Effect of Chronic Treatment of Spontaneously Hypertensive Rats With D 600

Catherine C. Y. Pang, Ph.D., and Morley C. Sutter, M.D., Ph.D.

SUMMARY The effects of chronic treatment of spontaneously hypertensive rats (SHR) with methoxyverapamil (D 600) on blood pressure (BP) and on the in vitro contractile response of aortic and portal vein strips of rats were examined. D 600, prepared as the free base and dissolved in sesame oil, was injected subcutaneously daily into SHR to maintain the systolic blood pressure (SBP) at less than 130 mm Hg for 24 hours after injections. The dose required increased progressively from 4 to 8.5 mg/day/rat. As controls, normotensive rats (WKY) and untreated SHR received daily injections of the vehicle. After 2 weeks, aortic and portal vein strips were prepared from each rat for studies of cumulative dose-response curves to norepinephrine (NE) in Krebs' solution containing normal (2.5 mM) and low (0.2 and 0.4 mM) calcium (Ca). Chronic treatment with D 600 restored to control values the ordinarily depressed contractile response to NE and increased the ED₅₀ values for NE (i.e., the NE dose that produces 50% of the maximum response) of aortic strips from SHR in normal and low Ca. Portal veins from SHR showed increased spontaneous activity, supernormal responses to NE, and decreased ED₅₀ values for NE that were all exaggerated by chronic D 600 treatment. These results imply that SHR developed a tolerance to D 600 associated with enhanced contractility of vascular smooth muscles. (Hypertension 3: 657-663, 1981)

Key Words • vascular smooth muscle • hypertension • contractility • calcium • norepinephrine

CONTRACTILITY and calcium (Ca) handling are both known to be altered in vascular smooth muscles obtained from spontaneously hypertensive rats (SHR). In physiological solutions containing normal or low concentrations of Ca, we have shown that aortic strips from SHR developed less contractility to norepinephrine (NE) than aortic strips from Wistar-Kyoto normotensive rats (WKY). In contrast, portal vein strips from SHR developed greater contractility to NE in normal and low Ca than portal vein strips from WKY. Moreover, in the presence of D 600 (methoxyverapamil), a Ca antagonist, aortic strips from SHR retained less contractility to NE in normal and low Ca than aortic strips from WKY. However, portal vein strips from SHR retained greater contractility in normal and low Ca in the presence of D 600 than portal vein strips from WKY. Thus, aortic strips from SHR demonstrated decreased contractility to NE and were more dependent on external Ca for contraction than aortic strips from WKY. Portal vein strips from SHR, on the other hand, contracted better and were less dependent on external Ca for contraction than portal vein strips from WKY.

To investigate further Ca handling by vascular smooth muscles from SHR and WKY, we chronically treated SHR with D 600 by daily subcutaneous injections of the drug as a free base dissolved in oil. Ca antagonists, namely, verapamil and nifedipine, had been reported to have antihypertensive effects. In this study, we first determined whether it was possible to lower the blood pressure (BP) of SHR with D 600 and, second, whether chronic treatment with D 600 prevented the derangements of contractility and Ca-dependence of vascular smooth muscles from SHR.

Methods

Animals

Three groups of rats (12-week-old, 250 g, Charles River, Lakeview, Massachusetts) were used for this study: eight WKY, eight untreated SHR, and eight SHR treated with D 600 for 2 weeks. D 600 prepared as a free base from the hydrochloride salt was dissolved in sesame oil and injected subcutaneously into SHR once daily (except on the day of the experiment).
to maintain systolic blood pressure (SBP) of these rats to less than 130 mm Hg at 24 hours after each injection. In an attempt to maintain the SBP of the SHR at this level, the dose of D 600 given to the SHR had to be progressively increased from an initial level of 4 mg/rat to 8.5 mg/rat by the end of 2 weeks of treatment. The WKY and untreated SHR received daily injections of sesame oil. The SBP of all rats was measured indirectly from the tail artery using a pneumatic pulse transducer (Narco Bio-Systems, Inc., Texas) prior to, during, and following 2 weeks of treatment. The heart rate was determined by counting the number of pulses over 6-second intervals from the BP record (with recording chart running at a high speed). On the day of the experiment, one rat from each group of rats was killed by a blow to the head. A longitudinal portal vein strip and a helical aortic strip (prepared by dissection, at 15° relative to the circular axis of the artery) was prepared from each rat for in vitro studies.

