Comparison of the Sympathetic Nervous System Activity between Spontaneously Hypertensive and Wistar-Kyoto Rats to Respond to Blood Pressure Reduction

Pablo Prados, Tomofumi Santa, Hiroshi Homma, Hisayoshi Doy, Hiroshi Narita, Benito Del Castillo, Mª Antonia Martin, and Kazuhiro Imai

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan, Pharmacological Research Laboratory, Tanabe Seiyaku Co., Ltd., Kawagishi, Toda-shi, Saitama 355, Japan, and Facultad de Farmacia, Universidad Complutense de Madrid, 28040-Madrid, Spain.

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Two types of calcium antagonists, diltiazem and nicardipine, were separately infused in 23–28 week-old spontaneously hypertensive (SHR) and age-matched normotensive Wistar-Kyoto (WKY) rats (under sodium thiobutabarbital anesthesia and ventilation, n=4) through the left femoral vein, resulting in the reduction of blood pressure. In each rat, mean arterial blood pressure, heart rate and the concentration of plasma catecholamines (CAs), norepinephrine (NE) and epinephrine (E), were concomitantly determined and the correlations between these three values were studied for each calcium antagonist. Plasma concentration of CAs were measured in blood samples collected during the infusion from the right femoral artery of each rat by the automatic sensitive and selective detection system. The reduction of blood pressure induced by the calcium antagonists brought about an increase in plasma CAs levels. The blood pressure correlated well with the logarithm of plasma NE and E concentration and the relations were expressed as Y = -a log(x) + m (Y, blood pressure; X, concentration of plasma NE or E; a, slope; and m, intercept). The zs of SHR rats were greater than those of WKY rats for the calcium antagonists employed, meaning that the increment of plasma CAs responding to a decrease in blood pressure was smaller in SHR than in WKY rats. It was concluded that the contribution of the sympathetic nervous system to maintaining blood pressure reduced by diltiazem and nicardipine is less in SHR than in WKY rats.

Key words calcium antagonist; rat plasma catecholamine; blood pressure; sympathetic nervous system

Many papers have reported the use of plasma catecholamines (CAs) as an appropriate means to evaluate baroreflex-mediated sympathetic nervous system (SNS) activity, because circulating CAs have been thought to consist mainly of CAs liberated from the synapses of the SNS in proportion to the degree of its activity. 1–10 We recently showed for the first time that the blood pressure reduction caused by the administration of diltiazem, verapamil and nicardipine to Sprague-Dawley (SD) rats correlated significantly with the increase of plasma norepinephrine (NE) and epinephrine (E) concentrations. 11,12 These findings revealed the great contribution of the SNS activity to the blood pressure regulation in SD rats. These data were obtained with the aid of our fully-automated system for the highly sensitive and selective determination of plasma CAs, NE and dopamine (DA), which requires only 25 μl of rat plasma. 13,14

Since the role of the SNS in the development and maintenance of hypertension in SHR rats remains controversial, 15–17 in this extension of our previous work we further studied baroreflex-mediated SNS activity in the hypertensive rats.

MATERIALS AND METHODS

Reagents and Animals CAs (norepinephrine, NE; epinephrine, E; dopamine, DA; and N-methyl dopamine, N-MeDA) were purchased from Sigma (St. Louis, MO, U.S.A.). Trifluoroacetic acid (TFA) was obtained from Pierce (Rockford, IL, U.S.A.). Acetonitrile, ethanol, dioxane, ethyl acetate and distilled water, all of HPLC grade, were purchased from Wako (Osaka, Japan). Hydrogen peroxide, bis[2-(3,6,9-trioxodecanoyloxy)carbonyl]-4-nitrophenyl]oxalate (TDPO) and thiobutabarbital sodium salt were also from Wako. Sodium octanesulfonate and imidazole (zone-refined) were obtained from Tokyo Kasei (Tokyo, Japan). Heparin sodium salt was obtained from Nacalai Tesque Inc. (Kyoto, Japan). For the calcium antagonists, we selected nicardipine as representative of the dihydropyridine type antagonists and diltiazem as representative of other types of antagonists. Diltiazem hydrochloride was kindly donated by Tanabe Seiyaku Co., Ltd. (Saitama, Japan). Nicardipine hydrochloride was purchased from Sigma. Male Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were supplied by Charles River Japan (Shizuoka, Japan). The age of the rats ranged from 23 to 28 weeks and the weights were between 330 and 430 g. Commercially available N-MeDA was purified as described previously. 13

