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Propranolol and bepridil attenuating levothyroxine-induced rat cardiac hypertrophy and mitochondrial Ca^{2+} Mg^{2+}-ATPase activity elevation

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KEY WORDS propranolol; bepridil; levothyroxine; heart mitochondria; Ca^{2+} Mg^{2+}-ATPase; heart hypertrophy

AIM: To study the effects of propranolol and bepridil on levothyroxine-induced rat cardiac hypertrophy and mitochondrial Ca^{2+} Mg^{2+}-ATPase activity elevation **METHODS**: Rat heart hypertrophy was induced by ip levothyroxine 1 mg $\cdot kg^{-1} \cdot d^{-1} \times 10$ d. Then rats were treated by ig propranolol (Pro) or bepridil (Bep) 10 mg $\cdot kg^{-1}$ daily. Ca^{2+} Mg^{2+}-ATPase activity and enzyme kinetic parameters were assayed. **RESULTS**: The activity and V_{max} of mitochondrial Ca^{2+} Mg^{2+}-ATPase isolated from hypertrophic left ventricle were 25 ± 4 and 35.1 ± 0.8 μmol $P_i \cdot h^{-1}$/mg protein, respectively, those of normal were 6.7 ± 1.8 and 10 ± 4 μmol $P_i \cdot h^{-1}$/mg protein, respectively. Apparent K_m of the hypertrophic group Ca^{2+} Mg^{2+}-ATPase was 0.4 ± 0 12 mmol $\cdot L^{-1}$ ATP, and that of normal was 0.59 ± 0.22 mmol $\cdot L^{-1}$ ATP. The total protein quantity of hypertrophic left ventricle was 80 ± 30 mg, and that of normal was 47 ± 9 mg. After treated with Pro or Bep (both 10 mg $\cdot kg^{-1}$ ig), the cardiac hypertrophy was attenuated, the enzyme activity and V_{max} as well as total protein quantity of hypertrophic left ventricle were reduced to normal level, but apparent K_m was not affected. **CONCLUSION**: Both Pro and Bep prevented the myocardium and its mitochondria from ischemia and overload calcium injury.

High dose of levothyroxine (Lev) which increased the cardiac mRNA and protein including Ca^{2+} Mg^{2+}-ATPase synthesis induced a heart hypertrophy[1,2] and uncoupled the mitochondria oxidative phosphorylation to decrease ATP synthesis, meantime, the oxygen consumption of heart kept still high. It made the myocardium short of ATP, and as a result, it accelerated the cardiac hypertrophy[1,2]. Therefore, the hypertrophic heart induced by Lev is a suitable model for studying ischemic myocardium. There existed a high level of calcium ion in Lev-induced hypertrophic myocardium. The effects of calcium antagonist bepridil (Bep)[3,4] and β-blocker propranolol (Pro) on Lev-caused ischemia hypertrophic myocardium, the left ventricle mitochondrial Ca^{2+} Mg^{2+}-ATPase activity, and the enzyme kinetic parameters were studied. Pro[5] was used as positive control drug in this paper.

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MATERIALS AND METHODS

Chemicals Pro was from Wuxi Fourth Pharmaceutical Factory (910516), Bep (901101) was from Changzhou Fourth Pharmaceutical Factory. Lev (sodium salt) and ATP (grade Ⅱ) were from Sigma. Ouabain (extra pure) was from Merck. Imidazle (99.0 % purity) was from Fluka (289 829 589). Other chemicals were AR.

Establishment and treatment of heart hypertrophic myocardium SD rats, ♀, $n = 40$, weighing 180 ± 27 g (121 - 226 g), were injected ip Lev 1 mg $\cdot kg^{-1} \cdot d^{-1}$ × 10 d. From d 11 to d 13, rats were treated by ig Pro or Bep 10 mg $\cdot kg^{-1}$ daily.

Preparation of left ventricle mitochondria Rat hearts were dropped into liquid nitrogen. The left ventricles were homogenized in 10 mL of imidazole buffer 10 mmol $\cdot L^{-1}$ containing sucrose 0.25 mol $\cdot L^{-1}$. The homogenates were assayed for protein content and centrifuged at 750 × *g* for 20 min, the supernatants were recentrifuged at 9000 × *g* for 20 min. The pellets, containing mitochondria, were resuspended in homogenizing medium for the study. All procedures were performed at 4 ℃.

