Propranolol and verapamil inhibit mRNA expression of RyR2 and SERCA in *L*-thyroxin-induced rat ventricular hypertrophy[1]

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ABSTRACT

AIM: To study the alteration in the mRNA level of cardiac ryanodine receptor 2 (RyR2) and sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in *L*-thyroxin-induced hypertrophy. **METHODS:** *L*-thyroxin (500 g/kg) daily was injected for 10 d. RT-PCR was used to determine mRNA expression. **RESULTS:** An increase in the relative amount of RyR2 (111 %) and SERCA mRNA (65 %) expression was observed in the hypertrophied rats (RyR2: 77±11; SERCA: 87±10, *n*=9) compared with the normal rats (RyR2: 36±10; SERCA: 53±10, *n*=9). Propranolol was effective to inhibit the increase in RyR2 (51±7) and SERCA (63±13) mRNA expression in hypertrophied rats, respectively. Verapamil also reduced RyR2 (62±5) and SERCA (75±8) mRNA expression. **CONCLUSION:** Both RyR2 and SERCA mRNA level in *L*-thyroxin-induced cardiac hypertrophy was over-expressed and propranolol or verapamil inhibited the alteration.

INTRODUCTION

An important mechanism contributing to the high mortality and sudden death in patients with cardiac hypertrophy is ventricular arrhythmias[1]. The most consistently observed abnormalities are: 1) prolongation of the action potential duration and refractoriness; 2) non-uniform prolongation of the action potential; 3) the impaired ability to handle intracellular calcium due to changes in ryanodine receptor (RyR) and sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA)[2,3].

Ryanodine receptor are the main intracellular Ca^{2+} channel residing in the SR and is responsible for the release of Ca^{2+} from the SR. Three different isoforms of ryanodine receptors, each encoded by different genes, have been characterized. The human ryanodine receptor 2 (RyR2) is abundantly expressed in myocardium, and serves to couple the excitation of myocardial cells with their contractile apparatus by a mechanism involving a calcium-induced calcium release. Ca^{2+}-induced release of Ca^{2+} by RyR2 is also related to the occurrence of delayed afterdepolarizations or early afterdepolarizations and dispersion of repolarization in ventricular myocardium thus, may contribute to triggered activity and ventricular arrhythmias[4-7].

Sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) performs the essential function of promoting

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muscle relaxation by rapidly removing Ca^{2+} from the cytosol to SR, but the overload of $[Ca^{2+}]$SR will increase the sensitivity of RyR to $[Ca^{2+}]_i$ which cause spontaneous Ca^{2+} release and arrhythmias[3,8,9].

A complex cardiac remodeling with hypertrophy and over-activity of sympathetic nervous system may be produced by *L*-thyroxin, which shows a tendency of arrhythmogenesis on the episode of occlusion/reperfusion coronary artery[10]. We have found that I_{Na} and I_{Kto} are reduced but I_{Ks}, I_{Kr}, and $I_{Ca\text{-}L}$ are increased in the cardiac remodeling, which may be responsible for the non-uniform prolongation of the action potential and proarrhythmias[11-13]. It will be interesting to explore the alterations of RyR2 and SERCA in this model.

Stress-induced arrhythmias can be effectively inhibited by β-antagonist such as propranolol. β-Adrenergic stimulation may result in functional modification of RyR through phosphorylation, which lead to more calcium release[3,13]. However, the effect of propranolol on the mRNA RyR2 expression is not known. Though the function of RyR is regulated by *L*-type calcium channel, it is interesting to investigate whether verapamil, a calcium antagonist, has any effect on the RyR2 mRNA expression. In this paper we intended to investigate the changes in mRNA of RyR2 and SERCA expression in *L*-thyroxin-induced hypertrophy and the effect of propranolol and verapamil on it.

