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Heart hypertrophy induced by levothyroxine aggravates ischemic lesions and reperfusion arrhythmias in rats

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AIM: To develop a cardiac hypertrophic model in rats. **METHODS**: Rats were ip levothyroxine 0.5 $mg \cdot kg^{-1} \cdot d^{-1} \times 10$ d. The action potentials of right papillary muscles were recorded by standard glass-microelectrode technique. The left coronary artery was ligated followed by reperfusion and the apparent infarcted zone (AIZ) was determined by tetracycline fluoresence, and the superoxide dismutase (SOD) activity and malondialdehyde (MDA) product in myocardium were also measured. **RESULTS**: In the rats treated by levothyroxine, the heart was hypertrophic and the action potential duration (APD) and effective refractory period (ERP) were prolonged, the APD_{20}, APD_{50}, APD_{90}, and ERP were prolonged by 80 %, 79 %, 74 %, and 68 %, respectively. No changes in resting potential (RP), action potential amplitude (APA), and V_{max} were produced. The incidence of heart arrest (8/8) and the risk of death (67 ± 0) induced by ischemia-reperfusion in rats with hypertrophic heart was higher than those in normal rats (4/10 and 44 ± 19, respectively). The AIZ was expanded markedly in hypertrophic heart, and attenuated by lidocaine and propranolol. **CONCLUSION**: Levothyroxine-induced heart hypertrophy is a suitable model for severe ischemia and arrhythmias in rats.

Heart hypertrophy is a common pathway of the final stage of many cardiopathies. As models of left ventricle hypertrophy, renal hypertension and partial ligation of abdominal aorta were widely used[1] but the experimental period was longer than the formation of these models. Meanwhile, heart rate, coronary efflux, and the ratio of nonperfused to perfused areas of the left ventricle did not differ between normal and hypertrophic hearts[2]. Heart mass was increased by levothyroxine which stimulated protein synthesis[3], enhanced oxygen comsumption, and decreased capillary density[4]. To develop an easier heart hypertrophic model in rats, levothyroxine was tried.

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MATERIALS AND METHODS

SD rats weighing 150 - 220 g, of either sex were used.

Chemicals and instruments Levothyroxine (Sigma), lidocaine (Lid, from Xuzhou Second Pharmaceutical Factory), propalenone (Prop, from Guanzhou Institute of Pharmaceutical Industry), and propranolol (Pro, from Wuxi Fourth Pharmaceutical Factory) were used. Other reagents were AR degree. DAAS-1 type of electrophysiological apparatus, ECG-6511, small animal respiratory apparatus (*Jiangwan type 1*), and ultraviolet lamp were used.

Heart hypertrophy Levothyroxine 0.5 $mg \cdot kg^{-1} \cdot d^{-1}$ [1,4] was injected ip for 10 d. After 24 h medication, the hearts were weighed to calculate the heart weight index (HW/BW) and ventricular weight index (VW/BW).

Reperfusion arrhythmias The left coronary artery underwent 10 min occlusion and 25-min reopening. ECG traces were recorded and arrhythmia was scored[5]. The risk of death was calculated according to survival and cardiac death[6]. Lid, Prop, and Pro were injected ip 30 min before ligation.

Action potentials The isolated papillary muscles from the normal and the hypertrophic hearts were used to record action potentials by standard glass-microelectrode of which the tip diameter was <0.5 μm with a resistance 10 - 30 MΩ. The electric signals were transferred to a micro-amplifier (FW-4B) and analyzed by DAAS-1, and from which electric impulses (1 Hz, 1 ms, 150 % threshold) were delivered to the preparations perfused by modified Krebs solution[7]. The resting potential (RP), action potential amplitude (APA), action potential duration (APD), effective refractory period (ERP), and the maximum depolarized velocity of phase 0 (V_{max}) were measured.

The apparent infarcted zone (AIZ) At the end of reperfusion the coronary artery was religated with iv 10 % tetracycline 1 $mL \cdot kg^{-1}$. The heart was removed 10 min

later and sliced into 6 – 8 pieces for fluoresence examination under $\lambda = 265$ nm. The nonstained area was referred as AIZ.

Myocardial Superoxide Dismutase (SOD) and Malondialdehyde (MDA) The isolated rat hearts (both normal and hypertrophied were subjected to global ischemia by perfusion with one-tenth of the original flow volume for 7 min followed by 10-min reperfusion. The SOD activity in myocardium was assayed by adrenaline autooxidation method (Yuan QS, Wang ZY, Weng QQ. Determination of superoxide dismutase-a method of epinephrine autooxidation. Biochem Pharm Manuf from Organs 1983; 3: 4 – 7), the MDA was assayed by thiobarbituric acid method[8].

Statistic analysis The t test was used.

RESULTS

Heart mass and HR The heart mass was increased after 10-d ip levothyroxine evaluated by HW, VW, heart weight index, and ventricular weight index ($P < 0.01$). HR was accelerated ($P < 0.01$) (Tab 1).

