Tempol attenuates the exercise pressor reflex independently of neutralizing reactive oxygen species in femoral artery ligated rats

Jennifer L. McCord, Hirotsugu Tsuchimochi, Katsuya Yamauchi, Anna Leal, and Marc P. Kaufman

Penn State Heart and Vascular Institute, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

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McCord JL, Tsuchimochi H, Yamauchi K, Leal A, Kaufman MP. Tempol attenuates the exercise pressor reflex independently of neutralizing reactive oxygen species in femoral artery ligated rats. J Appl Physiol 111: 971–979, 2011. First published July 7, 2011; doi:10.1152/japplphysiol.00535.2011.—In decerebrate rats, we reported previously that the exercise pressor reflex arising from a limb whose femoral artery was occluded for 72 h before the experiment was significantly higher than the exercise pressor reflex arising from a contralaterally freely perfused limb. These findings prompted us to examine whether reactive oxygen species contributed to the augmented pressor reflex in rats with femoral artery occlusion. We found that the pressor reflex arising from the limb whose femoral artery was occluded for 72 h before the experiment (31 ± 5 mmHg) was attenuated by tempol (10 mg), a superoxide dismutase (SOD) mimetic (18 ± 5 mmHg, n = 9, P < 0.05), that was injected into the arterial supply of the hindlimb. In contrast, the pressor reflex arising from a freely perfused hindlimb (20 ± 3 mmHg) was not attenuated by tempol (17 ± 4 mmHg, n = 10, P = 0.49). Nevertheless, we found no difference in the increase in 8-isoprostaglandin F2α levels, an index of reactive oxygen species, in response to contraction between freely perfused (3.76 ± 0.82 pg/ml, n = 19) and 72-h occluded (3.51 ± 0.92 pg/ml, n = 22, P = 0.90) hindlimbs. Moreover, tempol did not reduce the 8-isoprostaglandin F2α, levels during contraction in either group (P > 0.30). A second SOD mimetic, tiron (200 mg/kg), had no effect on the exercise pressor reflex in either the rats with freely perfused hindlimbs or in those with occluded femoral arteries. These findings suggest that tempol attenuated the exercise pressor reflex in the femoral artery-occluded hindlimb by a mechanism that was independent of its ability to scavenge reactive oxygen species.

The term reactive oxygen species (ROS) refers to cascade of free oxygen radicals that begins with the superoxide anion, which in turn is neutralized by superoxide dismutase (36). ROS may play a role in augmenting the exercise pressor reflex seen in peripheral arterial disease. In fact, ROS have been shown to stimulate group IV muscle afferents, an effect which was attenuated by a superoxide dismutase mimetic (8). Moreover, ROS production was found to increase when skeletal muscles were contracted (8, 35). These findings prompted us to determine if ROS play a role in evoking the exercise pressor reflex in rats whose hindlimbs were freely perfused and in rats whose femoral arteries were ligated 72 h before the start of the experiment. We tested the hypothesis that ROS played a role in evoking the pressor response to contraction in rats whose femoral arteries were ligated, but did not play a role in evoking the reflex in rats whose femoral arteries were patent.

METHODS

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University, Hershey Medical Center. Adult male rats (Sprague-Dawley, n = 73 weighing between 345 and 510 g) were used in this study. The rats were housed in a temperature-controlled room (24 ± 1°C) with a 12:12-h light-dark cycle. Rats were fed a standard diet and tap water ad libitum. Seventy-two hours before an experiment, 37 of 73 rats underwent surgery to induce unilateral femoral artery occlusion according to the procedure described previously (34, 49). Briefly, rats were anesthetized with a mixture of 4% isoflurane balanced with oxygen; one femoral artery was isolated and then tightly ligated with 5–0 silk suture just distal to the inguinal ligament. Using radiolabeled microspheres, it has been shown that this femoral artery ligation procedure reduced blood flow reserve capacity to ~10–20% of normal but allowed sufficient blood flow to meet resting requirements (50). The rats recovered for 72 h before the experiments were started. Femoral artery occlusion has been reported to have no effect on normal cage activity (39).

