European Journal of Pharmacology 634 (2010) 101–106

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

The separate roles of endothelin receptors participate in remodeling of matrix metalloproteinase and connexin 43 of cardiac fibroblasts in maladaptive response to isoproterenol

Hong-Jun Peng, De-Zai Dai *, Hui Ji *, Yin Dai

Research Division of Pharmacology, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 22 April 2009
Received in revised form 10 January 2010
Accepted 2 February 2010
Available online 16 February 2010

Keywords:
Matrix metalloproteinase
Connexin43
Cardiac fibroblast
Endothelin receptor antagonist
NADPH oxidase
Isoproterenol

ABSTRACT

Stress may affect gap junction connexin 43 and matrix metalloproteinase-2/9 (MMP-2/9) in cardiac fibroblasts, potentially contributing to worsening cardiac function and arrhythmias. Cardiac fibroblasts isolated from neonatal rat were incubated with isoproterenol at 3×10^{-7} M to mimic stress and were treated with either PD156707 or IRL-1038 (selective antagonists for endothelin A and B receptor respectively) and CPU0213 (a dual endothelin A/B receptor antagonist) at 1×10^{-8} M, 3×10^{-8} M or 1×10^{-7} M. RT-PCR and Western blotting were conducted. Upregulation of the two endothelin receptors, MMP-2/9 and NADPH oxidase subunits (p22phox and p47phox), and downregulation of connexin 43 in cardiac fibroblasts were found in the presence of isoproterenol and were attenuated by the selective blockers PD156707 and IRL-1038 in a dose-dependent manner. IRL-1038 was less effective. CPU0213 appeared to be more effective than the two selective blockers in blocking these changes. Changes in cardiac fibroblasts in response to isoproterenol mediated by upregulation of the endothelin–NADPH oxidase pathway may play a role in deteriorating cardiac function and arrhythmias. The endothelin A receptor has a major role, relative to the endothelin B receptor, in the remodeling of cardiac fibroblasts during isoproterenol stimulation. CPU0213, a dual endothelin receptor A/B blocker, seems to be more effective in normalizing these changes than do the selective endothelin receptor antagonists.

1. Introduction

Malignant arrhythmias and cardiac failure are major medical problems contributing to a high mortality in patients with cardiovascular disease. Stress related triggering factors exacerbate arrhythmias and heart failure. Infarcted rat hearts stimulated with isoproterenol to mimic stress reveal exacerbated cardiac arrhythmias (Wang et al., 2004) and cardiac performance (Cheng et al., 2009). Chronic stress produced by high doses of L-thyroxin results in a high prevalence of ventricular fibrillation in rats (Feng et al., 2007a; Xia et al., 2006; Yu et al., 1997). Stress exaggerated cardiac arrhythmias are associated with an increased activity of the endothelin pathway and are relieved by darusentan, a selective endothelin receptor antagonist (Xia et al., 2006), or by CPU0213, a dual endothelin blocker (Feng et al., 2007a).

β-Adrenoceptor activation is frequently associated with worsened arrhythmias and cardiac performance, an impairment of gap junction communication with which myocardial MMP-2/9 (matrix metalloproteinase-2/9) is actively involved. Cardiac fibroblasts, as important components in the myocardium, may play a role in arrhythmogenesis through cardiac fibrosis, a maladaptive response of cardiac fibroblasts in response to stress related events. The junction communication of connexin 43 contributes to the generation of malignant arrhythmias (Saffitz and Douglas, 2006). Cardiac fibrosis is directly related to upregulation of MMP-2/9, and maintenance of atrial fibrillation (Polyakova et al., 2008). It has been suggested that an increased risk of arrhythmia may be, at least in part, attributable to fibrosis resulting from cardiac fibroblast abnormalities (Batlle et al., 2007; Roldan et al., 2008). Remodeling of gap junction components, principally connexin 43, is central to arrhythmogenesis (Zhong et al., 2007). Reduced expression of connexin 43 is thought to affect the spatial and temporal coordination of the electrical activity in the heart, accounting for the occurrence of ventricular arrhythmias (Poelzing et al., 2004). Thus, cardiac fibroblasts are critical in exacerbating arrhythmogenesis and cardiac failure in the presence of triggering factors related to stress.

Oxidative stress is an important factor contributing to the risk of many cardiovascular diseases, including arrhythmias (Shimano et al., 2009; Rodrigo et al., 2009). Reactive oxygen species are mainly generated through activated NADPH oxidase. A positive feedback loop links activation of endothelin receptors with excessive reactive oxygen species production by activated NADPH oxidase, and the endothelin-reactive oxygen species pathway has been suggested to react to myocardial insults, possibly in response to isoproterenol (Li et al.,

* Corresponding authors. Tel.: +86 25 83271270; fax: +86 25 83302827.
E-mail addresses: dezaidai@vip.sina.com (D.-Z. Dai), Huijicpu@163.com (H. Ji).

0014-2999/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2010.02.001

2008). Thus, a blockade of endothelin receptors leads to attenuation of oxidative stress and the resultant relief of cardiac arrhythmias (Zhang et al., 2008).

CPU0213, a dual endothelin receptor antagonist is effective in suppressing the generation of reactive oxygen species (Cui et al., 2009), in association with relieving the abnormal matrix metalloproteinases (MMPs) in the myocardium (Liu and Dai, 2004). However, it is not known whether CPU0213 can eliminate the maladaptive responses of cardiac fibroblasts to stress by suppressing NADPH oxidase. It is also unknown whether endothelin A and B receptors exert separate roles during the remodeling of cardiac fibroblasts in response to stress.

