EFFECT OF REGRESSION OF CARDIAC HYPERTROPHY ON ISCHEMIC MYOCARDIAL DAMAGE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Effects of reduction of blood pressure and regression of left ventricular hypertrophy following hydralazine and captopril therapy on ischemic cardiac function and myocardial metabolism were studied in spontaneously hypertensive rats (SHR). Hydralazine (1.5 or 3 mg/kg/day) and captopril (50 or 100 mg/kg/day) were administered to SHR from 19 to 26 weeks of age. Both hydralazine and captopril significantly decreased arterial blood pressure in SHR, but only captopril significantly reduced left ventricular weight. The percentage of V₃ myosin isozyme significantly decreased in captopril-treated SHR compared to hydralazine-treated SHR. At the end of long-term treatment, hearts were removed and perfused for 15 min by the working heart technique, and then global ischemia was induced for 30 min. The ischemic heart was reperfused for 30 min. In hydralazine-treated SHR and captopril-treated SHR, the pressure-rate product and extent of recovery of the coronary flow during reperfusion following 30 min of ischemia were higher than those in control SHR, but this difference was significant only in captopril-treated SHR. Hydralazine and captopril treatment improved the restoration of the levels of ATP, creatine phosphate, total adenine nucleotide and energy charge potential in SHR after reperfusion following 30 min of ischemia, but only captopril had a significant effect. In conclusion, regression of left ventricular hypertrophy is more important than lowering of blood pressure in order to improve the ischemic myocardial damage.

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LEFV ventricular (LV) hypertrophy, as well as elevation of blood pressure, is an important prognostic indicator in patients with hypertension^{1–3}. When blood pressure is decreased, the morbidity of hypertensive patients is decreased. Regression of LV hypertrophy along with decreased blood pressure reduces cardiovascular risks even further⁴. Therefore, both reduction of blood

pressure and regression of LV hypertrophy contribute to reduced morbidity when hypertensive patients with LV hypertrophy are treated with antihypertensive drugs. Many studies have been done that involve the drug therapy of hypertension in humans and animals with cardiac hypertrophy. However, the results are not consistent. In spontaneously hypertensive rats (SHR), alpha-methyldopa reduced ventricular weight with only moderate blood pressure control, whereas the vasodilators, hydralazine and minoxidil, did not reduce ventricular hypertrophy despite lowering of blood

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pressure to normotensive levels^{5,6}. These discrepancies between lowering of high blood pressure and regression of cardiac hypertrophy by various antihypertensive agents suggest that cardiac hypertrophy in hypertension is regulated by many factors in addition to pressure overload.

In previous studies, we reported that the isolated perfused heart of SHR is more susceptible to irreversible ischemic damage than that of the normotensive Wistar-Kyoto rat (WKY)? Diltiazem, bunazosin and atenolol reduced LV hypertrophy, but only bunazosin and atenolol lowered the blood pressure. However, the three drugs improved ischemic myocardial metabolism in SHR to the same extent^{8,9}. These results indicate that regression of cardiac hypertrophy is more important than lowering of blood pressure to improve the ischemic damage in SHR.

Therefore, the present experiments were undertaken to clarify whether reduction of high blood pressure or regression of LV hypertrophy had a larger effect on ischemic damage in SHR. First, did long-term treatment with hydralazine, a vasodilator, and captopril, an angiotensin converting enzyme (ACE) inhibitor, lower systolic blood pressure, decrease LV hypertrophy and change the myosin isozyme pattern in SHR? Second, did these drugs improve ischemic damage as measured by the mechanical function and metabolism of the isolated perfused hearts of SHR?

METHODS

Experiment 1

Eight male normotensive WKY (WKY/NCrj, Charles River Japan Inc.) and 37 male SHR (SHR/NCrj, Charles River Japan Inc.) aged 19 weeks were divided into control WKY, control SHR, hydralazine-treated SHR (1.5 or 3 mg/kg/day) and captopril-treated SHR (50 or 100 mg/kg/day). Either hydralazine or captopril was administered through a gastric tube to the SHR from 19 to 26 weeks of age. Tap water was given to control WKY and control SHR. All rats were housed under the same conditions and were fed rat chow (Nihon Clea, CE-2). Systolic blood pressure was measured in the conscious state at 19 weeks and 26 weeks of age by the tail plethysmography

(Narco, PE-300) after 20 min of prewarming at 37 °C.

