α-AR mediated up-regulation of RXFP1 expression. RXFP1 expression was not altered in the Tg hearts with cardiomyocyte-restricted overexpression of β_2-AR versus NTg littermates (Fig. 3c and d).

We then examined effects of the β-agonists isoproterenol, dobutamine and zinterol on expression of RXFP1 in cardiomyocytes. Zinterol as a selective β_2-agonist showed only a weak inhibition on RXFP1 expression by 25 %

Fig. 2 Upregulation of RXFP1 expression in left ventricular tissues from transgenic (Tg) mice with cardiac-restricted overexpression of α_{1A}-AR or constitutively active mutant α_{1B}-AR. **a** and **c** mRNA expression of RXFP1 measured by qRT-PCR; **b** and **d** Protein expression of RXFP1 measured by immunoblotting. *NTg* non-transgenic littermate; *$P<0.05$ and ***$P<0.001$. *NS* not significant
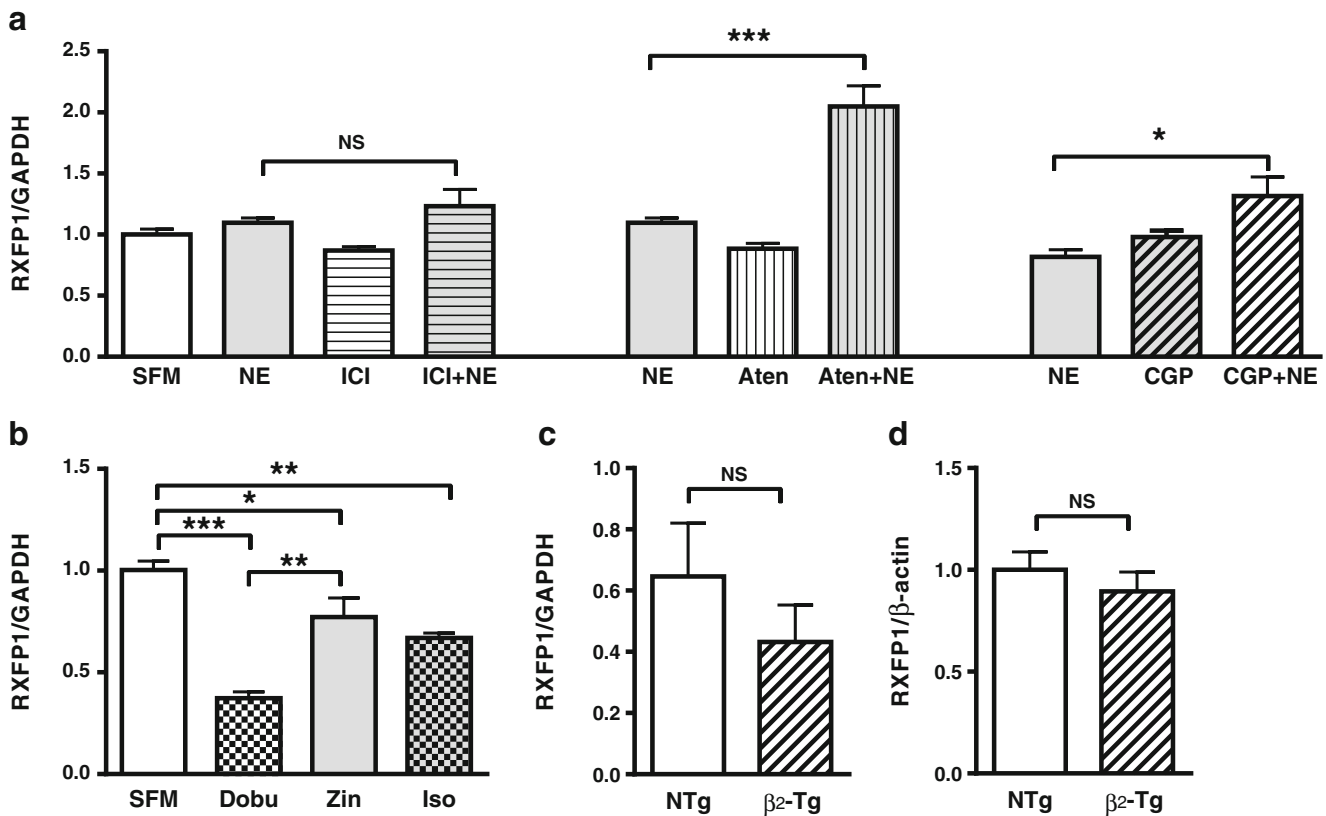
Fig. 3 Opposing action of β-AR on α₁-AR in regulation of RXFP1 mRNA expression in cardiomyocytes. **a** Simultaneous activation of α- and β-ARs with norepinephrine (NE, 10 μM) did not alter expression of RXFP1. Addition of the β₁-antagonist CGP20712A (CGP, 0.1 μM) or atenolol (Aten, 1 μM), but not the β₂-antagonist ICI-115881 (ICI, 1 μM), together with NE revealed α-AR mediated up-regulation of RXFP1. **b** Stimulation of β₁-AR with dobutamine (Dobu, 10 μM) or isoproterenol (Iso, 10 μM) potently suppressed RXFP1 expression while stimulation of β₂-AR with zinterol (Zin, 1 μM) resulted in only a weak reduction. Expression of RXFP1 at mRNA (**c**) and protein levels (**d**) was not significantly altered in left ventricles of transgenic mice with cardiac restricted overexpresion of β₂-AR (β₂-Tg) compared to that of non-transgenic (NTg) littermates. *$P<0.05$, **$P<0.01$ and ***$P<0.001$. *NS* not significant, *SFM* serum-free media

