NIFEDIPINE IN DIVIDED DOSES DOES NOT REVERSE LEFT VENTRICULAR HYPERTROPHY IN SPONTANEOUSLY HYPERTENSIVE RATS

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This study investigated whether nifedipine administered in divided daily doses would diminish left ventricular hypertrophy (LVH) in spontaneously hypertensive rats (SHR). We administered nifedipine (12 mg/kg/day) in 3 divided doses by gastric gavage to 15-week-old male SHR (n=10) for 4 weeks. Age- and sex-matched SHR served as controls (n=10). Left ventricular (LV) function was evaluated by LV catheterization and cardiac output was determined by the thermodilution method. Plasma renin activity (PRA) and plasma norepinephrine levels were measured. Nifedipine significantly decreased blood pressure (p<0.01), shortened time constant T (p<0.05), and increased cardiac output (p<0.05). Nifedipine did not impair the LV systolic and diastolic indices during acute afterload elevation with angiotensin II. LV weight was similar in the 2 groups of rats. While PRA was unaltered, plasma norepinephrine levels were higher in the nifedipine-treated rats (p<0.05). These data indicate that nifedipine in 3 divided doses reduced blood pressure in SHR without compromising cardiac function but did not reverse LVH. The short hypotensive duration of nifedipine and its enhancement of sympathetic nervous activity may be responsible for the failure to reverse LVH, despite adequate blood pressure control. (Jpn Circ J 1992; 56: 255–261)

LEFT ventricular hypertrophy (LVH) develops progressively in spontaneously hypertensive rats (SHR). LVH has been reported to cause impaired left ventricular (LV) relaxation in both humans and animals and may eventually lead to depressed LV systolic function. Clinically, LVH is associated with such cardiovascular risk factors as increased ventricular ectopy, decreased coronary reserve and sudden death in hypertensive patients. Reversal of LVH is therefore an important goal of antihypertensive therapy.

Vasodilators such as hydralazine and minoxidil did not diminish LVH in SHR; minoxidil even increased LV weight. This finding can be explained by volume overload due to water and sodium retention associated with these vasodilators. In contrast to conventional vasodilators, nifedipine has been reported to reverse LVH in SHR. However, in these studies nifedipine was administered mixed with food, whereas clinically this drug is given in divided doses.

The purpose of this study was to determine whether the administration of nifedipine in divided daily doses would lead to regression of LVH comparable to that seen with its administration mixed with food. We

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investigated the effect of divided dosage regimen on blood pressure, LV function, LVH, and neurohumoral factors in adult SHR with established hypertension and LVH. Left ventricular function was assessed by LV catheterization using a high fidelity catheter-tip micromanometer, and cardiac output was measured by the thermodilution method. Plasma renin activity (PRA) and plasma norepinephrine levels were also measured.

MATERIALS AND METHODS

Rats
Forty male SHR were obtained from Charles River Breeding Laboratory (Osaka, Japan) at 12 weeks of age. Rats were housed 2 to a plastic cage and given a standard rat chow and water ad libitum. They were placed in a quiet room at a constant temperature (20–22°C) and humidity (50–60%). Following a 3-week acclimatization period, the rats were randomly divided into 2 groups: 20 rats were treated with nifedipine and 20 served as controls. Each group was further randomly subdivided into 2 groups of 10 animals for the hemodynamic and hormonal studies.

Drug Administration
Nifedipine was mixed with a solvent containing 96% ethanol (15%), polyethylene glycol 400 (15%), and distilled water (70%), in a dark atmosphere and stored in a light-proof bottle. The drug was given by gastric gavage at a dose of 12 mg/kg/day in 3 equally divided doses 6h apart (7:00, 13:00 and 19:00 h) by the same experienced personnel. This dosage was chosen from our preliminary experiment (data not shown). The control rats received the same amount of vehicle by the same method. The treatment began when rats were 15 weeks old and continued for 4 weeks. This age was chosen because by this time both hypertension and LVH are well established. This duration of treatment was based on a report in which 3-week administration of nifedipine led to regression of LVH in 6-month-old SHR (older than those in our study).

Conscious Blood Pressure and Pulse Rate
During the 4-week treatment period, systolic blood pressure and pulse rate were measured weekly about 2–3 h after the morning dose of nifedipine in conscious animals by the tail-cuff method after heating the rats at 37°C for about 15 min (UR-1,000, Ueda Inc., Japan). Three measurements obtained were averaged. In addition, the animals’ body weights and appearance were monitored regularly.

