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Original Article

Effect of propranolol on atrial fibrillation in hyperthyrodism

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Abstract: The aim of this study was to investigate the effect of propranolol on atrial fibrillation in hyperthyroidism and explore the related mechanisms. A hyperthyroid rat model was established by thyroidectomy. Thyroidectomized rats were randomly divided into hyperthyroid group (n=9) and propranolol group (n=9). Rats received triiodothyronine (T3) and T3 + propranolol for 2 weeks, respectively. Serum level of thyroid hormone and heart rates were evaluated. The expression of L-type calcium channel (LLC), type I collagen and change of downstream signaling pathways were examined to explore the mechanisms. Propranolol treatment has no effect on the serum level of T3, L-thyroxine (T4), and thyrotropin. However, significantly decreased heart rates were observed in model rats with propranolol treatment. Propranolol treatment could significantly increase the expression of LCC α1C-subunit and decrease type I and type III collagen. Decreased phosphor-Erk and increased phosphor-Akt were found in propranolol group compared with hyperthyroid group. Consistent results were found in atrial fibroblasts treated with T3 and propranolol. Propranolol protects hyperthyroid rat from atrial fibrillation through upregulation of the expression of LCC α1C-subunit and downregulation of collagen. MAPK and Akt related pathways were involved in the mechanisms.

Keywords: Propranolol, atrial fibrillation, hyperthyroidism, L-type calcium channel, type I collagen

Introduction

Atrial fibrillation (AF), with an overall prevalence of 1% to 2%, is the most common sustained cardiac arrhythmia associated with a higher morbidity and mortality in clinical practice [1]. Therefore, the effective treatment and prevention of AF has important clinical significance.

AF is considered to have a very complex pathophysiology and multiple mechanisms can result in AF, including ion channel dysfunction, Ca(2+)-handling abnormalities, structural remodeling, and autonomic neural dysregulation [2]. A recent study by Zhang et al has shown that AF is associated with thyroid dysfunction including both hyperthyroidism and hypothyroidism [3]. Moreover, successful management of hyperthyroidism could lead to restoration of sinus rhythm in most of patients [4].

Propranolol, a noncardio selective β-blocker, has been used to treat hypertension and heart rate regulation for more than 50 years [5-7]. Burggraaf et al previously showed that propranolol treatment could improve the endothelial function in patients with hyperthyroidism [8]. However, the effects and mechanisms of propranolol on atrial fibrillation in hyperthyroidism have not been exploredyet.

Here, we established a hyperthyroid rat model and aimed to investigate the effect of propranolol on atrial fibrillation in hyperthyroidism and explore the related mechanisms.

Methods and materials

In vivo rat model

In accordance with the previously described techniques [3], adult male Wistar rats were

Table 1. Primer sequence of genes

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
LCC α1c subunit	5'-GCCAACATGAATGCCAATGC-3'	5'-GGAACTGACGGTAGAGATGG-3'
LCC β1 subunit	5'-GTTGGCTACAATCCGTCTC-3'	5'-CGAAGGCTGTCCAGTTTG-3'
LCC β3 subunit	5'-GA CTGGTGGATCGGGAGG-3'	5'-GCTTCTGCTTGGCTAGAG-3'
LCC α2d subunit	5'-GGCACAGATTACAGTTTGG-3'	5'-GCAGTATTCCCTTGGTGC-3'
GAPDH	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTGCAGCGAACTT

anesthetized by intraperitoneal injection of ketamine 100 mg/kg and received thyroidectomy. Each rat was given 1% calcium lactate in drinking water after thyroidectomy. Two weeks after thyroidectomy, the rats were randomly assigned to receive a daily injection of either 10 μg/100 g body weight T3 (Sigma, St. Louis, MO; dissolved in 2.5 mmol/L NaOH, hyperthyroid group) or T3 + propranolol (Sigma; pro group) for 2 additional weeks. The third group taking sham operation was designated as euthyroid controls. Serum T3 and thyrotropin (TSH) levels were assayed by radioimmunoassay to document euthyroid or hyperthyroid status. The dosage of hormone and drugs were used according to previous description [9]. All animal experiments in this study were performed in accordance with the Institutional Animal Care and Use Committee Guide for the Care and Use of Laboratory Animals.