We hypothesized that β-adrenoceptor stimulation by isoproterenol causes changes in Cx43 and MMP-2/9 of cardiac fibroblasts. We investigated, using selective and non-selective endothelin receptor antagonists, the roles of endothelin A and B receptors in abnormal MMP-2/9, Cx43, and NADPH oxidase expression during isoproterenol stimulation.

2. Materials and methods

2.1. Isolation and culture of fibroblasts

Animal handling procedures were conducted in accordance with the Laboratory Animal Regulations of the Scientific Bureau of Jiangsu Province, China.

Primary cultures of neonatal rat cardiac fibroblasts were prepared according to the previously published literature with minor modification (Feng et al., 2007b). Briefly, hearts were removed under aseptic conditions from 1- to 3-day-old neonatal Sprague–Dawley rats from the Animal Center of Nanjing Medical University (Nanjing, China). Ventricular tissue was isolated and minced into 2- to 3-mm^3 fragments followed by digestion in 0.25% trypsin solution. The dissociated cells were collected by centrifugation at 1000×*g* for 10 min and re-suspended in media containing DMEM (pH 7.4), 5.5 mM D-glucose (NG), 20% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cell suspension was plated into a flask (Falcon) and incubated at 37 °C in a humidified atmosphere (5% CO_2/95% air) for 90 min. This allowed preferential attachment of non-cardiomyocytes to the flask. Cardiac fibroblasts were maintained in the media and incubated in a humidified atmosphere of 5% CO_2 at 37 °C until fully confluent. Cells were treated with 0.05% trypsin-EDTA for 2–3 times before use. The purity of cultured cardiac fibroblasts was >95% as determined by morphological characterization of positive staining for fibronectin and negative staining for smooth muscle actin.

2.2. Cardiac fibroblasts incubated with isoproterenol

Cardiac fibroblasts were cultured at 37 °C in MEM/EBSS serum-free medium. Four hours later, isoproterenol and tested compounds at 3 concentrations were added to the chambers, containing 0.2 mL DMSO each, as follows. (1) control; (2) isoproterenol (3×10^{-7} M); (3)(4) and (5): isoproterenol 3×10^{-7} M with combined compound PD156707 at 1×10^{-8} M, 3×10^{-8} M, and 1×10^{-7} M, respectively; (6)(7) and (8): isoproterenol 3×10^{-7} M with IRL-1038 at 1×10^{-8} M, 3×10^{-8} M, and 1×10^{-7} M; (9)(10)and (11): isoproterenol 3×10^{-7} M with CPU0213 at 1×10^{-8} M, 3×10^{-8} M, and 1×10^{-7} M. The incubation was conducted for 24 h before the following procedures were carried out.

2.2.1. Chemicals

Isoproterenol was purchased from Shanghai Hefeng Medicine Company (Shanghai, China); CPU0213 (1-butyl-3-(2,4-dis-(para-chlorobenzyl-oxy)-phenyl)-pyrazole-5-carboxylic acid, purity>98%), a dual endothelin receptor antagonist with IC_{50} values of 2.57 nM and 93 nM for endothelin A and B receptors, respectively (Dai et al., 2004), was from the Department of Medicinal Chemistry of the China Pharmaceutical University (Nanjing, China); and PD156707 (sodium 2-benzo[1,3] dioxol-5-yl-4-(4-methoxy-phenyl)-4-oxo-3-(3,4,5-trimethoxy-benzyl)-but-2-enoate), a selective endothelin A receptor blocker (Maguire and Davenport, 1999), and IRL-1038 ([Cys^{11}–Cys^{15}]-Endothelin-1 (11–21)), a selective endothelin B receptor blocker (Kelso et al., 1998), were from Sigma and Genscript Corporation, USA, respectively.

2.3. Semi-quantitative determination of mRNA by RT-PCR

The total mRNA extracted from cardiac fibroblasts was determined, using Trizol reagent and reversely transcribed to cDNA with AMV reverse transcriptase (Promega, USA) (Na et al., 2007). Briefly, cDNA was amplified under the following conditions. Initial denaturalization was at 94 °C for 5 min, then cycling and denaturing at 94 °C for 40 s, annealing for 40 s extending at 72 °C for 1 min. The annealing temperature and cycle number of endothelin A receptor, endothelin B receptor, MMP-2/9, connexin 43, p22phox, p47phox and 18 S rRNA were 64 °C, 34 cycles; 61 °C, 32 cycles; 63 °C, 30 cycles; 58 °C, 30 cycles; 54 °C, 36 cycles; 60 °C, 30 cycles; 60 °C, 30 cycles; and 65 °C, 30 cycles, respectively. It was followed by a final extension at 72 °C for 10 min. Specific primers for endothelin A receptor were: sense, 5′-ATCGCTGACAATGCTGAGAG-3′ and antisense, 5′-CCACGATGAAAATGGTACAG-3′; for endothelin B receptor: sense, 5′-GTGAAGGCAGGAAGGAA-3′ and antisense, 5′-GCAGCCAACAGAAGATAAG-3′; for MMP-2: sense, 5′-CTATTCTGTCAGCACTTTGG-3′ and antisense, 5′-CAGACTTTGGTTCTCCAACTT-3′; for MMP-9: sense, 5′-CGTGGCCTAGTGACCTATG-3′ and antisense, 5′-GGATAGCTCGGTGGTGTCCT-3′; for connexin 43: sense, 5′-TGTAACACTCAACAACCTGGC-3 and antisense, 5′-GGTTTTCTCCGTGGGACGTGA-3′; for p22phox: sense, 5′-GCTCATCTGTCTGCTGGAGTA-3′ and antisense, 5′-ACGACCTCATCTGTAACTGGA-3′; for p47phox: sense, 5′-TCACCGAGATCTACGAGTTC-3′ and antisense, 5′-ATCCCATGAGGCTGTTGAAGT-3′; and for 18S rRNA: sense, 5′-GCTGCTGGCACCAGACTT-3′ and antisense, 5′-CGGCTACCACATCCAAGG-3′, respectively.

