of the neurotransmitter, together with a decrease in NE and E in adrenal by over secretion, might form a phenomenon of an increased tension of sympathetic nervous system.

ACEI Cap and calcium antagonist Ver could be used not only to reduce the cardiac hypertrophy but also to exert an action against hyperthyroidism in clinical medicine.

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普萘洛尔、维拉帕米和卡普托利对 *L*-甲状腺素性心肌肥厚的消退及抑制线粒体钙泵的作用

陈丁丁 于 锋 章 涛 戴德哉

(中国药科大学药理学研究室，南京 210009)

摘 要 大鼠连续口服 *L*-甲状腺素 4 mg·kg^{-1}×7 d 可形成心肌肥厚。高甲状腺素状态下，心肌去甲肾上腺素(NE)增加，交感神经兴奋而使细胞内 Ca^{2+} 增加，其表现为线粒体 Ca^{2+}，Mg^{2+}-ATP 酶活性增高。大鼠连续 3 天口服普萘洛尔 10 mg/kg，维拉帕米 5，10 mg/kg 及卡普托利 5 mg/kg 可使肥厚心肌消退，并可降低线粒体钙泵活性。

关键词 *L*-甲状腺素；心肌肥厚；钙泵；线粒体；去甲肾上腺素；普萘洛尔；维拉帕米；卡普托利

Reducing *L*-Thyroxine Cardiac Hypertrophy and Suppressing Mitochon-drial Calcium Pump by Propranolol, Verapamil and Captopril

Chen Dingding, Yu Feng, Zhan Tao, Dai Dezai
Research Division of Pharmacology, China Pharmaceutical University, Nanjing 210009

Abstract A cardiac hypertrophy model was produced by po l-thyroxine 4 mg/kg for 7 d, evidenced as an increased cardiac mass in terms of HW/BW and LVW/BW against normal. Rats were grouped for administering Pro 10, Ver 5 & 10 and Cap 5 mg/kg po for 3 d vs saline group to assess a reduction of hypertrophied myocardium and inhibition in mitochondrial calcium pump activity. All of the three drugs were effective to reduce hypertrophic mass and increased mitochondrial Ca^{2+}, Mg^{2+}-ATPase activity which reflects a surplus of Ca^{2+} presented in the affected cytosol caused by an over-activity of sympathetic nervous system by which myocardial NE increased and adrenal NE & E decreased due to over-secretion. Ver and Cap could be useful in treating hyperthyroidism.

Key words l-Thyroxine; Cardiac hypertrophy; Calcium pump; Mitochondria; Norepinephrine; Propranolol; Verapamil; Captopril

Ventricular hypertrophy of a heart always causes various dysrhythmias[1], cardiac ischemia and insufficiency, recognized as a common way stemmed from the final stage of varying cardiopathies[2,3]. Regression of hypertrophic heart, nowaday, is a main focus for drug evaluation and developing new drugs, e. g a lowering of blood pressure is no longer satisfied if a tested agent is not able to impact on remodelling of the cardiovascular system[2]. There are several models of cardiac hypertrophy used in laboratories. In this paper we are testing the efficacy of propranolol, verapamil and captopril by affecting the cardiac mass and mitochondrial calcium ATPase using l-thyroxine induced hypertrophic ventricular model, and mearsuring norepinephrine and epinephrine levels in myocardium and adrenal gland to evidence an over-activity of sympathetic nervous system existing in hyperthyroidism.

1 Materials and Methods

1.1 *Animal*

Rats, SD, either sex, weighing 180～230 g were used and offered by Animal House of the University.

1.2 *Chemicals and solutions*

l-thyroxine (l-thy) from Sigma, propranolol (Pro) from Wuxi No. 14 Pharmaceutical Factory, verapamil (Ver) from Tianjin Institute of Pharmaceutical Industry, captopril (Cap) from Changzhow Pharmaceutical Factory and ouabaine from Merck were used. Other chemicals were purchased from the market.

The homogenizing solution for isolating mitochondria was composed as follows: sucrose 0.25 mol/L, in imidazole-HCl buffer solution pH 7.5. The substrate buffer solution was composed as(mmol/L): EDTA 0.125, $MgCl_2$ 7.5, $CaCl_2$ 0.375, ATP-Na2 1.25, ouabaine 1.25 in imidazole-HCl buffer solution at pH 7.4.