Muscle Bath Studies

Portal vein strips from all three groups of rats were mounted in the same organ bath for isometric recording from Grass FT-03-C force-displacement transducers at a passive force of 5 mN. Aortic strips from all groups of rats were mounted in another organ bath for isometric recording at a passive force of 10 mN. The strips were equilibrated at 37° C for at least 1 hour in normal Krebs' solution that consisted of, in mM: NaCl, 112; KCl, 4.5; NaHCO₃, 26.2; KH₂PO₄, 1.2; MgCl₂, 1.2; CaCl₂, 2.5; EDTA, 0.026; and glucose, 11.1. The solution was continuously bubbled with a gas mixture of 5% CO₂ and 95% O₂.

After equilibration, cumulative doses of NE were added to the baths to provide concentrations of 10⁻⁴, 10⁻³, 10⁻², and 10⁻¹ M. Each dose was allowed in the bath for 2 minutes for the portal vein strips and for approximately 6 minutes for the aortic strips (to obtain steady-state responses) before the addition of a higher concentration of drug. After obtaining a cumulative dose-response curve to NE, the bath fluid was altered to one containing 0.4 mM Ca. Following 30 minutes of equilibration, another dose-response curve to NE was obtained in 0.4 mM Ca. The bath fluid was then changed to one containing 0.2 mM Ca to obtain another dose-response curve to NE. The bath fluid was changed back to one containing 2.5 mM Ca. After equilibration, the strips were exposed to a depolarizing solution containing 80 mM potassium (K) (obtained by substituting Na by K) in 2.5 mM Ca. Finally, the strips were equilibrated in 0.4 mM Ca prior to stimulation by a high K (80 mM) depolarizing solution containing 0.4 mM Ca.

At the end of the experiments, the baths were lowered, and the length of the strips were measured by a vernier caliper (resolution, ±0.05 mm). The strips were removed by cutting away the tied ends, blotted dry, and dried overnight at 90°C. The dry weight of the strips were obtained the following day.

Calculations

Force developed to NE and 80 mM K by portal vein strips was recorded on a Grass Polygraph. Force signal from portal vein strips was integrated electronically by an integrator (Grass, Model 7P10) over 1-minute intervals on a separate channel. The average integrated responses (mN) obtained over the 2 minutes of exposure to Na or K were recorded and expressed as stress (force/cross-sectional area, mN/mm²). The apparent cross-sectional area of the strips was obtained by dividing the dry weight by the length of the strips (table 1). The contractile responses of the aortic strips to NE and 80 mM K were calculated as maximum stress developed during the steady-state response to NE.

Responses of the strips to NE and 80 mM K in low Ca were also expressed as percent of the maximum response in normal Ca to NE and 80 mM K, respectively. The E₀ values for NE (the dose of NE that produces 50% of maximum responses) in each strip in each Ca solution were obtained graphically from each individual dose-response curve.

Statistics

Contractile responses of aortic or portal vein strips from all three groups of rats were analyzed by analysis of variance/covariance. To obtain homogeneity of variances, all data of stress were logarithmically transformed. The heart rate and BP of the three groups were analyzed by analysis of variance, completely random design. Duncan's Multiple Range Test was used to compare group means. A probability of error of less than 0.05 was selected as the criterion for statistical significance.

Results

Blood Pressure and Heart Rate

The SBP and heart rate of the three groups of rats are shown in table 2. Before treatment, SHR and SHR to be treated with D 600 had the same SBP of 151 mm Hg, which was significantly higher than the SBP of WKY of 110 mm Hg. The heart rates of all three groups of rats were not different from each other.