Hemodynamic Study
At the end of the treatment period, the animals were anesthetized by pentobarbital (45 mg i.p.) and were placed on their backs with limbs gently extended and taped in position. A 3F Mikro-Tip® catheter (Model PR-249, Millar Instrument, USA) was introduced through the right carotid artery into the ascending aorta. After recording aortic pressure, the catheter was advanced into the left ventricle and LV pressure, LV end-diastolic pressure (LVEDP), and rate of pressure change (dp/dt) were recorded. A 5-cm venous catheter (PE 50) was inserted via the right jugular vein into the right atrium to be used for administering infusions.

The animals were allowed to rest for at least 30 min following the surgical preparation. Hemodynamic parameters were recorded at a paper speed of 100 mm/sec on a multi-channel recorder (Rectigraph 8K, San-ei Inc., Japan). LVEDP was displayed at a high sensitivity on an additional channel. Mean arterial pressure (MAP) was calculated by adding one-third the pulse pressure to diastolic pressure. Time constant T, an index of LV isovolumic relaxation was obtained as described previously! To assess the LV hemodynamic response to acute afterload elevation, angiotensin II (ANG II) 0.125 mg in 100 ml normal saline (Hypertensin; Ciba-Geigy, Basle, Switzerland) was infused intravenously, as described elsewhere.

Cardiac output (CO) was determined by the thermodilution method (Cardiotherm 500, Columbus Instruments, USA). After the baseline hemodynamic measurement and ANG II infusion study, the tip-manometer was replaced by a thermistor-tipped catheter which was placed in the ascending aorta just above the aortic valve. Through the venous catheter, 0.1 ml of normal saline at room temperature was injected rapidly. CO was measured both at rest and during afterload.
TABLE I  BODY AND HEART WEIGHTS

<table>
<thead>
<tr>
<th></th>
<th>treated</th>
<th>untreated</th>
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<tbody>
<tr>
<td>BW (g)</td>
<td>345±20</td>
<td>354±10</td>
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<tr>
<td>HW (g)</td>
<td>1.18±0.06</td>
<td>1.20±0.05</td>
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<tr>
<td>HW/BW (mg/g)</td>
<td>3.42±0.17</td>
<td>3.38±0.17</td>
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<tr>
<td>LV (g)</td>
<td>0.95±0.04</td>
<td>0.97±0.04</td>
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<tr>
<td>LV/BW (mg/g)</td>
<td>2.75±0.17</td>
<td>2.74±0.14</td>
</tr>
<tr>
<td>RV (g)</td>
<td>0.23±0.05</td>
<td>0.23±0.03</td>
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<tr>
<td>RV/BW (mg/g)</td>
<td>0.66±0.12</td>
<td>0.62±0.07</td>
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TABLE II  BASELINE HEMODYNAMICS

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>HR (beats/min)</td>
<td>440±22*</td>
<td>399±39</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>139±12**</td>
<td>211±20</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.7±0.5</td>
<td>3.8±0.6</td>
</tr>
<tr>
<td>(+) dp/dt (mmHg/s)</td>
<td>2417±211*</td>
<td>2989±224</td>
</tr>
<tr>
<td>(−) dp/dt (mmHg/s)</td>
<td>2754±249</td>
<td>2974±328</td>
</tr>
<tr>
<td>T (ms)</td>
<td>19.3±1.52*</td>
<td>25.1±3.81</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>404±73*</td>
<td>281±66</td>
</tr>
<tr>
<td>SI (μmol/kg)</td>
<td>916±191*</td>
<td>751±198</td>
</tr>
<tr>
<td>TPR (mmHg/ml/min/kg)</td>
<td>2.55±0.48**</td>
<td>5.59±0.56</td>
</tr>
</tbody>
</table>

HR: heart rate, SBP: systolic blood pressure, LVEDP: left ventricular end-diastolic pressure, T: time constant, CI: cardiac index, SI: stroke index, TPR: total peripheral resistance index.
*: P<0.05, **: P<0.01 versus untreated rats.

Fig.1. Conscious systolic blood pressure during nifedipine treatment for 4 weeks in the treated (−) and untreated (―) SHR. Indicated are mean ±SD.

Systolic Blood Pressure

—70°C until assayed. PRA was measured by radioimmunoassay and plasma norepinephrine concentration by high performance liquid chromatography.