Amplification products were separated by agarose gel electrophoresis, stained with ethidium bromide, visualized under UV light, and digitally scanned (Syngene, England). Band density was determined by a gel imaging analysis system (Genegenus, Syngene, England), and the relative density of each DNA band was obtained by dividing that by the band of 18S rRNA.

2.4. Western blot

After 24 h incubation, cardiac fibroblasts were washed twice with cold PBS (137 mM NaCl, 1.47 mM KH_2PO_4, and 8.9 mM Na_2HPO_4, pH 7.4) and put into 500 μL lysing buffer containing 50 mM Tris–HCl, 1% Triton X-100, 150 mm NaCl, 1 mM EDTA, 0.5% SDS, and 1 mM phenyl-methyl-sulfonyl fluoride. Homogenates were centrifuged for 10 min at 10,000×*g* at 4 °C and supernatants were collected. Cell lysates (40 mg) were analyzed by 10% SDS-PAGE at 100 °C for 2 h. After transfer, nitrocellulose membranes were incubated in a blocking buffer (50 mM Tris–HCl, 200 mM NaCl, 0.2% Tween 20, and 5% nonfat dried milk) for 1 h at room temperature, then incubated with the appropriate dilution (1/100–1/600) of primary antibody (monoclonal anti-endothelin A and B receptor, and polyclonal rabbit anti-connexin 43 and polyclonal rabbit anti-β-actin from Wuhan Boster Biological Technology, China; polyclonal rabbit anti-MMP9 from the Life Science School of the University, and, monoclonal rabbit anti-MMP2 from Santa Cruz, USA; polyclonal rabbit anti-p22phox and polyclonal rabbit anti-p47phox from Uscnlife, USA). The reactions were in the same buffer containing 1% nonfat dry milk for 2 h at room temperature and the membranes were washed and then incubated for another 1 h with rabbit anti-goat antibody (Wuhan Boster Biological Technology, Wuhan, China). Immunoreactive bands were visualized by enhanced chemiluminescence detection reagent (Wuhan Boster Biological Technology, Wuhan, China) and were determined quantitatively by densitometry as compared to internal reference.

2.5. Statistical analysis

Data were presented as mean ± S.D. The homogeneity of the data was tested by one-way ANOVA and differences between means were tested for statistical significance by Bonferroni's multiple comparison tests. Differences were considered statistically significant at $P<0.05$.

3. Results

3.1. Endothelin A and B receptors in cardiac fibroblasts

Expression analysis of the endothelin A and B receptors in cardiac fibroblasts was measured: upregulation of mRNA and protein abundance was significant following exposure to isoproterenol (Fig. 1). Treatment with PD156707 and CPU0213, a selective endothelin A receptor antagonist and a dual antagonist respectively, decreased strongly the over-expression of endothelin A receptor. IRL-1038, a selective antagonist of the endothelin B receptor, was less effective in this regard. Upregulation of the endothelin B receptor was reduced significantly by both IRL-1038 and CPU0213, but PD156707 was without effect. IRL-1038 was more potent than CPU0213 in this regard. Although IRL-1038 also suppresses the upregulated endothelin A receptor to some extent, PD156707 is without effect on the elevated expression of endothelin B receptor.

3.2. Over-expression of MMP-2/9

MMP-2/9 mRNA and protein expression were significantly upregulated, relative to control, in cardiac fibroblasts incubated with isoproterenol ($P<0.01$). PD156707 attenuated the upregulation of MMP-2/9 mRNA and protein in cardiac fibroblasts in a dose-related manner ($P<0.01$), relative to isoproterenol alone (Fig. 2). IRL-1038 at three dose levels was less effective than PD156707 in ameliorating isoproterenol induced changes in MMP-2/9 expression ($P<0.01$). At 3 dose levels CPU0213 reduced significantly the exaggerated expression of MMP-2 (Fig. 2A and B) and MMP-9 (Fig. 2C and D) in a dose-related manner and was more potent ($P<0.01$) than either PD156707 or IRL-1038.

3.3. Downregulated connexin 43

To investigate whether expression of junction proteins in cardiac fibroblasts was altered under stress connexin 43 levels were measured. Expression of mRNA and protein levels of connexin 43 was high in normal cardiac fibroblasts and was dramatically downregulated, relative to control ($P<0.01$). Down regulation of connexin 43 was blocked in dose-dependent manner by the endothelin A antagonist PD 156707, which was more effective than the endothelin B antagonists IRL 1038. CPU0213 at 0.01, 0.03 and 0.1 μM concentrations was more potent than the selective endothelin antagonists in blocking the downregulation of connexin 43 mRNA and protein levels in cardiac fibroblasts (Fig. 3). These data indicate a primary role of the endothelin A receptor and a secondary role for the endothelin B receptor in the down regulation of connexin 43.