The 26 week-old rats were killed by decapitation. Blood samples were collected in chilled glass tubes containing ethylene diamine tetraacetic acid disodium. The plasma was immediately separated by centrifugation and stored at -20 °C until plasma renin activity was measured by radioimmunoassay¹⁰. Seven or 8 hearts in each group were perfused by the Langendorff technique with Krebs-Henseleit bicarbonate buffer that was equilibrated with a gas mixture of 95% O₂-5% CO₂ (37 °C) and contained 5.9 mM potassium chloride and 11 mM glucose. After 10 min of perfusion, the perfusate was switched to that containing high-potassium (20 mM) in order to arrest the hearts. After dissecting the aorta and vessels and blotting on filter paper, the ventricles were weighed. In control WKY, control SHR, hydralazine-treated SHR (3 mg/kg/day) and captopril-treated SHR (100 mg/kg/day), a part of the left ventricular wall was excised and fixed in 10% formalin to measure myocyte diameter. The remainder of the ventricle was stored at -80 °C to measure the myosin isozyme pattern.

After the heart was immersed in 10% formalin for several days, the specimens were dehydrated and embedded in paraffin. Transverse tissue sections (5 µm-thick) were stained with hematoxylin and eosin. The longitudinal profile of muscle fibers in the midwall layer of the LV free wall was used for the measurement of myocyte size. Myocytes with nuclei and clear cell margins were selected from several tissue sections. The short axis diameters were measured with a microscope with ×25 magnification.

Pyrophosphate gel electrophoresis was carried out as described^{11,12}. Native myosin from the LV was extracted with a solution that contained Na₄P₂O₇ (pH 8.8), 1, 4-dithiothreitol, ethylene glycol bis-(β-aminoethyl ether) N, N, N', N' tetraacetic acid and leupeptin. The gel contained 3.8% acrylamide and 0.12% N, N'-methylene-bisacrylamide, and the electrophoresis buffer was 20 mM Na₄P₂O₇ (pH 8.8) in the presence of 10% glycerol. Electrophoresis was carried out for 24 h at 2 °C, and the voltage gradient was 13.3 V/cm. The gels were stained for 30 min in 0.025% Coomassie

TABLE 1 EFFECTS OF LONG-TERM TREATMENT WITH HYDRALAZINE OR CAPTOPRIL ON BODY WEIGHT, SYSTOLIC BLOOD PRESSURE AND VENTRICULAR WEIGHT

	WKY		SHR			
	Control (n=8)	Control (n=8)	Hydralazine-treated (1.5 mg/kg/day) (n=8)	Hydralazine-treated (3 mg/kg/day) (n=7)	Captopril-treated (50 mg/kg/day) (n=7)	Captopril-treated (100 mg/kg/day) (n=7)
Body weight (g)	407±4	356±8**	346±7**	350±6**	350±7**	337±5**
Blood pressure (mmHg)	128±2	204±7**	161±5**##§	145±3**##	156±3**##§	144±3**##
Whole heart weight (mg)	1220±39	1288±35	1287±25	1264±16	1186±23#§	1125±21#§§
LV weight (mg)	989±33	1083±33	1048±23	1037±11	956±20#§§	908±18#§§
RV weight (mg)	231±8	205±4*	239±6#	227±11	231±7#	217±8
$\frac{\text{Whole heart weight}}{\text{Body weight}} \times 10^3$	2.997±0.084	3.622±0.055**	3.723±0.043**	3.618±0.039**	3.391±0.036**##§§	3.339±0.026**##§§
$\frac{\text{LV weight}}{\text{Body weight}} \times 10^3$	2.429±0.071	3.045±0.056**	3.030±0.038**	2.968±0.031**##	2.732±0.041***##§§	2.696±0.028***##§§
$\frac{\text{RV weight}}{\text{Body weight}} \times 10^3$	0.568±0.019	0.577±0.008	0.693±0.020***#	0.650±0.029*#	0.659±0.016***##	0.643±0.021*#

LV weight=left ventricular weight; RV weight=right ventricular weight, Values are means±S.E.; ()=number of observations.

* $p<0.05$, ** $p<0.01$, compared with control WKY

$p<0.05$, ## $p<0.01$, compared with control SHR

§ $p<0.05$, §§ $p<0.01$, compared with hydralazine-treated SHR (3 mg/kg/day).

