Involvement of Endogenous Endothelin-1 in Exercise-Induced Redistribution of Tissue Blood Flow
An Endothelin Receptor Antagonist Reduces the Redistribution

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Background—Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor peptide. Exercise results in a significant redistribution of tissue blood flow, which greatly increases blood flow in active muscles but decreases it in the splanchnic circulation. We reported that exercise causes an increase of ET-1 production in the internal organ and then hypothesized that ET-1 participates in the exercise-induced redistribution of tissue blood flow. We investigated the effects of acute endothelin-A (ETA)-receptor blockade on regional tissue blood flow during exercise in rats.

Methods and Results—Regional blood flow in the kidney, spleen, stomach, intestine, and muscles was measured using the microsphere technique before and during treadmill running of 30 minutes duration at 30 m/min after pretreatment with either an ETA-receptor antagonist (TA-0201; 0.5 mg/kg) or vehicle in rats. Blood flow in the kidney, spleen, stomach, and intestine was decreased by exercise, but the magnitude of the decrease after pretreatment with TA-0201 was significantly smaller than that after pretreatment with vehicle. Furthermore, the increase in blood flow to active muscles induced by exercise was significantly smaller in rats pretreated with TA-0201 than those pretreated with vehicle.

Conclusions—The present study revealed that ET-1–mediated vasoconstriction participates in the decrease of blood flow in the internal organs of rats during exercise, and therefore, that these actions of endogenous ET-1 partly contribute to the increase of blood flow in active muscles during exercise. The data suggest that endogenous ET-1 participates in the exercise-induced redistribution of tissue blood flow. (Circulation. 2002;106:2188-2193.)

Key Words: endothelin – exercise – blood flow – receptors

Exercise results in a significant redistribution of tissue blood flow, which greatly increases blood flow in active muscles but decreases it in the splanchnic circulation.1 Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by vascular endothelial cells.2 We previously reported that the circulating plasma ET-1 concentration is increased by exercise in humans,3 and that the expression of ET-1 mRNA in the kidneys was markedly higher in exercised rats than in control rats.4 These findings suggest that endogenously generated ET-1 may contribute to the regulation of vascular tone during exercise. To provide direct evidence that ET-1 participates in exercise-induced blood flow redistribution, the present study investigated whether the administration of the endothelin-A (ETA)-receptor antagonist TA-02012 or the endothelin-A/B (ETA/B) dual receptor antagonist SB2096702 affects blood flow changes induced by exercise in rats.

Methods

Animals and Training
Male Wistar rats (10 weeks old; Institute for Animal Reproduction, Ibaraki, Japan) were cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration, 1964. Rats were familiarized with running on treadmill over 2-week period.

Surgical Procedures for Microsphere Technique of Tissue Blood Flow Measurement
Surgical procedures were performed according to the method described by Laughlin et al.1 After the training period, each rat was instrumented with 2 permanently implanted polyurethane catheters in the ascending aorta via the right carotid artery and in the descending aorta via the left renal artery.

Experimental Protocol
Rats were placed on the treadmill and blood pressure and heart rate were monitored. After the intra-arterial administration of TA-02012
Control group

Heart rate, beats/min 349±10 339±9 431±12† 448±11† 463±9†
Systolic blood pressure, mm Hg 116±2 116±2 137±4† 134±3† 133±3†
Diastolic blood pressure, mm Hg 93±3 92±3 108±4† 104±3* 101±3*
Mean blood pressure, mm Hg 103±2 100±2 117±4† 114±3† 112±3†

TABLE 1. Heart Rate and Blood Pressure Before and During Exercise

<table>
<thead>
<tr>
<th></th>
<th>Before infusion</th>
<th>After infusion</th>
<th>10</th>
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<tr>
<td>Control group</td>
<td></td>
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<td>Heart rate, beats/min</td>
<td>349±10</td>
<td>339±9</td>
<td>431±12†</td>
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<td>137±4†</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
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<td>108±4†</td>
<td>104±3*</td>
<td>101±3*</td>
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<tr>
<td>Mean blood pressure, mm Hg</td>
<td>103±2</td>
<td>100±2</td>
<td>117±4†</td>
<td>114±3†</td>
<td>112±3†</td>
</tr>
</tbody>
</table>

†Significantly different vs before infusion, P<0.05.
‡Significantly different vs before infusion, P<0.01.

TA-0201 group

Heart rate, beats/min 334±10 336±7 449±9† 459±10† 475±10†
Systolic blood pressure, mm Hg 112±2 114±2 136±3† 137±2† 136±3†
Diastolic blood pressure, mm Hg 91±3 90±2 105±4† 104±3† 102±3*
Mean blood pressure, mm Hg 98±3 96±2 115±3† 115±2† 113±3†

Values are mean±SEM.
*Significantly different vs before infusion, P<0.05.
†Significantly different vs before infusion, P<0.01.