3.4. Upregulated p22phox and p47phox

The expressions of p22phox and p47phox mRNA and protein were measured in the presence and absence of isoproterenol, and the effects of endothelin receptor antagonists were determined. Consistent with the previous observations, upregulation of p22phox and

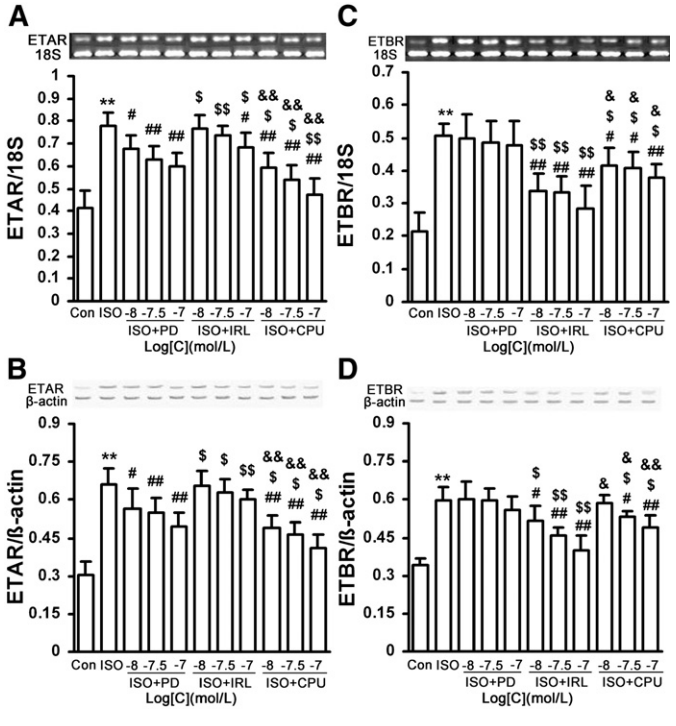

Fig. 1. Effects of endothelin receptor antagonists on expression of ET_A receptor and ET_B receptor in cardiac fibroblasts. The high expression of ET_A receptor (ETAR) (A, B) in cardiac fibroblasts by ISO was potently relieved by PD156707 and CPU0213. Upregulated expression of ET_B receptor (ETBR) (C, D) in cardiac fibroblasts by isoproterenol (ISO) was markedly suppressed by IRL-1038 and CPU0213. CPU0213 is more effective in relieving the over-expression of ET_AR, compared to PD156707. $n=5$. $^{**}P<0.01$ vs. Control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. ISO; $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. PD156707; $^{\&}P<0.05$, $^{\&\&}P<0.01$ vs. IRL-1038.

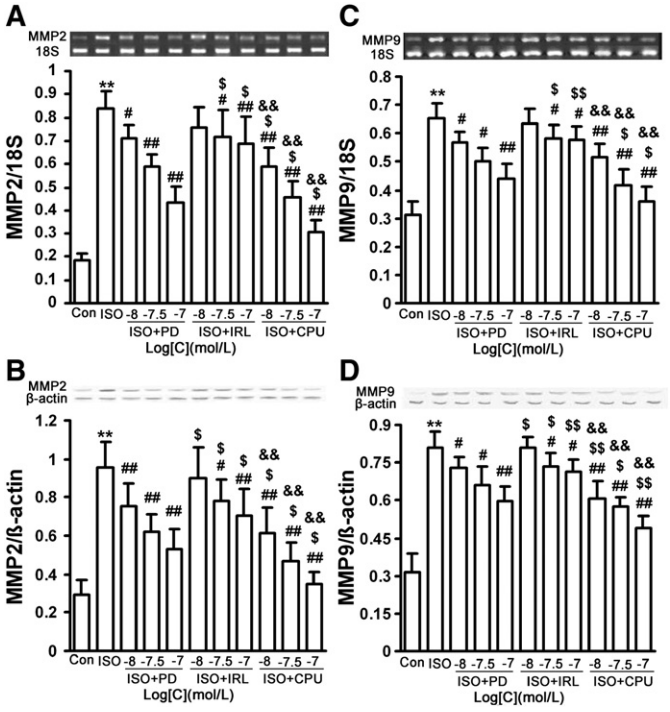

Fig. 2. Reversibility of endothelin receptor antagonists on expression of MMP2/9 in cardiac fibroblasts. Upregulated mRNA and protein expression of MMP2 (A, B) and MMP-9 (C, D) were found in cardiac fibroblast responsive to isoproterenol. PD156707 significantly relieved these changes in dose-dependent manner and was more effective as compared with IRL-1038. CPU0213 was predominant in suppressing these changes, more potent as compared with either PD156707 or IRL-1038. $n=5$. $^{**}P<0.01$ vs. Control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. ISO; $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. PD156707; $^{\&\&}P<0.01$ vs. IRL-1038.

