Pharmacological Reports
2008, 60, 524–531
ISSN 1734-1140

CPU0213, a novel endothelin receptor antagonist, suppresses the upregulation of matrix metalloproteinases and connexin 43 in hyperthyroid myocardium

Xiao-Yun Tang*, Qing Liu*, De-Zai Dai, Yin Dai

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

Correspondence: De-Zai Dai, e-mail: dezaidai@vip.sina.com

Abstract:
It has been verified that endothelin-1 (ET-1) activates matrix metalloproteinase (MMP) in the process of myocardial remodeling. Sustained high thyroid hormone level gives rise to left ventricular hypertrophy, in which the ET-1 system is probably involved. We attempted to study the effects of CPU0213, a novel endothelin receptor antagonist, on myocardial remodeling induced by high dose L-thyroxin. Adult male Sprague-Dawley rats were treated with L-thyroxin (0.4 mg/kg/d, *sc*) for 10 days to induce left ventricular hypertrophy. A subset of rats was given CPU0213 from day 6 to 10. Alterations in MMP, tissue inhibitor of metalloproteinase (TIMP), and connexin 43 were measured by reverse transcription polymerase chain reaction (RT-PCR), zymography, and Western blot assays. L-thyroxin treatment resulted in increased mRNA expression and MMP-2 and MMP-9 activities, along with decreased TIMP-1 and TIMP-2 mRNA expression. CPU0213 suppressed the increased activity of MMP, and prevented the downregulation of TIMP expression. The expression of connexin 43 was upregulated at both mRNA and protein levels after L-thyroxin treatment, which was attenuated by CPU0213. In addition, L-thyroxin caused upregulation of mRNA expression of preproET-1 (ppET-1) and endothelin converting enzyme (ECE). These results suggest that the ET receptors mediate high dose L-thyroxin induced myocardial remodeling by changing MMP, TIMP activities and connexin 43.

Key words:
L-thyroxin, MMPs, connexin 43, endothelin receptor antagonist, CPU0213

Abbreviations: ANOVA – analysis of variance, EB – ethidium bromide, ECE – endothelin converting enzyme, ECM – extracellular matrix, ET-1 – endothelin-1, GJIC – gap junction intercellular communication, MMP – matrix metalloproteinase, ppET-1 – preproET-1, RT-PCR – reverse transcription polymerase chain reaction, SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electropheresis, TIMP – tissue inhibitor of metalloproteinase

Introduction

Changes in thyroid hormone level have a profound impact on cardiovascular system. In hyperthyroid patients and experimental animals, increased thyroid hormone level gives rise to cardiac hyperfunction

* Drs. Xiao-Yun Tang and Qing Liu are the co-first author to the paper.

(e.g. increased resting heart rate, elevated cardiac output, and enhanced myocardial contractility) and left ventricular remodeling [4, 11]. Matrix metalloproteinase (MMP) is a family of zinc-dependent proteases. The interaction between MMPs and its intrinsic antagonist, tissue inhibitor of metalloproteinase (TIMP), plays a crucial role in cardiac remodeling by modulating the extracellular matrix (ECM) e.g. collagen, elastin, fibronectin, and laminin [3, 25]. It was reported that thyroid hormone induced myocardial matrix degradation by activating MMP-1 [14], which is a major reason for hyperthyroidism-induced cardiac remodeling. High-dose thyroid hormone also changes intercellular communication through gap junction channels formed by connexin [15, 35]. As a transcription factor, the intranuclear thyroid hormone receptor has the property of binding to the connexin 43 promoter, by which it modulates connexin 43 gene transcription [32].

Endothelin-1 (ET-1), a 21-amino acid peptide with potent vasoconstrictive effect, has extensive impact on different organs and is implicated in pathologic processes of various diseases [19, 26, 33, 37]. Influences of ET-1 on cardiac MMPs [2, 21] and gap junction proteins have been reported by several experiments. Activation of endothelin receptor A (ETA) results in upregulated mRNA expression of arterial MMPs and cardiac connexin 43 [10, 24]. Furthermore, there is a substantial evidence that cardiac hypertrophy induced by tri-iodothyronine treatment can be significantly attenuated by introducing a structural alteration to the ET-1 gene [31]. It is likely that ET-1 system is involved in the mechanism of myocardial remodeling caused by increased thyroid hormone level. The disturbed homeostasis of ECM and gap junction intercellular communication (GJIC) probably plays an important role in this pathologic process.

CPU0213 (Fig. 1) is a novel dual ETA/ETB antagonist and exhibits a similar pharmacological profile to bosentan [8]. In the present experiment, we attempted to study if ET receptor blockade by CPU0213 can attenuate high dose L-thyroxin-induced cardiac injury through intervening in MMP, TIMP, and connexin 43.