TABLE II EFFECTS OF LONG-TERM TREATMENT WITH HYDRALAZINE OR CAPTOPRIL ON PLASMA RENIN ACTIVITY

	WKY	SHR		
	Control (n=8)	Control (n=8)	Hydralazine-treated (n=7)	Captopril-treated (n=7)
plasma renin activity (ng/ml/h)	1.33±0.19	2.41±0.23*	1.80±0.12	5.34±0.50** ## §§

Values are means±S.E.; ()=number of observations.

* $p<0.05$, ** $p<0.01$, compared with control WKY.

$p<0.01$, compared with control SHR.

§§ $p<0.01$, compared with hydralazine-treated SHR (3 mg/kg/day).

TABLE III EFFECTS OF LONG-TERM TREATMENT WITH HYDRALAZINE OR CAPTOPRIL ON MYOCYTE DIAMETER

	WKY	SHR		
	Control (n=8)	Control (n=8)	Hydralazine-treated (n=7)	Captopril-treated (n=7)
Myocyte diameter (nm)	16.8±0.2	20.4±0.2**	20.3±0.2**	18.8±0.2**2 # §§

Values are means±S.E.; ()=number of observations.

** $p<0.01$, compared with control WKY.

$p<0.01$, compared with control SHR.

§§ $p<0.01$, compared with hydralazine-treated SHR (3 mg/kg/day).

brilliant blue (G-250), sulfuric acid, KOH and trichloroacetic acid!¹³ The destained gels were scanned with a high resolution densitometer (Herena, CLINISCAN 2), and the areas of the peaks were evaluated. The relative amount of individual myosin isozymes were expressed as a percentage of the total area in the three isozymes.

Experiment 2

The next series of experiments was undertaken to determine whether lowering of blood pressure or regression of cardiac hypertrophy plays a more important role in ischemic myocardial damage of SHR. Twenty WKY and 59 SHR that were 19 weeks of age were divided into control WKY, control SHR, hydralazine-treated SHR (3 mg/kg/day) and captopril-treated SHR (100 mg/kg/day). Either hydralazine or captopril was administered to SHR for 7 weeks.

Rats (26 weeks of age) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.).

Hearts were rapidly excised and perfused by the Langendorff technique with Krebs-Henseleit bicarbonate buffer (37°C) that contained 11 mM glucose and was equilibrated with 95%O₂-5%CO₂. The pulmonary artery and left atrium were cannulated, and both the caval veins were ligated. After 10 min of Langendorff perfusion, hearts were perfused by the working heart technique¹⁴ with a left atrial filling pressure of 9 mmHg and a hydrostatic aortic pressure of 60 mmHg. After 15 min of the working heart perfusion, global ischemia was induced for 30 min by lowering aortic pressure from 60 to 0 mmHg. Reduction of aortic pressure resulted in almost zero coronary flow and cessation of the heart beat!¹⁵ When the heart was reperfused, aortic pressure was raised to 60 mmHg again. The period of reperfusion was 30 min. Peak aortic pressure and heart rate were recorded with a recorder (Nihon Koden, RJG-4121) and a pressure transducer (Nihon, Koden, MPU-0.5A). Coronary flow was determined by collecting the

Fig.1. Representative pyrophosphate gels and densitometrical scans of myosin isoforms in rat hearts. These gels show the myosins extracted from left ventricles of control WKY, control SHR, hydralazine-treated SHR (3 mg/kg/day) and captopril-treated SHR (100 mg/kg/day).

perfusate that dripped from the pulmonary arterial cannula. Twenty four hours after the last administration, hearts from hydralazine-treated SHR or captopril-treated SHR were used for perfusion.

At the end of perfusion, the heart was quickly frozen by clamping between blocks of aluminum chilled in liquid nitrogen. The frozen heart tissue was pulverized in a mortar cooled with liquid nitrogen. An aliquot of pulverized myocardial tissue powder was weighed before and after heating at 80°C for 24 h, and from the difference in weight between before and after heating, the water content in the myocardium was calculated. The remainder of the tissue powder was used for determination of the tissue contents of adenosine triphosphate (ATP), adenosine

diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CrP) and lactate. These metabolites were analyzed enzymatically in neutralized 6% perchloric acid extract¹⁶. Total adenine nucleotide (TAN) was calculated as the sum of ATP, ADP and AMP. Energy charge potential (ECP)¹⁷ was calculated according to the following equation,

$$ECP = \frac{([ATP] + 1/2[ADP])}{([ATP] + [ADP] + [AMP])}$$

Statistics

All values are expressed as the mean $\pm$ SE. Analysis of variance was used for multiple group comparisons, using either Student's t-test or the Dunnett multiple range test, as appropriate. Statistical significance was defined as $p < 0.05$.