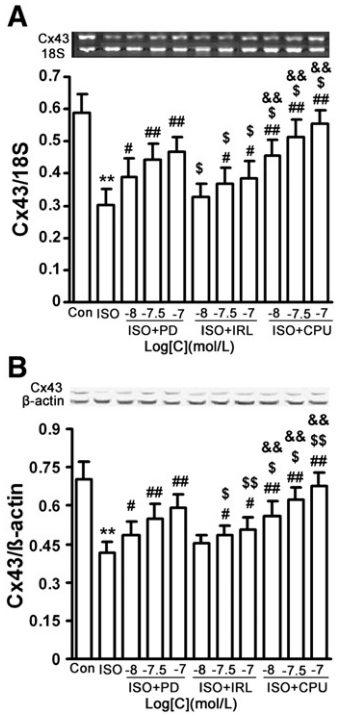

Fig. 3. Reversibility of endothelin receptor antagonists on expression of connexin 43 (Cx43) in cardiac fibroblasts. Downregulation of mRNA (A) and protein (B) expression of Cx43 in cardiac fibroblasts induced by isoproterenol is attenuated by selective ETAR antagonist PD156707 in concentration-dependent way. IRL-1038 is less effective. However, a recovery by CPU0213 is more dominant as compared with either PD156707 or IRL-1038. $n=5$. $^{**}P<0.01$ vs. Control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. ISO $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. PD156707; $^{\&\&}P<0.01$ vs. IRL-1038.

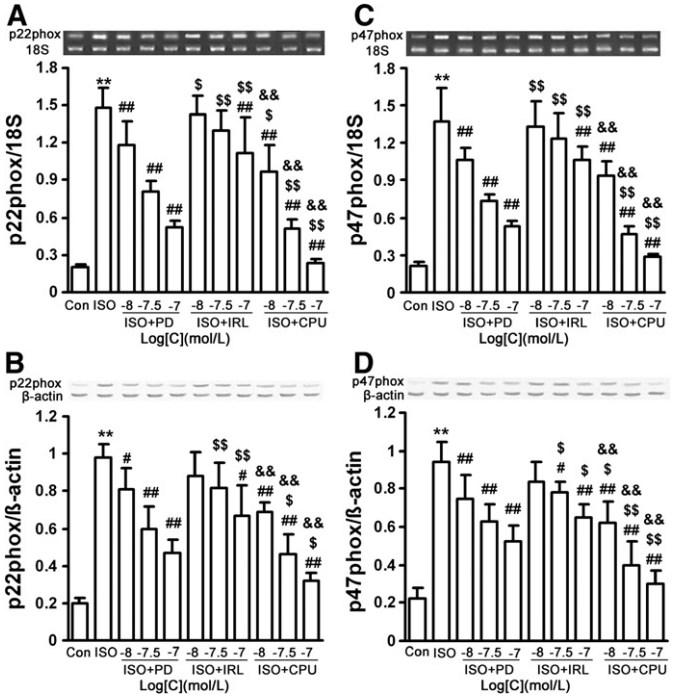

Fig. 4. Effects of endothelin receptor antagonists on expression of p22phox and p47phox in cardiac fibroblasts. The high expression of p22phox (A, B) and p47phox (C, D) by isoproterenol is potently relieved by PD156707, and less recovered by IRL-1038. CPU0213 was more effective in relieving these changes as compared to either PD156707 or IRL-1038. $n=5$. $^{**}P<0.01$ vs. Control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. ISO; $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. PD156707; $^{\&\&}P<0.01$ vs. IRL-1038.

p47phox was significant in the presence of isoproterenol and was suppressed by the three endothelin receptor antagonists. PD156707 was more potent than IRL-1038 in normalizing these changes. A dual receptor antagonist, CPU0213 also suppressed these changes (Fig.4A and B, C and D) and was more effective than either selective receptor antagonist alone, thus paralleling the effects of antagonists on connexin 43 expression.

4. Discussion

To date, the separate roles for the endothelin A and B receptors in the remodeling of cardiac fibroblasts have not been explored. In the present study, upregulation of MMP-2/9, and NADPH oxidase p22phox and p47phox and downregulation of connexin 43 were found in cardiac fibroblasts stimulated by isoproterenol. These changes were effectively blocked by both selective and non-selective endothelin receptor antagonists.

Abnormal matrix metalloproteinases adversely impact cardiac function (Liu and Dai, 2004), and are involved in such diabetic complications as cardiomyopathy (Na et al., 2007), nephropathy (Liu et al., 2008) and diabetic testopathy (Zhang et al., 2009), due to extracellular matrix remodeling (Atance et al., 2004). MMP-2/9 is a gelatinase that degrades basement membrane proteins (Malla et al., 2008; Ahmed et al., 2006) and abnormal MMP-2/9 in the myocardium impairs electrical signal conduction between neighboring cells, contributing to arrhythmogenesis by expanding spatially dispersed biomarkers (Cheng et al., 2009). In the present study, upregulation of MMP-2/9 in cardiac fibroblasts following exposure to isoproterenol was significant and substantially reduced by the three endothelin receptor antagonists, a finding consistent with the reversal by CPU0213 of abnormal MMP-2/9 expression in thyroxin-induced cardiomyopathy (Liu and Dai, 2004).