Fourteen days later, WKY and untreated SHR still had the same SBP and heart rate as on Day 0. Prelim-

### Table 1. Apparent Cross-Sectional Areas (mm²) of Aortic and Portal Vein Strips from WKY, SHR, and D 600-Treated SHR (Mean ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aorta strips</th>
<th>Portal vein strips</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>0.080 ± 0.006</td>
<td>0.116 ± 0.007</td>
</tr>
<tr>
<td>SHR</td>
<td>0.080 ± 0.006</td>
<td>0.087 ± 0.007*</td>
</tr>
<tr>
<td>SHR-D 600</td>
<td>0.079 ± 0.006</td>
<td>0.073 ± 0.007*</td>
</tr>
</tbody>
</table>

n = 8 in each group of rats.

*Significantly different from WKY.
**TABLE 2. Systolic Blood Pressure and Heart Rate of WKY, SHR, and D 600-Treated SHR (Mean ± SE)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Rat group</th>
<th>SBP (mm Hg)</th>
<th>Heart rate (beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>WKY</td>
<td>110 ± 5</td>
<td>397 ± 37</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>151 ± 4*</td>
<td>442 ± 37</td>
</tr>
<tr>
<td></td>
<td>SHR-D 600</td>
<td>151 ± 10*</td>
<td>457 ± 19</td>
</tr>
<tr>
<td>14</td>
<td>WKY</td>
<td>102 ± 9</td>
<td>412 ± 15</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>153 ± 6†</td>
<td>423 ± 31</td>
</tr>
<tr>
<td>Average (day 1-14)</td>
<td></td>
<td>102 ± 9</td>
<td>412 ± 15</td>
</tr>
<tr>
<td></td>
<td>SHR-D 600</td>
<td>153 ± 6†</td>
<td>423 ± 31</td>
</tr>
</tbody>
</table>

n = 8 in each group of rats.

*Significantly different from WKY on Day 0.
†Significantly different from WKY on Day 14.
jSignificantly different from SHR-D 600 on Day 0.

**Porton Vein Strips**

**Spontaneous Contractile Activity**

In 2.5 mM Ca, portal vein strips from SHR developed 2.3 times the spontaneous stress developed by portal vein strips from WKY (table 3). Fourteen days of treatment with D 600 further increased the spontaneous stress developed by portal vein strips from SHR (5.0 times the activity of WKY). In 0.4 mM Ca, only portal vein strips from SHR-D 600 developed spontaneous contractile activity. In 0.2 mM Ca, none of the strips from any groups of rats developed spontaneous contractile activity.

**NE Dose-Response Curves**

Analysis of whole curves by analysis of variance/covariance showed that in all Ca solutions (2.5, 0.4 and 0.2 mM) portal vein strips from SHR developed greater stress to different concentrations of NE than portal vein strips from WKY (fig. 1). Chronic treatment with D 600 further increased the contractile response to NE of portal vein strips from SHR.

When the responses to NE in all concentrations of Ca were plotted as a percent of the maximum response to NE in 2.5 mM Ca, it was found that, in low (0.4 and 0.2 mM) Ca, portal vein strips from SHR produced a greater percent of its maximum response than strips from WKY. Chronic treatment with D 600 further increased the percent maximum response developed by portal vein strips from SHR.

**ED<sub>50</sub> Values for Norepinephrine**

In all concentrations of Ca, portal vein strips from SHR had decreased ED<sub>50</sub> values for NE compared to strips from WKY (table 4). Treatment of SHR with D 600 further reduced the ED<sub>50</sub> values for NE.

**Contractile Response to Potassium**

Stress developed by portal vein strips to 80 mM K from each of the three groups of rats in 2.5 mM Ca were similar to the maximum stress developed to NE (10<sup>-5</sup> M) in normal Ca (table 5 and fig. 1). The strips from SHR developed greater stress to 80 mM K than strips from WKY; D 600 further increased the stress developed by portal vein strips from SHR.

