Effects of Prolonged Treatment with β-Adrenoceptor Antagonist, Carteolol on Systemic and Regional Hemodynamics in Stroke-Prone Spontaneously Hypertensive Rats

Kouichi HASUI,* Takashi OHMOTO,* Toshiaki TAMAKI,** Kiyoshi FUKUI,** Hiroshi IWAO,** and Youichi ABE***

Departments of Neurological Surgery* and Pharmacology,** Kagawa Medical School, Miki-cho, Kita-gun, Kagawa, 761-07, Japan

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The present study was designed to determine the regional hemodynamic effects of prolonged β-adrenergic receptor inhibition in conscious stroke-prone spontaneously hypertensive rats (SHRSP) using a radioactive microsphere method. When the regional blood flow was compared between 10 and 30 weeks of age, the age-related changes in organ blood flow were observed in several organs, i.e., the reduction of flow rate in kidney, adrenal gland and intestines. The reduction of flow rate in these organs contributes strongly to the age-related rise of total peripheral resistance.

Carteolol, a β-adrenoceptor antagonist, was given at a dose of 10 mg/kg/d from 10 to 30 weeks of age. These animals gained more weight than the untreated control SHRSPs, and heart rate was reduced significantly. Blood pressure was not affected. However, the prolonged treatment with carteolol prevented the age-related reduction of the blood flow rate in the kidney, adrenal gland and intestines. Thus, our findings indicate that carteolol had appreciable and beneficial effects on the maintenance of flow rates in the above organs of SHRSP without any change in blood pressure.

Keywords — carteolol; stroke-prone spontaneously hypertensive rat; regional hemodynamics; microsphere; renal blood flow; age-dependent hemodynamic change

Introduction

A stroke-prone strain of spontaneously hypertensive rat (SHRSP) was isolated in 1974 by Okamoto et al. from spontaneously hypertensive rat (SHR) substrains. The SHRSP showed a more abrupt rise of blood pressure than SHR, especially at an early stage of hypertension. This abnormal high blood pressure may come from regional hemodynamic disturbances or, in turn, may cause the regional hemodynamic abnormalities, since we have previously reported that the regional distribution of cardiac output in SHR significantly differed from that in WKY. Yamori and Horie observed low cerebral blood flow in conscious SHRSP when the systemic blood pressure exceeded 200 mmHg. Nagaoka et al. also reported the low renal blood flow in anesthetized SHRSPs without any significant difference in glomerular filtration rate. Thus, these findings may indicate the regional hemodynamic abnormality in SHRSP as well as in SHR and in renal hypertensive rat.

Beta-adrenergic receptor blocking drugs have been used clinically and experimentally for the treatment of hypertension for over a decade. In the experiments using SHR and SHRSP, Ozaki and colleagues observed that β-adrenergic receptor blockers were not only effective in controlling blood pressure but also protected against the secondary unfavorable events related to hypertension in SHR and SHRSP. Thus, it can be considered that the β-adrenergic receptor blockers may normalize the regional hemodynamic abnormality in SHRSP.

The present study was designed, therefore, to determine the regional hemodynamic effects of prolonged β-adrenergic receptor inhibition in SHRSP, clarifying the beneficial effects of this drug from a hemodynamic point of view.

Methods

Animal and Drug Administration — All studies were performed in male SHRSP (Otsu-
ka Strain, Japan). The animals were divided into the following groups. A: Twelve rats were given carteolol daily in a dose of 10 mg/kg/d from 10 to 30 weeks of age, B: Thirteen rats were followed without drug administration and served as the controls (during the 20-week experimental period, one animal was lost and 12 rats were used for the measurement of the regional hemodynamics), C: Ten rats 10 weeks of age were used for measurement of the pretreatment basal parameters. Carteolol was added to the drinking water. The volume of drinking water was measured once a week. The volumes in the control and carteolol treated rats were about 28—34 ml/d and 24—30 ml/d, respectively. Carteolol was dissolved at a concentration at which the intake would be about 10 mg/kg/d. Standard rat diet (F2, Funabashi Farm, Japan) was freely available.

Systolic blood pressure was measured indirectly using an electrophygmonanometer after prewarming the tail for 10 min at 38 °C. Body weight was measured once a week, blood pressure and heart rate were measured every 5 weeks throughout the experimental period.

