摘要 用离体兔胸主动脉淋浴式灌注方法探讨左旋精氨酸对内、外源性 OFR 损伤血管内皮功能的保护作用. 结果：用二乙二硫氨基甲酸盐(DETC)产生的内源性 OFR 与电解缓冲液产生的外源性 OFR 均可明显抑制血管内皮依赖性扩张，并使血管壁 MDA 含量增加. 左旋精氨酸能对抗内、外源性 OFR 所致 MDA 增加与内皮依赖舒血管功能损害.

关键词 精氨酸；二乙基二硫代氨甲酸酯；一氧化氮；电解；活性氧；胸主动脉

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Sympatholytic effect of captopril in regression of cardiovascular remodeling in spontaneously hypertensive rats[1]

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ABSTRACT Fifty-eight spontaneously hypertensive rats (SHR) at 12 wk of age were divided into 3 groups: A) captopril (Cap) 20 mg · kg^{-1} · d^{-1}; B) clonidine (Clo) 30 μg · kg^{-1} · d^{-1}; C) Clo 30 μg · kg^{-1} · d^{-1} + Cap 20 mg · kg^{-1} · d^{-1} orally for 24 wk. Concomitant administration of Cap and Clo did not result in more lowering of the systolic blood pressure (SBP) than that by Cap alone. Regression of left ventricular hypertrophy (LVH) were remarkable in Groups A and C, but not to the extent in that of WKY. No significant difference between these two groups was found. Cap alone resulted in a greater decrease of myocardial norepinephrine (NE) than that of Groups B and C. The wall/lumen ratio and the number of smooth muscle cell (SMC) layers of renal artery decreased in Groups A and C, but little difference was found between them. It seemed that combined blockade of renin-angiotensin-aldosterone (RAA) system and sympathetic nervous system (SNS) did not produce more significant BP reduction and reversal of cardiovascular remodeling than Cap alone did. The sympathetic inhibitory effect of angiotensin converting enzyme inhibitor (ACEI) was not enhanced by sympatholytic treatment.

KEY WORDS inbred SHR rats; inbred WKY rats; captopril; clonidine; blood pressure; myocardium; norepinephrine; calcium; hydroxyproline; renal artery

Cardiovascular remodeling always accompanies hypertension[1-2]. Both sympathetic nervous system (SNS) and renin-angiotensin-aldosterone (RAA) system were involved in the process of cardiovascular remodeling, besides the hemodynamic effect of hypertension. Hypertensive cardiovascular remodeling regressed after angiotensin converting enzyme inhibitor (ACEI) treatment, which blocked RAA directly or inhibited the SNS at different levels indirectly[1-3]. Sympatholytic drugs prevented or regressed the LVH[1,2,4], but it is un-

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clear which system is more important in cardiovascular remodeling and what role does the SNS play in the presence of ACEI. The present study was designed to compare the effects of clonidine (Clo), captopril (Cap), and Cap + Clo on the blood pressure (BP), regression of cardiovascular remodeling and the myocardial norepinephrine (NE), hydroxyproline in SHR, aiming at clarifying the relative importance of SNS and RAA system in the regression of cardiovascular remodeling.

MATERIALS AND METHODS

SHR and normotensive untreated Wistar Kyoto rats (WKY), 12 wk of age, were housed in groups of 4—6 per cage at temperature of 22 ± 2 ℃, humidity of 55 ± 5 %, and photoperiod (12 h light/dark cycle; 07:00-19:00), and were fed standard rat chow and water *ad lib*. Following 3 determinations of systolic blood pressure (SBP), 58 SHR were divided into 4 groups: A) Cap (Sino-American Shanghai Squibb Pharmaceuticals Ltd) 20 $mg \cdot kg^{-1} \cdot d^{-1}$ ($n = 19$); B) Clo (Danyang Pharmaceuticals, Jiansu) 30 $\mu g \cdot kg^{-1} \cdot d^{-1}$ ($n = 12$); C) Cap 20 $mg \cdot kg^{-1} \cdot d^{-1}$ + Clo 30 $\mu g \cdot kg^{-1} \cdot d^{-1}$ ($n = 11$); D) untreated ($n = 16$). WKY rats ($n = 12$) were used as control. Drugs were given once a day orally mixed in the vehicle (0.5 g milk powder). Blood pressure was measured using tail-cuff plethysmography with ECG as recorder every 4 wk. Heart rate and body weight were measured. Rats were decapitated after 24 wk of medication.

