RESPONSE OF ISOLATED PERFUSED HEART TO ISCHEMIA AFTER LONG-TERM TREATMENT OF SPONTANEOUSLY HYPERTENSIVE RATS WITH DILTIAZEM

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The effects of long-term treatment with diltiazem on the heart in normotensive (WKY) and spontaneously hypertensive rats (SHR) were studied. Diltiazem was added to the drinking fluid (900 mg/liter) and given ad libitum from 19 to 26 weeks of age, whereas tap water was given to the control animals. Although diltiazem did not decrease blood pressure in SHR, it decelerated the increase in their left ventricular weight ($p < 0.01$).

Hearts were removed and perfused by the working heart technique for 15 min, and then global ischemia was induced for either 10 or 30 min. The ischemic heart was reperfused for 30 min. The extent of recovery of coronary flow after reperfusion, following 30 min of ischemia in the diltiazem-treated SHR, was higher than that in the control SHR ($p < 0.01$). The levels of adenosine triphosphate (ATP), creatine phosphate (CrP), and energy charge potential in the SHR heart reperfused after 30 min of ischemia were lower than those in the reperfused WKY heart ($p < 0.01$, respectively). Diltiazem improved the restoration of ATP and CrP and prevented the decrease in energy charge potential in SHR after reperfusion following 30 min of ischemia ($p < 0.01$, respectively).

In conclusion, long-term treatment of SHR with diltiazem may protect the myocardium when myocardial ischemia occurs.

HYPERTENSION causes left ventricular hypertrophy as an adaptation to the increased systemic blood pressure. Treatment of hypertension with antihypertensive drugs regresses hypertrophy toward normal¹⁻⁵. This regression is associated with an improvement of cardiac pump performance^{3,4}. When the pressure overload is further prolonged, however, several histological irreversible changes occur in the hypertrophied heart^{1,6}, leading to cardiac decom-

pensation. In the hypertrophied myocardium, blood supply and/or oxygen supply to the tissue may be decreased by atherosclerosis of the coronary artery and by reduced oxygen diffusion due to an increase in the myofibril diameter. Although the cardiac mass increases in the hypertrophied heart, the ratio of mitochondria/myofibril-volume in the cell decreases⁷. These findings suggest that the hypertrophied myocardium is more susceptible to oxygen deficiency, such as ischemia, than the normal myocardium.

Ichihara and Abiko⁸ have reported that diltiazem, a calcium channel blocker, protects the perfused heart from ischemic damage *in vitro*. However, they used normotensive

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TABLE I EFFECTS OF LONG-TERM TREATMENT WITH DILTIAZEM ON BODY WEIGHT, SYSTOLIC BLOOD PRESSURE, AND VENTRICULAR WEIGHT

	WKY		SHR	
	Control (n = 5)	Diltiazem-treated (n = 5)	Control (n = 5)	Diltiazem-treated (n = 5)
Body weight (g)	368.0 ± 4.5	371.0 ± 5.6	352.2 ± 6.9	333.4 ± 4.7§§
Blood pressure (mmHg)	119.2 ± 3.6	128.2 ± 2.8	163.4 ± 1.6###	173.0 ± 4.1§§
Whole heart weight (mg)	1126.5 ± 10.1	1118.5 ± 33.1	1277.4 ± 27.7###	1118.7 ± 28.9**
LV weight (mg)	892.5 ± 10.8	869.9 ± 24.1	1051.3 ± 25.7###	913.1 ± 24.9**
RV weight (mg)	234.0 ± 11.1	248.6 ± 14.6	226.1 ± 3.2	205.6 ± 10.3§
$\frac{\text{Whole heart weight}}{\text{Body weight}} \times 10^3$	3.063 ± 0.042	3.013 ± 0.063	3.627 ± 0.035###	3.364 ± 0.054**§§
$\frac{\text{LV weight}}{\text{Body weight}} \times 10^3$	2.428 ± 0.052	2.343 ± 0.037	2.985 ± 0.036###	2.745 ± 0.043**§§
$\frac{\text{RV weight}}{\text{Body weight}} \times 10^3$	0.635 ± 0.026	0.670 ± 0.038	0.642 ± 0.080	0.619 ± 0.031

LV weight = left ventricular weight; RV weight = right ventricular weight.

Values are means ± S.E. (n = number of animals).