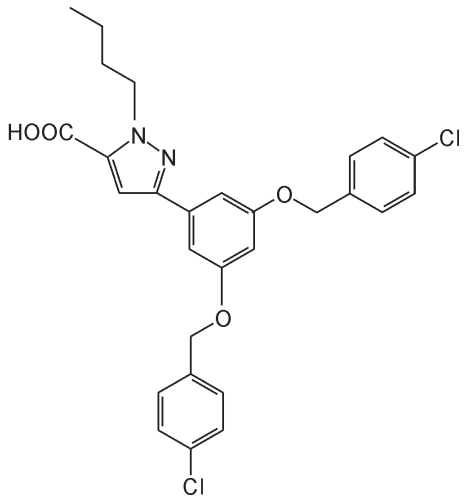

Fig. 1. The chemical structure of CPU0213, MW 525

Materials and Methods

All the procedures performed on the animals in the present study were conducted in accordance with the Laboratory Animal Regulations of Jiangsu Province.

Animals and treatment

CPU0213 was supplied by the New Drug Center of China Pharmaceutical University. Male Sprague-Dawley rats (n = 30, 220 ± 10 g) were obtained from Nanjing Qinglongshan Experimental Animal Center, and were given free access to food and water. The temperature (25°C) and humidity (70%) in the animal room were well controlled. CPU0213 and L-thyroxin (Sigma, USA) were suspended in 0.9% NaCl solution for administration. Hyperthyrodism was induced by subcutaneous injection of L-thyroxin (0.4 mg/kg/d) for 10 days, and a subset of rats (n = 10) was simultaneously treated with CPU0213 (100 mg/kg/d, *po*) from day 6 to 10. Rats of normal control (n = 10) were given vehicle. On day 11, animals were sacrificed by exsanguination through carotid artery after anesthetizing with urethane (1.5 g/kg, *ip*). Excised hearts were rinsed with ice-cold 0.9% NaCl solution, and carefully divided into right and left ventricles (including septum) to measure the weight.

Biochemical measurement of collagen in myocardium

Total collagen in left ventricle was determined by measuring hydroxyproline according to the previously described method [6] using a commercial kit

(Jiancheng Bioengineering Institute, Nanjing, China). Collagen content was estimated by multiplying hydroxyproline level by a factor of 7.46, since collagen contains an average of 13.4% hydroxyproline.

mRNA expression of MMP_2, MMP_9, $TIMP_1$, $TIMP_2$, connexin 43, ppET-1, and ECE

mRNA extraction from myocardial tissues of left ventricle and reverse transcription polymerase chain reaction (RT-PCR) were performed according to the previously described method [26]. Primers of MMP-2 (sense 5'-CCCAGAAAAGATTGACGC-3', antisense 5'-CGACAGCATCCAGGTTAT-3'), MMP-9 (sense 5'-CGTGGCCTAGTGACCTATG-3', antisense 5'-GGATAGCTCGGTGGTGTCCT-3'), TIMP-1 (sense 5'-GCTGCGGTTCTGGGATT-3', antisense 5'-CCTCTGGCATCCTCTTGTT-3'), TIMP-2 (sense 5'-GAAGAAAGGAGGTTGCAGT-3', antisense 5'-TCCAGGAAGGGATGTCAAAG-3'), connexin 43 (sense 5'-TGTAACACTCAACAACCTGGC-3', antisense 5'-GGTTTTCTCCGTGGGACGTGA-3'), ppET-1 (sense 5'-AGCAATAGCATCAAGGCATC-3', antisense 5'-TCAGACACGAACACTCCCTA-3'), ECE (sense 5'-CGTAGCGATAGTCTTAGCAC-3', antisense 5'-GTGCCACACCAAAACTACAG-3'), and GAPDH (sense 5'-GCTGGGGCTCACCTGAAGG-3', antisense 5'-GGATGACCTTGCCCACAGCC-3') were used, and GAPDH was the inner control. PCR reaction was performed in 25 μl final volume with the following profile: 40 s of melting at 94°C; 40 s of annealing (MMP-2: at 60°C; MMP-9: at 64°C; TIMP-1: at 60°C; TIMP-2: at 60°C; connexin 43: at 60°C; ppET-1: at 64°C; ECE: at 54°C; GAPDH: at 56°C); and 60 s of extension at 72°C. The cycle numbers for MMP-2, MMP-9, TIMP-1, TIMP-2, connexin 43, ppET-1, ECE, and GAPDH were 33, 35, 30, 25, 30, 30, 30, and 30, respectively. After the last cycle, the polymerization step was extended for a further 10 min. The PCR products were electrophoresed on 2% agarose gel followed by staining with ethidium bromide (EB) and analyzed with a gel image analysis system (UVP, USA).

