BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jan; 19 (1): 54 - 57

Effects of 1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenylethylamino) propane hydrochloride on heart function, lactate dehydrogenase and its isoenzymes in rats with cardiac hypertrophy

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KEY WORDS left ventricular hypertrophy; heart function tests; 1-(2, 6-dimethylphenoxy)-2-(3, 4-dimethoxyphenylethylamino) propane hydrochloride; lactate dehydrogenase; lactate dehydrogenase isoenzymes

AIM: To investigate the effects of 1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenylethylamino) propane hydrochloride (DDPH) on cardiac systolic and diastolic function, lactate dehydrogenase (LDH) activity, and LDH isoenzymes in rats with cardiac hypertrophy. **METHODS**: The cardiac hypertrophy of rats was induced by partly occluding abdominal aorta. The rats were given ig DDPH for 8 wk 4 wk after operation, and isolated working heart was made. **RESULTS**: Eight wk later, in model group, left ventricle systolic pressure (LVSP), LV + dp/dt_{max}, - dp/dt_{max} and aorta pressure (AP) decreased by 20.2 %, 20.0 %, 41.4 %, and 13.6 %, respectively. Left ventricle ending diastolic pressure (LVEDP) increased by 173.9 %. The hemodynamic study showed that flowing liquid of aorta (AF) and coronary (CF) and cardiac output (CO) decreased by 49.4 %, 41.2 %, and 48.9 %, respectively. After the rats were given ig DDPH, the all above-mentioned parameters recovered to different degrees. Under condition of cardiac hypertrophy, LDH isoenzymes and subunits changed significantly. Isoenzymes LDH_3, LDH_4, and LDH_5, especially LDH_5 increased, LDH_1 decreased, subunit M in hypertrophied heart increased 1.69 times than that in nomal heart. DDPH could decrease subunit M and increase subunit H. **CONCLUSION**: DDPH can increase cardiac function, coronary flow and reverse changes of LDH isoenzymes in rats with cardiac hypertrophy.

When cardiac hypertrophy occured, cardiac diastolic and systolic functions decreased[1,2], and some enzymes related to energic metabolism such as myosin heavy chain (MHC), LDH changed[3,4]. LDH isoenzymes have two types of subunits: H and M. Under normal condition, subunit H predominates in left ventricle tissue. But when heart becomes hypertrophic, subunit M increases[4]. Up to now, many antihypertensive drugs such as angiotensin converting enzyme inhibitors (ACEI), calcium antagonist, and α_1 adrenoceptor blocker have been found to be able to reverse cardiac function of hypertrophied heart[5,6,7], and some of them can reverse MHC changes[8]. But it is not clear whether these drugs can reverse LDH changes when they reverse cardiac function. 1-(2,6-Dimethylphenoxy)-2-(3, 4-dimethoxyphenylethylamino) propane hydrochloride (DDPH) is a compound synthesized by China Pharmaceutical University. In our previous studies, it was found that DDPH had α_1 adrenoceptor and weak calcium antagonistic effects[9], and antihypertensive effect[10]. The aim of present study is to investigate the effects of DDPH on cardiac function and LDH isoenzymes in rats with cardiac hypertrophy.

MATERIALS AND METHODS

Materials DDPH was provided by professor XIA Lin and NI Pei-Zhou, Department of Organic Chemistry, China Pharmaceutical University (chemical purity more than 99 %). When used, it was dissolved in distilled water. Wistar rats (n = 28, clean), ♂, body weight 150 - 200 g, were purchased from Laboratory Animal Center, Tongji Medical University. Pentobarbital sodium (Foshan Chemical Factory), benzylpenicillin (Huabei Pharmaceutical Company), agarose (Serva). nitroblue tetrazolium (NBT, Serva), nicotinamide adenine dinucleotide (NAD^+, Sigma), *N*-methylphenazinium (PMS, Sigma).

