LEFT VENTRICULAR FUNCTION OF THE HEART REGRESSED BY NIFEDIPINE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Left ventricular (LV) performance of the pharmacologically regressed heart in hypertension is still unclear. We compared LV function of the heart regressed by nifedipine with that of the hypertrophied heart in spontaneously hypertensive rats (SHR). Nifedipine (30 mg/kg/day in food) was given to 15-week-old male SHR for 20 weeks ($n=12$). Age- and sex-matched SHR served as controls ($n=12$). LV catheterization was performed using a micromanometer and cardiac output was determined by the thermodilution method. Hemodynamic studies were performed after washout of nifedipine (24 h), when blood pressure had returned to the untreated level. Peak pumping ability was assessed during acute volume loading with saline. Nifedipine significantly decreased blood pressure in conscious animals (222 ± 11 to 201 ± 12 mmHg, $p<0.01$) and reduced LV weight (1.20 ± 0.07 to 1.07 ± 0.05 g, $p<0.01$). After washout of nifedipine, LV systolic and end-diastolic pressures, $dp/dt_{\max}$ and cardiac output determined under pentobarbital anesthesia were similar in the treated and untreated groups. Peak pumping ability during acute preload elevation was also similar in the 2 groups. Plasma norepinephrine was unaltered, and plasma renin activity was significantly lower in the treated rats ($p<0.05$). These results indicate that nifedipine regressed LVH with a minimal reduction of blood pressure and without evidence of neurohumoral activation or volume retention. In conclusion, LV function of the heart regressed by nifedipine was preserved after a spontaneous rise in blood pressure and during acute preload elevation.

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HYPERTENSIVE left ventricular hypertrophy (LVH) is often associated with cardiac dysfunction^{1,2} and is an independent risk factor for cardiovascular morbidity and mortality³. The reduction of cardiac mass by drug intervention is therefore a logical and worthwhile goal of antihypertensive treatment. Various drugs have induced regression of LVH in both experimental^{2,4–10} and

human^{11–15} hypertension. However, the ability of the regressed left ventricle to respond to rapid increases in blood pressure has not been fully clarified¹⁶. Hypertensive episodes may develop when patients skip their antihypertensive medication, or during physical or mental stress. Some animal studies have indicated that reversal of LVH causes depressed left ventricular (LV) function during afterload elevation^{5,6,10} raising concerns that such regression may be deleterious. In these studies, however, afterload elevation was achieved abruptly by constricting the aorta^{6,10} or by administering a vaso-

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constrictor⁵ In the present study, cardiac function was evaluated after a spontaneous rise of blood pressure following discontinuation of nifedipine. It is believed that this method would more closely mimic clinical hypertensive episodes.

The purpose of this study was to compare LV function of the heart regressed by nifedipine with that of the hypertrophied heart in spontaneously hypertensive rats (SHR) after a spontaneous elevation of blood pressure to the untreated level, and also during acute preload elevation with saline. Plasma renin activity (PRA) and norepinephrine levels were measured at the end of the treatment.

MATERIALS AND METHODS

Animals and Nifedipine Administration

Forty four male SHR were obtained from Charles River Breeding Laboratories (Atsugi, Japan) at 12 weeks of age. Rats were housed 2 to a cage and given standard rat chow and water ad libitum. They were maintained in a room at constant temperature (20–22 °C) and humidity (50–60%). Following a 3-week acclimatization period, rats were randomly divided into 2 groups: 22 rats were given nifedipine and 22 served as controls. Each group was further subdivided in to 2 groups for hemodynamic (n=12 each) and hormonal (n=10 each) studies. We did not include normotensive rats in this protocol for 2 reasons: 1) several reports have already compared the hemodynamics in nifedipine-treated SHR with those in normotensive rats^{8–10}; and 2) the main purpose of this study was to see whether the left ventricle regressed by nifedipine could respond to acute afterload and preload elevation as well as the hypertrophied heart.