Both the hemodynamic and hormonal measurements were performed 3 h after the morning dose of nifedipine.

Heart Weight

Cardiac arrest was induced by the intravenous injection of 2% procaine. The heart was rinsed with saline solution and blotted dry. After careful removal of the atria, right and LV weights were measured. Absolute and relative (organ weight per body weight) values were used in the evaluation.

Statistics

All values are expressed as mean ±SD. Student’s t test was used for the analysis of paired and unpaired data. P values less than 0.05 were considered statistically significant.

RESULTS

Body and Heart Weights

While the body weight of the treated rats tended to be smaller than that of the untreated rats, the differences were not statistically significant (Table I). Left ventricular weight, either absolute or relative, was similar in the 2 groups, suggesting that nifedipine did not lead to regression of LVH. Right ventricular weight was unaltered.

Neurohumoral Measurements

Blood samples for the determination of PRA and plasma norepinephrine concentration were obtained from the carotid arteries of the anesthetized rats in which no hemodynamic study was performed. Samples were collected in chilled EDTA-treated tubes and centrifuged at 4°C. Plasma was stored at

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Fig. 2. Hemodynamic changes before and after the acute afterload elevation with ANG II. Systolic blood pressure was raised by about 30 mmHg. Peak positive dp/dt and TPRI were similarly increased in the 2 groups; the other parameters were unaltered by ANG II. See Table II for the abbreviations. Bars represent standard error.

**TABLE III  NEUROHUMORAL FACTORS**

<table>
<thead>
<tr>
<th></th>
<th>treated</th>
<th>untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>9.66 ± 6.19</td>
<td>9.03 ± 7.16</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>454 ± 115*</td>
<td>300 ± 77</td>
</tr>
</tbody>
</table>

PRA: plasma renin activity. *: P < 0.05 versus untreated rats.

**Conscious Blood Pressure and Pulse Rate**

Conscious systolic blood pressure declined significantly with nifedipine and remained reduced throughout the treatment period (Fig. 1). Mean pulse rate of the treated rats was significantly more rapid than that of controls during the entire period of treatment (443 ± 27 vs 402 ± 33 beats/min, p < 0.05 at the end of the treatment phase).

**Baseline Hemodynamics**

Table II shows baseline hemodynamic data obtained under pentobarbital anesthesia. Heart rate was higher and LV systolic blood pressure and peak positive dp/dt were lower in the treated rats than in their control counterparts. LVEDP did not differ between the treated and untreated SHR. While peak negative dp/dt was unaltered, time constant T, an index of LV diastolic property, was significantly shorter in the treated rats. Nifedipine augmented CI and decreased TPRI significantly compared with those in the untreated SHR.

**Hemodynamic Changes with ANG II**

After systolic blood pressure was raised by about 30 mmHg by the ANG II infusion, the hemodynamic parameters were recorded (Fig. 2). The ANG II infusion did not change CI, SI, peak negative dp/dt, and time constant T in either group. LVEDP did not change significantly in the treated and untreated rats (4.2 ± 0.9 vs 4.3 ± 0.8 mmHg). Peak positive dp/dt and TPRI were similarly increased in both groups.

**Neurohumoral Data**

At least 30 min were allowed to elapse following the insertion of the catheter into the right carotid artery to allow for stabilization. While PRA was unaltered, plasma norepinephrine concentration was higher in the treated rats than in their untreated counterparts (Table III).
DISCUSSION

In the present study, split-dose nifedipine produced a sustained decrease in blood pressure in mature SHR. LV function as quantified by Cl was enhanced in the nifedipine-treated SHR, as reported by others.\textsuperscript{13,15} This improved LV function was preserved during acute afterload elevation with ANG II. The blood pressure lowering effect of nifedipine and its preservation of LV pumping ability are due primarily to afterload reduction through arteriolar dilation.\textsuperscript{15} We further found that nifedipine significantly shortened time constant T, suggesting that LV relaxation was also improved, an observation also reported clinically.\textsuperscript{17} Thus, in addition to its LV unloading effect, improvement of LV relaxation with nifedipine may have contributed to the enhanced pumping ability by increasing LV compliance.