Surgical models[11] The rats were anesthetized with

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Received 1996-08-12 Accepted 1997-07-08

pentobarbital sodium (45 mg·kg^{-1}, ip). The abdominal aorta just above the left kidney artery was occluded with No 7 needle, then the needle was drawed out. As to the control group, the aorta was only separated but not ligated. After operation, each rat was given ip benzylpenicillin 5×10^4 unit to prevent infection. All the rats were divided into four groups at random. 1) Control: 4 wk after operation, the rats were given ig 3 mL distilled water as DDPH groups; 2) model: the same as control except that abdominal aorta was occluded; 3) low dosage of DDPH: 4 wk after operation, the rats were given DDPH (25 mg·kg^{-1}·d^{-1}, ig for 8 wk); 4) higher dosage of DDPH: the same as 3), except for DDPH 50 mg·kg^{-1}·d^{-1}.

Measurement of cardiac function The rat was stunned, isolated working heart was made [12]. The fulling pressure of left ventricle was 2.0 kPa, static water pressure of aorta was 9.3 kPa. Perfusate liquid was modified Krebs-Henseleit (KH) solution (mmol·L^{-1}: NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 2.55, KH_2PO_4 1.2, Na_2EDTA 0.5, glucose 5.0, Napyruvate 2.0). One polyethylene tube was inserted into the aorta, and the opposite end was connected to LMS-2B (Chengdu Medical Equipment Factory) physical recording apparatus with pressure transducer to record aorta pressure (AP); another polyethylene tube was inserted into the left ventricle, and the opposite end was connected to SJ-42 (Shanghai Medical Equipment Factory) physical recording apparatus with another pressure transducer to record left ventricle systolic pressure (LVSP), LV dp/dt_{max}, and left ventricle ending diastolic pressure (LVEDP). The isolated heart was perfused from left atrium for 10 – 20 min, then was perfused from aorta. The heart was kept stable for 10 – 20 min, the above-mentioned parameters were measured. Flowing liquid of aorta (AF) and coronary (CF) in per minute was gathered. Cardiac output (CO) was obtained by AF + CF. Through conversion, the values in per gram left ventricle tissue were obtained.

Measurements of LDH activity and isoenzymes Left ventricle tissue 150 mg was homogenated with 1.5 mL cold PBS buffer, the homogenate was centrifuged (2×10^4 g) under 4 ℃ for 30 min, the supernate was used to measure LDH activity and analyse LDH isoenzymes. LDH activity was measured as method[13] with UV-visible recording spectrophotometer UV-240 (Shimadzu, Japan). To analyse LDH isoenzymes, one 0.9 % agarose plate (10 cm × 10 cm) was prepared with barbital sodium-HCl buffer (pH 8.4) 50 μmol·L^{-1}. Supernate of each sample 2 μL was undertaken electrophoresis (SCR-4 electrophoretic apparatus) with barbital sodium-HCl buffer 75 μmol·L^{-1} under 150 – 200 V. Agarose 0.5 % 4 mL, 0.2 mL sodium lactate 1 moL·L^{-1}, 0.8 mL visible agent (0.2 % NBT, 0.5 % NAD^+, and 0.05 % PMS) were mixed and poured onto the 0.9 % agarose plates 60 – 90 min after electrophoresis. At 37 ℃, 30 min later, the plates were scanned at 540 nm with CS-930 scanner (Shimadzu, Japan) to obtain relative content of five isoenzymes. Subunit H was achieved by $LDH_1 + 3/4 \cdot LDH_2 + 1/2 \cdot LDH_3 + 1/4 \cdot LDH_4$; subunit M was achieved by 100 % – H

Statistics Data were compared with t test.

RESULTS

Effects of DDPH on hemodynamics in rats with cardiac hypertrophy In model group, LVSP, LV + dp/dt_{max}, -dp/dt_{max}, and AP decreased by 20.2 % ($P < 0.01$), 20.0 % ($P < 0.01$), 41.4 % ($P < 0.01$), and 13.6 % ($P < 0.01$) compared with control group. But LVEDP increased by 173.9 % ($P < 0.01$).

AF, CF, and CO in per gram of heart in model group decreased by 49.4 % ($P < 0.01$), 41.2 % ($P < 0.01$), and 48.9 % ($P < 0.01$) compared with control group, respectively.