Cardiac fibroblasts are coupled through gap junctions to each other as well as to myocytes (Kohl et al., 2005). Normal gap junctional communications depends on connexin 43 which conducts electrical excitation between adjacent cells. Heterogeneous expression of connexin 43 is associated with heart rhythm disturbance (Kohl, 2003) and downregulated connexin 43 appears to be the most prominent features of gap junction remodeling linked with high risk of malignant arrhythmias. Downregulation of connexin 43 in cardiac fibroblasts by isoproterenol may play a partial role in cardiac dysrhythmia by distorting the electrophysiological properties of the myocardium. Emerging findings indicate that higher level of myocardial connexin 43 expression is linked with lower lethal arrhythmia susceptibility (Quan et al., 2009) and vice versa. The low expression of connexin 43 indicates insufficient intracellular communication leading to predisposition to life-threatening arrhythmias in spontaneously hypertensive rats (Knezl et al., 2008). Consequently, abnormal connexin 43 is connected to marked non-uniform anisotropy causing an increase in dispersion of repolarization, in favor of the genesis of impulse reentry eventually leading to severe arrhythmias. Intracellular communication in the myocardium is sensitive to oxidative stress (Berthoud and Beyer, 2009), and to change in endothelin receptors.

Endothelin A and B receptors, which have been identified in cardiac fibroblasts, are upregulated in diabetic cardiomyopathy (Qi et al., 2006) and nephropathy (Xu et al., 2009). Upon activation the endothelin receptors initiate intracellular signaling via protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) cascades (Husse et al., 2007; Yan-ping et al., 2008). These pathways are likely implicated in the maladaptive responses of cardiac fibroblasts to isoproterenol correlating to changes in the expression of MMPs and connexin 43. Connexin 43 is the target protein implicated in PKC, PKG, protein tyrosine kinases, and MAPK pathways (Schulz and Heusch, 2004). Endothelin induces production of reactive oxygen species in myocytes (Rich and McLaughlin, 2003; Dong et al., 2006) and in renal mesangial cells (Xu et al., 2009).

We highlight the modulating activity of endothelin receptors on NADPH oxidase through their antioxidant activity, in accord with findings in cultured cardiomyocytes (Li et al., 2003; Li et al., 2008). Activated endothelin receptors contribute separate roles to the remodeling of cardiac fibrosis, and possibly participate in pathologies of arrhythmogenesis and cardiac failure (Clozel and Salloukh, 2005).

Discussion continues as to whether selective blockade of endothelin A receptor or a non-selective blockade of both receptors is more beneficial. A potential role for endothelin B receptor in the pathologies of cardiovascular disease is thus of great interest. Under pathological conditions, upregulation of endothelin B receptor, which may exert adverse effects in the cardiovascular system, is always accompanied by upregulation of endothelin A receptor (Qi et al., 2006; Xu et al., 2008). In this work we found that both PD156707 and CPU0213 blunted the over-expression of endothelin A receptor, MMP2/9, p22phox and p47phox and the depressed level of connexin 43 during stimulation by isoproterenol. In this regard, CPU0213 is more effective than PD156707, possibly due to combination with a mild blockade of the endothelin B receptor. This phenomenon can be explained as follows: an auto-reinforcement mechanism is responsible for stimulating the endothelin system, and is mediated by endothelin B receptor rather than by the endothelin A receptor, possibly via the JNK pathway (Yamakaw et al., 2002). This emphasizes that the endothelin B receptor exerts a definite, but mild, role in pathologies implicated in stress. In this connection, knockout of both endothelin receptors was more effective in producing hypertension (Ge et al., 2008). Impaired cardiac contractility by an excess of leptin is blunted by BQ123 and BQ788, selective blockers for endothelin A and B receptor respectively, through suppression of the endothelin–NADPH oxidase pathway (Dong et al., 2006).

In conclusion, downregulation of connexin 43 and upregulation of MMP-2/9 is found in isoproterenol stimulated cardiac fibroblasts, and may contribute to arrhythmogenesis and cardiac insufficiency. These changes are possibly mediated via the endothelin–NADPH oxidase pathway. Activation of endothelin B receptor contributes, but is of lesser significance than the endothelin A receptor. In normalizing changes in cardiac fibroblasts to isoproterenol a dual endothelin receptor antagonism is likely more promising than a selective endothelin A receptor antagonism.

Acknowledgements

This study is supported by National Basic Research Program of China (973 Program. 2007CB512000/2007CB512006). We thank Professor David Triggle, from the State University of New York, for his help with English styling of the manuscript.

References

Ahmed, S.H., Clark, L.L., Pennington, W.R., Webb, C.S., Bonnema, D.D., Leonardi, A.H., McClure, C.D., Spinale, F.G., Zile, M.R., 2006. Matrix metalloproteinases/tissue inhibitors of metalloproteinases: relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease. Circulation 113, 2089–2096.

Atance, J., Yost, M.J., Carver, W., 2004. Influence of the extracellular matrix on the regulation of cardiac fibroblast behavior by mechanical stretch. J. Cell Physiol. 200, 377–386.

Batlle, M., Perez-Villa, F., Garcia-Pras, E., Lazaro, A., Orus, J., Roque, M., Roig, E., 2007. Down-regulation of matrix metalloproteinase-9 (MMP-9) expression in the myocardium of congestive heart failure patients. Transpl. Proc. 39, 2344–2346.

Berthoud, V.M., Beyer, E.C., 2009. Oxidative stress, lens gap junctions, and cataracts. Antioxid. Redox Signal. 11, 339–353.

Cheng, Y.S., Dai, D.Z., Dai, Y., 2009. Isoproterenol disperses distribution of NADPH oxidase, MMP-9, and pPKCepsilon in the heart, which are mitigated by endothelin receptor antagonist CPU0213. Acta. Pharmacol. Sin. 30, 1099–1106.