Tab 1. Weight of heart mass of levothyroxine-induced heart hypertrophy model compared with normal rats. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

	n	Control	Hypertrophy
BW (g)	8	194 ± 14	188 ± 11[a]
HW (mg)	8	690 ± 50	910 ± 30[c]
VW (mg)	8	620 ± 80	820 ± 90[c]
HW/BW (mg·g^{-1})	8	3.52 ± 0.20	5.3 ± 0.6[c]
VW/BS (mg·g^{-1})	8	3.06 ± 0.20	4.8 ± 0.6[c]
HR (bpm)	6	490 ± 22	580 ± 40[c]

Reperfusion arrhythmias Arrhythmias were exaggerated during reperfusion period. The incidence of heart arrest was higher in hypertrophic hearts than that of normal heart ($P < 0.05$). Arrhythmias in hypertrophic hearts were improved by Lid and Pro (Tab 2).

Risk of death The incidence of death in normal rats was 4/10, Lid (1 mg·kg^{-1} iv) and Prop (30 mg·kg^{-1} ig) reduced the risk of death ($P < 0.05$). Risk of death was markedly worse in levothyroxine-induced hypertrophic heart and was reduced approaching to that of normal by Lid and Pro ($P < 0.05$) (Tab 3).

AIZ The AIZ of normal rats was 42.7 ± 2.8 %, being effectively reduced by Lid ($P < 0.01$) and Prop ($P < 0.01$). In the hypertrophic model the apparent infarcted mass increased ($P < 0.01$), and the ratio of infarcted/VW was also higher than normal (Tab 4). Treatment with Lid and Pro reduced the AIZ. However, a larger AIZ *vs* normal remained ($P < 0.01$).

Action potentials Levothyroxine prolonged both APD (from 71 % to 80 %, $P < 0.01$) and ERP (68 %, $P < 0.01$). There were no changes

Tab 2. Reperfusion arrhythmias and therapeutic effects of lidocaine (Lid) and propranolol (Pro) in hypertrophic rat heart. $n = 8$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* hypertrophy untreated.

Time/ min	Control	Hypertrophic model/mg·kg^{-1} Untreated	Lid (1.5)	Pro (2.0)
Occlusion				
5	0.4 ± 0.6	1.4 ± 1.3[a]	0.7 ± 0.5[d]	0.7 ± 0.5[d]
10	0.9 ± 1.3	1.5 ± 1.1[a]	0.5 ± 0[e]	0.9 ± 1.2[d]
Reperfusion				
0.17	4.4 ± 2.1	5.8 ± 0.3[a]	4.3 ± 1.6[e]	3.3 ± 1.7[f]
0.33	5.2 ± 0.8	5.8 ± 0.5[a]	3.9 ± 1.7[f]	2.6 ± 2.1[f]
0.5	5.1 ± 0.8	6.0 ± 0.4[b]	3.1 ± 2.1[f]	1.3 ± 1.9[f]
1	1.7 ± 2.0	5.6 ± 1.5[c]	1.4 ± 0.9[f]	0.6 ± 0.8[f]
2	2.2 ± 1.6	4.9 ± 1.9[c]	1.6 ± 2.3[f]	0.4 ± 0.7[f]
3	1.9 ± 2.1	5.1 ± 1.9[c]	1.6 ± 2.3[f]	0.25 ± 0.27[f]
5	1.9 ± 2.1	5.6 ± 1.7[c]	1.6 ± 2.3[f]	0.25 ± 0.27[f]
6	1.8 ± 2.2	6.3 ± 1.2[c]	1.5 ± 2.3[f]	0.4 ± 0.5[f]
7	1.7 ± 2.2	6.9 ± 0.2[c]	1.5 ± 2.3[f]	0.2 ± 0.3[f]
10	2 ± 3	7.0 ± 0[c]	1.3 ± 2.3[f]	0.13 ± 0.23[f]
15	2 ± 3	7.0 ± 0[c]	1.3 ± 2.3[f]	0
25	2 ± 3	7.0 ± 0[c]	1.3 ± 2.3[f]	0

Tab 3. Influence of lidocaine (Lid), propafenone (Prop) and propranolol (Pro) on risk at death, mortality of reperfusion arrhythmias in hypertrophic heart. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline; $^fP < 0.01$ *vs* levothyroxine untreated.

Groups/ mg·kg^{-1}			n	Live	Died	Mortality (%)	Risk at death
Control							
Saline			10	6	4	40	44 ± 19
Lid	1	iv	10	10	0	0	29.5 ± 0[b]
	3	iv	10	10	0	0	29.5 ± 0[b]
Prop	10	po	10	8	2	20	37 ± 16[a]
	30	po	10	10	0	0	29.5 ± 0[b]
Levothyroxine							
Untreated			8	0	8	100	67.0 ± 0[c]
Lid	1.5	iv	8	7	1	12.5	34 ± 13[f]
Pro	2	iv	8	8	0	0	29.5 ± 0[f]

in RP, APA, and V_{max} (Tab 5).