Hemodynamic studies

Rats were anesthetized with ketamine 100 mg/kg. A calibrated 2 French micromanometer-tipped catheter (Millar Instruments, Houston, TX) was passed into the carotid artery, and heart rate was recorded on a strip-chart recorder (model 2400, Gould, Cleveland, OH), with the high frequency filter cut-off set at 300 Hz.

Culture of cardiac fibroblasts

Atrial fibroblasts were obtained from atria of adult Wistar rats and were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin as described previously [10, 11]. Cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Immunofluorescence

Immunofluorescence were performed using anti-collagen I (Abcam, Cambridge, MA), antibodies as primary antibody followed by Cy3- (red color, Chemicon, Temecula, CA) conjugated secondary antibody, and were visualized by confocal immunofluorescent microscope.

Western blot analysis

Cellular or tissue proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. After blocking with 5% non-fat milk Tris buffered Saline-Tween 20 (TBS-T), membranes were incubated with primary antibodies against anti-collagen I (Abcam), anti-collagen III (Abcam), anti-LCC α1C-subunit, anti-phospho-Akt, anti-Akt, anti-phospho-p44/42 mitogen-activated protein kinases (MAPK), and anti-p44/42 MAPK (Cell Signaling Technology) antibodies. β-actin (Sigma) was used as the loading control. The protein bands were detected with SuperSignalWest Pico Chemiluminescent Substrate (Pierce, Rockford, IL) on X-ray films (Kodak, Tokyo, Japan).

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD) and real-time quantitative RT-PCR was performed as described previously [12]. GAPDH mRNA was used as the internal control. The primers were listed in **Table 1**.

Statistical analyses

All statistical analyses were carried out using SPSS v21 (SPSS, Chicago, IL). Data are presented as mean ± standard deviation (SD). Student's t-test or one way ANOVA was used to examine differences two groups or multiple group comparison. $P<0.05$ was considered as statistically significant.

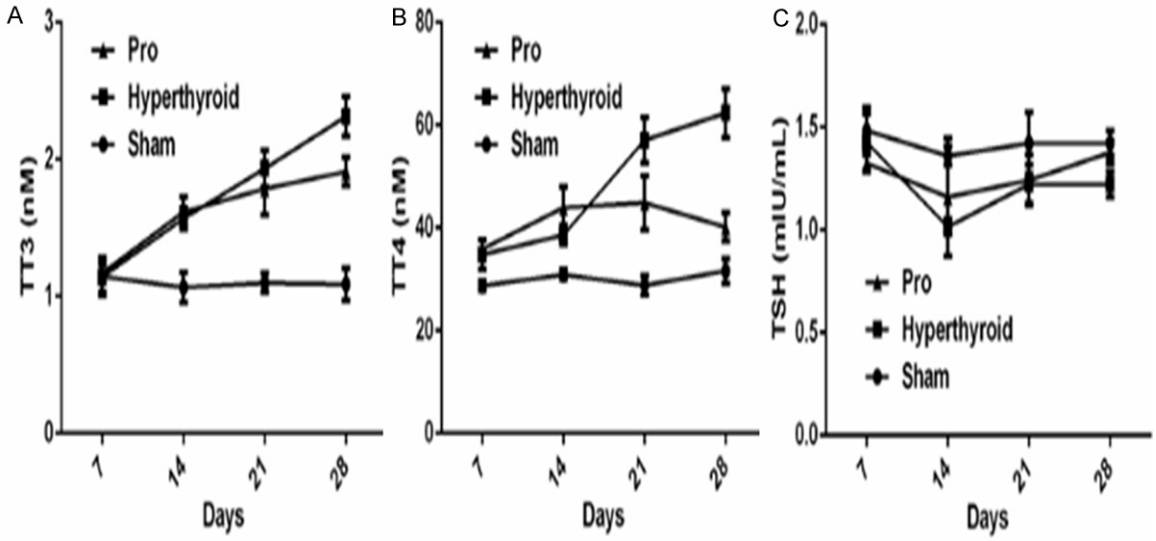

Figure 1. Effects of propranolol on serum triiodothyronine (T3) (A), L-thyroxine (T4) (B) and thyrotropin (C) in a rat model of hyperthyroidism-induced atrial fibrillation. *P<0.05 when compared with propranolol and hyperthyroid group.