Measurement of left ventricular mass (LVM) Both atria were excised along the atrioventricular groove. The aorta and the pulmonary artery were cut below the aortic and pulmonary valves. The wall of right ventricle was removed along the curve of the interventricular septum. The left ventricle was blotted dry and weighed.

Myocardial NE measurement Tissue samples were homogenized with a glass grinder in ice — cold perchloric acid 0.4 $mol \cdot L^{-1}$ (50 ml/g heart) containing edetic acid (EDTA) 3 $\mu mol \cdot L^{-1}$, and sodium metabisulfite 10 $\mu mol \cdot L^{-1}$. After centrifugation at 5000 × *g* for 20 min at 4 ℃, NE in the supernatant was extracted with activated alumine and measured with spectrofluorometer. The sensitivity was 10 ng/g heart, with recovery rate = 84 ± 3.2 %, CV = 3.8 %.

Myocardial hydroxyproline determination The samples of LV were dried to constant weight in a 40 ℃ oven. The ground tissue powder were heated in HCl 6 $mol \cdot L^{-1}$ at 100 ℃ overnight for hydrolysis of collagen. Hydroxyproline content was measured spectrophotometrically[5].

Measurement of intracellular free calcium (Ca_i^{2+}) of platelets Blood samples were obtained through a cannula placed in the abdominal aorta. Blood 5 ml was mixed with citrate-citric acid dextrose anticoagulant 0.7 ml. Platelet Ca_i^{2+} was measured with Fura-2[6]. Mean value was obtained after triplicate measurements in each rat.

DNA synthesis rate of aorta The aorta from the heart to the iliac bifurcation was excised. The deendothelized aorta was placed in a 25-ml Erlenmeyer flask with airtight cap containing 20 ml ice-cold Krebs-Henseleit buffer saturated with 95 % O_2 + 5 % CO_2. The flask was preincubated for 60 min at 37 ℃ in a shaking water bath. [^{3}H]Thymidine nucleotide (1.5 $PBq \cdot mol^{-1}$, 37 $TBq \cdot L^{-1}$, Shanghai Institute of Nuclear Research, Chinese Academy of Sciences) was added to achieve a final activity of 74 $MBq \cdot L^{-1}$ and the flask was incubated for another 60 min. DNA content and [^{3}H]Thymidine incorperation were determined[7].

Microscopic examination of intrarenal artery A segment of renal artery about 2 mm inside porta rems was fixed in 10 % formaldehyde for 24 h, and stained with HE. The wall / lumen ratio, the media / lumen ratio, and the number of smooth muscle cell (SMC) layers were determined morphormetrically.

Statistical analysis All data were expressed as $\bar{x} \pm s$. ANOVA and Newman-Keuls were used to determine differences in each treatment groups, and *t* test was used to evaluate the changes of body weight, heart rate and BP. Linear regression analysis was used to test the correlation between LVM and other factors.

RESULTS

Body weight Body weight increased with age, but the increase was lower in the SHR group. After 24 wk of treatment, no difference was found among the 3 treated groups (Tab 1).

Tab 1. Effects of captopril and clonidine treatment on the blood pressure (BP), body weight (BW), and heart rate (HR) of rats. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* untreated SHR; $^{d}P>0.05$, $^{e}P<0.01$ *vs* SHR treated with captopril; $^{f}P>0.05$, $^{g}P<0.05$, $^{h}P<0.01$ *vs* SHR treated with clonidine.