Surgical Preparation

On the day of the experiment, rats were anesthetized with a mixture of 4% isoflurane and 100% oxygen. The right jugular vein and common carotid artery were cannulated for the delivery of drugs and fluids and the measurement of arterial blood pressure, respectively. The carotid arterial catheter was connected to a pressure transducer (model P23 XL, Statham). Heart rate was calculated beat to beat from

Address for reprint requests and other correspondence: M. P. Kaufman, Penn State Heart and Vascular Institute, 500 Univ. Dr., Mail Code H047, Hershey Medical Center, Hershey, PA 17033 (e-mail: mkaufman@hmc.psu.edu).

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the arterial pressure pulse (Gould Biotach). The trachea was cannula-
ted, and the lungs were ventilated mechanically (Harvard Appara-
tus). Arterial blood gases and pH were measured by an automated
blood-gas analyzer (model ABL-700, Radiometer). PCO2 and arterial
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be contracted. The inflation was maintained for 5 min, after which it
injection of tiron to trap the injectate in the circulation of the limb to
the vascular occluder was inflated before injection of tempol to trap
that mimics the enzymatic activity of superoxide dismutase,
Exclusion of the dose used in these previous studies, which allowed tiron to
increase in muscle interstitial concentrations of 8-isoprostaglandin
in response to a 60-s static contraction before and after administering
tempol (200 mg/kg iv). Tempol was injected over a 10-min period and
causation of oxidative stress, immediately before and during a 60-s static contraction. In 20 rats, we measured the increase in interstitial concentrations of 8-isoprostaglandin F2α, in response to a 60-s static contraction before and after administering tempol (200 mg/kg iv). Tempol was injected over a 10-min period and caused a marked attenuation in baseline blood pressure. We waited until mean arterial pressure increased as close as possible to baseline blood pressure before initiating contraction again, usually 10–20 min after the start of the tempol injection. Muscle microdialysate samples were taken during both the baseline and contraction periods before and after injecting tempol. Dialysate samples were stored at −80°C until analysis with a commercially available enzyme immunoassay kit (8-isoprostaglandin F2α, Cayman Chemical, Ann Arbor, MI). 8-Isoprostaglandin F2α concentrations are expressed in picograms per milliliter. We did not measure interstitial concentration of 8-isoprostaglandin F2α, in response to tendon stretch.
To collect enough muscle interstitial fluid to run the enzyme immunoassay kit in triplicate for each sample, we needed eight probes inserted between the two triceps surae muscles and a collection period of 60 s. Because both hindlimbs would be in need of drug injection, we were not able to insert a femoral artery catheter as we did in the reflex protocol (see above). Therefore, to inject tempol into the circulation of both hindlimbs we injected this substance through the jugular vein catheter. In five rats, we injected blue dye into the jugular vein catheter and both triceps surae muscles turned blue, suggesting that the intravenous tempol injection reached the two hindlimbs. We did not determine the effect on tiron on the increase in muscle interstitial concentrations of 8-isoprostaglandin F2α, that was evoked by contraction.

In 19 rats, we injected tempol (10 mg; Sigma-Aldrich), a com-
pound that mimics the enzymatic activity of superoxide dismutase, retrogradely into contralateral femoral artery catheter. In these rats, the vascular ocluder was inflated before injection of tempol to trap the injectate in the circulation of the limb to be contracted. The inflation was maintained for 5 min, after which it was deflated and the hindlimb was reperfused. The dose of tiron used was based on previous experiments in which this agent was infused intravenously in a dose of 1 g/kg per hour (11, 40, 46). Because we injected this agent intravenously into one hindlimb, we used one-fifth of the dose used in these previous studies, which allowed tiron to travel throughout the entire body.