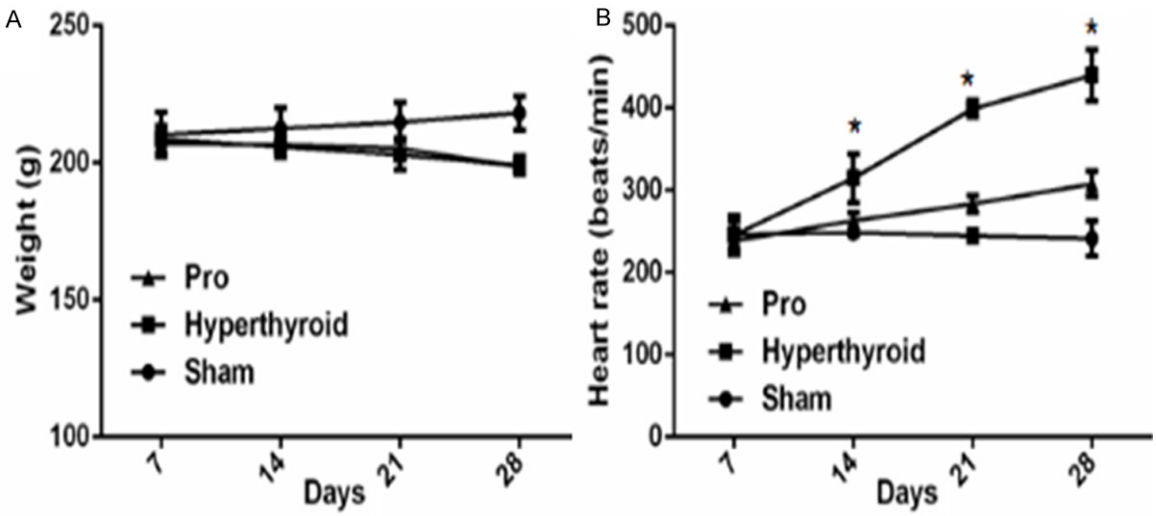

Figure 2. Effects of propranolol on weight (A) and heart rates (B) in a rat model of hyperthyroidism-induced atrial fibrillation. *P<0.05 when compared with propranolol group.

Results

Effects of propranolol on thyroid hormone and heart rates

A hyperthyroid rat model was established and the serum level of T3, T4 and thyrotropin (TSH) were tested at different time points. No effect was found on the serum level of T3, T4 and thyrotropin at 7, 14, 21 and 28 days in propranolol group compared with hyperthyroid group (**Figure 1**). However, we found significantly decreased heart rate in propranolol group compared with control group (**Figure 2A**). In addition, propranolol treatment exerted no effect on weight in different group (**Figure 2B**).

Effects of propranolol on the L-type calcium channel subunit and collagen expression

We firstly examined the expression of different LCC subunit and found that a significantly