Measurements of Systemic and Regional Hemodynamics — The animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Polyethylene catheters (PE-50) were placed in the femoral artery and in the left ventricle via the right carotid artery. Both catheters were filled with heparinized saline (100 IU/ml) and exteriorized through a cutaneous tunnel at the back of the neck after confirmation at the tip locations by pressure tracings. The animals were allowed to recover for 24 h before the initiation of the experimental procedures and could also take carteolol freely.

The catheterized SHRSP was placed in a small plastic chamber. The femoral arterial catheter was connected to the pressure transducer, and systemic arterial pressure was continuously recorded on a multichannel polygraph (Nihonkohden, Japan).

Radioactive microspheres were used to measure the cardiac output and regional blood flow according to the method of Tsuchiya et al.\textsuperscript{11} and Ishibe et al.\textsuperscript{12} The \textsuperscript{85}Sr labeled microsphere (New England Nuclear, USA), 15 ± 3 μm in diameter, was used. Following a 60 min stabilization period for the rat to adapt to the chamber, 0.25 ml of saline solution containing 75000 microspheres was injected into the left ventricle. The injection procedure was carried out over a 15 s period. Arterial blood samples for reference blood were obtained using a withdrawal pump at a rate of 0.70 ml/min starting immediately before the injection of the microspheres and ending 60 s later. After termination of the injection of microspheres the animal was killed by the injection of pentobarbital sodium. The brain, lungs, heart, liver, spleen, adrenal glands, kidneys, stomach, intestines (small and large intestine), skin and hindlimb skeletal muscle were removed and weighed. The radioactivity in the stock solution, reference blood and tissue samples was analyzed using a gamma scintillation counter.

The cardiac output was calculated as follows;

\[
\text{cardiac output (ml/min)} = \frac{\text{injected isotope counts (cpm)}}{\text{reference blood counts (cpm)}} \times 0.70 \text{ (ml/min)}
\]

Total injected radioactivity was obtained by subtracting the residual radioactivity from the radioactivity before injection. Total peripheral resistance (TPR) was calculated by dividing the mean blood pressure by the cardiac output and was expressed as mmHg · min/ml.

The organ blood flow was calculated as follows;

\[
\text{organ blood flow (ml/min)} = \frac{\text{organ isotope counts (cpm)}}{\text{reference blood counts (cpm)}} \times 0.70 \text{ (ml/min)}
\]

In the present paper the organ blood flow is expressed as ml/g of organ/min. The organ vascular resistance was calculated by dividing the mean blood pressure by the organ blood flow and was expressed as mmHg·g·min/ml.

Statistical Analysis: Results are expressed as means ± S.E.M. The data were evaluated using the paired or unpaired Student's \textit{t}-test and \textit{p} values less than 0.05 were regarded as significant.
Results

1. Body Weight, Systolic Blood Pressure and Heart Rate
The body weight of SHRSP treated with carteolol were significantly heavier from 20 weeks of age onwards, as compared to the age-matching controls (Fig. 1). At the beginning of the experiment the systolic blood pressure in all groups was around 185 mmHg and there were no significant differences between groups. The systolic blood pressure increased with age. The treatment with carteolol did not affect the systolic blood pressure throughout the experimental period (Fig. 1). The initial rates of heart beat were around 370 beats/min in both groups. Treatment with carteolol significantly reduced the heart rates from the age of 15 weeks (Fig. 1).

2. Systemic Hemodynamics
The data tabulated in Table I were obtained from the catheterized rats and the blood pressure is shown as the mean arterial pressure. In the non-treated SHRSP, the mean blood pressure rose from 171 ± 5 mmHg at 10 weeks of age to 200 ± 2 mmHg at 30 weeks of age. The heart rate tended to increase age-dependently,