Infusion of the Drugs, Sample Collection and Preparation WKY and SHR rats were anesthetized with sodium thiobutabarbital (100 mg/kg, intraperitoneally). Additional anesthesia (20 mg/kg) was injected after operation and before the infusion with drugs. Rats were under ventilation (60 strokes/min, 1 ml/100 g/stroke) until the end of the experiment using a Model SAR-830 ventilator (CWE Co., Pennsylvania, U.S.A.). Diltiazem and nicardipine were separately dissolved in saline at concentrations of 3.27 and 3.27 × 10⁻² mg/ml, respectively. The solutions of diltiazem and nicardipine were infused through the left femoral vein with a Model CFV-3100 infusion pump (Nihon Kohden, Tokyo, Japan) at flow rates of 5.1, 10.3, 20.6 and 51.0 μl/min. These flow rates corresponded to infusions at 16.7, 33.7, 67.5 and
167 mg/kg/min for diltiazem, and 0.33, 0.67, 1.35 and 3.34 mg/kg/min for nicardipine. Infusion started thirty minutes after surgery. The period of each infusion was 20 min. Saline was infused into the control rats at the same flow rate. Syringes containing the solutions of nicardipine were covered with aluminum film during the infusion in order to prevent solution decomposition by light. Arterial blood pressure and heart rate were measured through the left femoral artery with a Model DTX disposable transducer (Spectramed Inc., Oxnard, CA, U.S.A.), which was connected to a Model AP-621 G carrier amplifier for arterial blood pressure (Nihon Kohden) and a Model AT-601 G heart rate counter (Nihon Kohden), and were recorded on a Model WR-3701 recticorder (Graphtec, Tokyo, Japan).

Blood (0.20 ml) was collected through a catheter implanted into the right femoral artery, 10 and 5 min before the infusion of each calcium antagonist or saline, and 15 min after the beginning of each step of the infusion. Each calcium antagonist was infused in 4 WKY and 4 SHR rats, and 4 rats were infused with saline as a control. Plasma was diluted with the same volume of a sample dilution buffer, the composition of which was 10 mm glutathione, 10 mm citric acid, 0.1% Triton X-100, 100 mg/ml EDTA and 8 mm N-MeDA (pH 4.5). Fifty ml aliquots of the diluted mixture were then subjected to the fully-automated HPLC analyzer for CAs with PO-CL detection. [13]

**HPLC Detection System and HPLC Conditions**

The fully automated system consisted of four HPLC pumps, an autosampler, a rotatory six-way valve, a ternary gradient unit, a precolumn and an analytical column, two rotatory mixing devices, a reaction coil in a thermostatically controlled bath and a system controller, as previously reported. [13]

CAs extraction and HPLC conditions were: precolumn, serumout-Cex 10 × 4 mm i.d. (Sekisui Co., Osaka, Japan); buffer for delivering CAs in the precolumn, 10 mm potassium phosphate buffer (pH 7.5)/ethanol 92:8 (v/v), flow rate 1 ml/min; adsorption time in the precolumn, 2 min; eluent, 75 mm potassium acetate buffer (pH 3.2)/acetonitrile 81.7:4.3:14 (v/v) containing 7 mm sodium octanesulfonate, flow rate 0.5 ml/min; desorption time from the precolumn, 2 min; precolumn clean-up solution, 4% phosphoric acid/acetonitrile 50:50 (v/v), flow rate 1 ml/min; precolumn clean-up time, 5 min; analytical column, Crestpack, 150 × 4.6 mm i.d. (Jasco Co., Tokyo, Japan); column oven temperature, 40 °C; fluorogenic reagent solution, 120 mm ED and 175 mm imidazole in acetonitrile/ethanol/water 85:10:5 (v/v), flow rate 0.30 ml/min; reaction coil, Teflon tube 15 m × 0.5 mm i.d.; reaction temperature, 80 °C; chemiluminesogenic reaction solution, 0.25 mm TDPO, 150 mm H₂O₂ and 110 mm TFA in dioxane/ethyl acetate 50:50 (v/v), flow rate 1.4 ml/min.

**RESULTS**

The continuous infusion of diltiazem resulted in a gradual decrease in mean arterial blood pressure in both SHR and WKY rats (from 207 ± 5.0 to 68.25 ± 2.4 mmHg for SHR, from 124 ± 4.2 to 51 ± 4.6 mmHg for WKY) and a concomitant gradual increase in plasma NE concentration (from 0.4 ± 0.15 to 1.5 ± 0.28 pmol/ml for SHR, from 0.3 ± 0.07 to 2.2 ± 0.40 pmol/ml for WKY) (n = 4). A similar result was also obtained by the infusion of nicardipine: blood pressure, from 192 ± 15 to 78 ± 4.2 mmHg for SHR and from 120 ± 7.0 to 52 ± 3.8 mmHg for WKY; concentration of plasma NE, from 0.28 ± 0.03 to 1.36 ± 0.23 pmol/ml for SHR and from 0.27 ± 0.05 to 2.16 ± 0.48 pmol/ml for WKY. As shown in Fig. 1, the reduction in mean arterial blood pressure correlated well with the increase of the plasma NE concentration in both SHR and WKY rats. The relations were obtained using a Microsoft Excel version 4.0 program for Macintosh and were expressed as \[ Y = -a \log(X) + m \], where \( Y \) is the mean arterial blood pressure; \( X \), the concentration of plasma NE; \( a \), a slope; and \( m \), an intercept. As summarized in Table 1, good correlation was observed between the decrease of blood pressure and the increase of plasma NE concentration. The slopes (\( z \) values) were determined to be 232.3 for diltiazem and 175.9 for nicardipine in SHR.