Despite the significant reduction of blood pressure with nifedipine, LV weight did not decrease, a finding at variance with previous reports.\textsuperscript{10–15} Motz et al.\textsuperscript{13,14} proposed that afterload reduction played a major role in reversal of LVH with nifedipine in SHR. Our data, however, show that blood pressure reduction alone does not reverse LVH. The reason for this discrepancy is not clear. Unlike earlier studies,\textsuperscript{10–15} we administered nifedipine in 3 divided daily doses. Since the hypotensive effect of nifedipine is brief, blood pressure may not have been sufficiently reduced at night. Blood pressure elevation during the daytime seems unlikely because our preliminary experiment showed that a single 8 mg/kg dose of nifedipine given by gavage significantly decreased blood pressure for more than 6 h in adult SHR. An almost identical duration of hypotensive effect was reported with nifedipine, 3.15 mg/kg, in Dahl rats.\textsuperscript{11} Interestingly, Linz et al.\textsuperscript{18} have recently reported that nifedipine, 30 mg/kg/dose given once daily by gavage, did not cause regression LVH induced by aortic banding in rats despite good blood pressure control. A possible nocturnal elevation of blood pressure could explain the lack of regression observed with both the 3 times a day and once a day administration of nifedipine. The fact that rats are active mainly at night may have further increased nocturnal blood pressure. Further studies are needed to clarify this issue because we did not measure blood pressure at night. The duration of treatment may not be responsible for the persistence of LVH in our treated SHR, as Kazda and others\textsuperscript{22} reported reversal of LVH in 6-month-old SHR treated with nifedipine for only 3 weeks, although the reduction in blood pressure was smaller in their treated rats (10.4%) than in ours (34.1%).

Volume retention is a common finding with conventional vasodilators such as minoxidil and hydralazine, which is why these vasodilators did not cause regression of LVH. In contrast, nifedipine is known to possess natriuretic and diuretic effects that reduce volume retention.\textsuperscript{11,19} In the present study, development of volume overload with nifedipine is unlikely because LVEDP and PRA were similar in both groups of rats, and body weight of the treated rats tended to be lower. Several mechanisms are postulated for natriuretic effect of nifedipine, including an increase in glomerular filtration rate and an interference with the constriction of the afferent glomerular arteriole.\textsuperscript{19}

In this study, pressor response to ANG II was similar in the 2 groups, a finding at variance with the report in which nisoldipine depressed vascular responsiveness to ANG II.\textsuperscript{20} The reason for this discrepancy is not clear. The difference either in the amount of ANG II injected or in calcium antagonist used may be responsible. In addition to its vasoconstrictor action, ANG II may have a positive inotropic action.\textsuperscript{21} The precise mechanisms by which the myotropic effect of ANG II is mediated are not fully elucidated. Both direct action mediated by ANG II receptor in the myocardium and indirect action mediated by sympathetic nerve stimulation are postulated. Because of these complex interactions of ANG II, the results of this study should be interpreted with caution. However, the purpose of ANG II injection in the present study was to equally elevate afterload in the 2 groups and this was achieved.

In patients with essential hypertension, PRA rose after the acute administration of nifedipine; however, after chronic use PRA returned to near pretreatment level.\textsuperscript{22} In accordance with this observation, PRA in our nifedipine-treated rats was not increased.
despite the marked fall in blood pressure. This is in contrast to experience with such vasodilators as minoxidil and hydralazine which enhance the renin-angiotensin system\(^2\)\(^3\) probably leading to volume retention.

The cardiac sympathetic nerves have been implicated in the persistence or progression of LVH in animal experiments\(^2\)\(^4\)\(^5\) Both methyldopa and hydralazine lowered blood pressure to the same extent, but ventricular weight was decreased only in the methyldopa-treated rats\(^2\)\(^4\)\(^5\) Sen et al\(^2\) reported that hydralazine treatment of SHR was associated with a 20% increase in ventricular norepinephrine. Regression of LVH was observed when hydralazine was combined with propranolol in a dose that reduced the level of ventricular norepinephrine. In our study, the possibility of increased sympathetic nervous activity in the treated SHR is supported by the increases in both the plasma norepinephrine level and pulse rate in these animals. Increased sympathetic activity might have contributed to the failure in reversing LVH. Indeed, norepinephrine has been reported to increase the size of cultured rat myocardial cells via cardiac alpha\(_1\) receptors\(^2\)\(^7\) These findings suggest that, at least in SHR, adrenergic factors play a part in reversing LVH.

Acknowledgment

We thank Miss Kumiko Egawa for her excellent secretarial work.

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