1.3 *Hypertrophic heart by l-thy*[4]

Rats were medicated with l-thy 4 mg/kg orally for 7 days, thereafter, subdivided into the following groups treated with either saline or drug interventions for 3 days. Pro 10, Ver 5 & 10 and Cap 5 mg/kg were administered po once per day. No medication was offered on the day

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of experiment. The heart was taken and weighed after sacrificing animal by cervicle dislocation. Hearts were placed on a cold plate to separate and weighed the left ventricle. The heart index heart weight (mg)/body weight (g), HW/BW and the left ventricle index left ventricle weight (mg)/body weight (g), LVW/BW were obtained and compared with saline.

1.4 *Isolation of mitochondria*[5]

A portion of the left ventricle was placed in ten fold volume of homogenizing solution. After being homogenized, tubes then were centrifuged at 750 g for 30 min. Supernatant was centrifuged again at 9000 g for 20 min and the pellet was mitochondria redissolved in 5 ml of the homogenizing solution. The whole schedule was processed under 4℃.

1.5 *Assay of* Ca^{2+}, Mg^{2+}-*ATPase activity*[5,6]

An aliquot of 0.2 ml of ATPase containing liquid(mitochondrial portion) was added in a test tube, mixed with the substrate buffer solution 0.8 ml, starting the reaction at 37℃ and maintaining for 10 min. The reaction was ended by adding 15% TCA 0.5 ml. In the tissue blank tube, 15% TCA was added prior to the substrate for inactivating the enzyme. Following centrifugation at 750 g 10 min, the inorganic phosphate in 1 ml of supernatant was determined by spectrophotometric method at 660 nm. One unit(u) of the specific activity of the ATPase was defined as a micromole of inorganic phosphorus released from 1 mg mitochondrial protein within one hour($\mu mol\ P_i^{-1} \cdot mg\ protein^{-1} \cdot h^{-1}$).

1.6 *Protein assay*[7]

The protein assay of sample was after the method by Bradford with BSA as the standard.

1.7 *Assay of norepinephrine (NE) and epinephrine (E)*

The heart and adrenal gland were homogenized and assayed for NE and E as described in the previous paper[8].

1.8 *Statistics*

The significance of difference was set at $P>0.05$, $P<0.05$ *and* $P<0.01$ by t test with two tailed assay.

2 Results

2.1 *Ventricular hypertrophy by l-thy*

The weight of ventricle and heart increased in hypertrophic heart by l-thy, compared of the cardiac and ventricular index versus normal ($P<0.01$)(Tab 1).

2.2 *Reducing hypertrophic myocardium*

Three days treatment of the cardiac hypertrophy with Pro 10 mg/kg, Ver 5 and 10 mg/kg and Cap 5 mg/kg were sufficient to reduce the cardiac mass significantly. The extent of reduction of the heart and left ventricle index by Pro was −11.5%, −15.5%, and −14.8%, −15.8% by Ver 10 mg/kg and −10%, −10.6% by Cap. All of three tested drugs could not reduce the cardiac hypertrophy to normal(Tab 1).

Tab 1. Reduction of cardiac mass of *l*-thyroxine hypertrophic heart by propranolol, verapamil and captopril in rats. $\bar{x}\pm s$

Groups, mg/kg		n	HW/BW, mg/kg	LVW/BW, mg/kg
Normal		8	2.93±0.19	2.19±0.15
Hypertrophy				
Untreated		8	3.91±0.24***	2.84±0.35***
Propranolol	po			
10		7	3.46±0.06***###	2.40±0.07***###
Verapamil	po			
5		7	3.66±0.12***##	2.66±0.21***#
10		7	3.33±0.18***###	2.39±0.13***###
Captopril	po			
5		7	3.51±0.25***##	2.54±0.17***#

***$P<0.01$ *vs* normal, #$P>0.05$, ##$P<0.05$, ###$P<0.01$ *vs* untreated.

2.3 *Suppressing over-activity of mitochondrial Ca^{2+}, Mg^{2+}-ATPase*

The Ca^{2+}, Mg^{2+}-ATPase was measured after isolation of mitochondria in both the normal and pathological heart. There was an increment of 160% in mitochondria of l-thy hypertrophic heart over the normal. Lowering of Ca^{2+}, Mg^{2+}-ATPase was very impressed with Pro by a reduction of up to 58%. Two doses of Ver were also beneficial and a dose related decrease in activity of mitochondrial calcium pump was evidently noticed. Cap was able to inhibit the overactivity of the pump by 35% (Tab 2).