**p < 0.01, compared with the control SHR.

###p < 0.01, compared with the control WKY.

§p < 0.05; §§p < 0.01, compared with the diltiazem-treated WKY.

Sprague-Dawley rats, and added diltiazem to the perfusion medium directly. We⁹ have found that the isolated perfused heart removed from the spontaneously hypertensive rat (SHR) is more susceptible to ischemic damage than that from the normotensive Wistar-Kyoto rat (WKY). In the present study, therefore, we investigated the effects of long-term treatment with diltiazem on ischemic changes in metabolism and the mechanical function of the isolated perfused heart of SHR *in vivo*.

METHODS

Animals

Sixty-seven male normotensive Wistar-Kyoto rats aged 19 weeks (WKY/NCrj, Charles River Japan Inc.) and 67 male age-matched spontaneously hypertensive rats (SHR/NCrj, Charles River Japan Inc.) were divided into control (35 WKY and 35 SHR) and diltiazem-treated (32 WKY and 32 SHR) groups. Diltiazem was added to drinking fluid (900 mg/liter) and given ad libitum for 7 weeks, whereas tap water was given to the control animals. In

the diltiazem-treated groups, it was estimated that each rat received about 50–60 mg/kg of diltiazem daily. All rats were kept under the same conditions and fed rat chow (Nihon Clea, CE-2). Systolic blood pressure was measured at 19 weeks of age and 26 weeks of age by tail plethysmography (Narco, PE-300) after 20 min of prewarming at 37°C while conscious.

Heart perfusion

The 26 week-old rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The hearts were rapidly excised and perfused by the Langendorff technique with Krebs-Henseleit bicarbonate buffer (37°C) containing 11 mM glucose equilibrated with a gas mixture of 95% O₂–5% CO₂. The pulmonary artery and the left atrium were cannulated respectively and both the caval veins were ligated. Ten minutes after the Langendorff perfusion, the heart was perfused by the working heart technique¹⁰ with a left atrial filling pressure of 9 mmHg and a hydrostatic aortic afterload pressure of 60 mmHg. After 15 min of the working heart perfusion, ischemia (global ischemia) was induced for either 10 or 30

Fig.1. Effect of long-term treatment with diltiazem on recovery of pressure-rate product during reperfusion after ischemia. After 15 min of the working heart perfusion, ischemia was induced by lowering the afterload pressure from 60 to 0 mmHg. After either 10 or 30 min of ischemia, hearts were perfused for 30 min by the working heart preparation by raising the afterload pressure to 60 mmHg again. (●, control WKY; ○, diltiazem-treated WKY; ■, control SHR; □, diltiazem-treated SHR) Data were obtained from the same hearts shown in Table II. Values are means $\pm$ S.E.

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

min by lowering the afterload pressure from 60 to 0 mmHg, resulting in almost zero coronary flow and cessation of heart beat⁸. When the heart was reperfused, the afterload pressure was raised to 60 mmHg again. A period of reperfusion was fixed for 30 min. Peak aortic pressure and heart rate were recorded on a pressure recorder (Nihon Kohden, RJG-4124) via a pressure transducer (Nihon Kohden, MPU-0.5A) throughout the perfusion procedure. The coronary flow was determined by collecting the dripping perfusate from the pulmonary arterial cannula.

Medications were discontinued at least 20 hours before the perfusion experiment.

Biochemical assays

At the end of each perfusion, the heart was quickly frozen with freezing clamps chilled in liquid nitrogen. The frozen heart tissue was pulverized in a mortar cooled with liquid nitrogen. A part of the pulverized myocardial tissue powder was weighed, and then dried in an oven overnight for measurement of its dry weight. The remainder of the tissue powder was

used for determination of the tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CrP) and lactate, which were analyzed in 6% perchloric acid extract by a spectrophotometer (Gilford, System 2600) according to the enzymatic method of Bergmeyer¹¹. Energy charge potential (ECP)¹² was calculated according to the following equation,

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

Measurement of ventricular weight

To measure the left ventricular (including the ventricular septum) and the right ventricular weights, 5 hearts taken from each of the 4 groups were perfused using the Langendorff technique for 10 min, and weighed after dissecting the atria and vessels and blotting with filter paper.