DDPH could partly reverse changes mentioned above, to LVSP, AF, and CO, the effects of higher dosage of DDPH was more significant (Tab 1).

Effect of DDPH on LDH activity and change of isoenzymes There was no significant difference in LDH activity and LDH_2 isoenzyme among four groups, but other isoenzymes and subunits differed significantly. In model group, LDH_1 decreased, but LDH_3, LDH_4, and LDH_5, especially LDH_5 increased. Subunit M increased 1.69 times than that in control group. DDPH could reverse changes mentioned above (Tab 1, Fig 1).

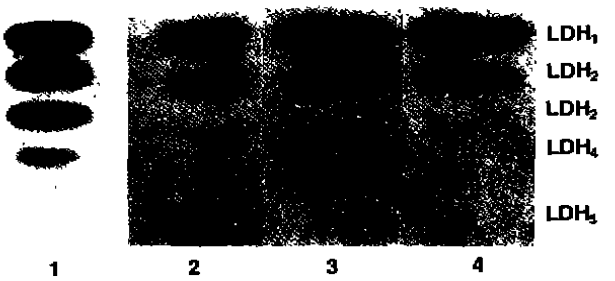

Fig 1. Electrophoresis of LDH isoenzymes of left ventricle tissue of rat. 1) Control; 2) model; 3) DDPH 25 mg·kg^{-1}·d^{-1}; 4) DDPH 50 mg·kg^{-1}·d^{-1}.

DISCUSSION

It was found that the systolic and diastolic functions of the heart with cardiac hypertrophy decreased significantly. AF, CF, and CO also

Tab 1. Effects of ig DDPH on hemodynamics, LDH, and isoenzymes in rats with cardiac hypertrophy. $x \pm s$. $^{a}P > 0.05$. $^{b}P < 0.05$. $^{c}P < 0.01$ *vs* control. $^{d}P > 0.05$. $^{e}P < 0.05$. $^{f}P < 0.01$ *vs* model. $^{g}P > 0.05$. $^{h}P < 0.05$, $^{i}P < 0.01$ *vs* DDPH 25 mg·kg^{-1}·d^{-1}.

	Control	Model	DDPH 25 mg·kg^{-1}·d^{-1}	DDPH 50 mg·kg^{-1}·d^{-1}
n	8	6	7	7
HR, beat·min^{-1}	204 ± 14^{dfg}	202 ± 25^{ae}	203 ± 23^{ael}	202 ± 14^{adg}
LVSP, kPa	15.8 ± 1.4^{fg}	12.6 ± 0.8^{cf}	14.1 ± 0.6^{cf}	15.0 ± 0.9^{afh}
LV + dp/dt_{max}, kPa·S^{-1}	612 ± 79^{fh}	489 ± 42^{cfh}	557 ± 67^{bcf}	589 ± 51^{afg}
LV − dp/dt_{max}, kPa·S^{-1}	378 ± 29^{fi}	223 ± 24^{cfh}	300 ± 34^{cfi}	306 ± 40^{bfg}
AP, kPa	12.6 ± 1.5^{fg}	10.9 ± 0.5^{cfg}	11.7 ± 0.5^{ac}	12.4 ± 1.3^{aeg}
LVEDP, kPa	0.46 ± 0.05^{fi}	1.26 ± 0.14^{cfi}	0.81 ± 0.04^{cfi}	0.52 ± 0.03^{befi}
CF, mL·min^{-1}	8.5 ± 0.5^{fh}	4.9 ± 0.6^{cfi}	7.7 ± 0.7^{bfi}	8.3 ± 1.1^{afg}
AF, mL·min^{-1}	27.8 ± 2.3^{fi}	14.1 ± 2.4^{cfi}	18.5 ± 2.9^{cfi}	21.5 ± 2.8^{cfh}
CO, mL·min^{-1}	37 ± 4^{fi}	18.9 ± 2.1^{cfi}	25.5 ± 2.7^{cfi}	29.0 ± 2.5^{cfh}
LDH, μmol·min^{-1}/g wet weight	196 ± 16^{dfg}	201 ± 19^{afg}	205 ± 24^{a}	199 ± 20^{adg}
LDH_1 (%)	57 ± 4^{fi}	44 ± 5^{cfh}	49.1 ± 1.6^{cfe}	53 ± 4^{bfh}
LDH_2 (%)	27 ± 3^{dfg}	25 ± 3^{afg}	24.6 ± 1.6^{adf}	25 ± 3^{adfg}
LDH_3 (%)	7.9 ± 1.8^{fi}	10.8 ± 2.1^{cfg}	11.7 ± 1.2^{cfi}	10.8 ± 2.4^{cdfg}
LDH_4 (%)	5.1 ± 1.4^{fi}	7.4 ± 0.4^{cfg}	7.8 ± 1.0^{cfi}	8.1 ± 1.1^{cdfg}
LDH_5 (%)	3.7 ± 1.2^{fi}	10.8 ± 1.1^{cfi}	7.0 ± 0.8^{cfi}	3.9 ± 0.8^{afi}
H (%)	82 ± 5^{fi}	70 ± 5^{cfh}	76 ± 7^{bfi}	79 ± 7^{afg}
M (%)	18.0 ± 2.2^{fi}	31 ± 5^{cfh}	24.3 ± 2.0^{cfi}	19.9 ± 2.4^{afi}
H/M	4.53	2.28	3.11	3.95