Treatment		WKY rats	SHR rats	Captopril	Clonidine	Cap+Clo
baseline	BP/kPa	15.3±1.0^{cfi}	27.3±1.9	26.7±1.6^{cg}	26.8±2.1^{bd}	27.8±2.7^{adg}
	BW/g	222±52^{adg}	210±38	199±32^{ag}	233±45^{ad}	252±51^{adg}
	HR/bpm	400±47^{adg}	385±30	390±34^{fg}	388±27^{ad}	401±40^{adg}
4 wk	BP/kPa	15.6±1.1^{cfi}	27.0±1.5	22.7±2.4^{cg}	24.9±3.3^{bd}	23.7±2.4^{cdg}
	BW/g	278±37^{adg}	242±38	241±45^{ag}	224±43^{ad}	247±50^{adg}
	HR/bpm	383±27^{adg}	383±52	362±34^{ag}	374±44^{ad}	355±27^{adg}
8 wk	BP/kPa	16.0±1.1^{cfi}	28.4±1.5	24.7±3.2^{cg}	24.8±1.4^{cd}	23.6±1.6^{cdg}
	BW/g	319±61^{adi}	272±54	262±52^{ag}	253±57^{ad}	245±60^{adg}
	HR/bpm	400±23^{adg}	407±49	368±49^{ag}	357±26^{ad}	354±38^{adg}
12 wk	BP/kPa	16.0±1.4^{cfi}	29.1±2.2	24.7±3.0^{cg}	25.9±2.1^{cd}	23.6±1.5^{cdg}
	BW/g	342±64^{cfi}	280±51	265±56^{ag}	258±58^{ad}	251±48^{adg}
	HR/bpm	379±34^{adi}	394±53	372±27^{ai}	328±32^{cf}	324±26^{cfg}
16 wk	BP/kPa	16.5±1.6^{cfi}	28.2±3.5	24.9±2.8^{cg}	27.4±2.9^{ad}	23.2±1.6^{cdh}
	BW/g	361±76^{cfi}	291±61	272±57^{ag}	272±57^{ad}	265±48^{adg}
	HR/bpm	387±23^{adi}	384±60	363±26^{ai}	316±43^{cf}	319±25^{cfg}
20 wk	BP/kPa	16.7±2.4^{cfi}	30.4±3.4	25.3±3.0^{cg}	27.3±2.9^{bd}	22.7±1.5^{cdh}
	BW/g	366±71^{cfi}	304±66	271±56^{ag}	270±56^{ad}	263±44^{adg}
	HR/bpm	350±41^{adi}	385±51	381±43^{ai}	309±26^{cf}	312±17^{cfg}
24 wk	BP/kPa	17.3±1.9^{cfi}	32.8±3.4	25.2±2.9^{cg}	27.3±2.9^{cd}	22.9±1.6^{cdg}
	BW/g	376±72^{cfi}	319±70	289±65^{ai}	267±55^{ad}	255±52^{adg}
	HR/bpm	358±27^{adi}	387±46	418±37^{ag}	309±26^{cf}	302±21^{cfg}

BP After 4 wk of medication, the SBP decreased in all treated groups, but still remained at a higher level than that of WKY (Tab 1). Thereafter, SBP stabilized at a lower level compared to that in untreated SHR, and increased slightly with age. After the 24-wk treatment, the SBP returned to baseline in groups A and B, lower than that in control SHR, but not to the extent of WKY rats. While no difference was found between group A and C after 24-wk treatment, SBP of group C remained at a markedly lower level compared with the baseline and that of group B. No difference was found between the SBP of groups A and C, indicating that Cap + Clo did not lower the SBP more than that obtained by Cap alone.

Heart rate Heart rate decreased after medication in groups B and C and kept at the low level during the rest of the experiment, but no difference was found between them (Tab 1). No change of heart rate was found in group A compared to untreated SHR and WKY.

LVM LVM and LVM/BW decreased in groups A and C, compared to control SHR. but no differences were found between A and C, indicating that combined blockade of SNS and RAA system did not cause more regression of LVH than Cap alone. Clo alone did not regress LVH in SHR (Tab 2). LVM/BW closely correlated to SBP ($r = 0.74$, $P<0.01$).

Myocardial NE Compared with untreated SHR, myocardial NE concentration and content were reduced in the 3 treated groups, particularly in group A. Combined blockade of SNS and RAA system did not cause a

Tab 2. Effects of captopril and clonidine on myocardium, aorta, and intrarenal artery in rats. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* **untreated SHR;** $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* **SHR treated with captopril;** $^{g}P>0.05$, $^{h}P<0.05$, $^{i}P<0.01$ *vs* **SHR treated with clonidine.**

	WKY rats	SHR rats	Cap	Clo	Cap+Clo
LVM/g	0.75±0.13^{cdi}	1.03±0.19	0.84±0.16^{c}	0.93±0.16^{ad}	0.82±0.12^{cdg}
LVM, g/kg BW	2.05±0.09^{cdi}	3.58±0.37	2.97±0.21^{c}	3.50±0.36^{ad}	3.16±0.22^{cdi}
NE content/μg	0.92±0.21^{adfg}	1.25±0.29	0.55±0.18^{c}	0.96±0.19^{cf}	0.75±0.10^{cegh}
hydroxyproline/μg	468.8±66.0^{cdh}	811.8±182.5	549.6±91.2^{c}	608.6±104.8^{cd}	497.6±96.0^{cdg}
DNA synthesis rate/ dpm·μg^{-1}·h^{-1}	44.5±12.6^{adg}	49.6±17.6	30.8±10.5^{c}	53.1±19.7^{af}	57.6±11.8^{afg}
Aorta, g/kg BW	0.30±0.04^{cdi}	0.46±0.07	0.33±0.05^{c}	0.51±0.07^{af}	0.43±0.01^{afi}
DNA, μg/mg protein	27.8±24.0^{cdi}	48.3±9.2	55.7±8.4^{b}	45.0±14.0^{af}	45.0±1.9^{afg}
Wall/lumen ratio	0.67±0.16^{cdh}	1.05±0.39	0.73±0.25^{c}	0.95±0.32^{ad}	0.73±0.18^{cdg}
Media/lumen ratio	0.34±0.06^{cdg}	0.48±0.14	0.34±0.12^{c}	0.42±0.15^{ad}	0.38±0.12^{cdg}
Number of SMC layer	3.7±0.7^{cdg}	4.9±0.8	3.9±0.6^{c}	4.1±0.7^{bd}	3.7±0.5^{cdg}