Statistics

All values are expressed as means $\pm$ SE. Either paired or unpaired Student's *t*-test was applied to determine significant differences between the

Fig. 2. Effect of long-term treatment with diltiazem on recovery of coronary flow during reperfusion after ischemia. Data were obtained from the same hearts shown in Fig. 1. Values are means $\pm$ S.E. Symbols are same as those in Fig. 1. * $p < 0.05$; ** $p < 0.01$, compared with control SHR. + $p < 0.05$; ++ $p < 0.01$, compared with diltiazem-treated SHR.

mean values. A p value of 0.05 or less was considered to be statistically significant.

RESULTS

Blood pressure and ventricular weight

The body weights of 19 week-old WKY and SHR were 348.3 ± 4.3 g ($n = 67$) and 325.4 ± 4.4 g ($n = 67$), respectively. The systolic blood pressures of these animals were 123 ± 3 mmHg (WKY) and 175 ± 4 mmHg (SHR). The body weight of SHR was significantly ($p < 0.01$) lower than that of WKY, and the systolic pressure of SHR was significantly ($p < 0.01$) higher than that of WKY. The animals, both WKY and SHR, were divided into 2 subgroups, and bred for 7 weeks with (diltiazem-treated WKY and diltiazem-treated SHR) or without diltiazem (control WKY and control SHR). In 26 week-old rats, the body weights of the control WKY, diltiazem-treated WKY, control SHR, and diltiazem-treated SHR were 381.0 ± 3.7 ($n = 35$), 367.6 ± 3.3 ($n = 32$), 349.6 ± 3.0 ($n = 35$), and 340.1 ± 2.9 g ($n = 32$), respectively. The body weights of the SHR groups were significantly ($p < 0.01$) lower than those of the WKY groups. The increases in body weight in both WKY and SHR were inhibited significantly by diltiazem ($p < 0.01$ and $p < 0.05$, respectively). The

systolic blood pressures of the control WKY, diltiazem-treated WKY, control SHR, and diltiazem-treated SHR were 122 ± 2 , 116 ± 2 , 179 ± 2 , and 174 ± 4 mmHg, respectively. The treatment with diltiazem for 7 weeks did not modify the systolic blood pressure either in WKY or in SHR. Both the whole heart (left and right ventricle) and the left ventricular weight in the control SHR were significantly ($p < 0.01$) higher than those in the control WKY (Table I). The ratio of the whole heart weight to the body weight and the ratio of the left ventricular weight to the body weight in the control SHR were significantly ($p < 0.01$) higher than those in the control WKY. The right ventricular weight and the ratio of the right ventricular weight to the body weight in the control SHR were not different from those in the control WKY. The treatment with diltiazem for 7 weeks decreased the ratio of the whole heart weight to the body weight and the ratio of the left ventricular weight to the body weight in SHR significantly ($p < 0.01$), but not in WKY.

Experiments of perfusion

Regardless of treatment with or without diltiazem, the pressure-rate products before ischemia (preischemia) in the SHR groups were not significantly different from those in the

TABLE II ATP LEVEL

	WKY		SHR	
	Control	Diltiazem-treated	Control	Diltiazem-treated
	(μmoles/g dry weight)			
Preischemia	17.7 ± 0.6 (n = 6)	17.4 ± 1.0 (n = 5)	17.6 ± 0.7 (n = 6)	17.5 ± 1.2 (n = 5)
Ischemia (10 min)	6.5 ± 1.1 (n = 6)	8.3 ± 0.8 (n = 5)	8.3 ± 0.8 (n = 6)	9.2 ± 0.6 (n = 5)
Ischemia (30 min)	3.0 ± 0.4 (n = 6)	2.5 ± 0.2 (n = 5)	2.6 ± 0.3 (n = 5)	4.1 ± 1.1 (n = 5)
Ischemia (10 min) + Reperfusion (30 min)	12.4 ± 0.9 (n = 5)	14.8 ± 0.7 (n = 6)	13.4 ± 0.8 (n = 6)	15.4 ± 0.3* (n = 6)
Ischemia (30 min) + Reperfusion (30 min)	7.7 ± 0.6 (n = 7)	8.8 ± 0.3 (n = 6)	3.9 ± 0.5## (n = 7)	6.8 ± 0.5**§§ (n = 6)

Values are means ± S.E. (n = number of animals)

*p < 0.05; **p < 0.01, compared with the control SHR.

##p < 0.01, compared with the control WKY.

§§p < 0.01, compared with the diltiazem-treated WKY.