MATERIALS AND METHODS

***L*-thyroxin-induced hypertrophy** Adult male and female rats (304±56 g) were divided into 4 groups randomly. Control group received ip injections of the solvent. Hypertrophied group was injected with *L*-thyroxin 0.5 mg/kg, ip, for 10 d. Propranolol group was injected with *L*-thyroxin 0.5 mg/kg for 10 d and propranolol (10 mg/kg) from d 7-d 10. Verapamil group was injected with *L*-thyroxin 0.5 mg/kg for 10 d and verapamil (10 mg/kg) from d 7-d 10. On d 10 after administration of *L*-thyroxin, hearts were rapidly removed and stored at -80 ºC until reversed transription-polymerase chain reaction (RT-PCR).

RNA preparation Total cellular RNA was isolated from each frozen tissue sample (100 μg) using Trizol reagent (life Technologies) according to the manufacture's instructions.

Semiquantitative determination of RyR2 and SERCA by RT-PCR A quantity of 0.4 μg of RNA was reacted in a 25 μL RT-PCR mixture containing 0.1 μg of primers, 3 U *Taq* polymerase, 3 U MLV reverse transriptase, together with 20 mmol/L $MgCl_2$, 200 μmol/L of each dNTP. DNA oligonucleotide primers were selected from the published sequence of the RyR2 gene. The sense primer was based on the sequence No X83933 (5'-GAATCAGTGAGTTACTGGGCA-TGG-3') and antisense primer was 5'CTGGTCTCTGAGTTCTCCA-AAAGC-3' [14]. For SERCA: forward (5'-ATGAGATCA-CAGCTATGACTGGTG-3'); reverse (5'-GACTTGCA-CATCTCTATGGTGACTAG-3')[15]. To fix the amount of initial mRNA, paralled-actin amplification was performed using the following oligonucleotides: 5'-GGTATGGGTCAGAAGGACTCC-3' (sense) and 5'-TGATCTTCATGGTGCTGCTAGGAGCC-3' (antisense). This reaction mixture was overlaid with 25 μL mineral oil and was incubated at 43 ºC for 45 min to initiate synthesis of cDNAs. Reverse transcription was inactivated at 94 ºC for 5 min. This mixture was then performed for PCR with 32 cyclings by a thermal cycler (BIO-RAD gene CyclerTM) using the following parameters: denaturation at 94 ºC for 45 s, annealing at 60 ºC for 1 min, extention at 72 ºC for 1.5 min, followed by a final incubation at 72 ºC for 8 min.

PCR products (4 μL each) were separated on 1.5 % agarose ethidium-bromide gel, visualized under ultraviolet light, and scanned by Magemaster VDS (Pharmcia Biotech). The density of the bands was computer analyzed by Matrox Innspector 2.0 (Matrox Electronic systems Ltd). The relative intensity of bands for each mRNA was divided by the intensity of the band for the internal control, β-actin.

Statistical analysis All Data are presented as Mean±SD. Comparisons between data were performed by a two-way analysis of variance (ANOVA) followed by *t*-test. Differences were considered significant at $P<0.05$.

RESULTS

Establishment of cardiac hypertrophy Ventricular weight normalized for body weight was increased significantly in the *L*-thyroxin-treated rats compared with the untreated control (Fig 1). The difference in the ratio of ventricular weight to body weight was due to both an increase in ventricular weight (control: 1.06 g±0.08 g, n=9; hypertrophied: 1.25 g±0.12 g, n=9) and a decrease in body weight (control: 304 g±51 g, n=9; hypertrophied: 261 g±42 g, n=9). Propranolol or verapamil 10 mg/kg for 3 d reduced the ratio of ven-