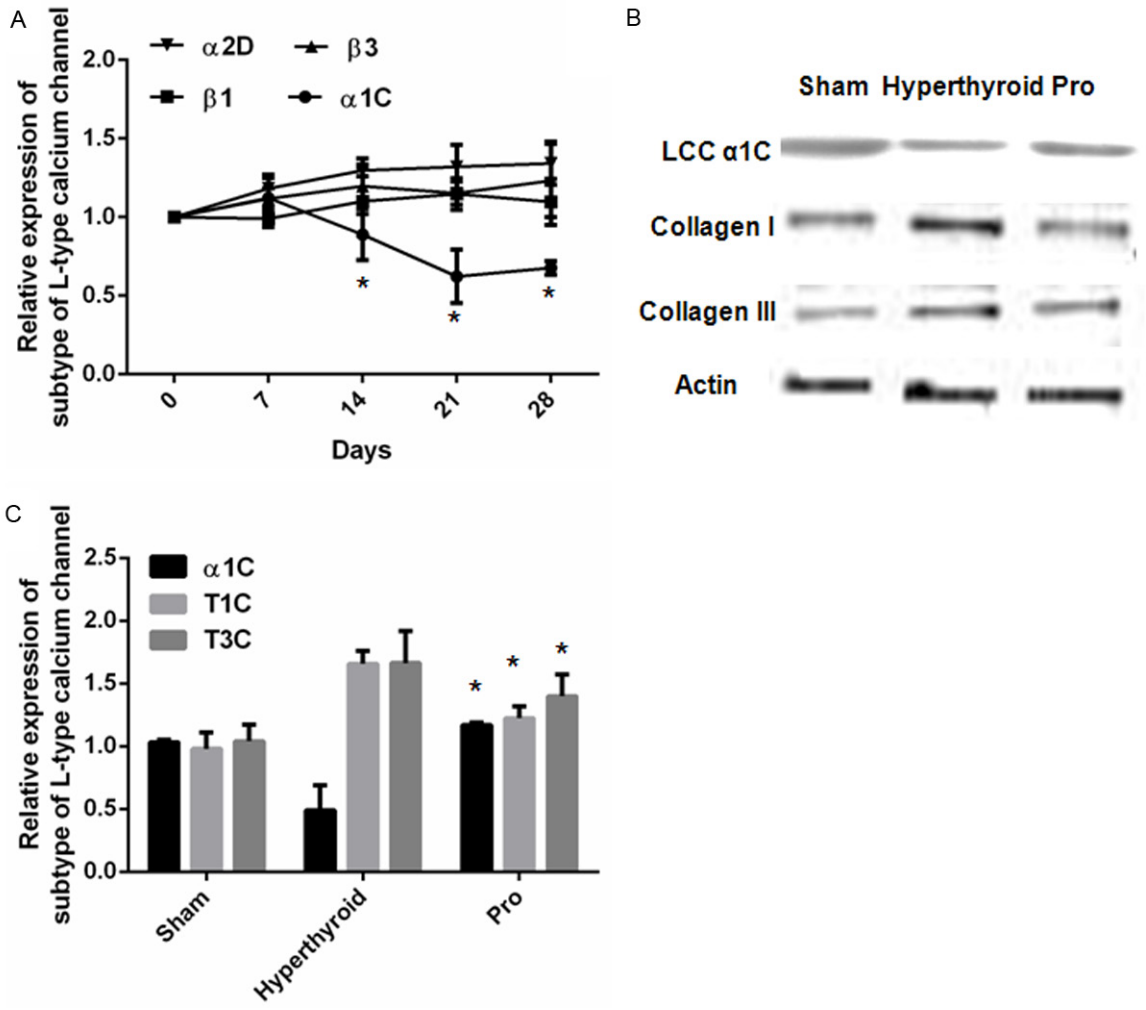

Figure 3. Effects of propranolol on the L-type calcium channel subunit (LCC) (A) and collagen expression (B) (A) The expression of LCC subunit α1C in hyperthyroid group at different time points, *P<0.05 when compared with start (Day 0) time point. (B) The expression of LCC subunit α1C, collagen I and collagen III in different group. *P<0.05 when compared with hyperthyroid group.

decreased LCC α1C-subunit in hyperthyroid group at 7, 14, 21 and 28 days after the thyroidectomy and T3 treatment. We therefore analyzed the expression of LCC α1C-subunit and collagen, the results showed that propranolol treatment could significantly decreased the expression LCC α1C-subunit, collagen I and collagen III (**Figure 3**).

