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Protective effect of the endothelin antagonist CPU0213 against isoprenaline-induced heart failure by suppressing abnormal expression of leptin, calcineurin and SERCA2a in rats

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Abstract

Heart failure (HF) may be produced by sustained β-adrenoceptor stimulation by causing changes in the expression of endothelin-1 (ET-1), the leptin system, calcineurin and sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) underlying cardiac dysfunction. The aim of this study was to verify whether isoprenaline (ISO)-induced HF is attributed to changes in the above molecular markers, and whether the dual ET-receptor antagonist CPU0213 could reverse the cardiac dysfunction caused by ISO treatment, focusing on these molecular markers. HF was induced in rats by administration of ISO (2 mg kg^{-1} s.c.) for 10 days. CPU0213 (30 mg kg^{-1} s.c.) and propranolol (4 mg kg^{-1} s.c.) were administered on days 7–10. HF developed after 10 days' ISO administration and was manifest as impaired cardiac performance, increased heart weight index, oxidative stress, elevated serum enzymes, and disordered expression of the endothelin system, leptin system, calcineurin and SERCA2a. All these abnormalities were significantly reversed by CPU0213, and the effectiveness of this ET-receptor antagonist was comparable to that of propranolol. Thus, antagonism of ET receptors by CPU0213 normalizes these changes in molecular markers, alleviating HF.

Introduction

Myocardial injury can be caused by sustained activation of β-adrenoceptors (β-AR) (Kiss et al 2004), which contributes to heart failure (HF) by impairing molecular biomarkers. Emerging data have suggested that pro-inflammatory cytokines are involved in the mechanisms that underlie HF resulting from β-AR activation.

Excessive endothelin-1 (ET-1) is involved in catecholamine-induced myocardial remodelling (Turner et al 2004) and plays an active role in the pathology of HF (Ostrowski et al 2003). It has been found that β-AR blockade reduces ET-1 release in coronary smooth muscle and vascular endothelial cells (Brehm et al 2000). Data collected in our previous study demonstrated that downregulation of phospholamban and FKBP12.6 (calstabin-2) by β-AR stimulation can be suppressed substantially by darusentan, a selective ET-receptor antagonist (Feng et al 2007). It is therefore suggested that an abnormal ET signalling pathway may mediate the biological molecular events linked to β-AR stimulation that produce the molecular markers in injured myocardium contributing to HF.

Leptin is secreted by adipocytes and acts on the leptin receptor (OB-Rb) in the hypothalamus to regulate appetite and energy metabolism (Ahima & Osei 2004). Leptin is also released by cardiomyocytes, and its long-form receptor OB-Rb has been identified in the myocardium (Purdham et al 2004). Clinical evidence has shown that leptin can be regarded as a molecular marker for the diagnosis of cardiovascular disease (Schulze et al 2003). We recently demonstrated that leptin protein and OB-Rb mRNA are upregulated in a failing rat heart created by coronary artery ligation (Na et al 2007). In addition, increased leptin can be modulated by sympathetic stimulation in obesity hypertension (Hall et al 2001). As a cytokine, leptin may stimulate upregulation of calcineurin (Morishita et al 1998), which is actively involved in hypertrophy and myocardial injury induced by β-AR stimulation (Macdonnell et al 2007). Leptin also activates the ET-1–reactive oxygen species (ROS) system to promote myocardial hypertrophy (Xu et al 2004). The increase in contractility of cardiomyocytes caused by leptin

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is probably mediated by the ET–ROS (NADPH oxidase) pathway (Dong et al 2006). Cardiomyocyte hypertrophy induced by ET-1 may be mediated by leptin (Rajapurohitam et al 2006). The ET-receptor antagonist bosentan decreases plasma leptin concentration and improves cardiac function in acutely infarcted heart (Ostrowski et al 2003).