Tab 4. Influence of lidocaine (Lid), propafenone (Prop), and propranolol (Pro) on the apparent infarcted zone (perfused area beyond the occluded coronary artery) of normal and hypertrophic heart. $\bar{x} \pm s$.
$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* levothyroxine untreated.

Groups/ mg·kg^{-1}			n	Infarcted /mg	Noninfarcted /mg	Infarcted /VW (%)
Control						
Saline			10	253 ± 29	350 ± 50	42.7 ± 2.8
Lid	1	iv	10	202 ± 25^c	480 ± 40^c	30.2 ± 1.9^c
	3	iv	10	138 ± 22^c	570 ± 30^c	20 ± 4^c
Prop	10	po	10	240 ± 50^a	330 ± 50^a	41 ± 3^a
	30	po	10	174 ± 18^c	362 ± 18^a	32.5 ± 1.5^c
Levothyroxine						
Untreated			8	500 ± 50^c	420 ± 60^b	61 ± 4^c
Lid	1.5	iv	8	398 ± 18^f	390 ± 40^d	53 ± 5^f
Pro	2	iv	8	400 ± 60^f	400 ± 140^d	48.4 ± 1.0^f

VW: Total ventricular weight.

Tab 5. Changes of action potential parameters in hypertrophic rat papillary muscles induced by levothyroxine. $\bar{x} \pm s$. **$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.**

	Control	Hypertrophic
n	6	5
RP/ms	83 ± 4	77 ± 5^a
APA/mV	107 ± 10	99 ± 7^a
V_{max}/V·s^{-1}	240 ± 70	240 ± 50^a
APD_{20}/ms	5 ± 1	9 ± 3^b
APD_{50}/ms	14 ± 4	25 ± 5^c
APD_{90}/ms	35 ± 6	61 ± 6^c
ERP/ms	38 ± 7	64 ± 5^c

SOD and MDA Seven-min low perfusion and reperfusion lowered SOD activity 0.76 ± 0.22 in hypertrophic ($P<0.01$) *vs* 1.6 ± 0.4 in normal rats, and raised MDA production 320 ± 60 nmol/g tissue in hypertrophic *vs* 200 ± 40 of normal ($P<0.01$).

DISCUSSION

Distance of oxygen diffusion from the center of a myocyte to capillary of coronary circulation is prolonged by hypertrophy, showing a tendency of myocardial ischemia existing in a hypertrophic heart. Under this condition an injury caused by reperfusion was severer against normal, resulting in more serious arrhythmias after reperfusion. An addition of two ischemic events, levothyroxine-induced ventricular hypertrophy and reperfusion, was crucial to develop life threatening arrhythmias.

Although the AIZ is a perfused zone of the occluded artery rather than a real infarcted mass where no visible collateral circulation can be detected by tetracycline staining, it can reflect to some extent the size of an infarct and responds to drug treatment sensitively. Pro and Lid[9] were able to reduce AIZ at dose effective for suppressing arrhythmias and Prop showed less potent. Our results showed the AIZ of levothyroxine-induced hypertrophic heart was significantly expanded, implying that a hypertrophic heart is at risk of enlarging an injured area by coronary insufficiency.

More serious arrhythmias caused by reperfusion in a hypertrophic heart[10] is still in doubt in its mechanisms[1]. A shortening of APD at an infarcted zone is worse to electrophysiological homogeneity attributing to an increased VF incidence emerged on reperfusing a hypertrophic heart.

This enlarged heart model by levothyroxine which was established faster than by partial ligation of abdominal aorta shares some characteristics e.g. the accelerated HR, arrhythmogenecity and ventricular hypertrophy. This hypertrophic heart is a simple and suitable pathological model for various research purposes.

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左甲状腺素诱导的大鼠心肌肥厚加重心肌缺血损害及再灌心律失常

R977.14

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关键词 左甲状腺素; 乳头状肌; 心肌肥厚; 心律失常; 心肌梗死; 动作电位; 超氧化物歧化酶; 丙二醛

心肌缺血 SOD

目的: 建立注射左甲状腺素(*L*-thy)造成的大鼠心肌肥厚模型. 方法: *L*-thy 0.5 mg·kg^{-1}·d^{-1} ip 10 d. 以缺血-再灌注方法, 测定心脏表观梗死区, 再灌性心律失常及超氧化物歧化酶活性(SOD)和丙二醛生成量(MDA), 并记录肥厚心脏乳头状肌的跨膜动作电位. 结果: 高甲状腺素使大鼠心肌肥厚、心率加快, 再灌注心律失常、室颤(VF)及致死危险性增加. 表观梗死区增大, 动作电位的 APD 及 ERP 明显延长, 而 RP, APA 及 V_{max} 无变化. *L*-thy 对大鼠心肌的损害与再灌注损害的叠加, 增加了心律失常的发生及严重程度. 结论: 注射 *L*-thy 可造成简易的心肌肥厚动物实验模型.

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