Clozel, M., Salloukh, H., 2005. Role of endothelin in fibrosis and anti-fibrotic potential of bosentan. Ann. Med. 37, 2–12.

Cui, B., Cheng, Y.S., Dai, D.Z., Li, N., Zhang, T.T., Dai, Y., 2009. CPU0213, a non-selective ET_A/ET_B receptor antagonist, improves pulmonary arteriolar remodeling of monocrotaline-induced pulmonary hypertension in rats. Clin. Exp. Pharmacol. Physiol. 36, 169–175.

Dai, D.Z., Huang, M., Ji, M., Liu, L.G., 2004. Endothelin receptor antagonist activity and selective blocking the ET_A and ET_B of compound 0213. J. Chinese Pharm. Univ. 35, 552–557.

Dong, F., Zhang, X., Ren, J., 2006. Leptin regulates cardiomyocyte contractile function through endothelin-1 receptor-NADPH oxidase pathway. Hypertension 47, 222–229.

Feng, Y., Dai, D.Z., Na, T., Cui, B., Wang, T., Zhang, Y., Dai, Y., 2007a. Endothelin receptor antagonist CPU0213 suppresses ventricular fibrillation in L-thyroxin induced cardiomyopathy. Pharmacol. Rep. 59, 306–314.

Feng, Y., Tang, X.Y., Dai, D.Z., Dai, Y., 2007b. Reversal of isoproterenol-induced downregulation of phospholamban and FKBP12.6 by CPU0213-mediated antagonism of endothelin receptors. Acta. Pharmacol. Sin. 28, 1746–1754.

Ge, Y., Bagnall, A., Stricklett, P.K., Webb, D., Kotelevtsev, Y., Kohan, D.E., 2008. Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. Am. J. Physiol. Ren. Physiol. 295, F1635–F1640.

Husse, B., Briest, W., Homagk, L., Isenberg, G., Gekle, M., 2007. Cyclical mechanical stretch modulates expression of collagen I and collagen III by PKC and tyrosine kinase in cardiac fibroblasts. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R1898–R1907.

Kelso, E.J., Spiers, J.P., McDermott, B.J., Scholfield, C.N., Silke, B., 1998. Receptor-mediated effects of endothelin on the L-type Ca^{++} current in ventricular cardiomyocytes. J. Pharmacol. Exp. Ther. 286, 662–669.

Knezl, V., Bacova, B., Kolenova, L., Mitasikova, M., Weismann, P., Drimal, J., Tribulova, N., 2008. Distinct lethal arrhythmias susceptibility is associated with sex-related difference in myocardial connexin-43 expression. Neuro. Endocrinol. Lett. 29, 798–801.

Kohl, P., 2003. Heterogeneous cell coupling in the heart: an electrophysiological role for fibroblasts. Circ. Res. 93, 381–383.

Kohl, P., Camelliti, P., Burton, F.L., Smith, G.L., 2005. Electrical coupling of fibroblasts and myocytes: relevance for cardiac propagation. J. Electrocardiol. 38, 45–50.

Li, L., Chu, Y., Fink, G.D., Engelhardt, J.F., Heistad, D.D., Chen, A.F., 2003. Endothelin-1 stimulates arterial VCAM-1 expression via NADPH oxidase-derived superoxide in mineralocorticoid hypertension. Hypertension 42, 997–1003.

Li, N., Jia, N., Dai, D.Z., Dai, Y., 2008. Endothelin receptor antagonist CPU0213 and vitamin E reverse downregulation of FKBP12.6 and SERCA2a: a role of hyperphosphorylation of PKCepsilon. Eur. J. Pharmacol. 591, 211–218.

Liu, Q., Dai, D.Z., 2004. Novel endothelin receptor antagonist CPU0213 suppresses expression of MMPs and TIMPs in hypertrophied myocardium caused by L-thyroxin. Chin. J. Clin. Pharmacol. Ther. 9, 281–284.

Liu, H.R., Tang, X.Y., Dai, D.Z., Dai, Y., 2008. Ethanol extracts of Rehmannia complex (Di Huang) containing no Corni fructus improve early diabetic nephropathy by combining suppression on the ET-ROS axis with modulate hypoglycemic effect in rats. J. Ethnopharmacol. 118, 466–472.

Maguire, J.J., Davenport, A.P., 1999. The therapeutic potential of PD156707 and related butenolide endothelin antagonists. Expert. Opin. Investig. Drugs 8, 71–88.

Malla, N., Sjoli, S., Winberg, J.O., Hadler-Olsen, E., Uhlin-Hansen, L., 2008. Biological and pathobiological functions of gelatinase dimers and complexes. Connect. Tissue Res. 49, 180–184.

Na, T., Dai, D.Z., Tang, X.Y., Dai, Y., 2007. Upregulation of leptin pathway correlates with abnormal expression of SERCA2a, phospholamban and the endothelin pathway in heart failure and reversal by CPU86017. Naunyn Schmiedebergs Arch. Pharmacol. 375, 39–49.

Poelzing, S., Akar, F.G., Baron, E., Rosenbaum, D.S., 2004. Heterogeneous connexin43 expression produces electrophysiological heterogeneities across ventricular wall. Am. J. Physiol. 286, H2001–H2009.