Pellets containing nifedipine (Bayer Inc, Wuppertal, Germany) at a concentration of 315 ppm were given as a substitute for normal food. The daily dose was about 30 mg/kg of body weight and the treatment was continued for 20 weeks. This dosage and duration of nifedipine were required to reduce blood pressure and reverse LVH in SHR^{8,9} Control rats received normal rat chow. The treated and untreated rats consumed similar amounts of food. The treatment was started at 15 weeks of age, when SHR develop established hypertension and

LVH!⁷ Subsequently, the degree of LVH becomes progressively more marked in SHR than in Wistar-Kyoto rats!^{1,9,17}

Hemodynamic Studies

Systolic blood pressure and pulse rate of conscious animals were measured every 4 weeks by the tail-cuff method⁴ using recorder (UR-1000, Ueda Inc., Tokyo, Japan). The means of 3 measurements were used for the evaluation.

At the end of the treatment period, the rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). Hemodynamic studies were performed more than 24 hours after washout of nifedipine because our preliminary experiments showed that blood pressure returned to the untreated level within 24 hours. Details of cardiac catheterization have been reported elsewhere⁴ Briefly, a 2F catheter-tip micro-manometer (Model PR249, Millar Instruments, Houston, Texas, USA) was introduced through the right carotid artery into the ascending aorta, and advanced to the left ventricle. Aortic and LV pressures, LV end-diastolic pressure (LVEDP), and the rate of LV pressure change (dp/dt_{max}) were recorded simultaneously (Rectigraph 8K, San-ei Inc, Tokyo, Japan). Heart rate was monitored by electrocardiogram. A 5-cm venous catheter (PE 50) was inserted via the right jugular vein into the right atrium to be used for injection. Mean arterial pressure (MAP) was calculated by adding one-third of the pulse pressure to diastolic pressure.

Cardiac output (CO) was determined by the thermodilution method as described previously⁴ A thermistor-tipped catheter (1F, Columbus Instruments, Columbus, Ohio, USA) was inserted through the right carotid artery and placed just above the aortic valve. Normal saline (100 μ l) at room temperature was injected through the catheter placed in the right atrium using a microsyringe (Micro-liter #710, Hamilton Co., Reno, Nevada, USA). Cardiac output was calculated with a Cardiotherm 500[®] computer (Columbus Instruments). Stroke volume (SV) and total peripheral resistance (TPR) were calculated as follows:

$SV (\mu l) = CO / \text{heart rate}$, $TPR (\text{mmHg/ml/min}) = MAP / CO$.

TABLE I BODY, HEART, AND KIDNEY WEIGHTS

	Weight (g)	
	Treated (n=11)	Untreated (n=10)
Body	415±11	420±17
Heart	1.33±0.07*	1.46±0.08
Left ventricle	1.07±0.05*	1.20±0.07
Right ventricle	0.26±0.03	0.26±0.03
Kidney	1.47±0.09	1.50±0.09

* $p < 0.01$ vs untreated rats.

To assess the ability of the heart to respond to acute volume load, normal saline warmed to 37°C was infused through the jugular vein at a rate of 2 ml/100 g body weight over a 1-minute period^{1,4}

Neurohumoral Determinations

On the same day as the hemodynamic study, blood samples (5 ml) for PRA and norepinephrine were drawn from the carotid artery of anesthetized treated and untreated rats, in which hemodynamic studies had not been performed. Samples were collected in chilled EDTA-treated tubes and centrifuged at 3,000 rpm. for 15 min, at 4°C. Plasma was stored at -70°C until assayed. PRA was determined by radioimmunoassay, and plasma norepinephrine levels were determined by high-performance liquid chromatography⁴. Blood samples were taken from the treated rats without discontinuing nifedipine.

Heart and Kidney Weight

Cardiac arrest was induced by intravenous injection of 2% procaine (1 ml). The heart was excised, rinsed with saline solution, and blotted dry. The great vessels and both atria were removed, and the right ventricular free wall was weighed to determine right ventricular mass. LV mass was determined as the weight of the left ventricle plus that of the interventricular septum. Weights of both kidneys were averaged.

Statistics

All values are expressed as mean±SD. Student's *t* test was used to analyze paired and unpaired data. *p* values of less than 0.05

Fig.1. Systolic blood pressure in conscious treated (●) and untreated rats (○). From 15 weeks of age (=0), blood pressure continued to rise significantly in the untreated rats ($p < 0.05$). This increase was prevented by nifedipine. Bars are mean ± S.D. * $p < 0.01$ vs untreated rats.

were considered to be statistically significant.