decreased. DDPH could markedly reverse these changes. To LVSP, LVEDP, AF, and CO, higher dosage of DDPH was more significant.

DDPH had weak calcium antagonistic effect[9], but in our experiment, heart rate didn't change in DDPH groups. This can't deny DDPH have negative inotropic action and negative chronotropic action to the heart, because DDPH also has antihypertensive effect[10], this can stimulate sympathetic nervous system reflexly, so the direct effect to the heart is covered. In our previous study, it was found that the length of sarcoplasm (LS) decreased in model group[14], this might be the reason of the decreased systolic function, because LS is the initial length of cardiac contraction. LS decreased, the initial length also decreased, so the contraction of the heart decreased.

Proliferation of fiber is an important reason of decreased diastolic function in rats with cardiac hypertrophy[15]. In our previous work, we also found that the collagen content increased significantly when the heart of the rats became hypertrophied[14]. When the collagen content increased, relax of the left ventricle decreased, and the stiffness of heart increased, these could reduce the diastolic function of the heart.

In model group, LDH_1 decreased, LDH_3, LDH_4, and LDH_5, especially LDH_5 increased. DDPH could reverse these changes. DDPH can reduce the collagen content[14] in the heart of model group, so it can reverse the changes of LDH isoenzymes and improve the diastolic function of the heart. The detailed mechanism awaited further investigation.

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1-(2,6-二甲基苯氧基)-2-(3,4-二甲氧基苯乙氨基)丙烷盐酸盐对心肌肥厚大鼠心功能、乳酸脱氢酶及其同工酶谱的影响

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关键词 左心室肥大；心脏功能试验；1-(2,6-二甲基苯氧基)-2-(3,4-二甲氧基苯乙氨基)-丙烷盐酸盐；乳酸脱氢酶；乳酸脱氢酶同工酶类

目的：研究 1-(2,6-二甲基苯氧基)-2-(3,4-二甲氧基苯乙氨基)-丙烷盐酸盐(DDPH)对心肌肥厚大鼠心功能及乳酸脱氢酶(LDH)及其同工酶的影响. **方法**：部分狭窄腹主动脉、术后 4 周，大鼠给予 DDPH. **结果**：DDPH ig 4 周明显改善大鼠心功能. 各组 LDH 活性及 LDH_2 无明显差异，但心肌肥厚组 LDH_3，LDH_4、LDH_5，特别是 LDH_5 增加，而 LDH_1 下降. *M* 亚基是对照组的 1.69 倍，DDPH 明显逆转上述变化. **结论**：DDPH 改善心肌肥厚时下降的心功能，同时逆转 LDH 同工酶谱的变化.