greater decrease of myocardial NE (Tab 2). LVM was positively related to myocardial NE content ($r = 0.60$, $n = 70$, $P<0.01$).

Hydroxyproline of left ventricle Myocardial hydoxyproline concentration and content were decreased in all 3 treated groups, and in groups A and C, to the extent of that of WKY (Tab 2). Among the 3 treated groups no significant difference was found ($P>0.05$).

Ca_i^{2-} concentration of platelets Ca_i^{2+} was reduced in groups A and C (A: 105±32, C: 100±30 *vs* untreated SHR: 136±27 nmol·L^{-1}, $P<0.01$), while no difference was found between A and C ($P>0.05$). Clo alone did not decrease the Ca_i^{2+} of platelets (148 ± 26 *vs* 136±27 nmol·L^{-1}, $P>0.05$).

Changes of aorta Compared to untreated SHR, DNA synthsis rate of aorta decreased in group A. Neither Clo nor Clo + Cap produced alteration of DNA synthysis rate of aorta in SHR. Aorta/BW was decreased after Cap treatment, which was sharply in contrast to the increase of aorta/BW by Clo treatment. Cap + Clo did not affect the aorta/BW of SHR. DNA/protein in SHR was higher than that of WKY, indicating hypertrophy of SMC of the aorta. Cap alone increased the DNA/protein markedly, while no change was noted after Clo or Clo + Cap (Tab 2).

Morphormetric examination Wall/lumen ratio and number of SMC layers of intrarenal artery decreased in groups A and C, but little difference was found between them (Tab 2). Media/lumen ratio decrease in group A. Slight, but no significant reductions of media/lumen ratio were noted in groups B an C. Number of SMC layers of intrarenal artery also reduced after Clo treatment, though no changes of wall/lumen and media/lumen were noted in this group. Wall/lumen, media/lumen, and number of SMC layers correlated poorly with SBP ($r = 0.52$; $r = 0.53$; $r = 0.59$) and Ca_i^{2+} of platelets ($r = 0.43$, 0.42, 0.52, $P<0.01$) in SHR (treated and untreated). Combined

blockade of SNS and RAA did not cause more changes of wall/lumen and number of SMC layers than Cap alone did.

DISCUSSION

The present study has examined the effect of Cap, centrally-acting sympatholytic agent Clo alone and concomitant treatment of Cap + Clo on cardiovascular remodeling. Originally, it was postulated that after the blockade of RAA system by Cap and the blockade of different levels of SNS by Cap + Clo, the LVH will be inhibited more markedly than either system blockade alone. To our surprise, blockade of both systems did not result in more prominent regression thao either drug alone did. Long term antihypertensive effects of Cap and Clo in the dosage used in this study were mainly manifested by prevention of BP increasing with age. However regression of LVH was showed only in groups A and C, while Clo alone did not cause regression of LVH. In previous studies we have demonstrated there was a close correlation between LVM/BW and SBP[8]. BP has been demonstrated repeatedly to play an important, but not the sole, role in the cardiovascular remodeling. The fact that Clo alone caused significant decrease of BP but no regression of LVH confirmed the suggestion.

Our data showed that both Cap and Clo decreased the myocardial NE, indicating both agents had sympatholytic effect. Cap alone cause greater decrease of myocardial NE than Clo and Cap + Clo, indicating the efficacy of sympatholytic effect of Cap, by blockade of different level of SNS from central to peripheral, was greater than that of central sympatholytic agent alone. Though a close correlation between myocardial NE and LVM was demonstrated, regression of LVH was inconsistent with the decrease of myocardial NE. It seemed that local NE may not be the decisive factor of LVH.