RESULTS

Effects of hydralazine and captopril on blood pressure, ventricular weight, myocyte diameter and myosin isozyme pattern

In 19 week-old WKY ($n=8$) and SHR ($n=37$), body weight of SHR was significantly ($p < 0.01$) lower than that of WKY (367 ± 2 or 327 ± 3 , respectively), and systolic blood pressure of SHR was significantly ($p < 0.01$) higher than that of WKY (125 ± 4 or 197 ± 2 , respectively).

In 26 week-old rats, body weights of all SHR groups were significantly ($p < 0.01$) lower than those of control WKY (Table I). Treatment with either hydralazine (1.5 or 3 mg/kg/day) or captopril (50 or 100 mg/kg/day) for 7 weeks decreased systolic blood pressure in SHR ($p < 0.01$). Both the ratio of the whole heart weight to body weight and the ratio of LV weight to body weight in control SHR were significantly ($p < 0.01$, respectively) higher than in control WKY. Treatment with hydralazine (1.5 or 3 mg/kg/day) for 7 weeks did not decrease these ratios in SHR, but treatment with captopril (50 or 100 mg/kg/day) decreased these ratios significantly ($p < 0.01$). These results show that both hydralazine and captopril decrease systolic blood pressure to the same extent in SHR, but only captopril decelerates cardiac hypertrophy. To characterize these animals further, plasma renin

TABLE IV EFFECTS OF LONG-TERM TREATMENT WITH HYDRALAZINE OR CAPTOPRIL ON MYOSIN ISOZYME PATTERN

	WKY		SHR	
	Control (n=8)	Control (n=8)	Hydralazine-treated (n=7)	Captopril-treated (n=7)
			(%)	
V1	58.5±1.1	35.3±2.9**	47.5±3.5*#	57.6±1.5###§
V2	19.2±0.8	24.5±0.2**	21.9±2.7	22.6±1.1*
V3	22.3±0.7	41.4±2.1**	30.6±1.4**##	19.8±1.7#####

Values are means±S.E.; ()=number of observations.

* $p<0.05$, ** $p<0.01$, compared with control WKY.

$p<0.05$, ## $p<0.01$, compared with control SHR.

§ $p<0.05$, §§ $p<0.01$, compared with hydralazine-treated SHR (3 mg/kg/day).

activity was measured in control WKY, control SHR, hydralazine-treated SHR (3 mg/kg/day) and captopril-treated SHR (100 mg/kg/day) (Table II). Plasma renin activity in control SHR was significantly ($p<0.05$) higher than that in control WKY. Hydralazine did not increase renin activity in SHR, but captopril raised it significantly ($p<0.01$).

In the next series of experiments, hearts were histologically evaluated to determine whether hydralazine and captopril actually affected myocyte size and myosin isozyme composition of LV. The myocyte diameter of control SHR was significantly ($p<0.01$) larger than that of control WKY (Table III). Hydralazine did not change myocyte diameter in SHR, but captopril decreased it significantly ($p<0.01$). These results show that regression of LV hypertrophy by long-term treatment with captopril in SHR is accompanied by a decrease of myocyte size.

Typical pyrophosphate gels are shown in Fig. 1. The percentage of myosin isozyme V₁ in control WKY was higher than that of myosin isozyme V₂ and V₃ (Table IV). The percentage of myosin isozyme V₁ in SHR was significantly ($p<0.01$) lower than that in control WKY, and that of myosin isozyme V₂ or V₃ were significantly ($p<0.01$) higher than that in control WKY. Both hydralazine and captopril significantly ($p<0.05$ and $p<0.01$, respectively) increased the percentage of myosin isozyme V₁ in SHR and significantly ($p<0.01$ or $p<0.01$, respectively) decreased that of myosin isozyme V₃.

These changes were significantly ($p<0.05$ and $p<0.01$, respectively) greater in captopril-treated SHR than in hydralazine-treated SHR. The percentages of myosin isozyme V₁ and V₃ in captopril-treated SHR were not different from these percentages in control WKY.