On completion of exercise, each rat was given pentobarbital sodium (50 mg/kg) for anesthesia. The right kidney, spleen, stomach, intestine, and muscles (tibialis anterior muscle, plantaris, and soleus) were removed.

We also attempted to investigate the interaction of endothelin-receptor antagonist and adrenergic blockade. Under α-adrenergic

TABLE 2. Systemic and Regional Vascular Resistances Before and After Exercise

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise</th>
<th>After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
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<td></td>
</tr>
<tr>
<td>Systemic resistance, mm Hg per mL · min⁻¹ · kg⁻¹</td>
<td>0.53±0.05</td>
<td>0.20±0.02*</td>
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<tr>
<td>Kidney, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>19.4±1.8</td>
<td>94.8±15.3*</td>
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<tr>
<td>Spleen, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>61.1±10.6</td>
<td>376.8±105.1*</td>
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<tr>
<td>Stomach, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>58.3±4.5</td>
<td>422.1±45.1*</td>
</tr>
<tr>
<td>Intestine, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>29.5±1.7</td>
<td>84.5±5.6*</td>
</tr>
<tr>
<td>Tibialis anterior muscle, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>248.6±21.2</td>
<td>61.9±5.4*</td>
</tr>
<tr>
<td>Plantaris, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>220.5±24.1</td>
<td>71.3±5.7*</td>
</tr>
<tr>
<td>Soleus, mm Hg per mL · min⁻¹ · g⁻¹</td>
<td>70.1±3.2</td>
<td>54.4±2.8*</td>
</tr>
</tbody>
</table>

TA-0201 group

Systemic resistance, mm Hg per mL · min⁻¹ · kg⁻¹ 0.49±0.04 0.18±0.01*
Kidney, mm Hg per mL · min⁻¹ · g⁻¹ 19.0±2.1 45.1±4.9*†
Spleen, mm Hg per mL · min⁻¹ · g⁻¹ 54.4±6.2 140.4±15.4*†
Stomach, mm Hg per mL · min⁻¹ · g⁻¹ 58.4±9.2 242.0±35.7*†
Intestine, mm Hg per mL · min⁻¹ · g⁻¹ 30.8±3.9 60.8±6.0*†
Tibialis anterior muscle, mm Hg per mL · min⁻¹ · g⁻¹ 246.1±19.2 98.1±8.3*†
Plantaris, mm Hg per mL · min⁻¹ · g⁻¹ 170.2±8.5 103.4±7.9*†
Soleus, mm Hg per mL · min⁻¹ · g⁻¹ 73.2±4.5 71.3±4.0*†

Values are mean±SEM.
*Significantly different vs before exercise, P<0.01.
†Significantly different vs control group, P<0.05.
‡Significantly different vs control group, P<0.01.
blockade (3 mg/kg phentolamine) or β-adrenergic blockade (6 mg/kg propranolol), however, the rats were unable to complete this 30-minute exercise.

Blood Flow Measurements
The stable isotope-labeled microsphere technique was performed according to the method described by Reinhardt et al.5 The microsphere suspension was injected into the aortic catheter. Simultaneously, a 1.5-minute reference blood sample was drawn at 0.8 mL/min from the renal arterial catheter. To test the adequacy of mixing by the aortic infusion, blood flows to bilaterally paired kidneys were compared in 5 other rats; no significant difference between the right and left kidney was seen (5.73 ± 0.44 versus 5.68 ± 0.48 mL·min⁻¹·g⁻¹, each of five rats). Cardiac output was determined as previously described.6

Data Analysis
Values are expressed as mean ± SEM. To evaluate the differences between before and during exercise, Fisher’s protected least significant difference (PLSD) test for multiple comparisons and Student’s t test for paired values were used. To evaluate the differences between the TA-0201 or SB209670 and control groups, Student’s t test for unpaired values was used. P<0.05 was accepted as significant.

Results
Effects of TA-0201 Infusion on Hemodynamics and Plasma Lactate
During exercise, blood pressure and heart rate increased significantly in both the control and TA-0201 groups, and there were no significant differences in the magnitude of an increase in these parameters between groups (Table 1). Total cardiac output was markedly increased by exercise in both the control and TA-0201 groups, and there was no significant difference between them (Figure 1A and 1B). Systemic vascular resistance was markedly decreased after exercise in both the control and TA-0201 groups, and there was no
The amount of muscle blood flow

A Tibialis anterior muscle  B Plantaris muscle  C Soleus muscle

Figure 3. The amount of muscle (A, tibialis anterior; B, plantaris; and C, soleus) blood flow before and after exercise and the percent changes in muscle (D, tibialis anterior; E, plantaris; and F, soleus) blood flow from resting values after exercise in control group (n=8) and TA-0201 group (n=8). Values are mean±SEM.