TABLE III ADP LEVEL

	WKY		SHR	
	Control	Diltiazem-treated	Control	Diltiazem-treated
	(μmoles/g dry weight)			
Preischemia	4.65 ± 0.27	4.62 ± 0.13	4.71 ± 0.23	4.61 ± 0.15
Ischemia (10 min)	5.66 ± 0.39	5.64 ± 0.26	6.15 ± 0.24	6.54 ± 0.35
Ischemia (30 min)	3.04 ± 0.24	3.27 ± 0.25	2.77 ± 0.32	3.49 ± 0.44
Ischemia (10 min) + Reperfusion (30 min)	3.27 ± 0.11	3.31 ± 0.23	3.17 ± 0.20	3.47 ± 0.31
Ischemia (30 min) + Reperfusion (30 min)	2.86 ± 0.10	2.73 ± 0.13	2.78 ± 0.10	2.91 ± 0.12

Data were obtained from the same hearts shown in Table II. Values are means ± S.E.

WKY groups. The preischemic coronary flow of the control WKY, diltiazem-treated WKY, control SHR, and diltiazem-treated SHR were 19.5 ± 0.6 , 19.0 ± 0.5 , 18.0 ± 0.3 , and 15.9 ± 0.4 ml/min, respectively. The coronary flow of the SHR groups were significantly ($p < 0.01$) lower than those of the WKY groups. After the onset of ischemia, the coronary flow rapidly became near 0 ml/min, and the pressure-rate product progressively decreased and became close to

0 mmHg/min within 5 min in both WKY and SHR. The pressure-rate product (Fig. 1) and the coronary flow (Fig. 2) are plotted as a function of the period of reperfusion. In the control heart made ischemic for 10 min, the extent of recovery of the pressure-rate product during reperfusion in SHR was higher than that in WKY, although the extent of recovery of the coronary flow was similar. In the control heart made ischemic for 30 min, however, the pressure-rate product in

TABLE IV AMP LEVEL

	WKY		SHR	
	Control	Diltiazem-treated	Control	Diltiazem-treated
	(μmoles/g dry weight)			
Preischemia	1.56 ± 0.15	1.48 ± 0.05	1.65 ± 0.17	1.63 ± 0.18
Ischemia (10 min)	5.80 ± 0.71	4.87 ± 0.71	5.18 ± 0.65	4.43 ± 0.17
Ischemia (30 min)	7.08 ± 0.48	7.43 ± 0.36	8.05 ± 0.21	7.02 ± 0.51
Ischemia (10 min) + Reperfusion (30 min)	1.84 ± 0.08	1.31 ± 0.06##	1.46 ± 0.10#	1.21 ± 0.05*
Ischemia (30 min) + Reperfusion (30 min)	1.77 ± 0.15	1.62 ± 0.10	3.55 ± 0.30##	2.29 ± 0.24**§

Data were obtained from the same hearts shown in Table II. Values are means ± S.E.

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

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

§ $p < 0.05$; compared with the diltiazem-treated WKY.

Fig.3. Changes in tissue total adenine nucleotides during ischemia and the following reperfusion. Hearts were frozen with liquid nitrogen immediately before the onset of ischemia (Preischemia), 10 or 30 min after ischemia, or 30 min after reperfusion following 10 or 30 min of ischemia. Data were obtained from the same hearts shown in Table II. Values are means ± S.E. C, control WKY and SHR; D, diltiazem-treated WKY and SHR.

SHR did not return at all, whereas that in WKY recovered, though incompletely. The extent of the coronary flow recovery in SHR was significantly ($p < 0.01$) lower than that in WKY. In the heart made ischemic for 10 min, the extent of recovery of the pressure-rate product in the diltiazem-treated WKY during reperfusion was greater than that in the control WKY. However, there was no significant difference in

the recovery of coronary flow between the control and the diltiazem-treated WKY hearts. The pressure-rate product and the recovery of the coronary flow in SHR during reperfusion following 10 min of ischemia were further improved by treatment with diltiazem. In the heart made ischemic for 30 min, diltiazem no longer improved the extent of recovery of the pressure-rate product during reperfusion in either

Fig.4. Changes in ECP during ischemia and the following reperfusion of control (C) WKY and SHR, and diltiazem-treated (D) WKY and SHR. Data were obtained from the same hearts shown in Table II. Values are means $\pm$ S.E.