Zymography assay of MMP-2 and MMP-9

SDS-PAGE zymography was performed according to the previously described method with some modifications [29]. Briefly, myocardial tissues of left ventricle were homogenized with the working buffer (50 mmol/l Tris-HCl, pH 7.6, 150 mmol/l NaCl, 5 mmol/l $CaCl_2$, 0.1% (v/v) Triton X-100, 10% (v/v) phenylmethyl sulfonyl fluoride). Homogenates were centrifuged at 12,000 × g at 4°C for 5 min and supernatants were collected. Protein contents were determined by Coomassie Brilliant Blue method. Aliquots of supernatants were mixed with loading buffer solution (400 mmol/l Tris-HCl pH 6.8, 5% SDS, 20% glycerol, and 0.03% bromophenol blue) and electrophoresed on an 8% SDS-PAGE gel polymerized with 0.1% gelatin. After electrophoresis, enzymes were renatured by washing the gel with 2.5% TritonX-100 solution at room temperature. Activities of MMPs were revealed by incubating the gel in the medium (50 mmol/l Tris-HCl, 10 mmol/l $CaCl_2$, 200 mmol/l NaCl, 2 mmol/l $ZnCl_2$, pH 7.5) at 37°C for 18 h. Gels were stained with 0.5% Coomassie brilliant blue and destained with the solution containing 10% acetic acid and 30% methanol until clear proteolytic bands appeared on the contrasting blue background.

Protein expression of connexin 43

Protein expression of connexin 43 was measured by Western blot assay according to the previously described method [26]. Briefly, the myocardial tissues were homogenized with the medium (20 mmol/l hydroxyethyl piperazine ethanesulfonic acid, 25 mmol/l NaCl, 2 mmol/l ethylene glycol tetraacetate, 1 mmol/l phenylmethyl sulfonyl fluoride, and 0.1% Triton X-100). Proteins were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane at 20 mA current for 1.5 h. After incubation with polyclonal rabbit anti-connexin 43 antibody and horseradish peroxidase conjugated second antibody (Boster Bioengineering Company, Wuhan, China), the bands of proteins were visualized by diaminobenzidine and analyzed with a gel image analysis system (UVP, USA).

Statistical analysis

Data were expressed as the mean ± SD. One-way analysis of variance (ANOVA) was performed, followed by Student-Newman-Keuls test as *post-hoc* analysis. $p < 0.05$ was considered statistically significant.

Results

Changes of weight index and collagen level in left ventricle

L-thyroxin treatment resulted in left ventricular hypertrophy shown by the significantly increased left ventricle weight relative to normal control (Tab. 1). The collagen content of left ventricle in hyperthyroid rats was by approximately 35% lower than that of normal control ($p < 0.05$). ET receptor blockade by CPU0213 significantly attenuated these changes (Fig. 2).

Tab. 1. Changes in left ventricle weight index before and after treatment with L-thyroxin and CPU0213

	Left ventricle weight (g)	Left ventricle weight/body weight (10^{-3})
Normal control (n = 10)	0.70 ± 0.07	2.97 ± 0.12
L-thyroxin (n = 10)	0.89 ± 0.05#	4.52 ± 0.19##
L-thyroxin + CPU0213 (n = 10)	0.78 ± 0.09	3.81 ± 0.20*

# $p < 0.05$, ## $p < 0.01$ *vs.* normal control, * $p < 0.05$ *vs.* L-thyroxin

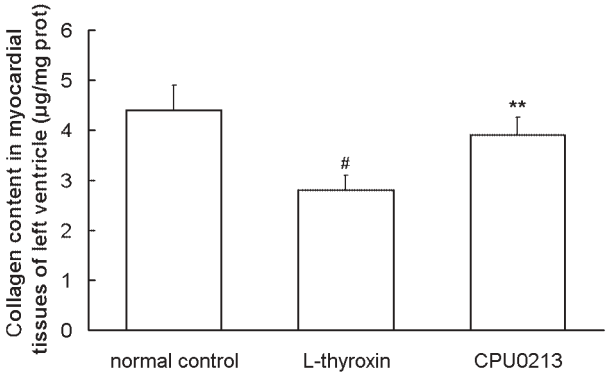

Fig. 2. L-thyroxin (0.4 mg/kg) treatment for 10 days caused a decrease in collagen content in left ventricle, which was significantly recovered after CPU0213 (n = 10). # $p < 0.05$ *vs.* normal control, ** $p < 0.01$ *vs.* L-thyroxin

Changes in ppET-1 and ECE expression in myocardial tissue of left ventricle

L-thyroxin treatment induced high expression of ppET-1 and ECE in left ventricle ($p < 0.01$), suggesting the activation of ET system. ET receptors blockade by CPU0213 had no effects on the upregulation of ppET-1 and ECE expression (Fig. 5).