β-blockers are used in the treatment of chronic heart failure (CHF). Myocardial injury can be induced experimentally by isoprenaline (ISO); abnormal ET pathways, leptin, calcineurin and sarcoplasmic reticulum (SR) Ca^{2+} ATPase 2a (SERCA2a) may be implicated in this damage. It is interesting to explore whether blocking the ET system could alleviate the deterioration in cardiac function induced by β-AR activation. We hypothesized that myocardial injury caused by sustained β-AR stimulation may be mediated via abnormal ET–ROS pathways, leptin, calcineurin and SERCA2a in the myocardium, and that these changes could be reversed by blockade of ET receptors. CPU0213 is a potent dual ET_AR and ET_BR antagonist (Dai et al 2004) and shows benefit in treating diabetic cardiomyopathy (Qi et al 2006) and pulmonary artery hypertension (Cui et al 2007). In this paper we intended to verify the above hypothesis by testing the effectiveness of CPU0213 in alleviating ISO-induced HF by suppressing the molecular changes listed above.

Materials and Methods

Animals and procedures

All experiments were carried out by personnel who were permitted to handle laboratory animals, and complied with Guidelines for the Care and Use of Laboratory Animal in Jiangsu Province, People's Republic of China.

Male Sprague–Dawley rats weighing 220–250 g were supplied by the Animal Center of Nanjing Medical University. Forty rats were randomly assigned into four groups of ten.

Heart failure (HF) was induced by ISO ($2\,mg\,kg^{-1}$ s.c. daily) for 10 days in three groups. One group of ISO-treated rats were given CPU0213 ($30\,mg\,kg^{-1}$ s.c.; provided by the Center of New Drug Discovery, China Pharmaceutical University) and another group were given propranolol ($4\,mg\,kg^{-1}$ s.c.) daily on days 7–10. Normal and ISO-treated rats were given injections of an equal volume of the vehicle (0.5% sodium carboxymethylcellulose). Rats were allowed free access to the regular chow and water. The operation was carried out on the 11th day.

Cardiac hypertrophy and haemodynamics

Rats were fasted overnight and the measurement of haemodynamics was conducted as described previously (Wang et al 2004). In brief, after surgery under urethane anaesthesia ($1.5\,g\,kg^{-1}$ i.p.), the right carotid artery was cannulated and connected to a pressure transducer (MAP-V, Second Military Medical University, Shanghai, China) to measure left ventricular (LV) systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), the maximal uprising rate of LV systolic pressure (LV+dP/dtmax) and the maximal declining rate of LV diastolic pressure (LV−dP/dtmax). The heart rate (HR) was monitored by lead II ECG.

After the experiment, blood samples were collected via the cannulae in the right common carotid, and hearts were dissected rapidly, rinsed with ice-cold saline and weighed. The whole heart and the left ventricle, including the septum, were weighed and the heart weight/body weight (HW/BW, $mg\,kg^{-1}$) and left ventricle weight/body weight (LVW/BW, $mg\,kg^{-1}$) ratios calculated as indices of ventricular hypertrophy. The left ventricle was snap-frozen in liquid nitrogen and stored until further processing.

Biochemical assays

Blood samples were centrifuged ($4000\,rev\,min^{-1}$, 10 min, 4°C) and a sample of serum was used for the assay (n = 10).

A portion of the left ventricle (100 mg) was homogenized in normal saline and the supernatant collected for biochemical measurements. Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), and myocardial malondialdehyde (MDA), superoxide dismutase (SOD) and nitric oxide (NO) were measured using commercially available kits (Jiancheng Bio-engineering Company, Nanjing, China) (Wang et al 2004).

mRNA expression by RT-PCR

Reverse transcriptase polymerase chain reaction (RT-PCR) was conducted as described previously (Na et al 2007). Reagents were from Promega Corporation (Madison, WI, USA). Oligonucleotides for the primers were synthesized by Invitrogen (Shanghai, China). Total RNA was extracted from frozen ventricular muscle (n = 5) using Trizol reagent, according to the manufacturer's instructions; 5 μg RNA was used to synthesize the first strand of cDNA used as a template in the following PCR reactions (Eppendorf Mastercycler, Germany): collagen I, prepro-endothelin-1 (ppET-1), endothelin A receptor (ET_AR), endothelin B receptor (ET_BR), OB-Rb, collagen 1, SERCA2a and calcineurin. Samples were then standardized by concomitant expression of 18S. The density of the bands was analysed with Labworks imaging acquisition and analysis software (Genegenus, Syngene, Cambridge, UK).