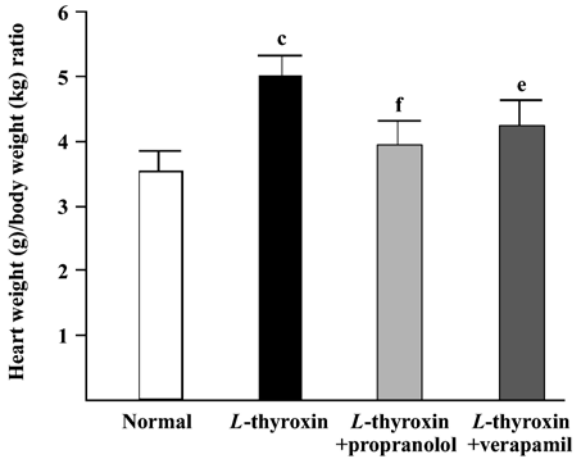

Fig 1. Comparison of heart weight to body weight (g/kg) ratios. *n*=9. Mean±SD. $^{c}P<0.01$ *vs* control. $^{c}P<0.05$, $^{f}P<0.01$ *vs* rat treated with chronic *L*-thyroxin.

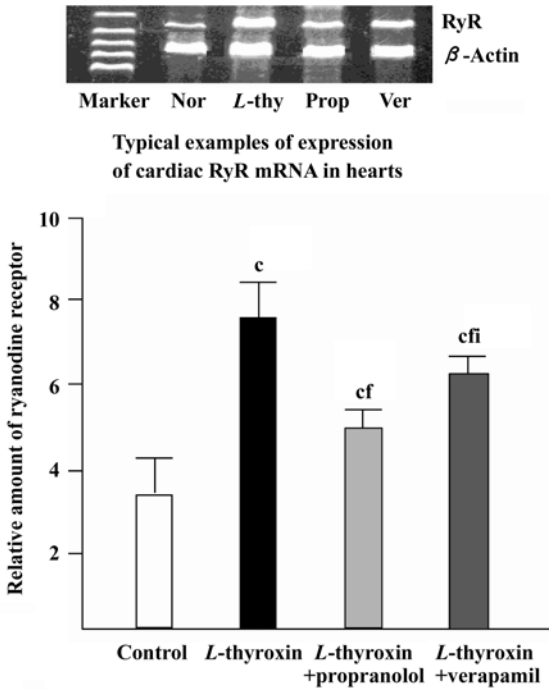

Fig 2. mRNA expression levels of ryanodine receptor in rat heart. These levels are normalized by expression of β-actin mRNA. *n*=9. Mean±SD. $^{c}P<0.01$ *vs* control. $^{f}P<0.01$ *vs* hypertrophied rat treated with chronic *L*-thyroxin. $^{i}P<0.01$ *vs* rat treated with *L*-thyroxin and propranolol.

tricular weight to body weight, respectively.

Effects of *L*-thyroxin and propranolol or verapamil on RyR2 mRNA expression Higher expression level (111 % increase) of RyR2 mRNA in the hypertrophied rats was observed. Propranolol 10 mg/kg or verapamil 10 mg/kg ip from d 7-d 10 after chronic treatment with *L*-thyroxin decreased the expression of RyR2 mRNA by 34 % and 19 %, respectively (Fig 2).

Effects of *L*-thyroxin and propranolol or verapamil on SERCA mRNA expression Higher expression level (65 % increase) of SERCA mRNA was observed in the hypertrophied rats (87±8) compared with control (53±13). Propranolol 10 mg/kg or verapamil 10 mg/kg ip from d 7-d 10 after chronic treatment with *L*-thyroxin decreased the expression of SERCA mRNA by 28 % and 14 %, respectively (Fig 3).

DISCUSSION

The roles of Ca^{2+} released by RyR2 from SR and Na^{+}-Ca^{2+} exchange in arrhythmogenesis have been gradually realized[2,3,8-12,16-18] in some arrhythmogenic models with hypertrophy. However, it is not clear whether the expression of RyR2 and SERCA mRNA is altered. Our previous results showed that rats had a potential tendency of arrhythmogenensis after *L*-thyroxin treatment[10-13]. The results presented here demonstrated that the expression of RyR2 mRNA in hypertrophied ventricle induced by *L*-thyroxin was increased. Na^{+}/Ca^{2+} exchange current, Ca^{2+} released from SR, and β-adrenergic responsiveness are key factors involved in triggered arrhythmogenesis in hypertrophied heart[3,4-8,16-18] (Fig 4). The increased Ca^{2+} release by RyR will lead to larger Ca^{2+}-activated transient inward currents (I_{ti}) which has been proposed to be entirely carried by Na^{+}/Ca^{2+} exchange current and is responsible for DADs or EADs and dispersion of repolarization[3-5,16-18]. In this model long term stimulation for beta-adrenergic receptor results in hyperphosphorylation of RyR2, leading to pathological hypersensitivity of RyR to release more Ca^{2+} in diastolic period[3,14]. Our finding indicates that the increased expression of RyR2 mRNA is involved in an enhanced arrhythmogenesis in hypertrophied rats after chronic treatment of *L*-thyroxin.

