Abnormal Renal Vascular Responses to Dipyridamole-Induced Vasodilation in Spontaneously Hypertensive Rats

Dinko Susic, Jasmina Varagic, Edward D. Frohlich

Abstract— The objective of this study was to determine whether there were differences in hemodynamic responses of different vascular beds to systemic administration of dipyridamole between spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. To this end, systemic hemodynamics and organ blood flows (using labeled microspheres) were determined in conscious rats before and 10 minutes after dipyridamole (4 mg·kg⁻¹·min⁻¹) infusion. In both the normotensive and hypertensive rats, the dipyridamole infusion reduced arterial pressure by ≈20 mm Hg, associated with a decreased total peripheral resistance and an increased cardiac output. Renal blood flow decreased significantly in SHR after dipyridamole but remained unchanged or increased slightly in the WKY rats. There were no other differences in regional hemodynamics, including those of brain, liver, skin, and muscle, between the WKY and SHR. Antihypertensive treatment completely restored normal renal vascular response to dipyridamole. Previous reports had demonstrated an abnormal coronary hemodynamic response of the SHR. Our data demonstrate that, as with coronary hemodynamics, hypertension selectively induced alterations in renal vasculature. These findings may be of importance in identifying the earliest hemodynamic evidence of developing hypertensive nephrosclerosis.

(Hypertension. 2001;37:894-897.)

Key Words: hypertension ■ renal vasculature ■ dipyridamole ■ regional hemodynamics

Sustained increase in arterial pressure alters function as well as structure of systemic vasculature.¹⁻³ These hypertension-induced vascular alterations eventually lead to target organ damage of brain, heart, and kidneys. Fortunately, antihypertensive therapy seems to ameliorate these hypertensive vascular changes, reducing morbidity and mortality from strokes and coronary heart disease.⁴⁻⁵ In contrast, the incidence and prevalence of end-stage hypertensive renal disease continue to increase.⁶ Moreover, renal vascular involvement in hypertension is seldom detected during its early stages. At the moment when clear clinical signs of renal insufficiency become apparent, renal damage is usually so extensive that therapy can only delay, but not prevent, the development of end-stage renal failure. For this reason, early signs of renal involvement in hypertensive disease are highly desirable.

In our studies dealing with the coronary circulation in spontaneously hypertensive rats (SHR), early hemodynamic alterations were demonstrated by dipyridamole infusion.⁷⁻¹⁰ We found that coronary vasodilation in response to dipyridamole infusion was significantly diminished in SHR and that antihypertensive therapy restored vasodilatory response of coronary vasculature. This report concerns the effects of dipyridamole on the other regional circulations of normotensive Wistar-Kyoto (WKY) rats and SHR. Our purpose was to determine whether there were differences between WKY and SHR in the hemodynamic responses of various vascular beds to the systemic administration of dipyridamole, an agent commonly used to assess vasodilatory capacity of coronary circulation.¹¹ The results of this analysis are presented herein.

Methods

This study includes unreported data from several previously published studies.⁷⁻¹⁰ Adult (older than 23 weeks) male WKY and SHR rats were uniformly used. All studies had been approved by our institutional Animal Care and Use Committee. All studies were designed initially to examine the coronary hemodynamic effects of various drugs in the WKY and SHR. Additionally, responses of cardiovascular mass and collagen content were followed in these same experimental protocols. In general, the rats were randomly divided into several groups, each consisting of 10 to 12 rats. Control groups were given an inert vehicle, whereas the experimental groups received various antihypertensive agents including enalapril (30 mg·kg⁻¹·d⁻¹), losartan (30 mg·kg⁻¹·d⁻¹), ramipril (1 mg·kg⁻¹·d⁻¹), felodipine (30 mg·kg⁻¹·d⁻¹), and candesartan (10 mg·kg⁻¹·d⁻¹) for either 3⁻⁹ or 12 weeks. All rats had received their respective therapy daily, by gastric gavage.

Hemodynamic Studies

At the end of each treatment period, the rats were anesthetized with pentobarbital (40 mg/kg) and then instrumented for the determination of systemic and coronary hemodynamics (using the reference
standard radiomicrosphere method) as detailed elsewhere.7-10 In brief, a jugular vein, femoral artery, and the left ventricle (via right carotid artery) were cannulated with polyethylene catheters (PE-50), filled with a heparinized 1% NaCl solution, which were exteriorized at the nape of the neck through a subcutaneous tunnel. Rats were returned to their nonrestrictive polyethylene cages, where they were allowed to recover for 3 to 5 hours.7-10