Western blotting

For the quantitative analysis of protein levels of ET_AR, leptin and SERCA2a in the myocardium, the LV tissue (100–200 mg) was homogenized in four volumes of extraction buffer and centrifuged at 10000 g for 10 min, as described previously (n = 5) (Na et al 2007). After determining the protein concentration, supernatants were stored at −20°C until use. A sample was heated to 95°C and size fractionated by SDS-PAGE. Following transfer to nitrocellulose and blocking with non-fat milk (5% w/v), plates were incubated first with antibody to ET_AR, leptin or SERCA2a (1:500, Santa Cruz Biotechnology, Inc, CA, USA) for 1 h. After three washes, the blot was incubated with the second antibody, horseradish peroxidase-conjugated goat immunoglobulin G (1:500, Santa Cruz Biotechnology) for 1 h. Antigen was detected with a 3,3′-diaminobenzidine kit (Wuhan Boster Biological Technology, Wuhan, China). A linear relationship between blot density and protein load was observed when 20, 40, 60, 80, and 100 μg of membrane protein were used per lane.

Statistical analysis

Data are expressed as mean ± s.d. One-way analysis of variance was performed, and the Student–Newman–Keuls test was used to determine the statistical differences between the two means, which was considered significant at $P < 0.05$.

Results

Cardiac hypertrophy and myocardial collagen

Significant ventricular hypertrophy occurred in ISO-treated rats, assessed by either HW/BW ($P < 0.05$) or LVW/BW ($P < 0.01$), compared with control rats. CPU0213 significantly inhibited this increase in ventricular mass ($P < 0.05$) compared with the untreated group, to an extent comparable to that with propranolol ($P < 0.01$ vs HF group) (Table 1).

Myocardial fibrosis may be involved in ISO-induced HF and was evaluated using expression of collagen I mRNA. Upregulation of collagen I mRNA was significant in HF rats ($P < 0.01$) compared with controls. Treatment with either CPU0213 ($P < 0.05$) or propranolol ($P < 0.01$) markedly decreased the upregulation of collagen I mRNA, compared with the untreated HF group (Figure 2A).

Heart rate and haemodynamics

Myocardial injury due to large doses of ISO was manifest as increase in HR by 12% compared with the control group ($P < 0.05$), and compromised cardiac performance, characterized by depressed systolic (LVSP and LV + dp/dtmax) and diastolic (LVEDP and LV – dp/dtmax) functions ($P < 0.01$) compared with control rats. These effects of ISO were reversed by propranolol ($P < 0.01$), confirming that they are due to sustained activation of β-AR. Interestingly, blockade of ET receptors by CPU0213 was sufficient to reverse the changes induced by β-AR activation ($P < 0.05$), compared with untreated HF rats, and the benefit were comparable to that seen with propranolol (Table 2).

Serum enzymes

Serum from ISO-induced HF rats showed significant increases in the activities of CPK and LDH, by 136% ($P < 0.01$) and 42% ($P < 0.01$) compared with the control rats, respectively (Table 1). These elevated enzyme activities in serum were markedly suppressed by CPU0213 ($P < 0.05$) and propranolol ($P < 0.01$).

The redox system

Changes in the redox system were significant, indicating a status of oxidative stress in ISO-injured myocardium. Production of MDA was increased in the untreated ISO-induced HF group by 142% ($P < 0.01$; Table 1), and the activity of SOD was decreased by 44% ($P < 0.01$; Table 1) compared with the control group. In addition, treatment with ISO resulted in a significant increase in the NO level relative to control rats ($P < 0.01$; Table 1). CPU0213 treatment markedly ameliorated the impaired redox system in the myocardium ($P < 0.01$).

Table 1 Changes in heart weight indices, serum/myocardial enzymes and the redox system in isoprenaline-induced heart failure (HF) in rats are improved by propranolol (Pro, 4 mg kg^{-1} s.c) or CPU0213 (30 mg kg^{-1} s.c.) daily for 4 days

Group	LVW/BW[a]	HW/BW	CPK	LDH	MDA	SOD	NO
Control	2.32 ± 0.30	3.31 ± 0.48	1.22 ± 0.34	4917.65 ± 528.76	2.30 ± 0.50	217.97 ± 11.18	1.29 ± 0.45
HF	2.99 ± 0.20**	3.87 ± 0.25**	2.88 ± 0.41**	7000.00 ± 603.34**	5.56 ± 0.47**	121.16 ± 29.55**	3.94 ± 0.44‡
HF + Pro	2.55 ± 0.24‡	3.41 ± 0.17‡	1.67 ± 0.41‡	5254.90 ± 550.14‡	3.71 ± 0.50‡	172.79 ± 20.87‡	2.29 ± 0.35‡
HF + CPU0213	2.62 ± 0.25†	3.51 ± 0.24†	2.22 ± 0.53†	6000.00 ± 769.67†	4.17 ± 0.47‡	158.35 ± 14.54†	2.67 ± 0.51‡