Effects of hydralazine and captopril on mechanical function and metabolism in perfused rat hearts

The peak aortic pressure×heart rate (pressure-rate) products before ischemia were not significantly different in control WKY, control SHR, hydralazine-treated SHR and captopril-treated SHR (23.7 ± 0.8 , 24.5 ± 0.8 , 24.1 ± 0.5 and $24.2\pm0.9\times10^3$ mmHg/min, respectively). The coronary flow before ischemia in control WKY was significantly ($p<0.05$) higher than that in control SHR, hydralazine-treated SHR and captopril-treated SHR (21.6 ± 0.8 , 17.3 ± 0.3 , 16.9 ± 0.4 and 17.2 ± 0.7 ml/min, respectively). The ratio of the coronary flow to heart weight, calculated by using the ratio of heart weight to body weight (Table I), was significantly ($p<0.01$) lower in control SHR than in control WKY (13.7 ± 0.3 or 19.7 ± 0.6 ml/min/g heart weight, respectively). Hydralazine did not improve the ratio, but captopril did ($p<0.01$) (13.6 ± 0.3 or 15.8 ± 0.7 ml/min/g heart weight, respectively).

After the onset of ischemia, coronary flow rapidly fell to near 0 ml/min, and the pressure-rate product progressively decreased to 0 mmHg/min within 5 min in both control WKY and control SHR. Both the

Fig.2. Effects of long-term treatment with hydralazine or captopril on peak aortic pressure $\times$ heart rate during reperfusion.

Symbols of $\square$, $\circ$, $\blacktriangle$ and $\bullet$ represent control WKY, control SHR, hydralazine-treated SHR (3 mg/kg/day) and captopril-treated SHR (100 mg/kg/day), respectively.

Values are means $\pm$ S.E. (vertical lines) of 6–8 hearts.

* $p < 0.05$, ** $p < 0.01$, compared with the control WKY.

Fig.3. Effects of long-term treatment with hydralazine or captopril on percentage recovery of coronary flow during reperfusion.

Data were obtained from the same hearts described in Fig. 2.

* $p < 0.05$, ** $p < 0.01$, compared with control WKY.

$p < 0.01$, compared with control SHR.

§ $p < 0.05$, compared with hydralazine-treated SHR (3 mg/kg/day).

extent of recovery of pressure-rate product and that of percentage recovery of the coronary flow during reperfusion in control SHR were significantly ($p < 0.01$) lower than those in control WKY (Fig. 2 and 3). Hydralazine did not improve the extent of recovery of pressure-rate product, but

captopril had a small effect. Hydralazine did not improve the extent of percentage recovery of coronary flow during reperfusion, but captopril did so significantly ($p < 0.05$).

Water content was not significantly different prior to ischemia or during ischemia in

TABLE V EFFECTS OF LONG-TERM TREATMENT WITH HYDRALAZINE OR CAPTOPRIL ON WATER CONTENT, ATP, ADP, AMP, TOTAL ADENINE NUCLEOTIDE (TAN), CREATINE PHOSPHATE AND LACTATE LEVELS IN MYOCARDIUM

		WKY	SHR		
		Control	Control	Hydralazine-treated	Captopril-treated
				(%)	
water content	Preischemia	84.0±0.2	84.1±0.3	84.2±0.2	84.0±0.2
	Ischemia (30 min)	81.0±0.5	81.6±0.2	81.5±0.3	81.7±0.4
	Ischemia (30 min)				
	+ Reperfusion (30 min)	85.0±0.4	86.6±0.4*	86.2±0.5	85.4±0.2 #
			(μmoles/g dry weight)		
ATP	Preischemia	18.7±0.4	18.8±0.4	18.4±0.4	18.4±0.4
	Ischemia (30 min)	2.2±0.3	2.4±0.2	2.7±0.4	2.5±0.3
	Ischemia (30 min)				
	+ Reperfusion (30 min)	9.6±0.9	4.8±0.6**	5.9±0.8*	7.6±0.7#
ADP	Preischemia	5.0±0.4	4.9±0.1	5.3±0.3	5.1±0.3
	Ischemia (30 min)	2.5±0.4	2.9±0.3	2.7±0.4	2.7±0.2
	Ischemia (30 min)				
	+ Reperfusion (30 min)	2.6±0.2	2.6±0.3	2.7±0.2	2.6±0.1
AMP	Preischemia	1.5±0.1	1.4±0.1	1.3±0.2	1.3±0.1
	Ischemia (30 min)	6.7±0.3	8.2±0.6*	7.5±0.3	6.7±0.4
	Ischemia (30 min)				
	+ Reperfusion (30 min)	1.3±0.1	3.3±0.3**	2.6±0.3*	2.0±0.3*##
TAN	Preischemia	25.1±0.8	25.1±0.5	25.3±0.6	24.8±0.5
	Ischemia (30 min)	11.4±0.6	13.4±0.9	12.9±0.6	11.9±0.6
	Ischemia (30 min)				
	+ Reperfusion (30 min)	13.5±1.0	10.3±0.7**	11.2±0.7*	12.1±0.6#
CrP	Preischemia	17.3±1.5	18.7±0.8	16.6±1.2	17.7±1.6
	Ischemia (30 min)	3.7±0.3	3.5±0.3	4.4±0.5	4.1±0.6
	Ischemia (30 min)				
	+ Reperfusion (30 min)	24.0±1.6	15.4±2.1**	18.0±2.0*	24.2±2.4 #
Lactate	Preischemia	1.8±0.6	2.1±0.6	1.4±0.5	1.9±0.7
	Ischemia (30 min)	43.4±2.6	62.3±4.3**	48.2±3.4 #	48.5±1.8 #
	Ischemia (30 min)				
	+ Reperfusion (30 min)	3.2±0.6	15.5±2.3 **	8.0±1.9#	7.4±1.5*#