Ca^{2+} Mg^{2+}-ATPase assay The Ca^{2+} Mg^{2+}-ATPase activity of mitochondria was measured[4,6].

Measurement of Ca^{2+} Mg^{2+}-ATPase apparent K_m and V_{max} According to Lineweaver-Burk double-reciprocal plot method, the apparent K_m and V_{max} of Ca^{2+} Mg^{2+}-ATPase were calculated by linear regression[7]. Protein was determined[8].

Statistical analysis Data were compared with *t* test.

RESULTS

The heart weight (HW)/body weight (BW), left ventricle weight (LVW), and LVW/BW were increased by 39 %, 33 %, and 51 %, respectively ($P<0.01$). After treatment with Pro and Bep, all of the 3 parameters returned to normal (Tab 1).

Tab 1. Effects of propranolol and bepridil 10 mg·kg^{-1} ig on mass parameters of hypertrophic rat hearts induced by levothyroxine 1 mg·kg^{-1} ip.
$n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* normal.

Groups	HW/BW, mg·g^{-1}	LVW, mg	LVW/BW, mg·g^{-1}
Normal	3.58±0.16	360±40	2.43±0.23
Hypertrophy			
Untreated	4.97±0.14^c	480±70^a	3.7±0.5^c
Propranolol	3.65±0.19^a	370±70^a	2.6±0.3^a
Bepridil	3.63±0.18^a	400±70^a	2.38±0.18^a

The total protein contents of left ventricles of untreated rats were increased by 70 % ($P<0.05$). After treated with Pro and Bep, total protein contents were decreased to normal (Tab 2).

Tab 2. Effects of propranolol and bepridil 10 mg·kg^{-1} ig on total protein quantity in left ventricle of hypertrophic rat hearts induced by levothroxine 1 mg·kg^{-1} ip. $\bar{x}\pm s$.
$^aP>0.05$, $^bP<0.05$ *vs* normal.

Groups	n	Total protein quantity/ mg
Normal	10	47±9
Hypertrophy		
Untreated	10	80±30^b
Propranolol	8	43±5^a
Bepridil	10	45±10^a

In left ventricles, mitochondria Ca^{2+} Mg^{2+}-ATPase activities and maximal velocities (V_{max}) of untreated rats were elevated by 273 % and 251 %, respectively ($P<0.01$), whearas the enzyme apparent Michaelis constants (K_m) of untreated rats were reduced by 32 % below that of normal ($P<0.01$). After treated with Pro and Bep, both of activities and V_{max} were decreased to normal, but no significant difference for apparent K_m were observed (Tab 3).

Tab 3. Effects of propranolol and bepridil 10 mg·kg^{-1} ig on activity and kinetic parameters of mitochondria Ca^{2+} Mg^{2+}-ATPase in left ventricle of hypertrophic rat hearts induced by levothyroxine 1 mg·kg^{-1} ip. $\bar{x}\pm s$.
$^aP>0.05$, $^cP<0.01$ *vs* normal.

Groups	n	Activity/ u*	V_{max}/ u*	Apparent K_m/ mmol·L^{-1} ATP
Normal	6	6.7±1.8	10±4	0.59±0.22
Hypertrophy				
Untreated	9	25±4^c	35.1±0.8^c	0.40±0.12^a
Propranolol	8	6.9±0.9^a	9.1±1.3^a	0.39±0.19^a
Bepridil	10	6.4±0.6^a	11±3^a	0.45±0.17^a

*: u = μmol P_i·h^{-1}/mg protein.

DISCUSSION

The results suggested that Lev could stimulate the biosynthesis of Ca^{2+} Mg^{2+}-ATPase in rat left ventricle mitochondria and agreed with reports[2,9]. When the heart hypertrophy induced by Lev, as a compensatory mechanism for maintenance cardiac calcium homeostasis, the quantity of mitochondria Ca^{2+} Mg^{2+}-ATPase was simultaneously elevated for preventing myocardium from ischemia overload calcium damage. But on the other hand, Ca^{2+} Mg^{2+}-ATPase consumed a great number of ATP when it pumped the Ca^{2+}, the load of energy and oxygen of myocardium were enhanced, and then, the lethal recurrence would take place.