[a]LVW/BW, left ventricle weight/body weight ratio (mg kg^{-1}); HW/BW, heart weight/body weight ratio (mg kg^{-1}); CPK, creatine phosphokinase (serum; U mol^{-1}); LDH, lactate dehydrogenase (serum; U L^{-1}); MDA, malondialdehyde (myocardial; nmol mg^{-1} protein)); SOD, superoxide dismutase (myocardial; U mg^{-1} protein); NO, nitric oxide (myocardial; μmol g^{-1} protein).
Data are mean ± s.d. (n = 10). *$P < 0.05$, **$P < 0.01$ vs control; $^{†}P < 0.05$, $^{‡}P < 0.01$ vs HF group.

Table 2 Changes in heart rate and haemodynamics in isoprenaline-induced heart failure (HF) in rats are improved by propranolol (Pro, 4 mg kg^{-1} s.c.) or CPU0213 (30 mg kg^{-1} s.c.) daily for 4 days

Group	HR (beats min^{-1})	LVSP (Kpa)	LVEDP (Kpa)	LV + dP/dtmax (Kpa s^{-1})	LV – dP/dtmax (Kpa s^{-1})
Control	350 ± 37	16.8 ± 2.3	0.94 ± 0.13	1037 ± 91	–661 ± 97
HF	392 ± 17*	13.4 ± 1.3**	1.52 ± 0.21**	846 ± 79**	–476 ± 66**
HF + Pro	354 ± 26†	15.8 ± 1.3‡	1.15 ± 0.15‡	972 ± 86†	–612 ± 95†
HF + CPU0213	365 ± 22†	15.2 ± 1.3†	1.25 ± 0.16†	943 ± 58†	–579 ± 67†

HR, heart rate; LVSP, left ventricular (LV) systolic pressure; LVEDP, LV end-diastolic pressure; LV + dP/dtmax, the maximal uprising rate of LVSP; LV – dP/dtmax, the maximal declining rate of LV diastolic pressure.
Data are mean ± s.d. (n = 10) *$P < 0.05$,**$P < 0.01$ vs control; $^{†}P < 0.05$, $^{‡}P < 0.01$ vs HF group.

The endothelin signalling pathway

We explored changes in expression of ppET-1 and ET receptors in the myocardium following treatment with ISO. Expression of ppET-1 mRNA was upregulated by 247% ($P<0.01$), ET_AR mRNA by 128% ($P<0.01$), and ET_BR mRNA by 261% ($P<0.01$) relative to the control group (Figure 1). In addition, a separate experiment was conducted to measure levels of ET_AR protein in the myocardium, which was also significantly increased ($P<0.05$) relative to the control group (Figure 1D). Treatment with either CPU0213 or propranolol suppressed the increased expression of ppET-1 and the two ET receptors towards control levels (Figure 1).

The leptin system

The leptin system in the myocardium was targeted to explore the effects of ISO in worsening cardiac function that can be corrected with CPU0213 treatment. Myocardial expression of the leptin receptor OB-Rb mRNA was increased by 230% ($P<0.01$) in the ISO-injured myocardium compared with the control group (Figure 2B). Furthermore, Western blotting showed that levels of leptin protein were increased two-fold in ISO-induced cardiomyopathic heart ($P<0.01$) (Figure 2C). CPU0213 and propranolol treatment significantly attenuated abnormalities of the leptin system in the ISO-injured myocardium.