Changes of MMPs and TIMPs in myocardial tissues of left ventricle

MMP-2 and MMP-9 mRNA expression was significantly upregulated after L-thyroxin treatment, and TIMP-1 and TIMP-2 expression was reduced ($p < 0.01$). Zymography assay showed increased activities of MMP-2 and MMP-9, which was in accordance with the results of RT-PCR ($p < 0.01$). CPU0213 significantly suppressed MMPs activity, and relieved the downregulation of TIMPs expression (Fig. 3).

Changes in connexin 43 in myocardial tissues of left ventricle

L-thyroxin treatment significantly increased the connexin 43 mRNA expression in left ventricle ($p < 0.05$). Accordingly, the protein expression of connexin 43 was increased either ($p < 0.05$). CPU0213 partially attenuated the change of connexin 43 at both mRNA and protein levels (Fig. 4).

Discussion

In the present experiment, high-dose L-thyroxin treatment resulted in left ventricular hypertrophy. Metabolism of myocardial ECM seemed to be disturbed by L-thyroxin, since activities of MMP-2 and MMP-9 significantly increased, and expression of TIMP-1 and TIMP-2 was suppressed. The increased ECM degradation by MMPs led to the decline in myocardial collagen content. This change probably confers adverse influences to heart, since ECM not only serves as the scaffold of myocardium [17], but also transmits cellular signals through ECM receptors such as β1 integrin [28]. Recent studies have reported that loss of ECM (mainly collagen) causes myocyte slippage, ventricular dilation, progressive contractile dysfunction, and finally heart failure [7]. ECM remodeling is also associated with sustained atrial fibrillation [22]. Patients with hyperthyroidism have increased risk of heart