The baseline measurements of systemic and coronary hemodynamics were obtained while the rats were unrestrained, after full recovery from anesthesia. To this end, the femoral arterial catheter was connected to a pressure transducer (P23DD; Statham Instruments); and mean arterial pressure was recorded on a multichannel physiograph (R612; Sensor Medics) while, simultaneously, heart rate was derived through a tachometer coupler. Cardiac output was measured using the reference sample microsphere method as reported previously.7-10 Cardiac index was calculated from cardiac output and body weight and expressed in mL · min⁻¹ · kg⁻¹. Total peripheral resistance index (U/kg) was calculated by dividing mean arterial pressure by cardiac index. Blood flow to different organs, including heart, lungs, liver, kidneys, skeletal muscle, skin, and brain, was determined on the basis of percentage distribution of the radiolabeled (57 Co) microspheres to each organ at the end of the study.7-10 The method has been validated previously.12,13

After the baseline measurements were obtained, maximal coronary vasodilatation was produced by dipyridamole infusion (4 mg · kg⁻¹ · min⁻¹, IV for 10 minutes) using a Harvard Apparatus infusion/withdrawal pump. The hemodynamic studies were repeated using microspheres with a second radionuclide (113 Sn). In 1 study, hemodynamic measurements were also obtained during treadmill exercise, using microspheres with a third radionuclide (57 Co). At the conclusion of each study, the rat was killed with an overdose of pentobarbital, and immediately thereafter, the heart, aorta, lungs, liver, kidneys, brain, and samples of skin and skeletal muscle were removed and weighed. Tissue samples, as well as blood reference samples, were placed in plastic scintillation vials and were counted for 15 minutes in a deep-well gamma scintillation spectrometer (Packard) with a multichannel analyzer. Spillover correction between channels was achieved using matrix inversion software (CompuSphere; Packard). Organ blood flows were calculated by multiplying the fractional distribution of radioactivity to each organ by cardiac output. They were normalized for the wet weight of the respective organ and expressed as mL · min⁻¹ · g⁻¹. Regional vascular resistances were calculated by dividing the mean arterial pressure by the respective organ blood flows and then normalized for that organ weight (expressed as U/g). The hemodynamic response of each vascular bed to vasodilator infusion was calculated as the difference between the baseline and dipyridamole infusion flows. Minimal vascular resistance for each organ was defined as vascular resistance achieved by dipyridamole.

Statistical Analysis

Values are expressed as the mean±SEM. A 1-way ANOVA and Student-Newman-Keuls post hoc tests were used to test the significance of differences between the groups.14 The <5% confidence level was considered to be of statistical significance.

Results

Systemic hemodynamic indices of WKY and SHR rats under basal conditions and during dipyridamole infusion are presented in Table 1. Under basal conditions, mean arterial pressure and total peripheral resistance were greater in SHR (P<0.05); there were no differences in cardiac output and heart rate. During dipyridamole infusion, mean arterial pressure and total peripheral resistance decreased and cardiac output increased in both SHR and WKY, although the significant differences between the 2 strains persisted.

Basal organ blood flows and flows during dipyridamole infusion in WKY, SHR, and SHR treated with antihypertensive agents are presented in Table 2. Although renal blood flow remained unchanged in the WKY during dipyridamole infusion, it decreased (P<0.05) in the SHR. However, after the SHR were treated with various antihypertensive agents, their renal blood flow was similar to the WKY. There were no differences in other organ hemodynamics between WKY, SHR, and SHR treated with antihypertensive agents in response to the dipyridamole infusion.

The effects of treadmill exercise on renal blood flow and renal vascular resistance in WKY, SHR, and SHR treated with enalapril or losartan are presented in the Figure. There were no hemodynamic differences among these groups in response to treadmill exercise.

Discussion

The purpose of this analysis was to examine whether there were any organ hemodynamic differences between WKY and SHR rats and whether different vasculatures reacted differently to systemic dipyridamole infusion as they had responded with the coronary hemodynamics.7-10 If any differences were found, our second objective was to determine whether antihypertensive therapy could reverse this phenomenon. Attention was focused primarily on other target organs of hypertension (kidneys and brain).

The data of this study clearly indicate that the responses of the SHR renal vasculature to dipyridamole were significantly different from the responses of the WKY. Thus, in the SHR, renal blood flow became significantly reduced with dipyridamole, whereas it did not change significantly, or actually increased, in the WKY rats. There were no differences in other organ blood flows between WKY and SHR, except for our previously reported coronary flow changes.7-10 Thus, in most vasculatures, changes in blood flow paralleled changes in cardiac output. Furthermore, there were no renal hemodynamic differences in response to exercise between the WKY and SHR. The difference in renal vascular responses to dipyridamole and exercise between WKY and SHR may be related either to specific actions of the drug or specific secondary physiological adaptations to the drug or exercise such as sympathetic stimulation. Our findings suggest that the autoregulatory responses of renal vasculature to dipyridamole induced reduction of arterial pressure is altered in SHR; and that normal responses were restored with various modes of

| TABLE 1. Systemic Hemodynamic Indices Under Basal Conditions and After Dipyridamole |
|-----------------|-----------------|-----------------|
| Index           | Condition       | WKY             | SHR             |
| Mean arterial   | Basal           | 99±6            | 148±4*          |
| pressure, mm Hg | Dipyridamole    | 73±5            | 123±6*          |
| change, %       | change, %       | 23.1            | 17.3            |
| Cardiac output, | Basal           | 415±19          | 409±33          |
| mL · min⁻¹ · kg⁻¹ | Dipyridamole    | 461±27          | 449±27          |
| change, %       | change, %       | 11.2            | 9.6             |
| Total peripheral resistance, U | Basal | 0.240±0.015 | 0.385±0.029* |
| Dipyridamole    | change, %       | 0.155±0.018     | 0.271±0.023*    |
| change, %       | change, %       | 34.2            | 28.7            |