The Ca^{2+} handling system

Significantly compromised diastolic and systolic function in the ISO-injured myocardium may be due to changes in the uptake of intracellular calcium by the SR. We measured mRNA and protein levels of the Ca^{2+} handling system in the myocardium. Changes in mRNA (Figure 3B) and protein

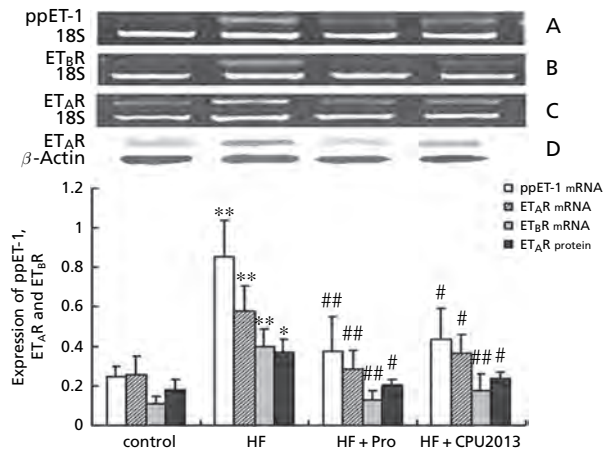

Figure 1 Upregulation of prepro-endothelin-1 (ppET-1; A), endothelin B and A receptors (ET_BR; B) and ET_AR; C) mRNA, and levels of ET_AR protein (D) in left ventricular tissue from rats with isoprenaline-induced heart failure (HF) was reversed by CPU0213 (30 mg kg^{-1} s.c. daily for 4 days) or propranolol (Pro; 4 mg kg^{-1} s.c. for 4 days). Data are mean ± s.d. (n = 5) of the ratio of the level of protein or mRNA to that of β-actin or 18S, respectively. $^{*}P<0.05$, $^{**}P<0.01$ vs control group; $^{\#}P<0.05$; $^{\#\#}P<0.01$ vs HF group.

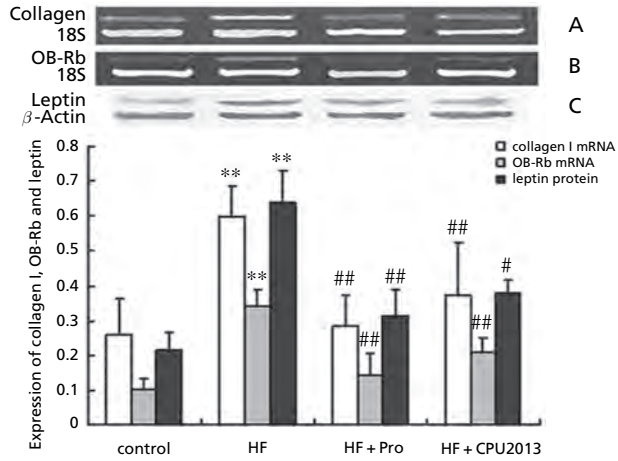

Figure 2 Upregulation of collagen I mRNA (A), OB-Rb mRNA (B) and leptin protein levels (C) in left ventricular tissue from rats with isoprenaline-induced heart failure (HF) was suppressed by CPU0213 (30 mg kg^{-1} s.c. daily for 4 days) or propranolol (4 mg kg^{-1} s.c. for 4 days). Data are mean ± s.d. (n = 5) of the ratio of the level of protein or mRNA to that of β-actin or 18s, respectively. $^{**}P<0.01$ vs control, $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs HF group.

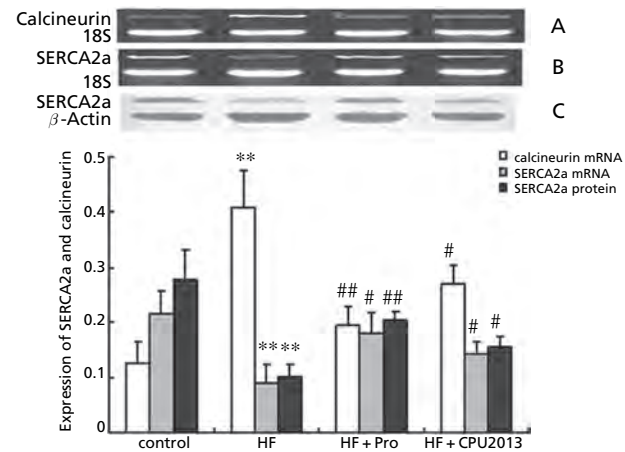

Figure 3 Changes in calcineurin mRNA (A), and mRNA expression (B) and protein expression (C) of sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) in rats with isoprenaline-induced heart failure (HF) were reversed by CPU0213 (30 mg kg^{-1} s.c. daily for 4 days) or propranolol (Pro; 4 mg kg^{-1} s.c. for 4 days). Data are mean ± s.d. (n = 5) of the ratio of the level of protein or mRNA to that of β-actin or 18S, respectively. $^{**}P<0.01$ vs control, $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs HF group.