![Fig. 1. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma NE Concentration Obtained with the Infusion of Diltiazem (a) or Nicardipine (b) into WKY (○) and SHR (●) Rats](image-url)

The experimental conditions are described in the text.
Table 1. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma NE Concentration (n=4)

<table>
<thead>
<tr>
<th></th>
<th>SHR rats</th>
<th></th>
<th></th>
<th>WKY rats</th>
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<tbody>
<tr>
<td></td>
<td>Diltiazem</td>
<td>Nicardipine</td>
<td>Control</td>
<td>Diltiazem</td>
<td>Nicardipine</td>
<td>Control</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>232.3 ± 21.3</td>
<td>175.9 ± 10.7</td>
<td>11.3 ± 4.5</td>
<td>76.4 ± 3.4</td>
<td>77.5 ± 4.3</td>
<td>-9.5 ± 3.2</td>
</tr>
<tr>
<td>$m$</td>
<td>123.8 ± 4.7</td>
<td>103.4 ± 2.3</td>
<td>124.8 ± 8.1</td>
<td>82.3 ± 1.1</td>
<td>77.8 ± 1.4</td>
<td>92.6 ± 4.8</td>
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<tr>
<td>$r$</td>
<td>-0.983</td>
<td>-0.994</td>
<td>-0.378</td>
<td>-0.995</td>
<td>-0.994</td>
<td>-0.673</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Comparisons of the $\alpha$s in the same rat group using the Student's $t$-test resulted in the following: SHR rats, diltiazem > control ($p<0.01$), nicardipine > control ($p<0.01$), diltiazem > nicardipine ($p<0.05$); WKY rats, diltiazem > control ($p<0.01$), nicardipine > control ($p<0.01$). Comparison of the $\alpha$s between SHR and WKY rats: SHR diltiazem > WKY diltiazem ($p<0.01$), SHR nicardipine > WKY nicardipine ($p<0.01$).

![Graph a) and b)](image)

Fig. 2. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma E Concentration Obtained with the Infusion of Diltiazem (a) or Nicardipine (b) into WKY (○) or SHR (●) Rats

The experimental conditions are described in the text.

Table 2. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma E Concentration (n=4)

<table>
<thead>
<tr>
<th></th>
<th>SHR rats</th>
<th></th>
<th></th>
<th>WKY rats</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diltiazem</td>
<td>Nicardipine</td>
<td>Control</td>
<td>Diltiazem</td>
<td>Nicardipine</td>
<td>Control</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>136.0 ± 8.9</td>
<td>131.7 ± 6.3</td>
<td>21.4 ± 6.7</td>
<td>70.1 ± 14.9</td>
<td>58.1 ± 3.4</td>
<td>18.1 ± 4.2</td>
</tr>
<tr>
<td>$m$</td>
<td>65.7 ± 5.5</td>
<td>68.7 ± 3.5</td>
<td>50.2 ± 4.6</td>
<td>41.5 ± 9.5</td>
<td>61.1 ± 1.9</td>
<td>48.4 ± 6.2</td>
</tr>
<tr>
<td>$r$</td>
<td>-0.991</td>
<td>-0.995</td>
<td>-0.556</td>
<td>-0.937</td>
<td>-0.993</td>
<td>-0.456</td>
</tr>
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</table>

Values are mean ± S.E. Comparisons of the $\alpha$s in the same rat group using the Student's $t$-test resulted in the following: SHR rats, diltiazem > control ($p<0.01$), nicardipine > control ($p<0.01$); WKY rats, diltiazem > control ($p<0.01$), nicardipine > control ($p<0.01$). Comparison of the $\alpha$s between rat groups: SHR diltiazem > WKY diltiazem ($p<0.01$), SHR nicardipine > WKY nicardipine ($p<0.01$).

rats, and 76.4 for diltiazem and 77.5 for nicardipine in WKY rats (Table 1).