**TABLE I. Systemic and Regional Hemodynamics in SHRSPs at 10 and 30 Weeks of Age, and Effects of Treatment from 10 to 30 Weeks of Age on Systemic and Regional Hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>10 weeks</th>
<th>30 weeks</th>
<th>Carteolol (10 mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg)</td>
<td>171 ± 5</td>
<td>200 ± 2(^a)</td>
<td>192 ± 4</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>123 ± 4</td>
<td>112 ± 4(^a)</td>
<td>104 ± 5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>388 ± 10</td>
<td>416 ± 10</td>
<td>315 ± 5(^b)</td>
</tr>
<tr>
<td>Total peripheral resistance (mmHg-min/ml)</td>
<td>1.58 ± 0.08</td>
<td>1.81 ± 0.09(^a)</td>
<td>1.79 ± 0.11</td>
</tr>
<tr>
<td>Organ blood flow (ml/g-min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.77 ± 0.05</td>
<td>0.81 ± 0.06</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>Lung</td>
<td>0.57 ± 0.13</td>
<td>0.51 ± 0.05</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>6.97 ± 0.03</td>
<td>6.82 ± 0.29</td>
<td>5.96 ± 0.23(^b)</td>
</tr>
<tr>
<td>Liver (hepatic artery)</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Liver (portal vein)</td>
<td>1.20 ± 0.11</td>
<td>0.89 ± 0.05(^a)</td>
<td>0.93 ± 0.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.28 ± 0.26</td>
<td>1.46 ± 0.12(^a)</td>
<td>1.57 ± 0.13</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.40 ± 0.45</td>
<td>4.87 ± 0.46(^a)</td>
<td>6.15 ± 0.23(^a)</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>6.63 ± 0.78</td>
<td>4.92 ± 0.33(^a)</td>
<td>6.61 ± 0.50(^b)</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.97 ± 0.06</td>
<td>0.70 ± 0.04(^a)</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.61 ± 0.15</td>
<td>1.10 ± 0.04(^a)</td>
<td>1.45 ± 0.10(^b)</td>
</tr>
<tr>
<td>Large intestine</td>
<td>1.27 ± 0.12</td>
<td>0.75 ± 0.05(^a)</td>
<td>1.03 ± 0.06(^b)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.232 ± 0.023</td>
<td>0.269 ± 0.023</td>
<td>0.150 ± 0.015(^b)</td>
</tr>
<tr>
<td>Skin</td>
<td>0.069 ± 0.005</td>
<td>0.035 ± 0.003(^a)</td>
<td>0.042 ± 0.002(^b)</td>
</tr>
</tbody>
</table>

All values are means ± S.E.M. \(^a\) Indicates significant difference between 10 and 30 weeks of age, and \(^b\) Indicates significant difference between SHRSPs at 30 weeks of age with and without carteolol treatment (\(p < 0.05\)).
but the cardiac output significantly decreased with age. As a result, the TPR significantly increased. The treatment with carteolol for 20 weeks tended to reduce the mean arterial pressure and the cardiac output, but these changes were not statistically significant (Table I). As a result, any significant changes in TPR were not observed after carteolol treatment. However, carteolol significantly reduced the heart rate.

3. **Regional Hemodynamics**

The age-dependent regional hemodynamic changes were observed in the non-treated SHRSP. As tabulated in Table I, the flow rates of the kidney, stomach, adrenal gland, spleen, intestines and skin decreased with age. However, the flow rates of the brain were not different be-

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**Fig. 2.** Organ Vascular Resistances in the Non-treated SHRSPs of 10 and 30 Weeks of Age
The organ vascular resistances of the spleen, kidney, small intestine and skin increased age-dependently (*a* *p* < 0.05). □, 10 weeks; ▪, 30 weeks.

**Fig. 3.** The Effects of Prolonged Treatment with Carteolol on the Organ Vascular Resistances in SHRSPs
The carteolol significantly reduced the vascular resistances of the kidney, small intestine and skin, and increased the vascular resistance of the skeletal muscle (*a* *p* < 0.05). □, control; ▪, carteolol (10 mg/kg/d).
between 10 and 30 weeks of age. All organ vascular resistance except that in the skeletal muscle tended to increase age-dependently along with the rise of blood pressure. The age-dependent changes of vascular resistance in the above organs among all organs were statistically significant as shown in Fig. 2, but the vascular resistance in the brain was not different between both groups.

The treatment with carteolol over the 20 weeks resulted in significant increases of blood flow in the kidney, adrenal gland and intestines, and significant decreases of blood flow in heart and skeletal muscle (Table I). The cerebral blood flow was not affected by the treatment with carteolol. The chronic treatment with carteolol reduced organ vascular resistance in the kidney, adrenal gland, intestines and skin (Fig. 3). However, the vascular resistance in the skeletal muscle significantly increased.

Discussion

In the SHRSRP the age-related changes in organ blood flow were observed in several organs, i.e., the kidney, adrenal gland and intestines. However, the flow rates in the brain and heart did not change age-dependently. Thus, the age-related reduction in flow rates of the kidney and intestines, which receive a high percentage of distribution of cardiac output, may have contributed to the age-related increase of blood pressure and total peripheral resistance. However, there was no significant difference between cerebral blood flow at 10 and 30 weeks of age. Fredriksson et al. also reported that the total and regional blood flow in SHRSRP were not different from those in the normotensive rat. There was no significant difference between the organ flow rates in SHRSRP and SHR at 10 weeks of age. In addition, the age-related organ blood flow changes were not observed in SHR (unpublished observations). Accordingly, these age-related changes may be specific in the SHRSRP.