abundance (Figure 3C) of SERCA2a were decreased by 58% and 63%, respectively, compared with the control group ($P<0.01$). An increase in Ca^{2+} during diastole is indicated by decreased expression of SERCA2a; this phenomenon is likely to be associated with a change in levels of calcineurin. Thus, we compared levels of calcineurin mRNA between the four groups. Calcineurin was increased by 1.4-fold compared with the control group ($P<0.01$) (Figure 3A). Following treatment with CPU0213 or propranolol, abnormal levels of SERCA2a mRNA and protein were completely abolished ($P<0.05$). In

addition, a reversal of calcineurin mRNA levels was also found with both CPU0213 ($P < 0.05$) and propranolol treatment ($P < 0.01$) compared with the HF rats.

Discussion

Heart failure is a syndrome typically characterized by a progressive loss in systolic (contractility and ejection fraction) and diastolic function, ventricular chamber dilatation and myocardial remodelling. Profound β-AR activation serves as an important aetiological factor in CHF, and β-blockers are widely used in the treatment of CHF (Yancy 2007). Sustained activation of β-AR by ISO impairs cardiac performance due to the induction of inflammatory factors and cytokines that mediate myocardial damage. In the present study, besides manifesting systolic and diastolic dysfunction in the ISO-treated heart, elevated production of collagen I may indicate an early sign of myocardial fibrosis by upregulation of collagen 1 mRNA in conjunction with an increase in cardiac weight index. These were reversed by treatment with propranolol, indicating that these changes are the consequence of β-AR stimulation. The ET-receptor antagonist CPU0213 proved to have comparable efficacy to propranolol in relieving cardiac insufficiency. In preclinical studies, selective ET_AR antagonists as well as non-selective (dual) $ET_{A/B}R$ antagonists have been shown to be effective in essential hypertension, pulmonary hypertension, heart failure and atherosclerosis (Iqbal et al 2005; Motte et al 2006).

Activation of the ET pathway may underlie the molecular mechanisms of cardiac insufficiency mediated via β-AR activation. In the present study, we demonstrated that upregulation of ppET-1 and two subtypes of ET receptors was associated with compromised cardiac performance. These changes are in line with findings following myocardial infarction (Na et al 2007). Myocardial ET_BR mRNA was also increased, but less than ET_AR, which is in accordance with previous reports (Motte et al 2006). It may indicate synergism between activation of the ET pathway and the β-AR–cAMP pathway in the ISO-affected myocardium. We demonstrated that ISO could downregulate mRNA and protein levels of phospholamban in cardiomyocytes, effects that are reversed by darusentan, a selective blocker, or CPU0213, a dual non-selective ET blocker (Feng et al 2007). Thus, activation of the β-AR–cAMP pathway may synergize with activation of the ET system in the myocardium.

Activation of OB-Rb and upregulation of leptin, which are attributed to activation of the β-AR–cAMP pathway, do harm to the myocardium in the development of HF. Leptin is released in response to ISO treatment and exerts negative effects on the myocardium via activation of OB-Rb, in agreement with a previous report (Purdham et al 2004). The activity of adenylate cyclase is profoundly affected by leptin, indicating that leptin plays a significant part in pathologies relating to β-AR stimulation (Illiano et al 2002). Changes in leptin and OB-Rb in damaged myocardium are in line with hyperleptinaemia caused by myocardial infarction (Xia et al 2006; Na et al 2007). Interestingly, the elevated expression of leptin and its receptor are prevented by CPU0213 treatment, as with propranolol. The results further support the theory that ET-1 may stimulate leptin production in both non-cardiac (Xiong et al 2001) and cardiac tissue (Rajapurohitam et al 2006). It has been suggested that nuclear factor (NF)-κB and the MAPK family are involved in hypertrophic responses of the myocardium to ET-1 or leptin (Cook et al 2003; Rajapurohitam et al 2003; Irukayama-Tomobe et al 2004; Lappas et al 2005). As a pro-inflammatory factor, leptin regulates cardiac function in a paracrine or autocrine fashion in both physiological and pathophysiological states. Leptin induces cardiomyocyte hypertrophy through various intracellular signalling cascades, including the JAK/STAT, p38 MAPK, ERK and PI3K/Akt pathways (Nickola et al 2000; Irukayama-Tomobe et al 2004; Xu et al 2004). However, the biological effects of leptin on the myocardium are probably varied. Leptin administration to leptin-deficient obese ob^-/ob^- mice reduces cardiac hypertrophy, indicating that leptin may have an anti-hypertrophic effect (Barouch et al 2003). Thus, leptin may exert a dual effect on the myocardium. At low concentrations it is essential to maintain the normal structure of the myocardium. However, excessive leptin has exerted adverse effects by promoting the genesis of ET-1 and ROS (Xu et al 2004).