An increase in the concentration of plasma E was also observed upon the decrease in blood pressure induced by the infusion of the calcium antagonists: diltiazem, from 0.08 ± 0.008 to 0.89 ± 0.04 pmol/ml for SHR and from 0.16 ± 0.05 to 0.68 ± 0.28 pmol/ml for WKY; nicardipine, from 0.12 ± 0.02 to 0.91 ± 0.07 pmol/ml for SHR and from 0.08 ± 0.007 to 1.23 ± 0.4 pmol/ml for WKY. The blood pressure also correlated well with the logarithm of plasma E concentration in both rat strains (Fig. 2) and the relations were expressed in a similar way to those for the plasma NE concentrations (Table 2). The slopes ($\alpha$s) were: for SHR rats, 136.0 for diltiazem and 131.7 for nicardipine; for WKY rats, 70.1 for diltiazem and 58.1 for nicardipine.

**DISCUSSION**

**Selection of the Anesthetic Agent** In our previous works, we used sodium pentobarbital (50 mg/kg i.p. dose and a further 0.16 mg/kg/min continuous infusion) as an anesthetic for SD rats. However, when SHR and WKY rats were anesthetized with pentobarbital, respiratory distress and an unstable blood pressure base line occurred throughout the experiment; therefore, we employed sodium thiobutabarbital, which is widely considered as a good alternative in the experiments with SHR rats. With thiobutabarbital the blood pressure base line was stable at normal levels in control SHR and WKY rats for at least two hours, making it possible to compare the SNS activity in the two rat strains. Moreover, since thiobutabarbital was administered intraperitoneally, plasma volume was not affected and more accurate measurement of plasma CAs was achieved.
Levels of CAs in SHR and WKY Rats. We observed similar basal plasma NE and E concentrations in adult rats of both types (23–28 weeks). This result agrees well with the generally accepted idea that these concentrations are higher in SHR rats than in WKY rats only during the pre- or early phases of hypertension (0—6 weeks), whereas in the established phase of hypertension in adult SHR rats the values are similar or slightly lower than in WKY rats.6–10,18,19

Plasma Levels of NE and Blood Pressure after Infusion of Calcium Antagonists. The data presented in this report show that there is a good correlation between the decrease of blood pressure and the increase of plasma NE concentration in both rat strains.

The slopes (x values) were similar for each rat strain, 232.3 for diltiazem and 175.9 for nifedipine in SHR rats and 76.4 for diltiazem and 77.5 for nifedipine in WKY rats, suggesting that the values represent the inherent sensitivity of the baroreflex arc mediating SNS activation upon blood pressure decrease. The former observation contrasts with our previous data for SD rats12—anesthetized with pentobarbital, which showed that less SNS contribution (greater slope z) was observed when nifedipine was used as compared with diltiazem or verapamil. The slight increase of the heart rate at the first stage observed in SD rats upon infusion of nifedipine12 might contribute in part to sustain the blood pressure and attenuate the stimulation of the SNS. In the present study, however, the heart rate did not change markedly in WKY or SHR rats anesthetized with thiobutabarbital.

Plasma Levels of E and Blood Pressure after Infusion of Calcium Antagonists. It was earlier reported4–6 that plasma E levels did not markedly increase in a normal physiological situation to compensate a decrease in blood pressure, except in the case of damage in the noradrenergic SN endings. The result suggested the insignificant role of plasma E in the control of the blood pressure. However, no detailed reports on the evolution of plasma E concentration during controlled acute hypotension have been published, except for our previous papers.11,12 Our present data on plasma E concentration also showed that in both SHR and WKY rats, the SNS stimulates the adrenomedullary secretion of E to help maintain the blood pressure at normal levels as we previously described for SD rats.12 The data also suggested that E secretion could contribute to blood pressure homeostasis in the event of controlled hypotension.

Baroreflex Function in SHR Compared with WKY Rats. As shown in Figs. 1 and 2, the slopes (x) of the curves of blood pressure versus logarithms of NE or E concentrations for SHR rats were greater than those of the corresponding curves for WKY rats. A greater slope corresponds to a lower baroreflex sensitivity. The data showed that with the same reduction in blood pressure there was a smaller evoked rise in plasma CAs in SHR rats than in WKY rats, indicating that the reduced sensitivity of the baroreflex-mediated SN response was evident in adult SHR rats as compared with age-matched normotensive WKY rats (23–28 weeks) once the chronic phase of hypertension was established. A further experiment has to be done in the prehypertensive and early phases of hypertension in young rats since high activity of the SNS as well as high levels of plasma CAs was suggested.18,19

On the other hand, in patients undergoing short-term treatment with nitrendipine,20 nifedipine21 or nicardipine,22 plasma NE showed a tendency to increase as the blood pressure was lowered by the action of the drugs, probably as a result of baroreflex-mediated increase in SNS activity.23–25 However, diltiazem26 and verapamil27 were reported not to alter substantially the plasma NE levels. To clarify this discrepancy we are now exploring the correlations in humans using the technique described in the present work.

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REFERENCES