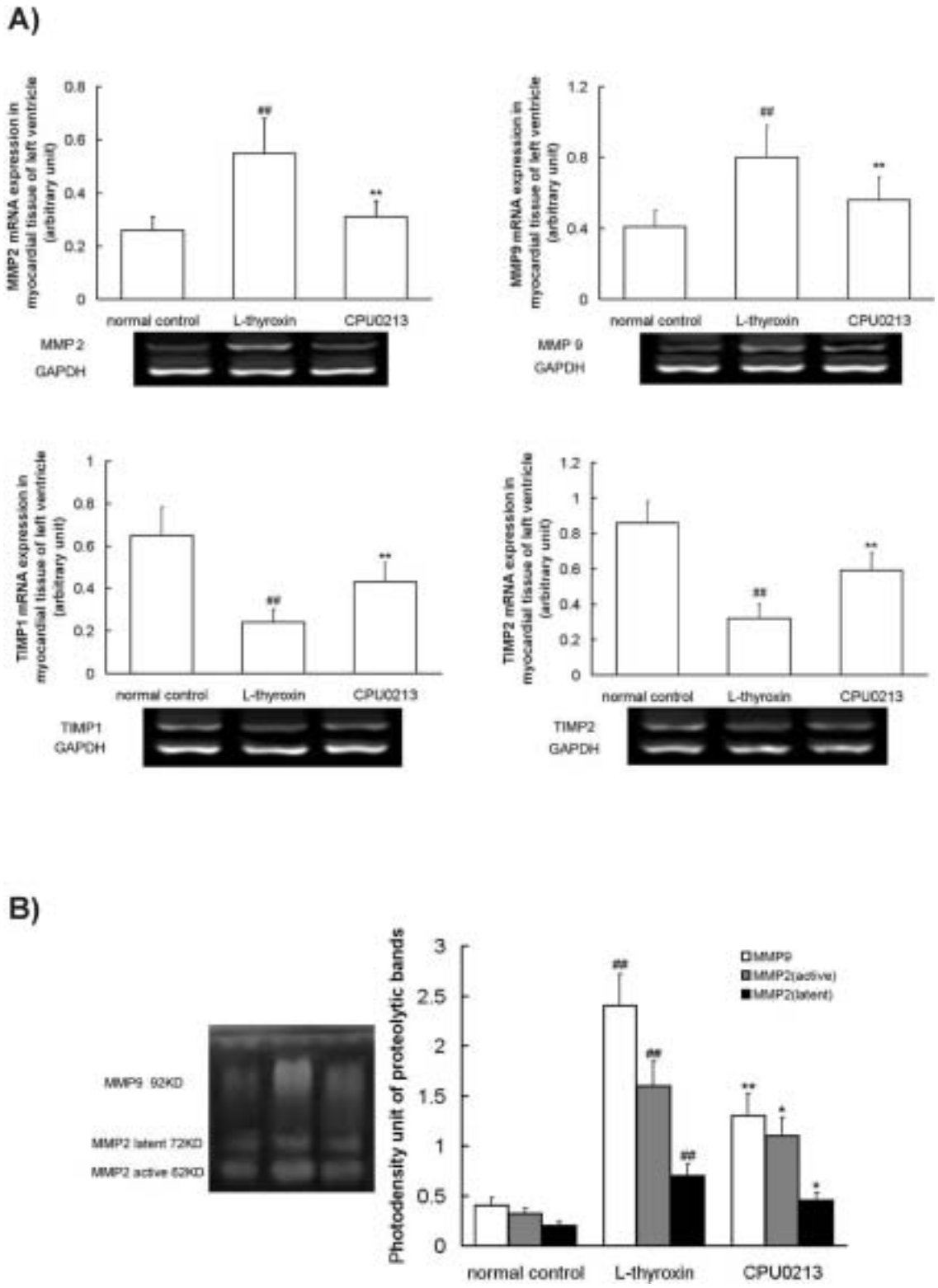

Fig. 3. (A) MMP-2 and MMP-9 mRNA expression in left ventricle tissue was significantly upregulated after 10 days of L-thyroxin (0.4 mg/kg) treatment, along with decreased expressions of TIMP-1 and TIMP-2. These changes were recovered in part after CPU0213 (n = 6). **(B)** Increased activities of MMP-2 and MMP-9 in L-thyroxin group were shown by zymography, which is in accord with the changes in mRNA expression. This was significantly suppressed by CPU0213 (n = 4). $^{\#\#}$ $p < 0.01$ *vs.* normal control, * $p < 0.05$, ** $p < 0.01$ *vs.* L-thyroxin

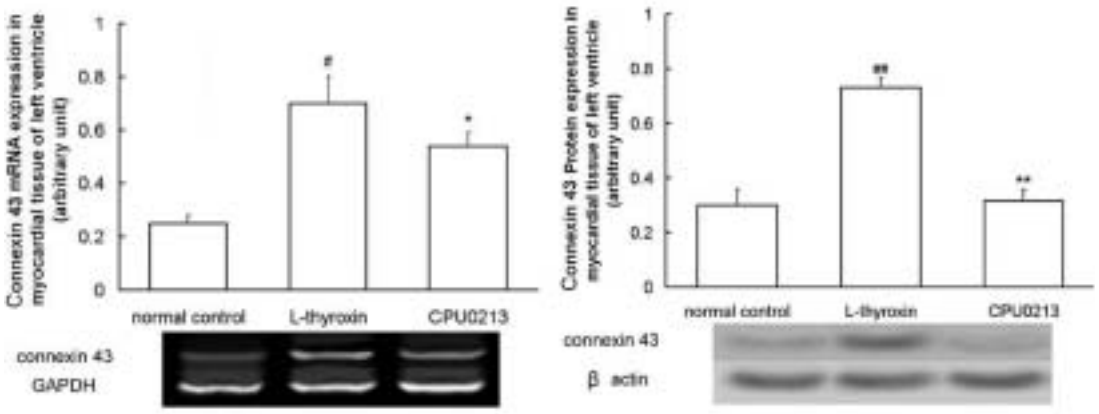

Fig. 4. L-thyroxin (0.4 mg/kg) treatment for 10 days significantly increased the left ventricle connexin 43 expression in both mRNA (n = 6) and protein (n = 4) levels, which was suppressed significantly by CPU0213. # $p < 0.05$ *vs.* normal control, * $p < 0.05$, ** $p < 0.01$ *vs.* L-thyroxin