The redox system of the myocardium is greatly disturbed by ISO treatment, exhibiting an increase in MDA and a reduction in SOD. Increased NO may result from an increase in the expression and activity of inducible NO synthase (iNOS) in the myocardium, as discovered in diabetic cardiomyopathy (Qi et al 2006). Thus, elevation of NO levels via iNOS may also indicate an increase in cytokines and oxidants in the affected myocardium (Sun et al 2005). The upregulation of the ET pathway contributes to oxidative stress by producing more hydroxyl radicals (Mundy et al 2007). Thus, the attenuation of oxidative stress by antioxidants offers a relief to injury caused by chronic β-AR stimulation (Ishizawa et al 2006); antioxidant activity mediated by ET receptor blockade by CPU0213 reduces ROS, attenuating the myocardial damage due to ISO. As a consequence, enzyme leak into serum was dramatically diminished by CPU0213 and propranolol.

The impairment of cardiac systolic and diastolic function is associated with an abnormal calcium handing system of the myocardial SR (Yano et al 2005). The activity of SERCA2a is crucial in maintaining a low level of calcium in diastole by pumping calcium into the SR (Gianni et al 2005). Downregulation of SERCA2a results in elevated intracellular calcium, which impairs diastolic function and reduces the SR calcium store. Thus, less calcium can be released from the SR to initiate excitation–contraction coupling, leading to compromised systolic function. CPU0213 elevated the expression of depressed SERCA2a, contributing to restoration of cardiac function resulting from chronic β-AR stimulation. The beneficial effect of CPU0213 in normalizing the depressed SERCA2a is in line with observations in diabetic cardiomyopathy (Qi et al 2006) and acute heart failure caused by septic shock (He et al 2006).

ISO, which promotes transcription of ET-1 (Morimoto et al 2001), increases calcium levels in the myocardium, presumably by activating the L-type calcium channels via the cAMP/protein kinase A pathway. The upregulation of calcineurin serves as an intermediate step to link stimulation of the β-AR with upregulation of the ET-1 pathway. In fact, calcineurin/nuclear factor of activated T cell (NFAT) takes an

active part in pathologies of ventricular hypertrophy (Ennis et al 2007) involving inflammatory factors such as leptin, ROS and ET-1. Cyclosporin A, an inhibitor of calcineurin, improves ventricular hypertrophy. However, many factors that reduce intracellular calcium levels are effective in improving myocardial hypertrophy, such as the ET-receptor antagonist CPU0213 and inhibitors of Na^+/Ca^{2+} exchangers (Ennis et al 2007). Calcium influx into cytosol can be facilitated by ET-1 in various cells (Kawanabe et al 2006; Geneau et al 2007), and cardiomyocyte proliferation, which causes myocardial hypertrophy, correlates with upregulation of calcineurin and the ET pathway (Kuhlmann et al 2005). The suppression of an increased abundance of calcineurin mRNA by the ET-receptor blocker CPU0213 contributes to a reversal of ventricular hypertrophy induced by ISO.

Conclusion

Downregulation of SERCA2a and upregulation of calcineurin can be taken as biomarkers of cardiac insufficiency caused by injury from an excess of cytokines (leptin), paracrine (ET-1) and inflammatory factors (ROS). These changes are induced by chronic stimulation of β-AR. Interestingly, the recovery of these changes can be achieved by antagonism of ET receptors by CPU0213.

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