Propranolol treatment decreased Erk and increased Akt phosphorylation

We further analyzed downstream signaling pathways involved in the effects of propranolol and the results showed that propranolol treatment could significantly decrease the expression of Erk phosphorylation and increased the level of Akt phosphorylation (**Figure 4**).

Effect of propranolol treatment on cardiac fibroblast in vitro

We also isolated the cardiac fibroblast and test the effect propranolol. The results showed that 10-100 μM T3 could significantly decrease the expression of LCC α1C-subunit (**Figure 5A**), and propranolol treatment could significantly decreased the expression of LCC α1C-subunit (**Figure 5B**).

Moreover, we also examined the expression of different type of collagen. The results showed

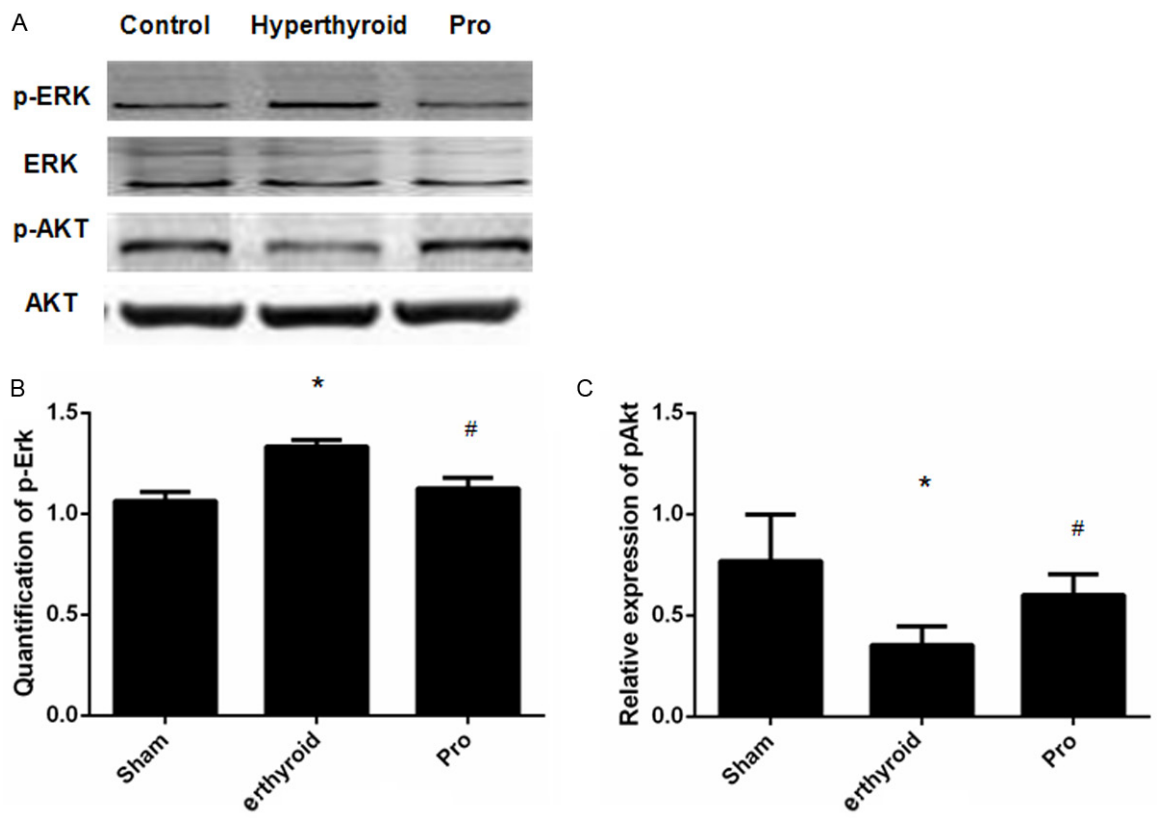

Figure 4. Signaling pathways involved in the effects of propranolol in a rat model of hyperthyroidism-induced atrial fibrillation. A. Western-blot analyses of Erk and Akt phosphorylation. B. Quantification of Erk and Akt phosphorylation. *P<0.05 when compared with sham group, # P<0.05 when compared with hyperthyroid group.

that propranolol treatment could significantly decrease the expression of collage I and collagen III (**Figure 6**).