($P<0.05$, Fig. 3b). Isoproterenol, a non-selective β-agonist, suppressed the expression by about 40 % ($P<0.01$, Fig. 3b). The selective β₁-agonist dobutamine was potent in the inhibition of RXFP1 expression by 60 % relative to untreated cardiomyocytes ($P<0.001$, Fig. 3b).

Signalling Pathways Involved in Regulation of RXFP1 by α₁-AR

To explore signalling pathway(s) involved in upregulating RXFP1 by α₁-AR, inhibitors specific to PKC or MEK/ERK were respectively tested for their effects on mRNA levels of RXFP1 in cardiomyocytes stimulated with phenylephrine.

A near complete blockade of upregulated RXFP1 expression was observed in cardiomyocytes treated with PKC inhibitors (bisindolylmaleimide and Ro-32-0432, both $P<0.001$, Fig. 4a). Conversely, activation of PKC with PMA stimulated the expression by 2.3-fold ($P<0.01$, Fig. 4b). Significant reduction of RXFP1 expression in cardiomyocytes was also observed with the treatment of MEK/ERK inhibitors (PD98050 and U0126, both $P<0.01$, Fig. 4c).

Discussion

The present study confirmed that RXFP1 expression in cardiomyocytes is oppositely regulated by α₁-AR and β-AR (mainly β₁-AR) [18]. Specifically, RXFP1 expression is enhanced by α₁-AR stimulation but suppressed by β₁-AR activation. Importantly, α₁-AR mediated RXFP1 upregulation was inhibited when β₁-AR was simultaneously activated using the non-selective agonist norepinephrine. Our findings suggest that the PKC-MEK/ERK pathway is involved in the α₁-AR mediated upregulation of RXFP1.

RXFP1 upregulation mediated by α₁-AR activation was similarly observed with the use of different α₁-agonists including norepinephrine (in the presence of a β-antagonist),