The antihypertensive effect of the \( \beta \)-adrenoceptor antagonist has been extensively assessed in the SHR. In the present experiment, we have evaluated the effects of carteolol on the regional hemodynamics in SHRSRP. Carteolol is a potent \( \beta \)-adrenoceptor antagonist widely prescribed for patients as an antihypertensive, anti-anginal and an anti-arrhythmic agent. This drug has non-cardioselective \( \beta \)-blocking ability, and weak intrinsic sympathomimetic actions. The animals treated with carteolol gained more weight than did the untreated controls during the 20-week treatment period. The heart rate significantly decreased 5 weeks after the start of treatment. These results indicate that the drug manifested a \( \beta \)-blocking action. However, the blood pressure lowering effects of carteolol were weak even with a dose of 10 mg/kg/d. This effect of the \( \beta \)-adrenoceptor antagonist on blood pressure is not unique. Nishiyama et al. found that the long-term treatment by \( \beta \)-adrenergic receptor blockers such as propranolol and timolol did not reduce the high blood pressure of SHR, despite a 30% reduction in cardiac output. Nevertheless, the present observations that the long term treatment with carteolol had prevented the age-related flow reductions in the kidney, adrenal gland and intestines, were noteworthy.

Yamashita et al. and Nagaoka et al. reported that the intensive renal vascular wall changes such as arteriolosclerotic or proliferative changes and thickening of the cortical zona glomerulosa in the adrenal gland were always noted in the SHRSRP. Such histological changes in these organs might cause the age-related reduction of blood flow in these organs. It has been generally considered that these histological changes might be induced by the exposure to high blood pressure. However, the present findings may indicate the existence of other factors linked to the pathophysiology of hypertension, since the chronic treatment with carteolol prevented the age-related reduction of flow rates in several organs without any significant change in blood pressure. Yamashita et al. also found that chronic treatment with carteolol prevented the development or aggravation of lesions in the kidney and the adrenal gland. Our results support their findings on the basis of regional hemodynamics.

Concerning the age-related reduction of organ blood flow, the following mechanisms can be considered. 1) Moritoki et al. reported the
age-associated reduction in the release of an endothelial derived relaxing factor from endothelial cells and reduction in the guanosine 3', 5'-cyclic monophosphate (cyclic GMP) formation in rat blood vessels, indicating the damage of endothelial cells. 2) Hyland et al. 20) and Sawyer and Docherty 21) reported that vasodilation induced by β-agonists has been found to decrease age-dependently in animals. 3) The renin–angiotensin system participates in malignant hypertension of SHRSP that shows proteinuria and renovascular changes. 22) However, the former two systems among the above possible mechanisms may not relate to the preventing action of carteolol in the age-related changes of organ blood flow. The damage to endothelial cells may be induced secondarily by hypertension, but the treatment with carteolol did not reduce the blood pressure. The vasodilation via the β-adrenoceptor should be blocked by the β-blocker. Thus, it is likely that the inhibition of renin release and the presynaptic inhibition of norepinephrine release by carteolol may have participated in this prevention. Another possibility is that carteolol caused a redistribution of the cardiac output from the skeletal muscle to the kidneys, adrenal glands and intestines, since carteolol significantly reduced the flow rate in skeletal muscle. However, the oral administration of the β-blocker, atenolol, also reduced the flow rate in the skeletal muscle, but the flow rates in the kidney and intestines were not affected (unpublished observations). In addition, Janczewski et al. 23) have recently reported that carteolol facilitates the abluminal release of endothelium-dependent relaxing factor caused by α-2 adrenergic activation, and causes the intraluminal release of vasodilator prostaglandins. Thus, the release of endothelium-dependent relaxing factor by carteolol may also have participated in its preventive action. Furthermore, the vasodilation via the intrinsic sympathomimetic action of carteolol and an unknown pathway involving the metabolic changes induced by the β-blocker must also be considered. However, on the basis of the present results, we could not define the exact mechanisms responsible for the prevention of the age-related reduction of renal blood flow with carteolol.

The present experiments showed that carteolol did not exert the definite antihypertensive action in the SHRSP, but prevented the age-related reduction of the flow rate in the kidney, adrenal gland and intestines. Thus, it can be concluded that carteolol had appreciable and beneficial effects on the maintenance of flow rates in the above organs of SHRSP without any change in blood pressure.

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References