Polyakova, V., Miyagawa, S., Szalay, Z., Risteli, J., Kostin, S., 2008. Atrial extracellular matrix remodelling in patients with atrial fibrillation. J. Cell Mol. Med. 12, 189–208.

Qi, M.Y., Xia, H.J., Dai, D.Z., Dai, Y., 2006. A novel endothelin receptor antagonist CPU0213 improves diabetic cardiac insufficiency attributed to up-regulation of the expression of FKBP12.6, SERCA2a, and PLB in rats. J. Cardiovasc. Pharmacol. 47, 729–735.

Quan, X.Q., Bai, R., Lu, J.G., Patel, C., Liu, N., Ruan, Y., Chen, B.D., Ruan, L., Zhang, C.T., 2009. Pharmacological enhancement of cardiac gap junction coupling prevents arrhythmias in canine LQT2 model. Cell Commun. Adhes. 23, 1–10.

Rich, S., McLaughlin, V.V., 2003. Endothelin receptor blockers in cardiovascular disease. Circulation 108, 2184–2190.

Rodrigo, R., Vinay, J., Castillo, R., Cereceda, M., Asenjo, R., Zamorano, J., Araya, J., Castillo-Koch, R., Espinoza, J., Larrain, E., 2009. Use of vitamins C and E as a prophylactic therapy to prevent postoperative atrial fibrillation. Int. J. Cardiol. 14 Epub ahead of print.

Roldan, V., Marin, F., Gimeno, J.R., Ruiz-Espejo, F., Gonzalez, J., Feliu, E., 2008. Matrix metalloproteinases and tissue remodeling in hypertrophic cardiomyopathy. Am. Heart J. 156, 85–91.

Saffitz, J.E., Douglas, P., 2006. Zipes Lecture. Biology and pathobiology of cardiac connexins: from cell to bedside. Heart Rhythm 3, 102–107.

Schulz, R., Heusch, G., 2004. Connexin 43 and ischemic preconditioning. Cardiovasc. Res. 62, 335–344.

Shimano, M., Shibata, R., Inden, Y., Yoshida, N., Uchikawa, T., Tsuji, Y., Murohara, T., 2009. Reactive oxidative metabolites are associated with atrial conduction disturbance in patients with atrial fibrillation. Heart Rhythm 6, 935–940.

Wang, H.L., Dai, D.Z., Gao, E., Zhang, Y.P., Lu, F., 2004. Dispersion of ventricular mRNA of RyR2 and SERCA2 associated with arrhythmogenesis in rats. Acta. Pharmacol. Sin. 25, 738–743.

Xia, H.J., Dai, D.Z., Dai, Y., 2006. Up-regulated inflammatory factors endothelin, NFkappaB, TNFalpha and iNOS involved in exaggerated cardiac arrhythmias in L-thyroxine-induced cardiomyopathy are suppressed by darusentan in rats. Life Sci. 79, 1812–1819.

Xu, J., Li, N., Dai, D.Z., Yu, F., Dai, Y., 2008. The endothelin receptor antagonist CPU0213 is more effective than aminoguanidine to attenuate isoproterenol-induced vascular abnormality by suppressing overexpression of NADPH oxidase, ETA, ETB, and MMP9 in the vasculature. J. Cardiovasc. Pharmacol. 52, 42–48.

Xu, M., Dai, D.Z., Dai, Y., 2009. Normalizing NADPH oxidase contributes to attenuating diabetic nephropathy by the dual endothelin receptor antagonist CPU0213 in rats. Am. J. Nephrol. 29, 252–256.

Yamakaw, K., Kitamura, K., Nonoguchi, H., Takasu, N., Miller, R.T., Tomita, K., 2002. Galpha13 induces preproET-1 gene expression via JNK. Hypertens. Res. 25, 427–432.

Yan-ping, H., Lian-you, Z., Qiang-sun, Z., Shao-wei, L., Xiao-yan, Z., Xiao-long, L., 2008. Mitogenic effect of arginine vasopressin on adult rat cardiac fibroblast: involvement of PKC-erk1/2 pathway. J. Cardiovasc. Pharmacol. 52, 72–81.

Yu, F., Dai, D.Z., An, L.F., Guo, X.F., 1997. Heart hypertrophy induced by levothyroxine aggravates ischemic lesions and reperfusion arrhythmias in rats. Zhong guo yao li xue bao 18, 71–74 fromer name for Acta Pharmacol Sin.

Zhang, Y., Huang, Z.J., Dai, D.Z., Feng, Y., Na, T., Tang, X.Y., Dai, Y., 2008. Downregulated FKBP12.6 expression and upregulated endothelin signaling contribute to elevated-diastolic calcium and arrhythmogenesis in rat cardiomyopathy produced by L-thyroxin. Int. J. Cardiol. 130, 463–471.

Zhang, Q., Liu, H.R., Ying, H.J., Dai, D.Z., Tang, X.Y., Dai, Y., 2009. Strontium fructose 1, 6-diphosphate alleviates early diabetic testopathy by suppressing abnormal testicular matrix metalloproteinase system in streptozocin-treated rats. J. Pharm. Pharmacol. 61, 229–236.

Zhong, J.Q., Zhang, W., Gao, H., Li, Y., Zhong, M., Li, D., Zhang, C., Zhang, Y., 2007. Changes in connexin 43, metalloproteinase and tissue inhibitor of metalloproteinase during tachycardia-induced cardiomyopathy in dogs. Eur. J. Heart Fail. 9, 23–29.