Discussion

In present study, we demonstrated that propranolol treatment has no effect on the serum level of thyroid hormone. However, a significantly effect on heart rates decreasing was observed in the hyperthyroid rats with propranolol treatment. On the mechanisms, propranolol treatment could significantly decrease the expression of LCC α1C-subunit and type I collagen. Furthermore, decreased phosphor-Erk and increased phosphor-Akt were found in propranolol group compared with hyperthyroid group. To the best knowledge, this is the first study to show the possible mechanism about the action of propranolol in hyperthyroid induced AF.

According to previous reports, AF is the most common sustain arrhythmia in patients with hyperthyroidism [13-15]. However, the pathogenesis of AF has not been fully explored yet. It is generally accepted that the abbreviation of action potential duration (APD) due to a decreased LCC current, a response that potentially protects the myocyte against intracellular Ca^{2+} overload, resulting in shortening of atrial refractoriness [16-19]. Shortening of atrial effective refractory period could decrease wave length and result in increased intra-atrial reentry, thereby contributing to the development of AF [16-21]. Earlier study in animal model of hyperthyroidism has demonstrated that thyroid hormone downregulates LCC expression at the mRNA level [22]. Here, we examined the expression of different subunits of LCC and found that only LCC subunit α1c was significantly decreased at Day 14, 21 and 28 after model establishment. We also assessed the expression of protein level of LCC subunit α1c in hyperthyroid group and propranolol group, and the results showed that reduced level of α1c in hyperthyroid group and increased level in propranolol group.