In 41 rats, we measured interstitial concentrations of 8-isoprostaglandin F2α (also known as 8-isoprostane, 8-epiprostaglandin F2α, iPF2α-III or 15-F2-Isop), an index of oxidative stress, immediately before and during a 60-s static contraction. In 20 rats, we measured the increase in interstitial concentrations of 8-isoprostaglandin F2α, in response to a 60-s static contraction before and after administering tempol (200 mg/kg iv). Tempol was injected over a 10-min period and caused a marked attenuation in baseline blood pressure. We waited until mean arterial pressure increased as close as possible to baseline blood pressure before initiating contraction again, usually 10–20 min after the start of the tempol injection. Muscle microdialysate samples were taken during both the baseline and contraction periods before and after injecting tempol. Dialysate samples were stored at −80°C until analysis with a commercially available enzyme immunoassay kit (8-isoprostaglandin F2α, Cayman Chemical, Ann Arbor, MI). 8-Isoprostaglandin F2α concentrations are expressed in picograms per milliliter. We did not measure interstitial concentration of 8-isoprostaglandin F2α, in response to tendon stretch.
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Intravenous control. In 12 rats, tempol (10 mg) was injected into the jugular vein to test whether our findings with intra-arterial injec-
tion of tempol could be explained by its circulation to the spinal cord.
Static contraction and tendon stretch were evoked at least 15 min after
the injection. All contractions lasted for 30 s.

Microdialysis protocol. Microdialysis can be used to introduce and
remove ions, molecules, and drugs of interest to, or from, the inter-
istitial space of skeletal muscle (12). We manufactured microdialysis
probes by gluing both ends of a 2-cm length of capillary microdialysis
membrane (0.20 mm in diameter, with a 13-kDa molecular cutoff)
into nylon tubing. The nylon tubing was attached to a Luer tip adapter
stub that connected the probe and the perfusate-filled syringe. Each rat
had four microdialysis fibers placed in each triceps surae muscles, for
total of eight fibers; the fibers were separated by ~0.25 cm. The
probes were inserted into the muscles via a 20-gauge cannula inserted
parallel to muscle fiber orientation. The insertion and exit points in
and out of the muscle were ~3 cm apart. The microdialysis probe was
threaded through the internal lumen of the needle. The needle was
withdrawn, leaving the membrane in place. The luer tip adapter stub
was attached to a syringe for administration of saline through a
perfusion pump (model 402, CMA) at 20 μl/min. After insertion of
the eight microdialysis probes, we waited 2 h for equilibration.

The tibial nerve was placed on shielded stimulating electrodes. Each
calcaneal tendon was attached to a force transducer (model FT 10,
Grass), which in turn was attached to a rack-and-pinion. The tendon was
stretched so that baseline tension was set between 80 and 110 g. Static contraction was evoked by electrically stimulating (40 Hz, 0.025 ms, ~2 times motor threshold) the tibial nerve. Muscle contraction lasted for 60 s.

Experimental Protocols

Reflex protocol. The peripheral cut ends of the L4 and L5 ventral
roots were placed on shielded stimulating electrodes. Each calcaneal
tendon was attached to a force transducer (model FT 10, Grass),
which in turn was attached to a rack-and-pinion. The tendon was
stretched so that baseline tension was set between 80 and 110 g. Static contraction was evoked by electrically stimulating (40 Hz, 0.025 ms, ~2 times motor threshold) the cut peripheral ends of the L4 and L5 ventral roots. A muscle mechanoreceptor reflex was evoked by
stretching the triceps surae muscles by manually turning the rack-and-
pinion that was attached to the calcaneal tendon (38). Baseline tension
was set between 80 and 110 g. Both muscle contraction and tendon
stretch lasted for 30 s. The order of presentation of the two stimuli was
varied randomly.