TABLE V CREATINE PHOSPHATE LEVEL

	WKY		SHR	
	Control	Diltiazem-treated	Control	Diltiazem-treated
	(μmoles/g dry weight)			
Preischemia	16.0 $\pm$ 1.0	15.7 $\pm$ 1.5	18.0 $\pm$ 1.8	18.2 $\pm$ 1.3
Ischemia (10 min)	4.6 $\pm$ 0.8	6.3 $\pm$ 0.8	4.8 $\pm$ 0.4	6.1 $\pm$ 0.6
Ischemia (30 min)	4.3 $\pm$ 0.5	4.0 $\pm$ 0.4	3.2 $\pm$ 0.3	4.7 $\pm$ 0.4*
Ischemia (10 min) + Reperfusion (30 min)	26.5 $\pm$ 0.3	28.1 $\pm$ 1.7	28.4 $\pm$ 1.1	28.3 $\pm$ 1.6
Ischemia (30 min) + Reperfusion (30 min)	23.6 $\pm$ 1.6	25.6 $\pm$ 1.5	10.8 $\pm$ 1.5###	21.5 $\pm$ 1.9**

Data were obtained from the same hearts shown in Table II. Values are means $\pm$ S.E.

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

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

WKY or SHR, although it significantly ($p < 0.01$) increased the extent of recovery of the coronary flow in SHR.

At the end of each perfusion experiment, the heart was frozen and prepared for determination of the tissue levels of ATP, ADP, AMP, CrP, and lactate. The tissue level of ATP is shown in Table II. There were no significant differences in ATP level before ischemia among the 4 groups. In both WKY and SHR ischemia decreased the level of ATP depending on the time period of ischemia with or without diltiazem. Reperfusion

after 10 min of ischemia recovered the ATP level to 70–90% of the preischemic level. Treatment with diltiazem improved the restoration of the ATP level. When the period of ischemia was prolonged to 30 min, the level of ATP after reperfusion became less than 50% of the preischemic level. Although the level of ATP, recovered by reperfusion, in the control SHR was only 22% of the preischemic level, the level in the diltiazem-treated SHR was 39%, higher than that in the control SHR ($p < 0.01$). The level of ADP is shown in Table III. The pre-

TABLE VI LACTATE LEVEL

	WKY		SHR	
	Control	Diltiazem-treated	Control	Diltiazem-treated
	(μmoles/g dry weight)			
Preischemia	0.8 ± 0.6	2.0 ± 1.1	1.1 ± 0.7	3.5 ± 1.5
Ischemia (10 min)	22.8 ± 2.6	23.5 ± 3.5	36.5 ± 2.9##	28.5 ± 3.1
Ischemia (30 min)	31.2 ± 4.4	37.7 ± 4.4	44.1 ± 3.7	39.7 ± 3.1
Ischemia (10 min) + Reperfusion (30 min)	3.3 ± 1.4	0.8 ± 0.4	2.5 ± 1.2	1.6 ± 0.5
Ischemia (30 min) + Reperfusion (30 min)	2.1 ± 1.0	1.9 ± 0.8	12.1 ± 3.2#	4.9 ± 1.9

Data were obtained from the same hearts shown in Table II. Values are means ± S.E.

#p < 0.05; ##p < 0.01, compared with the control WKY.

ischemic level of ADP was 4.6–4.7 μmoles/g in all 4 groups. The ADP level slightly increased after 10 min of ischemia, and then decreased after 30 min of ischemia. Reperfusion after either 10 or 30 min of ischemia slightly decreased the ADP level. Diltiazem did not affect the changes in ADP levels due to ischemia and reperfusion. Table IV shows the level of AMP. The level of AMP in the preischemic heart was about 1.6 μmoles/g in all 4 groups. Ischemia increased the AMP level, depending on the time period of ischemia. After 30 min of reperfusion, the AMP level that had been increased by ischemia decreased toward the preischemic level. In the heart reperfused after 30 min of ischemia, however, the levels of AMP in the SHR groups were significantly ($p < 0.01$ for control and $p < 0.05$ for diltiazem-treated) higher than those in the WKY groups. The AMP level in the diltiazem-treated SHR was significantly lower ($p < 0.01$) than that in the control SHR. The total adenine nucleotide level calculated as the sum of the concentration of ATP, ADP, and AMP is illustrated in Fig. 3. The level of total adenine nucleotides in all 4 groups decreased during ischemia. Reperfusion after ischemia did not restore the total adenine nucleotides, but decreased them further in some groups. Particularly, in the control SHR heart made ischemic for 30 min, the level of total adenine nucleotides was significantly ($p < 0.01$) decreased by reperfusion. Fig. 4 shows changes in ECP caused by ischemia and reperfusion. Preischemic value of ECP was constant at around 0.84 in