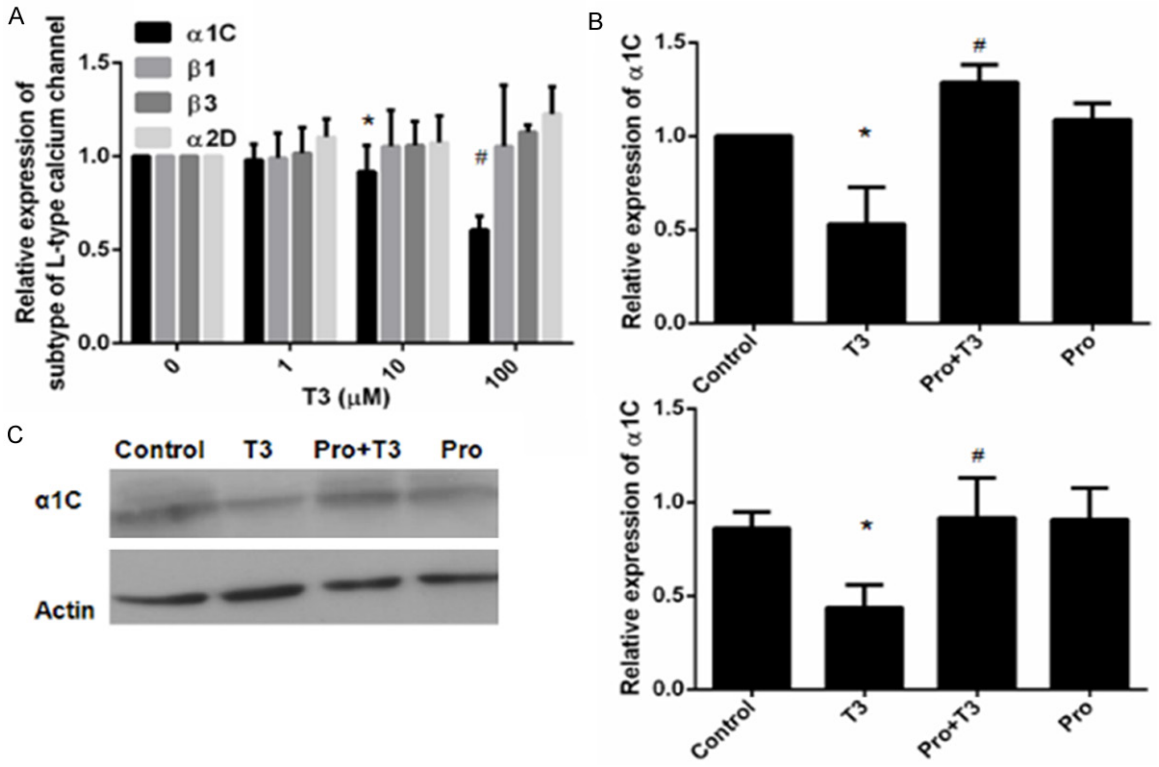

Figure 5. Effects of propranolol on L-type calcium channel subunit expression incardiac fibroblast stimulated by thyroid hormone. A. Expression of L-type calcium channel subunit. Cardiac fibroblasts were stimulated by different concentration of L-thyroid hormone. *P<0.05 when compared with control, #P<0.01 when compared with control. B. Relative expression of L-type calcium channel α1C mRNA in cardiac fibroblasts with different treatment. *P<0.05 when compared with sham group, #P<0.05 when compared with hyperthyroid group. C. Expression of L-type calcium channel α1C protein in cardiac fibroblasts with different treatment. *P<0.05 when compared with sham group, #P<0.05 when compared with hyperthyroid group.

From a previous review, thyroid hormone exerts a key role in the regulation of heart rate, heart rhythm, blood pressure, cardiac contractility, and cardiac hypertrophy by modulating the cardiac related gene expression, including alpha myosin heavy chain fusion (MHC-α), MHC-β, sarcoplasmic reticulum calcium-activated ATPase (SERCA), β1 adrenergic receptor, phospholamban (PLB), calcium (Ca^{2+}) transporter proteins, cardiac troponin I, atrial natriuretic peptide (ANP), adenylyl cyclase (IV and V), and the sodium $(Na)^{+}$-Ca^{2+} antiporter [23]. Thyroid hormone also exerts a modulation role on SERCA, which plays an important role in both normal systolic and diastolic function regulation [23, 24]. For example, the increasing in $SERCa^{2+}$ ATPase and the ryanodine channel, and decreased PLB phosphorylation and $SERCa^{2+}$ pump activity inhibition could be fulfilled by thyroid hormone. These $SERCa^{2+}$ and PLB changes can be linked to a decrease in the rate of diastolic relaxation [24]. TH regulates the expression of specific cardiac genes related to heart rhythm, such as plasma membrane sodium potassium (K^{+}) ATPase and voltage-activated K1 channel genes including Kv4.2, Kv4.3, and Kv1.5. Here, we analyzed the expression of LCC α1C-subunit and collagen, the results showed that propranolol treatment could significantly decreased the expression LCC α1C-subunit, collagen I and collagen III.

The signaling pathways such as mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) have been reported to be involved in the cardiac functional changes induced by hyperthyroidism. For example, activated serum kinases contribute to increased sinoatrial activity, lowering of the threshold for atrial activity, and shortened atrial repolarization resulting in heart rate increases in thyrotoxic-dilated cardiomyopathy [23, 24]. Hyperthyroidism also