CrP=creatine phosphate, Values are means±S.E. of 6–8 hearts.

* $p<0.05$, ** $p<0.01$, compared with control WKY.

$p<0.05$, ## $p<0.01$, compared with control SHR.

any of the 4 groups (Table V). The water content in control WKY after reperfusion following 30 min of ischemia was significantly ($p<0.05$) lower than that in control SHR.

Treatment with hydralazine in SHR did not decrease the water content, but treatment with captopril significantly did so.

ATP levels were not significantly different

Fig.4. Effects of long-term treatment with hydralazine or captopril on energy charge potential.

* $p < 0.05$, ** $p < 0.01$, compared with control WKY.

$p < 0.01$, compared with control SHR.

(□; control WKY. ■; control SHR. ▨; hydralazine-treated SHR (3 mg/kg/day).

▩; captopril-treated SHR (100 /mg/kg/day)).

Fig.5. The relationship between heart weight and pressure-rate product after reperfusion following 30 min of ischemia. Data were obtained from the same heart ($n=32$) described in Fig. 2. ($Y=70.0-51.9x$, $r=0.753$, $p < 0.01$)

prior to ischemia or during ischemia in any of the 4 groups (Table V). Reperfusion after 30 min of ischemia in control WKY resulted in recovery of the ATP level to 51% of the preischemic level. In control SHR, however, ATP recovered to only 26% of the preische-

mic level. ATP levels during reperfusion were significantly ($p < 0.01$) lower in control SHR than in control WKY. Reperfusion after 30 min of ischemia in hydralazine-treated SHR and captopril-treated SHR resulted in recovery of ATP levels to 32% and 41% of

the respective preischemic levels. Hydralazine slightly improved recovery of the ATP level in SHR, but captopril improved this value significantly ($p < 0.05$). The preischemic levels of TAN were 24.8–25.1 $\mu\text{moles/g}$ dry weight in all 4 groups. The levels of TAN in all 4 groups decreased during ischemia by 45–55%. Reperfusion after 30 min of ischemia did not restore these values. After 30 min of reperfusion following 30 min of ischemia, the level of TAN in control SHR was significantly ($p < 0.05$) lower than that in control WKY (10.7 ± 0.7 or 13.5 ± 1.0 $\mu\text{moles/g}$ dry weight, respectively). Treatment with hydralazine in SHR did not improve the restoration of TAN, but treatment with captopril significantly ($p < 0.05$) did so (11.2 ± 0.7 or 12.1 ± 0.6 $\mu\text{moles/g}$ dry weight, respectively). Fig. 4 shows the change in ECP caused by ischemia and reperfusion. Preischemic values of ECP were constant at approximately 0.84 in all 4 groups. ECP decreased during ischemia and was restored by reperfusion. In hearts reperfused after 30 min of ischemia, ECP in control SHR was significantly ($p < 0.01$) lower than that in control WKY (0.56 ± 0.11 or 0.80 ± 0.05 , respectively). Values of ECP after reperfusion following 30 min of ischemia in hydralazine-treated SHR and captopril-treated SHR (0.64 ± 0.10 or 0.72 ± 0.08 , respectively) were higher than that in control SHR, but the effect was significant only in captopril treated SHR ($p < 0.01$).