Tab 2. Reduction in enhanced mitochondrial Ca^{2+}, Mg^{2+}-ATPase activity of l-thyroxine hypertrophic left ventricle by propranolol, verapamil and captopril in rats. $\bar{x}\pm s$

Groups, mg/kg po	n	Ca^{2+}, Mg^{2+}-ATPase activity, μmol Pi • mg protein^{-1} • h^{-1}
Normal	8	3.5±0.22
Hypertrophy		
Untreated	8	9.1±0.5***
Pro 10	7	3.81±0.10***###
Ver 5	7	6.19±0.08***###
10	7	3.98±0.11***###
Cap 5	7	5.90±0.13***###

***$P<0.01$ *vs* normal, ###$P<0.01$ *vs* untreated.

2.4 *Norepinephrine and epinephrine levels in the heart and adrenal gland*

Under a state of hyperthyroidism catecholamine levels in the heart was assayed to show an increase in NE and E. An amount of catecholamines in the adrenal was very concentrated up to a level of ug per gram of tissue and a significant decrease was observed by hyperthyroidism in rats(Tab 3).

Tab 3. Influence of hyperthyroidism on levels of NE and E in the heart and adrenal gland. $\bar{x}\pm s$

Groups	Heart ($n=9$)		Adrenal ($n=6$)	
	NE	E, ng/g	NE	E, ug/g
Normal	450±150	10±30	71±17	670±120
Hyprethyroid	620±150**	50±30***	47±8**	280±30**

**$P<0.05$, ***$P<0.01$ *vs* normal

3 Discussion

l-thy cardiac hypertrophy shows arrhythmogeneity creating as more severe arrhythmias on reperfusion[4], which, besides a change in APD and ERP[4], an elevation in Ca^{2+} ion in the cytosol reflected by an in direct signal of an enhanced Ca^{2+}, Mg^{2+}-ATPase activity may play a role to contributing to arrhythmogeneity.

In the case of l-thy hypertrophy triggering synthesis of mRNA and binding of high-affinity nucleus receptor with l-thy are contributive to the increased protein systhesis. Effectiveness of Pro comes from a blockade of β-adrenoceptor on cardiac membrane rather than interfering mRNA transferring system in myocytes. It is true, however, that signal transduction transversing membrane is altered by an enhanced coupling between β-receptor and the stimulatory guanine nucleotide regulatory protein by chronic treatment of Pro[9]. A large amount of Ca^{2+} in myocardium is likely a consequense of over-activity of sympathetic nervous system and it in turn stimulates the ventricular hypertrophy by increased contractility. Pro[10], Ver and Cap showed negative effects on Ca^{2+}, Mg^{2+}-ATPase activity in parallel with a reduction in cardiac mass. Intracapillary distance increased by hypertrophic myocardium causing a longer way than usual for oxygen diffusion is reduced by Ver which improves myocardial microperfusion by dilating large coronary arterioles but not the terminal arterioles and capillaries[11].

Some beneficial actions of Cap other than inhibiting ACE in plasma and tissues as free radical scavenging and suppressing platelat aggregation are involved[12].

Under physiological conditions, Ca^{2+} is mainly pumped out of cytosol during diastole by calcium pump of sarcoplasmic reticulum[13], however, the main burden of a surplus of Ca^{2+} in pathological situation is taken over by mitochondrial Ca^{2+}, Mg^{2+}-ATPase to pump into its matrix keeping a stable intracellular enviroment for normal function. An over-load of calcium deposited in matrix can be visible under electro-microscope[14] and compromised mitochondrial function in energetics is expected.

Depletion of myocardial NE is a consequense of denervation by infarction, and impact of an over-activity of peripheral sympathetic nerve system on cardiac myocyte is an over-load

of the neurotransmitter, together with a decrease in NE and E in adrenal by over secretion, might form a phenomenon of an increased tension of sympathetic nervous system.

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