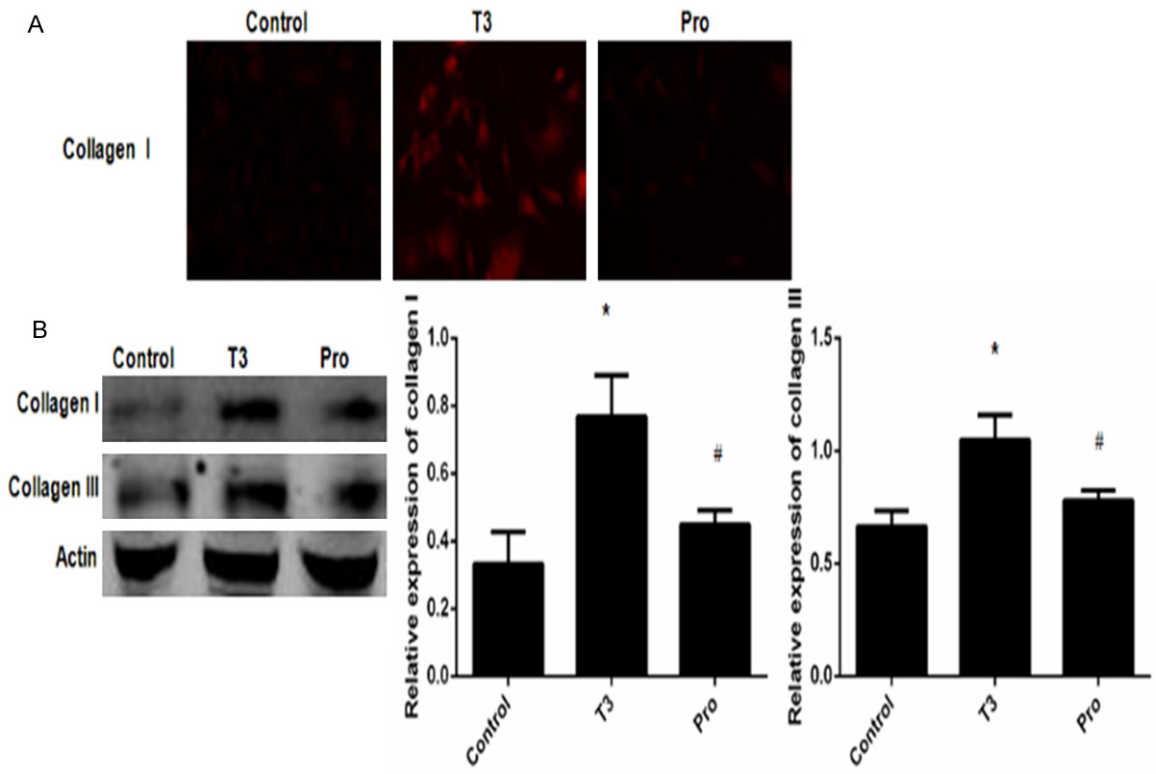

Figure 6. Effects of propranolol treatment on collagen expression in cardiac fibroblasts with thyroid hormone stimulation. A. Immunofluorescence staining of type I collagen. B. Expression of L-type calcium channel α1C protein in cardiac fibroblasts with different treatment. *P<0.05 when compared with sham group, #P<0.05 when compared with hyperthyroid group.

affects expression of cardiac connexins, which can "talk" to each other, directly exchange ions and messenger molecules between adjacent cardiomyocytes and play important roles in the promotion of arrhythmogenesis [23]. Wang et al provided a novel molecular mechanism underlying eccentric cardiac hypertrophy and myocardial degeneration in DCM, in which locally increased T3 in the heart by transcriptional up-regulation of Dio2 causes physiological and pathological cardiac hypertrophy through activating two distinct non-nuclear TH-signalling pathways, TRα1-PI3K-Akt-mTOR-S6K1 and TRα1-TAK1-p38 MAPK, respectively [25]. We also analyzed downstream signaling pathways involved in the effects of propranolol and the results showed that propranolol treatment could significantly decrease the expression of Erk phosphorylation and increased the level of Akt phosphorylation.

In conclusion, we found here that propranolol could protect hyperthyroid rat from AF through upregulation of the expression of LCC α1C-subunit and downregulation of collagen. MAPK and Akt related pathways were involved in the mechanisms.

Acknowledgements

All research involving human participants was approved by the Institutional Review Board of Our University. Written informed consent was obtained from the participating individual. The study protocol was approved by the Institutional Animal Care and Use Committee of our hospital as which is adherent to generally accepted international guidelines for animal experimentation. The health and family planning commission of Chongqing, China (No. 2012-1-066).

Disclosure of conflict of interest

None.

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