In 0.4 mM Ca, strips from all groups of rats developed less stress and produced less percent maximum stress to 80 mM K compared to 10<sup>-5</sup> M NE (table 5 and fig. 1). The stress developed by portal vein strips from SHR to 80 mM K in 0.4 mM Ca was greater than that developed by strips from WKY. Treatment with D 600 further increased the stress developed by strips from SHR to 80 mM K and increased the percent maximum response developed in 0.4 mM Ca (table 5).

**Aortic Strips**

**Norepinephrine Dose-Response Curves**

In all Ca concentrations, aortic strips from SHR developed less stress to different doses of NE compared to aortic strips from WKY (fig. 2). Chronic treatment of SHR with D 600 increased the contractile responses of aortic strips to different doses of NE to similar levels as those developed by strips from WKY in 2.5 and 0.4 mM Ca. In 0.2 mM Ca, aortic strips from D 600-treated SHR produced greater stress to NE than strips from WKY. When the response in low Ca (0.2 and 0.4 mM) were shown as the percent of maximum response produced in 2.5 mM Ca, it was found that aortic strips from SHR...
TABLE 4. ED$_{50}$ Values (Mean ± SE) for NE in Portal Vein and Aortic Strips from WKY, SHR, and D 600-Treated SHR

<table>
<thead>
<tr>
<th>Conc. Ca (mM)</th>
<th>Portal veins: NE ED$_{50}$ ($\times 10^{7}$ M)</th>
<th>Aortas: NE ED$_{50}$ ($\times 10^{9}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>2.5</td>
<td>9.7 ± 0.4</td>
<td>5.2 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>3.1 ± 0.2*†</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>24.2 ± 1.0</td>
<td>12.1 ± 0.5*†</td>
</tr>
<tr>
<td></td>
<td>8.3 ± 0.4*†</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>35.5 ± 1.4</td>
<td>20.2 ± 0.9*†</td>
</tr>
<tr>
<td></td>
<td>14.5 ± 0.6*†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td></td>
<td>3.2 ± 0.2</td>
<td>6.2 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>2.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.2 ± 0.4</td>
<td>23.1 ± 1.2*†</td>
</tr>
<tr>
<td></td>
<td>6.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.5 ± 0.8</td>
<td>51.2 ± 2.5*†</td>
</tr>
<tr>
<td></td>
<td>14.9 ± 0.8</td>
<td></td>
</tr>
</tbody>
</table>

n = 8 in each group of rats.
*Significantly different from WKY.
†Significantly different from SHR.

ED$_{50}$ Values

Aortic strips from SHR had greater ED$_{50}$ values for NE than aortic strips from WKY in all Ca concentrations (table 4). Treatment of SHR with D 600 decreased the ED$_{50}$ values for NE to similar values obtained in aortic strips from WKY.

Contractile Response to Potassium

In 2.5 mM Ca, aortic strips from SHR and SHR-D 600 developed less stress to 80 mM K than strips from WKY (table 5). In 0.4 mM Ca, aortic strips from SHR but not SHR-D 600 developed less stress to 80 mM K than strips from WKY. Aortic strips from

FIGURE 1. Contractile response to nor-epinephrine (NE) of portal vein strips from WKY, SHR, and D 600-treated SHR in normal and low Ca. The points and vertical bars are mean ± se from eight rats.
TABLE 5. Stress Developed by Portal Vein and Aortic Strips to 80 mM Potassium in 2.5 and 0.4 mM Calcium