all 4 groups. ECP was decreased by ischemia, and then restored by reperfusion. In the heart reperfused after 30 min of ischemia, ECP in the control SHR was significantly ($p < 0.01$) lower than that in the others. Table V shows the level of CrP. The preischemic levels of CrP were between 15 and 18 μmoles/g and there were no significant differences among them. Ten or 30 min of ischemia decreased the CrP levels to 20–40% of the preischemic level. The level of CrP that had been decreased by 10 or 30 min of ischemia was recovered by reperfusion far beyond the preischemic level (over 100% of the preischemic level), except in the control SHR heart made ischemic for 30 min. In this SHR group, the level of CrP was recovered by reperfusion to only 60% of the preischemic level. The level of lactate is listed in Table VI. It increased more in the SHR groups during either 10 or 30 min of ischemia than in the WKY groups. The raised level of lactate was decreased during reperfusion and returned to the preischemic level. Although the lactate level in the control SHR heart made ischemic for 30 min was also decreased by reperfusion, it was still significantly ($p < 0.05$) higher than that in the control WKY.

DISCUSSION

The effect of antihypertensive drugs on cardiac hypertrophy has been extensively studied in the experimental hypertensive rat. Sen et al^{1,2} have demonstrated that effective control of

arterial blood pressure of the mature SHR with methyl dopa for 6 weeks decreases ventricular weight, whereas more apparent reduction of the pressure with hydralazine or minoxidil has no effect and may even accelerate the hypertrophy. However, treatment of the mature SHR with hydralazine for 6 months, a longer period, decreases ventricular weight/body weight ratio.³ Propranolol that does not lower blood pressure decreases ventricular weight in SHR.² These findings suggest that a reduction of the afterload is not essential for the inhibition of ventricular hypertrophy development. In the present study, the treatment of SHR with diltiazem (50–60 mg/kg daily) for 7 weeks did not change the arterial blood pressure, but reduced the ventricular weight (Table I). Diltiazem was administered in the drinking water, because this route and the dosage of administration had been shown to lower the blood pressure without undue stress to the animal,^{1,13,14} and because Sen et al² had shown that at least 6 weeks of treatment were required for distinct changes in cardiac weight to appear. However, Narita et al¹⁵ have shown that the antihypertensive effect of the orally administered diltiazem (30 mg/kg daily) on SHR is recognized after 6 weeks of the treatment, and Tubau et al¹⁴ have reported that the treatment of the mature SHR with diltiazem (30 mg/kg daily) evokes the arterial blood pressure rise during the initial month of administration. In both studies, additional prolonged treatment with diltiazem decreases the blood pressure and blocks hypertrophy progression. Thus, the duration of treatment of SHR for 7 weeks, in the present study, may be not enough to achieve the apparent antihypertensive effect. Nevertheless, it is widely accepted that combinations of dosage, route applied, and age of animals influence the experimental results in this kind of trial. Furthermore, studies are also required on the antihypertensive and antihypertrophic effects of diltiazem. Although care was taken to ensure that food and fluid intake were not substantially different between the control and diltiazem-treated animals, a gain in body weight in the latter group was slightly inhibited on the average. It may be ascribed to the somewhat bitter taste of the drinking water.

Calcium channel blockers are capable of reducing hypertrophy to some extent, though it is still under investigation whether or not the reduced ventricular mass is due to their antihypertensive effect. It is generally accepted that