The damage of overload calcium for myocardium was mainly caused by calcium "bomb" mitochondria[10]. It would seriously inhibit ATP synthesis and lead to arrhythmias and cardiac infarct[10]. Tab 2 and 3 showed that the quantity of mitochondrial Ca^{2+} Mg^{2+}-ATPase in hypertrophic left ventricle was elevated, the enzyme apparent K_m was significantly decreased *vs* normal, that is, the enzyme affinity for ATP was increased, the ability of pump calcium was significantly enhanced. These results supported the hypothesis of overload calcium "bomb" mitochondria and provided evidence for treatment of hypertrophic cardiomyopathy.

The report didn't show whether Pro affects the mitochondrial Ca^{2+} Mg^{2+}-ATPase in Lev-induced hypertrophic heart. Tab 3 showed that Pro significantly inhibited the mitochondria Ca^{2+} Mg^{2+}-

ATPase activity elevation in hypertrophic left ventricle induced by Lev, it proved that Pro could prevent myocardium mitochondria from overload calcium damage and from ATP depletion, *ie*, Pro could prevent myocardium from ischemic damage.

Bep, a novel type of calcium antagonist, not only blocks the calcium channels of cell surface membrane greatly lowering intracellular free calcium level, but also enters cytosol to block calmodulin and inhibit calmodulin-stimulated Ca^{2+} Mg^{2+}-ATPase activity[4]. We observed that Bep inhibited the mitochondrial Ca^{2+} Mg^{2+}-ATPase activity and V_{max} in Lev-induced rat hypertrophic left ventricle, however, the apparent K_m was not affected. The results showed that Bep non-competitively, *in vivo*, inhibited mitochondrial Ca^{2+} Mg^{2+}-ATPase. We postulated that Bep, after entering cytosol, could inhibit Ca^{2+} Mg^{2+}-ATPase activity through blocking calmodulin, thus, ATP depletion of myocardium and the overload calcium damage mitochondria were avoided.

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普萘洛尔和苄普地尔消除左甲状腺素诱发的大鼠心脏肥厚和线粒体 Ca^{2+} Mg^{2+}-ATP 酶活力升高

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关键词 普萘洛尔；苄普地尔；左甲状腺素；心脏线粒体；Ca^{2+} Mg^{2+}-ATP 酶；心脏肥厚

目的：研究普萘洛尔和苄普地尔对左甲状腺素诱发的大鼠心脏肥厚及其线粒体 Ca^{2+} Mg^{2+}-ATP 酶活力升高的影响。**方法**：ip 左甲状腺素 1 mg·kg^{-1}·d^{-1} × 10 d，诱发大鼠心脏肥厚，然后 ig 普萘洛尔或苄普地尔 10 mg·kg^{-1}·d^{-1} × 3 d 治疗。Ca^{2+} Mg^{2+}-ATP 酶活力及其酶动力学参数测定。**结果**：肥厚左室线粒体 Ca^{2+} Mg^{2+}-ATP 酶活力和 V_{max} 分别为 25 ± 4 和 35 1 ± 0.8 μmol P_i·h^{-1}/mg protein，正常组相应值分别为 6 7 ± 1 8 和 10 ± 4 μmol P_i·h^{-1}/mg protein，肥厚组 Ca^{2+} Mg^{2+}-ATP 酶表观 K_m 为 0 40 ± 0.12 mmol·L^{-1} ATP，正常组为 0 59 ± 0 22 mmol·L^{-1} ATP。肥厚左室蛋白总量为 80 ± 30 mg，正常组的为 47 ± 9 mg。经 ig 普萘洛尔和苄普地尔 10 mg·kg^{-1} 治疗后，心脏肥厚被消除，肥厚左室酶活力和 V_{max} 以及蛋白总量降低至正常水平，但表观 K_m 未受影响。**结论**：普萘洛尔和苄普地尔均可保护心肌及其线粒体免受缺血和超负荷钙损伤

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