<table>
<thead>
<tr>
<th>Strip</th>
<th>WKY</th>
<th>SHR</th>
<th>SHR-D 600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal vein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mM Ca</td>
<td>94 ± 5</td>
<td>143 ± 7*</td>
<td>174 ± 9**</td>
</tr>
<tr>
<td></td>
<td>(32 ± 2)</td>
<td>(33 ± 2)</td>
<td>(47 ± 2)**</td>
</tr>
<tr>
<td>0.4 mM Ca</td>
<td>30 ± 2</td>
<td>46 ± 2*</td>
<td>77 ± 4*</td>
</tr>
<tr>
<td></td>
<td>(33 ± 2)</td>
<td>(33 ± 2)</td>
<td>(47 ± 2)**</td>
</tr>
<tr>
<td>Aortic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mM Ca</td>
<td>98 ± 7</td>
<td>79 ± 5*</td>
<td>81 ± 6*</td>
</tr>
<tr>
<td></td>
<td>(70 ± 2)</td>
<td>(71 ± 2)</td>
<td>(78 ± 2)**</td>
</tr>
<tr>
<td>0.4 mM Ca</td>
<td>69 ± 5</td>
<td>56 ± 4*</td>
<td>63 ± 4</td>
</tr>
</tbody>
</table>

Values in parentheses represent percent (mean ± SE) of the maximum stress developed by each strip to 80 mM K in 2.5 mM Ca. All other values are stress + SE (transformed back from log values). n = 8 in each group of rats.

*Significantly different from the same strips from WKY at the same concentration of Ca.
†Significantly different from the same strips from SHR at the same concentration of Ca.

SHR-D 600 retained a greater percent of contracture in 0.4 mM Ca compared to the strips from WKY and SHR.

Discussion

In this study, an attempt was made to normalize the BP of SHR by treatment with D 600, a drug that inhibits the slow inward current carried predominantly by Ca** in the myocardium. D 600, prepared as the free base and dissolved in oil, was injected subcutaneously into SHR once daily. The BP of the rats fluctuated from a minimum value of 82 ± 8 mm Hg at 2 hours postinjection to a maximum value of 128 ± 8 mm Hg at 24 hours postinjection. Heart rate was not changed at any time. Since a reflex tachycardia is to be expected from a reduction in BP brought about by a drug that causes smooth muscle relaxation (peripheral vasodilatation), it seems likely that a reflex increase in heart rate did not occur due to the opposing effect of direct myocardial depression caused by a Ca antagonist. To control the BP of SHR, the dose of D 600 given to SHR had to be increased progressively with time. Thus, tolerance to D 600 developed readily in SHR. It should be mentioned that a fourth group of rats, namely, D 600-treated WKY, was originally included in the study. However, the treatment consisting of once-daily injections of WKY with the same dose of D 600 that was given to the SHR (progressive increase of dose with time) resulted in severe hypotension and death in four of a total of 25.
four rats within 1 week of treatment. Thus, we were unable to complete the study on this fourth group of rats.

Treatment of SHR with D 600 partially restored the reduced contractility of aortic strips to NE in normal Ca and completely restored reduced contractility to NE in low Ca. However, reduced contractility to potassium-induced contracture in aortic strips from SHR was less effectively prevented by D 600. We have previously shown that changes in the contractility of aortic strips from hypertensive rats are secondary to the effect of high BP. Normalization of BP by chronic treatment with hydralazine of both DOCA-salt-treated rats and SHR completely prevented reduced contractility of aortic strips to NE. In this study, however, the BP of SHR was less effectively controlled by daily injections of D 600 compared to the BP of the hypertensive rats treated with hydralazine in the previous study. It may be for this reason that we were unable to completely restore the reduced contractility of aortic strips to NE and K.

The ED₅₀ values of NE in aortic strips from WKY were approximately twice those of strips from WKY in normal Ca and three times those of strips from WKY in low Ca solutions. Increased ED₅₀ values for NE may be caused by a number of factors, such as a reduction in the affinity of NE for its receptor; or a decrease in the number of NE receptors in the aortas of SHR compared to the aortas of WKY; or a decrease in the permeability of Ca following activation by NE, thereby resulting in less efficient excitation-contraction coupling. Treatment with D 600 completely restored the ED₅₀ values for NE in the aortas of SHR.