RESULTS

The overall mortality rate in the treated and untreated rats were 9% and 18%, respectively. No specific cause of death was determined, and most deaths occurred toward the end of the protocol.

Body, Heart and Kidney Weights (Table I)

Body weights of 35-week-old treated and untreated rats were similar. Nifedipine significantly reduced LV weight (11%) and the LV weight/body weight ratio (10%) (from 2.86 ± 0.15 to 2.58 ± 0.09 mg/g, $p < 0.01$), but had no effect on right ventricular or kidney weight.

Resting Hemodynamic and Neurohumoral Data (Fig. 1, Table II)

Blood pressure in the conscious, untreated SHR continued to rise during the 20-week observation period, but nifedipine completely prevented this increase (Fig. 1). As a result, the treated rats had significantly lower blood pressure than the untreated rats throughout the treatment period. The final tail-cuff pressure in the treated rats was 9%

TABLE II RESTING HEMODYNAMIC AND NEUROHUMORAL DATA

	Treated (n=11)	Untreated (n=10)
Heart rate (beats/min)	424±38	435±23
LV systolic pressure (mmHg)	222±12	220±14
Mean arterial pressure (mmHg)	192±9	190±10
LVEDP (mmHg)	3.8±0.7	3.9±0.6
dp/dt _{max} (mmHg/s)	3115±320	3140±312
Cardiac output (ml/min)	118±18	124±16
Stroke volume (μl)	278±38	286±30
TPR (mmHg/ml/min)	1.68±0.35	1.53±0.13
Plasma renin activity (ng/ml/hr)	9±4*	15±5
Norepinephrine (pg/ml)	375±155	388±176

LV=left ventricular; LVEDP=LV end-diastolic pressure; TPR=total peripheral resistance. * $p<0.05$ vs untreated rats.

lower than that in their untreated counterparts (201 ± 12 vs 222 ± 11 mm Hg), although it was still markedly hypertensive (Fig. 1). Pulse rates in conscious treated and untreated rats were similar (final pulse rates were 446 ± 41 and 441 ± 38 beats/min, respectively). Table II summarizes resting hemodynamic and neurohumoral data obtained under pentobarbital anesthesia. After a 24-hour washout of nifedipine, blood pressure rose to the untreated level. Under this condition, the hemodynamic parameters in the treated and untreated groups were similar. Plasma norepinephrine concentrations were similar, but PRA was significantly lower in the treated rats ($n=9$) than in their untreated counterparts ($n=8$).

Hemodynamic Responses to Preload Elevation (Fig. 2)

Cardiac output peaked within 1 min after the initiation of volume loading and the plateau lasted for about 30–45 sec. Saline infusion significantly decreased LV systolic pressure, dp/dt and TPR, and increased LVEDP, cardiac output and stroke volume. As shown in Fig. 2, all of these changes were similar between the 2 groups. Heart rate tended to decrease during volume loading in the treated (422 ± 34 to 398 ± 30 beats/min) and untreated rats (428 ± 29 to 401 ± 34 beats/min), however, these changes were

Fig. 2. Hemodynamic changes during preload elevation. Saline infusion caused significant and similar decreases in systolic blood pressure (BP), dp/dt_{max}, and total peripheral resistance (TPR), and increases in left ventricular end-diastolic pressure (LVEDP), cardiac output (CO), and stroke volume (SV) in the treated (▨) and untreated (□) rats. vol=volume loading. Bars are mean±S.D. * $p<0.01$ vs hemodynamics at rest.

not statistically significant.

DISCUSSION

In this study, nifedipine reduced blood pressure long-term in conscious SHR and decreased LV weight. After a spontaneous rise in blood pressure, LV function in the treated and untreated rats was comparable. Peak pumping ability during acute preload elevation was also similar in the 2 groups. Neurohumoral factors determined under anesthesia were not activated in the treated rats.

The effects of reversal of LVH on LV function are still unclear, particularly during acute blood pressure elevation¹⁶ This condition may develop when hypertensive patients forget to take their medications, or when they are exposed to physical or mental stress. Cardiac function is the product of the interaction of the hemodynamic load on the heart and myocardial contractility. Since antihypertensive agents have been shown to

change both of these factors,^{18,19} we evaluated hemodynamics after washout of nifedipine, which we believed would closely mimic hypertensive episodes in patients.