The content of Ca^{2+} in SR is an important factor in determining Ca^{2+} release from SR and proarrhythmias[3,8,16]. Overload of $[Ca^{2+}]$SR will increase the sensitivity of RyR to $[Ca^{2+}]_i$ which cause spontaneous Ca^{2+} release and arrhythmias[3,5,11]. In the very end of heart failure, arrhythmias are rarely seen when the expression of SERCA decreased, which lead to reduced content of Ca^{2+} in SR[3,16]. In this paper the expression of SERCA mRNA in hypertrophied ventricle induced by *L*-thyroxin was increased, which demonstrated that SERCA was also involved in proarrhythmia induced by chronic treatment of *L*-thyroxin.

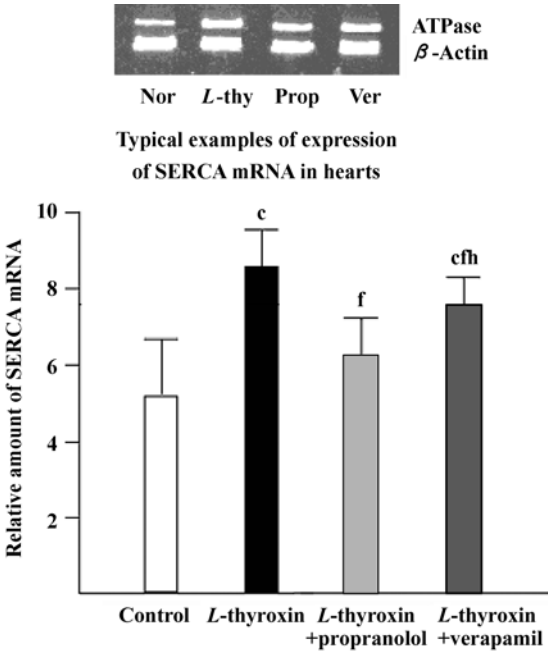

Fig 3. mRNA expression levels of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in rat heart. *n*=9. Mean±SD. $^{c}P<0.01$ *vs* control. $^{f}P<0.01$ *vs* rat treated with chronic *L*-thyroxin. $^{h}P<0.05$ *vs* rat treated with chronic *L*-thyroxin and propranolol.

Arrhythmias is often induced by stress. The clinical picture of hyperthyroidism is related with increased sympathetic activity. Long term stimulation by elevated serum catecholamines results in PKA hyperphosphorylation of many ion channel proteins including RyR[2,3,16-20] and enhances hypertrophy by stimulating RNA and protein synthesis. Propranolol inhibited the elevated expression of RyR and SERCA mRNA induced by *L*-thyroxin by antagonizing β-adrenergic receptor to cause less phosphorylation of RyR.

Verapamil is a calcium antagonist with antiarrhythmic effects. Verapamil reduced the elevated expression of cardiac RyR and SERCA mRNA in the hypertrophied heart with chronic treatment of *L*-thyroxin. The mechanism is assumed that verapamil ameliorated hypertrophy by inhibiting I_{Ca} and Ca^{2+}-induced Ca^{2+} release from SR.

In summary both RyR2 and SERCA mRNA in *L*-thyroxin-induced cardiac hypertrophy was over-expressed and propranolol or verapamil inhibited the alteration.

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