Similar to the results from a previous study, portal vein strips from SHR developed greater spontaneous contractile activity and greater stress in response to NE in normal and low Ca solutions compared to portal vein strips from WKY. Treatment with D 600 further increased spontaneous contractile activity and stress developed to NE in normal and low Ca solutions. Moreover, portal vein strips from D 600-treated SHR retained greater percentage of their maximum contractile activity in low Ca solutions than portal vein strips from WKY and untreated SHR. The treatment also caused similar increases in the contractile responses of portal vein strips, in normal and low Ca, to depolarizations caused by K. Thus, treatment of SHR with a Ca antagonist resulted in further enhanced contractile responses of portal vein strips to NE and K as well as reduced Ca requirement for contraction. The changes obtained in the portal vein strips from SHR treated with D 600 were drastically different from the changes obtained from the portal vein strips from SHR treated with hydralazine in which there was no increase in the contractile response and no decrease in the dependence of Ca associated with the treatment.

In all Ca solutions, portal vein strips from SHR had decreased ED₅₀ values for NE compared to portal vein strips from WKY. Treatment with D 600 caused further reduction of the ED₅₀ values for NE. Therefore, the treatment resulted in a further increase in the apparent affinity of NE for receptors in portal veins from SHR and/or a further increase in the efficiency of excitation-contraction coupling.

What remains to be answered is this: What is responsible for the rapid development of tolerance to the hypotensive effect of D 600? Obviously, tolerance to the hypotensive effect of D 600 is likely to be a result of tolerance to the vasodilator effect as well as the myocardial depressant effect of D 600. Tolerance to D 600 was found to develop in conjunction with changes in the contractile response of portal veins from SHR, namely, enhanced spontaneous contractile activity, enhanced response to NE- and K-induced depolarizations, reduced ED₅₀ values for NE, and reduced Ca dependence for contractile activity of vascular smooth muscles.

One explanation for the observation is that Ca deprivation caused by the treatment with a Ca antagonist may result in a compensatory response resulting in an increase in Ca permeability, a decrease in Ca dependence, and an increase in contractility of vascular smooth muscles. This obviously leads to a decrease in the vasodilator effect of the drug. It is feasible that a similar compensatory response had taken place in the myocardium leading to a decrease in the cardiac depressant effect and, consequently, a decrease in the hypotensive effect of D 600 as well. Unfortunately, we did not examine the contractility of cardiac tissue from SHR chronically treated with D 600. If a compensatory increase in Ca permeability and a reduction in Ca dependence is responsible for the development of tolerance to D 600, it remains to be seen whether this compensatory response is unique to SHR, which are known to have abnormal Ca handling compared to normotensive rats. The observation that treatment of WKY with the same increasing dose of D 600 given to SHR resulted in the death of the rats suggests that perhaps tolerance develops less readily in WKY compared to SHR. More studies are needed to determine whether chronic treatment of normotensive rats with D 600 causes similar changes in the contractility and Ca dependence of vascular smooth muscles.

Another possibility that may be responsible for the development of tolerance is that denervation supersensitivity occurred following chronic treatment of D 600. Since Ca antagonists are known to possess local anesthetic properties and since the release of NE by sympathtic nerve stimulation is Ca-dependent, it is possible that treatment with D 600 may result in a suppression of sympathetic nervous activity resulting in a development of denervation supersensitivity of vascular smooth muscles similar to that obtained after the treatment of animals with reserpine or 6-hydroxydopamine and following surgical sympathectomy. We are not able to tell from this study whether denervation supersensitivity did occur in the SHR. Chronic denervation of rats by ganglionection (celiac and superior mesenteric) was reported to cause denervation supersensitivity of portal vein strips to NE but no increase in spontaneous contractile activ-
ity or contractile response to acetylcholine. Thus, it appears unlikely that denervation supersensitivity is the mechanism responsible for the development of tolerance to D 600. Further experiments are needed to find out the cause for the development of tolerance to D 600 in SHR.

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