After an acute rise in blood pressure, LV function of the heart regressed by nifedipine was comparable to that of the hypertrophied heart. This finding disagrees with previous animal studies using methyldopa⁵, captopril⁶, and nifedipine¹⁰. The cause of this discrepancy is not clear. One possibility is that our method of afterload elevation was spontaneous and more gradual than those of previous studies which used either aortic constriction^{6,10} or phenylephrine⁵. Differences in the method for evaluating LV function may also have produced different results. The other investigators^{5,6,10} used electromagnetic flowmetry in open-chest and artificially ventilated rats, whereas we used the thermodilution method in closed-chest and spontaneously breathing rats. Recent clinical investigations have shown that cardiac function of the heart regressed by several anti-hypertensive agents is normal during exercise or cold pressor test.^{11–15} Most of these studies^{11–14} were conducted several weeks after the discontinuation of therapy, when blood pressure had increased to the pretreatment level. Our results and these clinical findings indicate that the regressed heart can respond well to acute elevation of blood pressure.

Cardiac response to rapid volume overload is a reliable means of assessing LV pumping ability in rats^{2,5,6,20}. In this study, peak pumping ability of the once-hypertrophied heart was not depressed, as evidenced by similar cardiac output and stroke volume obtained at comparable preload (LVEDP) and afterload (systolic LV pressure) in the 2 groups (Fig. 2). These findings support the results of previous studies with nifedipine^{9,10}, methyldopa⁵ and captopril². Volume loading significantly decreased systolic blood pressure, dp/dt_{max} , and TPR, with a tendency to bradycardia, as noted previously^{2,20}. These changes are probably due to marked vasodilation, while increased stroke volume results from increased venous return. Similar hemodynamic changes were also found in a high-output state induced by abdominal aortocaval fistula in SHR²¹.

As previously reported^{7–10} nifedipine sig-

nificantly decreased LV weight in SHR. Our earlier study showed that dose of nifedipine equal to that used in this study significantly decreased the LV weight/body weight ratio in 35-week-old SHR, as compared with that in 15-week-old SHR²². This finding suggests that nifedipine induces regression of LVH. Although blood pressure was significantly lowered (9%), it still remained near 200 mm Hg in our treated rats. This finding disagrees with previous studies^{8,9} in which the same dosage of nifedipine produced a much greater blood pressure reduction (24–26%). The exact reason for this difference is not known. One explanation would be that our untreated SHR had considerably higher blood pressures than the SHR in the other studies (222 vs 194 mm Hg)^{8,9}. Interestingly, LV weight in our SHR was decreased to an extent similar to that previously reported (10% vs 9–10%)^{8,9} despite a smaller reduction of blood pressure in this study.

A discrepancy between the reduction of blood pressure and LV weight has been previously reported^{6,10} raising the possibility that factors other than afterload reduction play a role in regression of LVH. Angiotensin II²³ and norepinephrine²⁴ have been shown to cause myocardial hypertrophy independent of blood pressure. Chronic therapy with calcium antagonists does not seem to increase sympathetic activity or PRA^{7,25–28} as found in this study. Besides, there was no evidence of volume retention in the treated SHR, as indicated by similar body weight and LVEDP. These findings are in contrast to those found with other vasodilators such as hydralazine and minoxidil, which enhance neurohumoral responses and volume retention, but do not induce reversal of LVH despite afterload reduction^{7,28,29}. Although the results of this study do not identify mechanisms that are responsible for the absence of neurohumoral activation and volume retention, this finding may explain why nifedipine and the conventional vasodilators have different effects on LVH. The neurohumoral data in this study should be interpreted with caution because blood samples were taken under anesthesia.

In conclusion, nifedipine regressed LVH even while blood pressure remained severely hypertensive in mature SHR. There was no evidence of neurohumoral activation or

volume retention associated with nifedipine treatment. A heart regressed by nifedipine could respond to a spontaneous rise in blood pressure and acute volume loading as well as a hypertrophied heart.

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