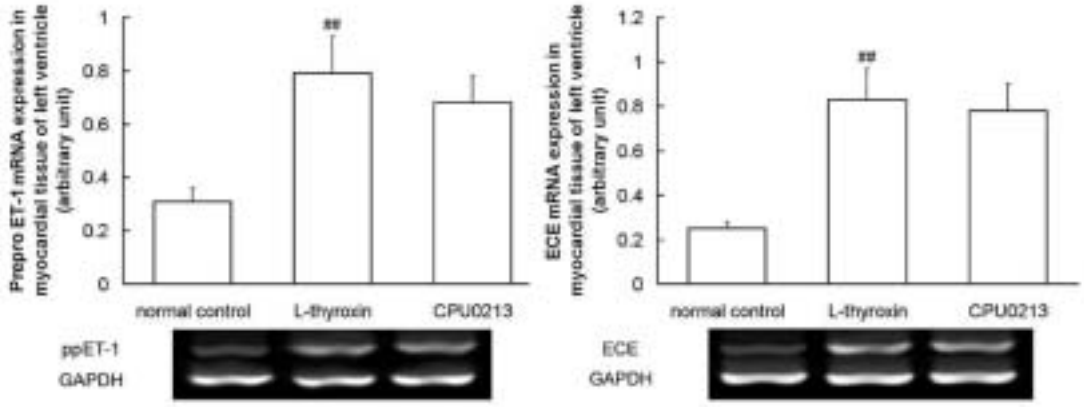

Fig. 5. Expression of ppET-1 and ECE mRNA in left ventricle increased significantly after L-thyroxin treatment (0.4 mg/kg) for 10 days. These increased expressions were not restored by CPU0213 (n = 6). ## $p < 0.01$ *vs.* normal control

failure and arrhythmia [16, 27]. In this process, the effects of MMPs should not be neglected.

Stock and Sies reported that thyroid hormone increases connexin 43 expression in rat liver, but not in heart [32]. However, Tribulova et al. proved the increase in connexin 43 expression in cultured cardiomyocytes treated with triiodo-L-thyronine [35]. The opposite results from cells and intact animals suggest that effects of thyroid hormone on cardiac connexin 43 are probably intervened by other neurohormonal factors. Differences in the dose of thyroid hormone, and in the duration of treatment between experiments may also account for the different results. Dynamic changes in connexin 43 expression during the process of experimental heart failure have been confirmed [1], suggesting the complexity in the regulation of connexin 43. In the present experiment, hypertrophied left ventricle exhibited upregulation of connexin 43 expression at both mRNA and protein levels. Cardiac gap junction channels have a critical role to play in electrical transduction and signal communication between myocardial cells. A single gap junction channel is composed of two hemi channels, each constructed from six connexin proteins. Connexin 43 is a member of connexin family, and is mainly expressed in ventricle. The important role of connexin 43 in maintaining normal cardiac function has been proved by series of experiments [34, 36]. Deficiency of connexin 43 is associated with arrhythmia and heart failure [23]. However, experiments also showed increased connexin 43

expression in some pathologic processes [1, 9], and gene transfer of connexin 43 mutants was considered to be a promising strategy to treat ventricular tachycardia by reducing intracellular electrical conduction [18]. Therefore, the excessive connexin 43 expression caused by L-thyroxin most likely underpins the enhanced cardiac performance, and may alter the normal electrical characteristics of cardiomyocytes. These changes probably accounts for the increased incidence of arrhythmia induced by high-dose L-thyroxin.

Our findings showed that ppET-1 and ECE were heavily expressed in hyperthyroid myocardial tissue, in conjunction with the changes of ECM and connexin 43. This is in agreement with the previous experiment, in which the close relationship between thyroid hormone and ET-1 was reported [5, 12]. Definitive evidence has been presented that ET-1 mediates structural remodeling by promoting MMPs expression in various diseases, e.g. pulmonary hypertension [20] and myocardial remodeling [21]. Effects of ET-1 on gap junction channels and connexin 43 were controversial [13, 30]. But in cardiomyocytes, exogenous ET-1 resulted in an increase in connexin 43 expression [24]. In this experiment, CPU0213 showed no effects on expressions of ppET-1 and ECE, but the changes in MMPs, TIMPs, collagen, and connexin 43 induced by high-dose L-thyroxin were partially attenuated by CPU0213. These results indicate that ET receptors most likely mediate the effects of thyroid hormone on ECM and GJIC. Blockade of ET receptors by CPU0213 nullifies the downstream signal of ET, however, the upstream of ET receptors e.g. ppET-1 and ECE were not affected.

In conclusion, ET receptors are implicated in the process of cardiac remodeling induced by high thyroid hormone level. Under this condition, changes in MMPs, TIMPs and connexin 43 are mediated by ET receptors. ET receptor is an important target for treating cardiac injury induced by elevated thyroid hormone.

Acknowledgment:
This work was supported by National Natural Science Foundation of China. Contract grant number: 30670760.