Fig. 4 Protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) are signalling molecules mediating α_1-AR induced RXFP1 upregulation in cardiomyocytes. **a** PKC inhibitors bisindolylmaleimide (BIM, 1 μM) or Ro-32-0432 (Ro, 10 μM) abolished phenylephrine (PE)-stimulated RXFP1 mRNA expression. **b** PKC activator phorbol-12-myristate-13-acetate (PMA, 0.1 μM) significantly stimulated RXFP1 mRNA expression. **c** α-AR mediated upregulation of RXFP1 expression was abolished by inhibitors for mitogen activated protein kinase (MEK) and ERK PD98050 (PD, 30 μM) or U0126 (10 μM). **d** Simplified illustration of a proposed signalling mechanism for α_1-AR regulation of RXFP1 expression. *SFM* serum-free media. **$**P<0.01$ and ***$P<0.001$. *SFM* serum-free media
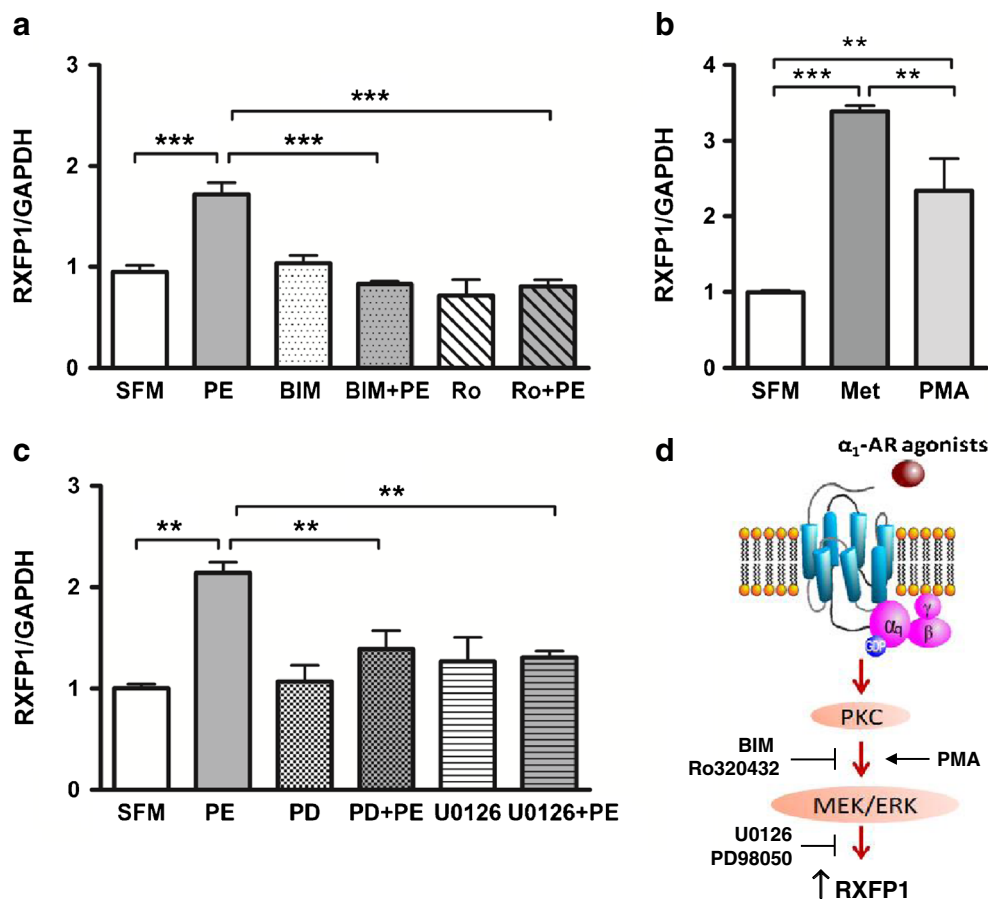
phenylnephrine and methoxamine, indicating a class-effect. The results from cultured cardiomyocytes were further confirmed by the finding of a significantly upregulated RXFP1, both at mRNA and protein levels, in LV tissues of both α_{1A}- and α_{1B}-AR Tg strains, albeit elevation of RXFP1 at the protein level was only seen in the α_{1B}-AR Tg hearts. Likewise, the significant inhibitory regulation by β_1-AR was documented by using isoproterenol or the β_1-agonist dobutamine and the use of specific antagonists. Whereas RXFP1 expression in the LV of β_2-AR Tg mice was unchanged, activation of β_2-AR in cardiomyocytes using the β_2-agonist zinterol showed a weak inhibition. Association of β_1-AR in the inhibition of RXFP1 was supported by a clear increase in the RXFP1 expression in the presence of NE and the β_1-antagonist CGP20712A or atenolol. Our findings from cardiomyocytes thus revealed that β-AR activation, mainly β_1-AR, suppressed RXFP1 expression.

RXFP1 expression was once considered to be limited to atrial cardiomyocytes based on early reports that showed relaxin binding sites in rat atria tissue but very low levels in ventricles [23]. With advancement of technology and methodology, RXFP1 expression has since been detected by RT-PCR not only in atrial but also in ventricular cardiomyocytes [13]. Our immunoblotting

results, in addition to RT-PCR, clearly confirmed expression of RXFP1 in ventricles. The anti-RXFP1 antibody used is a peptide-affinity purified rabbit immunoglobulin (Ig) raised against a portion of amino acid 50–100 of human RXFP1, which is 94 % homologous in mouse (IMGENEX). At the protein level, mouse and rat RXFP1 are 85.2 % and 85.7 % identical to human RXFP1 [24]. This antibody reveals a single protein band sized approximately 87 KDa on Western blots of human, mouse or rat heart lysates and the band disappears in the presence of immunizing peptide (IMGENEX). Consistently, it displayed a single ~87 KDa protein band in our Western blots of rat cardiomyotes or mouse LV. In the present study, we prepared cardiomyocytes from ventricular tissues of neonatal rats. RXFP1 expression thus is detectable in ventricular myocardium including ventricular cardiomyocytes.