The levels of CrP are shown in Table V. The preischemic levels of CrP were between 16.6 and 18.7 $\mu\text{moles/g}$ dry weight and there were no significant differences in any of the 4 groups. Ischemia decreased CrP levels to 19–27% of the preischemic levels in all 4 groups. The CrP level in control WKY recovered during reperfusion after 30 min of ischemia to values above the preischemic level. The CrP level in control SHR, which recovered to only 82% of the preischemic level, was significantly ($p < 0.01$) lower than that in control WKY. The CrP level in hydralazine-treated SHR was slightly higher than that in control SHR, but the CrP level in captopril-treated SHR was significantly ($p < 0.05$) higher than that in control SHR.

The levels of lactate are listed in Table V. There were no significant differences in

lactate levels prior to ischemia in any of the 4 groups. The lactate level increased during ischemia and decreased during reperfusion. The lactate level in control SHR after 30 min of ischemia was significantly ($p < 0.01$) higher than that in control WKY. Both hydralazine and captopril significantly decreased accumulation of lactate after 30 min of ischemia ($p < 0.05$). During reperfusion after 30 min of ischemia, the lactate level in control SHR was significantly ($p < 0.05$) higher than in control WKY. Both hydralazine and captopril significantly decreased the levels of lactate during reperfusion ($p < 0.05$).

DISCUSSION

This study shows different effects of antihypertensive therapy with hydralazine and captopril on lowering of blood pressure, regression of LV hypertrophy, change of myosin isozyme pattern of the LV and ischemic myocardial damage in SHR. Both of these antihypertensive drugs reduced blood pressure in SHR to the same extent, but only captopril decreased LV hypertrophy. The change in myosin isozyme pattern with hydralazine treatment was smaller than with captopril treatment. Antihypertensive treatment with hydralazine in SHR did not improve the extent of recovery of cardiac mechanical function or restoration of myocardial ECP during reperfusion after 30 min of ischemia, but treatment with captopril improved these parameters. These differences between hydralazine-treated SHR and captopril-treated SHR may be explained by different effects of these drugs on regression of LV hypertrophy and myocardial structure. Heart weight and pressure-rate product after reperfusion following 30 min of ischemia were well correlated ($p < 0.01$, Fig. 5). These results strongly suggest that regression of LV hypertrophy is more important than lowering of blood pressure in order to improve the ischemic myocardial damage in the hypertrophic heart with hypertension.

The development of cardiac hypertrophy in hypertension has been understood to be an adaptation to chronic pressure overload. However, lack of a linear correlation between blood pressure and the degree of cardiac hypertrophy in hypertension has been demonstrated both in experimental animal

models and in humans^{5,6,8,9,18-21} The mechanism of development of cardiac hypertrophy in hypertension is very complicated, and many factors are involved.²² Neurohumoral factors, such as catecholamine, angiotensin II, thyroxine, growth hormone, parathyroid hormone and insulin, influence the development and regression of cardiac hypertrophy. Sen et al²³ have reported that in hypertrophied myocardium there is a soluble factor which stimulates protein synthesis and that this factor may play a key role in modulation of myocardial structure during development or regression of myocardial hypertrophy in hypertension. The renin-angiotensin systems also participate in hypertensive cardiac hypertrophy. Katoh et al²⁴ have recently reported that angiotensin II stimulates the expression of the proto-oncogene, *c-fos*, in cultured rat heart myocytes. Angiotensin II directly stimulates protein synthesis and cell growth in chick heart cells^{25,26} and in abdominal aorta-constricted rats.²⁷ In the present study, long-term treatment with captopril decreased the ratio of LV weight to body weight and myocyte size in SHR. These results also confirm that angiotensin II plays an important role in the development and regression of cardiac hypertrophy in SHR.

The myosin isozyme pattern changes with development of LV hypertrophy caused by pressure overload and humoral stimulation. The pattern also changes when pressure overload is removed or drug therapy is performed. These changes of myosin isozyme pattern are correlated with a change of cardiac contractility and myosin ATPase activity in myocardium.²⁸⁻³⁰ WKY has a predominance of myosin isozyme V_1 , but SHR has a predominance of myosin isozyme V_3 .^{12,28} Rupp and Jacob³¹ have reported that hydralazine lowers blood pressure in SHR but does not change the myosin isozyme pattern. However, Nagano et al³² have reported that hearts from SHR treated with hydralazine experience a shift in myosin isozyme pattern with a decrease in myosin isozyme V_3 and an increase in myosin isozyme V_1 . In the present study, the percentage of myosin isozyme V_3 decreased and V_1 increased in SHR that were treated either by hydralazine or captopril therapy. The ratio of myosin isozyme V_3 to V_1 in captopril-treated SHR was much