cardiac hypertrophy can be regressed by a decrease in the rate of protein synthesis and/or by an increase in the rate of protein degradation. Because neurohumoral factors such as catecholamines (α_1 -adrenergic action) and angiotensin II accelerate protein synthesis in the myocardial cell,^{16–18} these factors may facilitate the progress of hypertrophy. Diltiazem does not produce reflex stimulation of sympathetic nerves due to decrease in blood pressure, and does not lead to an increase in the level of catecholamines in the blood.¹⁹ In addition, Nayler et al²⁰ have shown that diltiazem has some α_1 -adrenoceptor blocking activity. Therefore, the effect of the drug on neurohumoral factors may play an important role in regression of hypertrophy. Kobrin et al²¹ have reported that nitrendipine, a calcium channel blocker, decreases ventricular weight in WKY without significantly affecting systemic hemodynamics. They have speculated that nitrendipine inhibits the influx of calcium ions which can increase protein synthesis directly. Accordingly, it is likely that the inhibition of cardiac hypertrophy development without any decrease in the blood pressure which was obtained in the present study is due to the direct effect of diltiazem or the effect via neurohumoral factors, or both.

The level of ATP decreases after the onset of ischemia. If the period of ischemia is prolonged, the myocardial cell cannot maintain the intracellular homeostasis or its structure, leading to irreversible ischemic damage. After prolonged ischemia, reperfusion does not recover the ATP store, because of mitochondrial damage²² and/or the loss of adenine nucleotides.²³ Ichihara and Abiko⁸ have suggested that the extent of recovery of the ATP level reflects the degree of myocardial damage caused by ischemia. We⁹ have reported that in the heart reperfused after 20 or 30 min of ischemia, the ATP level in SHR is lower than that in WKY. This phenomenon was reproduced in the present study, i.e., the level of ATP of the control SHR heart reperfused after 30 min of ischemia was significantly ($p < 0.01$) lower than that of the control WKY heart (Table II). In addition, it was observed in the present study that the levels of total adenine nucleotides, ECP, and CrP in SHR after reperfusion were also lower than those in WKY (Figs. 3 and 4, and Table V), indicating that myocardial damage induced by ischemia is severer in SHR than in WKY. Diltiazem increased the extent of recovery of the ATP level

during reperfusion in SHR (Table II). These results, therefore, suggest that long-term treatment with diltiazem has a beneficial effect on the ischemic-reperfused hypertrophied heart. Acute effects of diltiazem on the ischemic myocardium have been studied by many investigators^{8,24,25}. Ichihara and Abiko⁸ have reported that in using the isolated perfused heart, diltiazem added to the perfusate not only preserves tissue stores of ATP and CrP during ischemia, but also accelerates recovery of the cardiac function from ischemic deterioration during reperfusion. Reibel et al²⁶ and Ichihara and Abiko⁸ have demonstrated that the recovery of the mechanical function of the heart made hypoxic or ischemic during reperfusion is closely related to the tissue level of ATP. When the level of ATP falls below a certain level (about 8–10 μ moles/g dry weight), the mechanical function never recovers. In the present study, the pressure-rate product recovered by reperfusion appeared to be proportional to the level of ATP recovered, and it did not recover at all when the ATP level was below 7 μ moles/g. On the other hand, Opie²⁷ has proposed the existence of a small pool of "contractile ATP", which is rapidly turning over, in the myocardial cell. When the supply of ATP to this pool is insufficient, in spite of total ATP level recovery, mechanical dysfunction of the myocardium due to ischemia may occur.

Ventricular myosin of the rat heart consists of V1, V2, and V3 isotypes separated by pyrophosphate gel electrophoresis²⁸. The functional consequences of these different isomyosins have not yet been fully understood. It has been demonstrated, however, that the V3 isomyosin is more efficient in doing mechanical work²⁹ and consumes less oxygen per gram per beat than the others³⁰. Tubau et al³¹ have reported that pressure overload may shift a type of isomyosin from V1 to V3 in the rat myocardium associated with increase in the ventricular wall thickness. In fact, the V1 isomyosin is predominant in mature WKY, whereas the V3 isomyosin is predominant in mature SHR³². In the present study, the extent of recovery of the pressure-rate product in the control SHR heart during reperfusion after 10 min of ischemia was higher than that in the control WKY heart (Fig. 1). The reason for this may be that the myocardium of SHR contains the V3 isomyosin more than that of WKY. However, concerning the functional improve-

ment of the treatment of WKY with diltiazem during reperfusion after 10 min of ischemia, further studies are needed as to the effects of antihypertensive drugs on the myocardial isomyosin and mechanical function.

In conclusion, although long-term treatment with diltiazem does not lower the arterial blood pressure of the mature SHR, it causes a considerable regression of the left ventricular hypertrophy, and protects the myocardium from induced ischemic damage.

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