††P<0.01 versus control.
**P<0.01 versus before exercise.

significant difference at rest or during exercise between the 2 groups (Table 2). Plasma lactate concentration was markedly increased by exercise in both the control and TA-0201 groups, and there was no significant difference between them (Figure 1C and 1D).

Effects of TA-0201 Infusion on Visceral and Muscle Blood Flows

Visceral blood flow in both the control and TA-0201 groups was markedly decreased by exercise, and the magnitude of the decrease was significantly smaller in the TA-0201 group than controls (Figure 2). Visceral vascular resistance was markedly increased after exercise in both the control and TA-0201 groups, and the magnitude of the increase was significantly smaller in the TA-0201 group than controls (Table 2).

Muscle blood flow in both the control and TA-0201 groups was markedly increased by exercise, and the magnitude of the increase was significantly smaller in the TA-0201 group than controls (Figure 3). After exercise, the muscle vascular resistance was markedly decreased in both the control and TA-0201 groups, and the magnitude of the decrease was significantly smaller in the TA-0201 group than controls (Table 2).

Effects of SB209670 Infusion on Visceral and Muscle Blood Flows

Visceral blood flow in both the control and SB209670 groups was markedly decreased by exercise, and the magnitude of the decrease was significantly smaller in the SB209670 group than controls (Figure 4A through 4D). Muscle blood flow in both the control and SB209670 groups was markedly increased by exercise, and the magnitude of the increase was significantly smaller in the SB209670 group than controls (Figure 4E and 4F).

Discussion

This study revealed that the magnitudes of decrease in the blood flow to visceral organs and increase in the blood flow to active muscles during exercise were significantly depressed by the administration of the ETA-receptor antagonist TA-0201. Therefore, it was suggested that ET-1–mediated vasoconstriction participates in the decrease of blood flow in visceral organs during exercise, thereby contributing to the
increase of blood flow in active muscles during exercise. Indeed, we previously observed that ET-1 mRNA expression was increased in visceral organs such as the kidney by exercise. Thus, the present results and the results from our past studies indicate that ET-1 participates in the exercise-induced redistribution of tissue blood flow.

The magnitude of the exercise-induced changes in visceral and muscle blood flows was of the same degree between the ETA-receptor blockade and ETA/B dual receptor blockade. Although the vascular ETB receptor system may cause constriction or dilation of vessels, the reversal of the redistribution caused by the ETA-receptor blockade was similar in degree to that of ETA/B receptor blockade in the present study.

Active muscles are supplied an efficient blood flow through exercise-induced redistribution of cardiac output, and thereby allow performance of intense exercise. Because the blood flow to active muscles in the TA-0201 group was lower than that in controls, the extent of anaerobic metabolism in active muscles might be more remarkable in the TA-0201 group than in controls. The plasma lactate concentration, however, was the same in the control group and TA-0201 group (4.9 versus 4.8 mmol/L). Because the present exercise intensity might be near the anaerobic threshold level in rats (4.0 mmol/L), it is possible that plasma lactate concentration during a more intense exercise might be higher in the TA-0201 group than in the controls.

ETA-receptor blockade caused no significant change in mean blood pressure and systemic vascular resistance during exercise, although the magnitudes of decrease in visceral blood flow and increase in visceral vascular resistance were significantly depressed by ETA-receptor blockade. On the other hand, the magnitude of decrease in active muscle vascular resistance during exercise was significantly depressed by ETA-receptor blockade. This unexpected finding implies that the ETA-receptor blockade might cause vasoconstriction in active muscle to maintain blood pressure during exercise. The finding that ETA-receptor blockade did not cause a decrease in systemic resistance suggests that alternate endogenous mechanisms caused vasoconstriction to compensate for the loss of ET-1-mediated vasoconstriction in the splanchnic organs, and that this effect was prominent in active skeletal muscle. An increase in adrenergic vasoconstriction after ETA-receptor blockade is a possible mechanism for the increased resistance to blood flow in the active muscle, as adrenergic vasoconstriction is known to compete with metabolic vasodilation.

In conclusion, the data suggest that ET-1 participates in the exercise-induced redistribution of tissue blood flow.

Figure 4. The amount of renal (A), splenic (B), gastric (C), intestinal (D), and muscle (E, tibialis anterior; F, plantaris) blood flow before and after exercise in control group (n=8) and SB209670 group (n=6). Values are mean±SEM. †P<0.05, ††P<0.01 versus control. **P<0.01 versus before exercise.
Acknowledgments
This work was supported by grants-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan (00006781, 11480003, 11557047, 12470417, 12670646) and a grant from University of Tsukuba Research Projects.

References
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Circulation. 2002;106:2188-2193
doi: 10.1161/01.CIR.0000038362.16740.A2
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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