lower than that in hydralazine-treated SHR. Our results indicate that both hydralazine and captopril decreased the blood pressure to the same extent, but that changes of the myosin isozyme pattern induced by these drugs were not the same. Different myosin isozyme patterns may reflect different sympathetic activities between hydralazine-treated SHR and captopril-treated SHR. Hydralazine activates the sympathetic nervous system secondary to a decrease in arterial pressure,³³ but captopril does not!⁸ Small doses of isoproterenol that induce cardiac hypertrophy shift the myosin isozyme pattern toward V_1 .³⁴ Swimming of Wistar rats shifts the myosin isozyme pattern toward V_1 with increased contents of norepinephrine both in left ventricle and adrenal gland.³⁵ Another explanation may be different angiotensin II availability in the two groups. The myosin isozyme V_1 in rat ventricle in angiotensin II-induced hypertension decreased with cardiac hypertrophy.³⁶ Therefore, a reversal of an abnormal isozyme profile of cardiac myosin might be explained by decreased blood pressure, a change in the cardiac sympathetic tone or a change in the renin-angiotensin system.

Wicker et al³⁷ have reported that the coronary flow per unit of LV mass decreases with the development of cardiac hypertrophy in renovascular hypertensive rats. Anti-hypertensive therapy with captopril decreased coronary vascular resistance with regression of cardiac hypertrophy in renovascular hypertensive rats³⁷ or SHR.³⁸ However, Anderson et al³⁹ reported that coronary flow improves during maximal vasodilation with adenosine without regression of cardiac hypertrophy after hydralazine treatment in SHR, but the basal coronary flow does not change. Canby et al³⁸ also reported that the myocardial perfusion of LV during maximal vasodilation with dipyridamole was slightly more improved after hydralazine treatment in SHR, but the basal perfusion flow was not. In the present study using perfused rat hearts, the basal ratio of the coronary flow to heart weight in control SHR was significantly lower than in control WKY. Long-term treatment with hydralazine did not improve this ratio in SHR, but treatment with captopril did so significantly.

As shown in Fig. 2, the extents of recovery of pressure-rate product during reperfusion after 30 min of ischemia in control SHR and hydralazine-treated SHR were significantly lower than in control WKY. Captopril improved the recovery of pressure-rate product in SHR slightly, but not significantly. However, as shown in Fig. 5, there was a significant ($p < 0.01$) negative correlation between heart weight and pressure-rate product. After reperfusion following 30 min of ischemia, the extents of recovery of coronary flow in control SHR and hydralazine-treated SHR were significantly lower than in control WKY (Fig. 3). Captopril significantly improved the recovery of the coronary flow in SHR. It has been reported in the anoxic perfused guinea pig heart that edema develops during anoxia;⁴⁰ thereby increasing coronary resistance. We therefore measured the water content in the myocardium. The water content in control SHR after reperfusion following 30 min of ischemia was significantly greater than that in control WKY. Treatment with hydralazine in SHR did not decrease the water content, but treatment with captopril significantly did so. Accordingly, there remains a possibility that progressive myocardial edema decreases the coronary flow. There were no differences in the myocardial ATP and CrP levels before ischemia and during ischemia among control WKY, control SHR, hydralazine-treated SHR and captopril-treated SHR. The levels of ATP and CrP after reperfusion following 30 min of ischemia in control WKY were significantly higher than in control SHR. These level in hydralazine-treated SHR were slightly higher than in control SHR, but the levels in captopril-treated SHR were significantly higher than in control SHR (Table V). Restoration of TAN and ECP during reperfusion in control SHR was significantly lower than in control WKY. That in captopril-treated SHR or hydralazine-treated SHR was significantly higher than in control SHR (Table V and Fig. 4). Our former reports^{8,9} and these results strongly suggest that regression of cardiac hypertrophy rather than lowering of high blood pressure improves ischemic damages as assessed both by mechanical function and myocardial metabolism.

In conclusion, long-term treatment with

either hydralazine or captopril in SHR reduced blood pressure, but only captopril induced regression of cardiac hypertrophy and improved coronary flow reserve. Captopril was more effective than hydralazine in reversal of the myosin isozyme pattern and in prevention of ischemic myocardial damage in perfused rat hearts. These results indicate that decreased blood pressure is not always correlated with regression of LV hypertrophy, and that regression of LV hypertrophy is more important than decrease in blood pressure in order to improve the ischemic myocardial damage. Angiotensin II, may play an important role in the development, maintenance and regression of myocardial hypertrophy.

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