References:

1. Akar FG, Nass RD, Hahn S, Cingolani E, Shah M, Hesketh GG, DiSilvestre D et al.: Dynamic changes in conduction velocity and gap junction properties during development of pacing-induced heart failure. Am J Physiol Heart Circ Physiol, 2007, 293, 1223–1230.
2. Ammarguellat FZ, Gannon PO, Amiri F, Schiffrin EL: Fibrosis, matrix metalloproteinases, and inflammation in the heart of DOCA-salt hypertensive rats: role of ET(A) receptors. Hypertension, 2002, 39, 679–684.
3. Anne W, Willems R, Roskams T, Sergeant P, Herijgers P, Holemans P, Ector H, Heidbuchel H: Matrix metalloproteinases and atrial remodeling in patients with mitral valve disease and atrial fibrillation. Cardiovasc Res, 2005, 67, 655–666.
4. Biondi B, Palmieri EA, Lombardi G, Fazio S: Effects of thyroid hormone on cardiac function: the relative importance of heart rate, loading conditions, and myocardial contractility in the regulation of cardiac performance in human hyperthyroidism. J Clin Endocrinol Metab, 2002, 87, 968–974.
5. Chu CH, Lee JK, Keng HM, Chuang MJ, Lu CC, Wang MC, Sun CC et al.: Hyperthyroidism is associated with higher plasma endothelin-1 concentrations. Exp Biol Med (Maywood), 2006, 231, 1040–1043.
6. Comporti M, Arezzini B, Signorini C, Sgherri C, Monaco B, Gardi C: F2-isoprostanes stimulate collagen synthesis in activated hepatic stellate cells: a link with liver fibrosis? Lab Invest, 2005, 85, 1381–1391.
7. D'Armiento J: Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. Trends Cardiovasc Med, 2002, 12, 97–101.
8. Dai DZ, Ji M, Huang M, Liu LG: Endothelin receptor antagonist activity and selective blocking the ETA and ETB of compound CPU0213 (Chinese). J Chin Pharm Univ, 2004, 35, 552–557.
9. De K, Ghosh G, Datta M, Konar A, Bandyopadhyay J, Bandyopadhyay D, Bhattacharya S, Bandyopadhyay A: Analysis of differentially expressed genes in hyperthyroid-induced hypertrophied heart by cDNA microarray. J Endocrinol, 2004, 182, 303–314.
10. Ergul A, Portik-Dobos V, Giulumian AD, Molero MM, Fuchs LC: Stress upregulates arterial matrix metalloproteinase expression and activity via endothelin A receptor activation. Am J Physiol Heart Circ Physiol, 2003, 285, 2225–2232.
11. Fazio S, Palmieri EA, Lombardi G, Biondi B: Effects of thyroid hormone on the cardiovascular system. Recent Prog Horm Res, 2004, 59, 31–50.
12. Feng Y, Dai DZ, Na T, Cui B, Wang T, Zhang Y, Dai Y: Endothelin receptor antagonist CPU0213 suppresses ventricular fibrillation in L-thyroxin induced cardiomyopathy. Pharmacol Rep, 2007, 59, 306–314.
13. Fischer R, Reinehr R, Lu TP, Schonicke A, Warskulat U, Dienes HP, Haussinger D: Intercellular communication via gap junctions in activated rat hepatic stellate cells. Gastroenterology, 2005, 128, 433–448.
14. Ghose RS, Mishra S, Ghosh G, Bandyopadhyay A: Thyroid hormone induces myocardial matrix degradation by activating matrix metalloproteinase-1. Matrix Biol, 2007, 26, 269–279.
15. Gilleron J, Nebout M, Scarabelli L, Senegas BF, Palmero S, Segretain D, Pointis G: A potential novel mechanism involving connexin 43 gap junction for control of sertoli