In hypertension, hypertrophy of the medial layer in the conducting arteries is due to hypertrophy of SMC, whereas in the resistant arteries, the primary change is hyperplasia of SMC. Lee[9] suggested that prevention of vascular changes in the large mesenteric arteries by Cap treatment was due to the sympatho—inhibitory action of Cap on the vessel wall, as treatment of SHR with Cap lowered the reactivity and the sensitivity of the vascular bed to NE. Our study showed that cardiovascular remodeling was regressed after ACEI treatment but not after sympatholytic treatment, confirmed the importance of RAA system in the development and regression of vascular remodeling.

Clo played an antihypertensive effect by stimulating α_2 receptor in the medulla oblongata, such as A_1 cell group of ventrolateral medulla[10], whereby outflow of SNS decreased. It is well known that α receptor exists widespreadly in the cardiomyocytes and the vascular SMC and other cells. Myocardial α_1 receptor has been showed to play a predominant role in the myocardial hypertrophy. Whether Clo of the dosage used in this paper may stimulate central α_2 and meanwhile activate myocardial and vascular α_1 receptor remains unclear. In addition, Clo resulted in increased DNA synthesis rate of aorta and aorta/BW in SHR. Our result showed a slight, but not significant, increase of platelet Ca_i^{2+} after Clo treatment. Changes of platelets Ca_i^{2+} may reflect the free calcium level in vascular SMC. The increase in the Ca_i^{2+} may increase the tension of vascular SMC and initiate various intracellular biochemical effects. Our data showed that Cap reduce Ca_i^{2+} significantly and that regression of LVH paralelled with reduction of Ca_i^{2+} of platelets.

Ca_i^{++} weekly correlated with wall/lumen and number of SMC layers in SHR (treated and untreated). It seemed that the changes of Ca_i^{++} of platelets may play a certain role in the cardiovascular remodeling.

In conclusion, combined blockade of SNS and RAA did not produce greater BP reduction and regression of cardiovascular structural and regional humoral alteration than Cap alone did. The sympathetic inhibitory effect of ACEI inhibitor appeared not to be enhanced by sympatholytic agent, such as Clo.

REFERENCES

1 Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium: Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991; **83**: 1849—65.

2 Rorive G, Carlier P, Smelten N, Zgheib A. Sympatholytic drugs and cardiac remodeling in human and experimental arterial hypertension. Basic Res Cardiol 1991; **86** Suppl 1: 141—8.

3 Berecek KH, Kirk KA, Nagahama S, Oparil S. Sympathetic function in spontaneously hypertensive rats after chronic administration of captopril. Am J Physiol 1987; **252**: H796—H806.

4 Lee RMKW, Gzik DJ. Sympatholytic interventions and vascular remodeling. Basic Res Cardiol 1991; **86** Suppl I, 55—64.

5 Bergman I, Loxley R. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal Chem 1963; **35**: 1961—5.

6 Xie LD, Chen DG, Wu DY, Chen HJ. Measurement of intracellular ionic calcium concentration of platelets with Fura-2. J Fujian Med Coll 1991; **25**: 167—8.

7 Loeb AL, Mandel HG, Straw JA, Bean BL. Increased aortic DNA synthesis precedes renal hypertension in rats: an obligatory step? Hypertension 1986; **8**: 754—61.

8 Xie LD, Chen DG, Zhang S, Wang HJ. [illegible] term low dose captopril on regression of L[illegible] neously hypertensive rats. Chin Med Sci J 1991; **6** Suppl: 90.

9 Lee RMKW, Berecek KH, Tsoporis J, Triggle CR. Prevention of hypertension changes by captopril treatment. Hypertension 1991; **17**: 141—50.

10 Quintin L, Ghignone M, Pujol JF. The ab adrenergic agonist clonidine to suppress cer ergic hyperactivity secondary to hemodynan mental stimuli. J Cardiovasc Pharmacol 1987; **10** Suppl 12

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卡托普利逆转高血压大鼠心血管重抗交感神经作用

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摘要 已有心血管肥厚的 SHR，给予(Cap) 20 $mg \cdot kg^{-1} \cdot d^{-1}$(A 组)；可 30 $\mu g \cdot kg^{-1} \cdot d^{-1}$(B 组)； Cap 20 mg 加 Clo 30 $\mu g \cdot kg^{-1} \cdot d^{-1}$(C 组)；死. 各组的 SBP 均明显下降，但水平. Cap 加 Clo 无更强降压作用 C 组的 LVH 明显逆转，两组间无差 Clo 不逆转 SHR 的 LVH. 各组的心显降低，单用 Cap 的作用最强. 说明抗交感神经作用，且不因联用 Clo 而

关键词 近交 SHR 大鼠；近交 WKY 托普利；可乐定；血压；去甲肾上腺钙；羟脯氨酸；肾动脉