Values are mean±SEM. *P<0.05 vs WKY.
antihypertensive therapy producing profound renal dilation. It should be noted that our study involved drugs that were deemed to offer better nephroprotection than, perhaps, other antihypertensive agents.15

Question may be raised as to whether our finding that the renal vascular response to dipyridamole may be compromised in hypertension has any biological significance or whether it is just another marker of hypertension. We believe that the altered responses of renal vasculature to dipyridamole may be one of the first signs of renal involvement in hypertension. The kidney is a prime target of hypertensive vascular disease and an important pathophysiological concern. The underlying mechanisms of hypertensive renal vascular disease are multifactorial, with early ischemia being one of the major contributing factors.15,16 Our finding that renal blood flow actually decreased in response to a fall in blood pressure in hypertensive animals may be of major importance since it may aggravate ischemia and exacerbate and generate further renal hemodynamic deterioration. Moreover, during periodic falls in arterial pressure in hypertensive subjects, such as nocturnal fall in pressure, renal ischemia may occur and in this way impaired autoregulation of renal blood flow may contribute to the further development and progression of hypertensive nephrosclerosis. Furthermore, as with coronary circulation, the renal response to dipyridamole infusion which we describe herein may be of value clinically in ascertaining early hemodynamic involvement and compromise.7–10

Our results do not point to the precise mechanism underlying impaired autoregulation of renal blood flow in hypertensive rats. Endothelial dysfunction of the renal vasculature is present in hypertensive subjects.17 We have been able to induce similar changes experimentally in young adult SHR with nitric oxide synthetase inhibition.18 Therefore, the increased sympathetic activity, triggered by a fall in arterial pressure through baroreceptors, may be unopposed by renal endothelial factors leading to vasoconstriction, increased vascular resistance, and reduced renal blood flow. It is also possible that the response of the local renal renin angiotensin system to arterial pressure reduction may be exaggerated in the SHR renal circulation. This may lead to vasoconstriction and a reduced renal blood flow. Endothelial dysfunction may further contribute to decreased renal blood flow through this mechanism.

### TABLE 2. Organ Blood Flows (Percent of Cardiac Output) Under Basal Conditions and After Dipyridamole

<table>
<thead>
<tr>
<th>Index</th>
<th>Condition</th>
<th>WKY</th>
<th>Losartan</th>
<th>Enalapril</th>
<th>Ramipril</th>
<th>Felodipine</th>
<th>Candesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, % increase after dipyridamole</td>
<td>Basal</td>
<td>0.3</td>
<td>3.2</td>
<td>4.0</td>
<td>3.9</td>
<td>4.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Basal</td>
<td>3.4</td>
<td>3.8</td>
<td>3.7</td>
<td>3.4</td>
<td>3.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Brain</td>
<td>Basal</td>
<td>2.6</td>
<td>2.8</td>
<td>2.7</td>
<td>2.6</td>
<td>2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Liver</td>
<td>Basal</td>
<td>3.4</td>
<td>3.6</td>
<td>3.7</td>
<td>3.5</td>
<td>3.7</td>
<td>3.6</td>
</tr>
<tr>
<td>Muscle, %/10/g</td>
<td>Basal</td>
<td>1.3</td>
<td>1.4</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Skin, %/10 g</td>
<td>Basal</td>
<td>1.6</td>
<td>1.7</td>
<td>1.3</td>
<td>1.3</td>
<td>1.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05 vs WKY.

Renal blood flow and renal vascular resistance in normotensive WKY and SHR at baseline conditions and after either treadmill exercise. SHR received either vehicle (control), enalapril (30 mg · kg⁻¹ · d⁻¹ by gastric gavage), or losartan (30 mg/kg) for 12 weeks (10 to 12 rats in each group). *Significantly different from baseline (P<0.05).
In conclusion, the present results demonstrate that autoregulation of renal blood flow in response to dipyridamole induced arterial pressure reduction is impaired in the SHR, possibly as a consequence of hypertension. This altered renal vascular response may contribute to the development of hypertensive nephropathy and may be reversed by various forms of antihypertensive therapy.

References

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Hypertension. 2001;37:894-897
doi: 10.1161/01.HYP.37.3.894

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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