cell proliferation by thyroid hormones. J Cell Physiol, 2006, 209, 153–161.
16. Howarth FC, Nowotny N, Zilahi E, El Haj MA, Lei M: Altered expression of gap junction connexin proteins may partly underlie heart rhythm disturbances in the streptozotocin-induced diabetic rat heart. Mol Cell Biochem, 2007, 305, 145–151.
17. Kim HE, Dalal SS, Young E, Legato MJ, Weisfeldt ML, D'Armiento J: Disruption of the myocardial extracellular matrix leads to cardiac dysfunction. J Clin Invest, 2000, 106, 857–866.
18. Kizana E, Chang CY, Cingolani E, Ramirez-Correa GA, Sekar RB, Abraham MR, Ginn SL et al.: Gene transfer of connexin43 mutants attenuates coupling in cardiomyocytes: novel basis for modulation of cardiac conduction by gene therapy. Circ Res, 2007, 100, 1597–1604.
19. Korzonek-Szlacheta I, Gwóźdź B: Effects of endothelin-1 on prevention of microvascular endothelium injuries in hemorrhagic shock in rats. Pharmacol Rep, 2007, 59, 98–106.
20. Matsui K, Takano Y, Yu ZX, Hi JE, Stetler-Stevenson WG, Travis WD, Ferrans VJ: Immunohistochemical study of endothelin-1 and matrix metalloproteinases in plexogenic pulmonary arteriopathy. Pathol Res Pract, 2002, 198, 403–412.
21. Murray DB, Gardner JD, Brower GL, Janicki JS: Endothelin-1 mediates cardiac mast cell degranulation, matrix metalloproteinase activation, and myocardial remodeling in rats. Am J Physiol Heart Circ Physiol, 2004, 287, 2295–2299.
22. Nakano Y, Niida S, Dote K, Takenaka S, Hirao H, Miura F, Ishida M et al.: Matrix metalloproteinase-9 contributes to human atrial remodeling during atrial fibrillation. J Am Coll Cardiol, 2004, 43, 818–825.
23. Poelzing S, Rosenbaum DS: Altered connexin43 expression produces arrhythmia substrate in heart failure. Am J Physiol Heart Circ Physiol, 2004, 287, 1762–1770.
24. Polontchouk L, Ebelt B, Jackels M, Dhein S: Chronic effects of endothelin 1 and angiotensin II on gap junctions and intercellular communication in cardiac cells. FASEB J, 2002, 16, 87–89.
25. Polyakova V, Hein S, Kostin S, Ziegelhoeffer T, Schaper J: Matrix metalloproteinases and their tissue inhibitors in pressure-overloaded human myocardium during heart failure progression. J Am Coll Cardiol, 2004, 44, 1609–1618.
26. Qi MY, Xia HJ, Dai DZ, Dai Y: 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, 2006, 47, 729–735.
27. Roffi M, Cattaneo F, Brandle M: Thyrotoxicosis and the cardiovascular system. Minerva Endocrinol, 2005, 30, 47–58.
28. Ross RS, Pham C, Shai SY, Goldhaber JI, Fenczik C, Glembotski CC, Ginsberg MH, Loftus JC: β_1 integrins participate in the hypertrophic response of rat ventricular myocytes. Circ Res, 1998, 82, 1160–1172.
29. Rouet BP, Buhler JM, Dreyfus P, Delcourt A, Dorent R, Perennec J, Crozatier B et al.: Altered balance between matrix gelatinases (MMP-2 and MMP-9) and their tissue inhibitors in human dilated cardiomyopathy: potential role of MMP-9 in myosin-heavy chain degradation. Eur J Heart Fail, 1999, 1, 337–352.
30. Rózyczka J, Figiel M, Engele J: Chronic endothelin exposure inhibits connexin43 expression in cultured cortical astroglia. J Neurosci Res, 2005, 79, 303–309.
31. Shohet RV, Kisanuki YY, Zhao XS, Siddiquee Z, Franco F, Yanagisawa M: Mice with cardiomyocyte-specific disruption of the endothelin-1 gene are resistant to hyperthyroid cardiac hypertrophy. Proc Natl Acad Sci USA, 2004, 101, 2088–2093.
32. Stock A, Sies H: Thyroid hormone receptors bind to an element in the connexin43 promoter. Biol Chem, 2000, 381, 973–979.
33. Tang X, Qi M, Dai D, Zhang C: Effects of CPU 86017 (chlorobenzyltetrahydroberberine chloride) and its enantiomers on thyrotoxicosis-induced overactive endothelin-1 system and oxidative stress in rat testes. Urology, 2006, 68, 455–461.
34. Thomas SP, Kucera JP, Bircher LL, Rudy Y, Saffitz JE, Kleber AG: Impulse propagation in synthetic strands of neonatal cardiac myocytes with genetically reduced levels of connexin43. Circ Res, 2003, 92, 1209–1216.
35. Tribulova N, Shneyvays V, Mamedova LK, Moshel S, Zinman T, Shainberg A, Manoach M et al.: Enhanced connexin-43 and -sarcomeric actin expression in cultured heart myocytes exposed to triiodo-L-thyronine. J Mol Histol, 2004, 35, 463–470.
36. Vaidya D, Tamaddon HS, Lo CW, Taffet SM, Delmar M, Morley GE, Jalife J: Null mutation of connexin43 causes slow propagation of ventricular activation in the late stages of mouse embryonic development. Circ Res, 2001, 88, 1196–1202.
37. Zhang TT, Cui B, Dai DZ, Su W: CPU 86017, p-chlorobenzyltetrahydroberberine chloride, attenuates monocrotaline-induced pulmonary hypertension by suppressing endothelin pathway. Acta Pharmacol Sin, 2005, 26, 1309–1316.

Received:
November 7, 2007; in revised form: May 26, 2008.