Regulation of RXFP1 expression by α_1- and β_1-AR appears to be restricted to cardiomyocytes. Our cultured cardiomyocytes had over 95 % purity and we performed similar testing in cultured cardiac fibroblasts from neonatal rat hearts and such regulation was not evident (data not shown). This was further indicated by our results from the two α_1-AR Tg lines, in which the transgenes were controlled

by the cardiomyocyte-specific promoter α-MHC [19–21]. Regulation of RXFP1 expression by estrogen and progesterone was recently reported in the reproductive tissues [25, 26]. The transcriptional mechanism controlling RXFP1 expression remains to be elucidated. We, however, explored possible signal mechanisms of RXFP1 upregulation associated with α_1-AR activation by using specific inhibitors of PKC or MEK/ERK, important signalling molecules. Inhibition of PKC or MEK/ERK effectively abolished the upregulation by α_1-AR stimulation whilst RXFP1 expression was enhanced by stimulating PKC with PMA. Specific inhibitors targeting PKA (H89 at 10 μM) were used to explore signalling of β-AR inhibition of RXFP1 (data not show). Unlike the role of PKC in α_1-AR mediated stimulation of RXFP1, blockade of PKA signalling was unable to significantly reverse the action of β-AR. Future studies to employ more specific molecular modulation other than pharmacological inhibitors may shed some light on the signalling of the β-AR mediated downregulation of RXFP1.

Some features of adrenergic regulation on RXFP1 expression merit discussion. *First*, our findings clearly show the opposing regulation of RXFP1 expression in cardiomyocytes, i.e. upregulation by α_1- and down-regulation with β-AR activation. In the setting of simultaneous activation of both α_1- and β-ARs (i.e. use of norepinephrine), such counter-regulation results in absence of significant change in RXFP1 expression. *Second*, our findings of upregulated RXFP1 mRNA in hearts of both α_{1A}- and α_{1B}-Tg lines, but the elevated protein levels in hearts of the α_{1B}-Tg mice only, suggests a more potent up-regulation by α_{1B}-subtype. However, it should be noted that two α_1-Tg strains differ in that the α_{1B}-Tg mice express CAM receptor whilst α_{1A}-Tg mice express wild-type receptor. Therefore the extent of activation of the down-stream signalling in the two models is not in proportion to the fold increase of their elevated α_1-AR density. *Third*, whilst both β_1- and β_2-AR mediate signalling through activation of PKA, activation of β_2-AR had only weak in vitro but no in vivo inhibitory effect on RXFP1 expression dissimilar to that by β_1-AR stimulation. Whilst predominance of β_1-AR in the myocardium [27] may explain part of this diversity, differences in the signalling pathways between β_1- and β_2-AR, in addition to the common stimulatory G-protein/PKA coupling, may be responsible for their differential regulation of RXFP1 expression. Recent studies clearly show that heterogeneous β_1-AR signalling could involve β-arrestin mediated transactivation of EGFR [27] and exchange protein activated by cAMP 2 (Epac2) mediated Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activation [28]. Relative to β_1-AR, β_2-AR utilises inhibitory G-protein Gi and non-classical signalling pathways to mediate compartmentalised rather than a global activation of cardiomyocytes [29–31].

The pathophysiological and therapeutic consequences of the interactions between RXFP1 and adrenoceptors that we reported here remain to be established. Delineation of the consequences of interaction at the cellular level in vitro or ventricular level in vivo are complicated by certain degrees of overlapping in the signalling effects by α_1-AR stimulation, β-AR blockade or by activation of RXFP1. RXFP1, relaxin receptor, are confirmed to express in atrial and LV tissues in rodents [13, 32] and atrial tissues in the human [33]. Recent work by Parikh et al. also demonstrates functional RXFP1 in human cardiomyocytes derived from induced pluripotent stem cells [34]. While it remains controversial with regard to whether RXFP1 stimulation enhances cardiac contractile function [33, 35], experimental studies have documented cardiac protection of relaxin both in vitro and in vivo [6, 8, 36, 37]. In the setting of cardiovascular diseases and heart failure, the sympathetic nervous activity and circulating levels of catecholamines are elevated [38, 39], resulting in a tonic stimulation of α- and β-ARs. Thus regulation of RXFP1 expression by α_1- and β_1-AR is likely to occur in the setting of heart failure, providing a mechanism by which the sympathetic nervous activity might impact RXFP1 signalling in cardiovascular cells stimulated with relaxin, such that β-blockers used as a standard therapy for cardiovascular diseases and heart failure [15] may unmask α_1-AR mediated RXFP1 elevation. In the recent RELAX-AHF trial, subgroup analysis indicated that in acute heart failure patients, β-blocker users and non-users were similarly benefited from relaxin therapy [40]. However, this conclusion is derived from a relatively small sample size and it needs to be assessed further in larger scale relaxin clinical trials.

Conclusion

The present study described for the first time the diverse regulation by α_1- and β_1-AR of RXFP1 regulation in the cardiomyocytes. Considering the strong evidence for beneficial cardiac actions of relaxin [1, 8], the importance of sympathetic nervous system in heart diseases and α_1- and β-AR being therapeutic targets, such regulation is potentially significant in cardiac pathophysiology. Further studies are required to explore pharmacological and pathological significance of the heterogeneous regulation of RXFP1 by subtypes of ARs.

Disclosure Statement All authors have nothing to declare.

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