In 19 rats, we injected tempol (10 mg; Sigma-Aldrich), a com-
pound that mimics the enzymatic activity of superoxide dismutase, retrogradely into contralateral femoral artery catheter. In these rats, the vascular ocluder was inflated before injection of tempol to trap the injectate in the circulation of the limb to be contracted. The inflation was maintained for 5 min, after which it was deflated and the hindlimb was reperfused. The dose of tempol used in our experiments was identical to that used by Koba et al. (22) and about twice that used by Wang et al. (43). Tempol tends to decrease arterial pressure
below baseline levels. Arterial pressure would then increase back to
baseline within 10–15 min. Thus we waited for a steady arterial pressure before evoking static contraction and tendon stretch again.

In 12 other rats, we injected tiron (0.2 g/kg; Sigma-Aldrich), another compound that mimics the enzymatic activity of superoxide dismutase, retrogradely into the contralateral femoral artery catheter. Like the rats given tempol, the vascular ocluder was inflated before
injection of tiron to trap the injectate in the circulation of the limb to
be contracted. The inflation was maintained for 5 min, after which it

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contraction increased mean arterial pressure and heart rate.

Effect of Tempol on Responses to Static Contraction

RESULTS

significance was set at

Then post hoc tests were performed with the ANOVA, two-way repeated-measures ANOVA, or a linear mixed-comparisons were performed with either one-way repeated-measures ANOVA, two-way repeated-measures ANOVA, or a linear mixed-effects model ANOVA. Statistical comparisons were performed with either one-way repeated-measures ANOVA, two-way repeated-measures ANOVA, or a linear mixed-effects model ANOVA. Then post hoc tests were performed with the Tukey test between individual means. The criterion for statistical significance was set at \( P < 0.05 \).

Data Analysis

Arterial blood pressure, heart rate, and muscle tension were recorded with a Spike 2 data acquisition system (CED, Cambridge). Mean arterial pressure (MAP) is expressed in millimeters Hg and heart rate (HR) in beats per minute (bpm). The initial 30-s values were used to compare the differences between baseline and the response to each maneuver. The tension-time index (TTI) was calculated by integrating the area between the tension trace and the baseline level within vertical bars. Means \( \pm \) SE. *Significant difference (\( P < 0.05 \)) between baseline and peak. †Significant difference (\( P < 0.05 \)) between before and after tempol. ‡Significant difference (\( P < 0.05 \)) between freely perfused and 72-h ligated.

Fig. 1. The pressor (A) and cardioaccelerator (B) responses to static contraction before (filled bars) and after intra-arterial injection of 10 mg of tempol (open bars) in rats whose hindlimbs were freely perfused and whose femoral arteries were ligated. Baseline mean arterial pressure and heart rate values are shown within vertical bars. Means \( \pm \) SE. *Significant difference (\( P < 0.05 \)) between baseline and peak. †Significant difference (\( P < 0.05 \)) between before and after tempol. ‡Significant difference (\( P < 0.05 \)) between freely perfused and 72-h ligated.

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Effect of Tempol on Responses to Stretch

In eight rats whose hindlimbs were freely perfused, tendon stretch increased mean arterial pressure above baseline levels both before and after tempol (\( P < 0.05 \); Fig. 2A). The magnitude of the pressor response to tendon stretch was attenuated by tempol (\( P < 0.05 \); Fig. 2A). Tendon stretch increased heart rate above baseline levels before tempol (\( P < 0.05 \)) but not after tempol (\( P = 0.07 \); Fig. 2B). The tendon stretch tension-time index was not different before and after tempol (\( P = 0.18 \); Table 1).

We found that the pressor response to tendon stretch was greater in rats whose femoral artery had been ligated 72 h before the start of the experiment (\( n = 8 \)) than that in rats whose hindlimbs were freely perfused (\( P < 0.05 \); Fig. 2A). There was no difference in the cardioaccelerator response to stretch between the two groups (\( P = 0.89 \); Fig. 2B). In the rats whose femoral artery was ligated, stretch increased mean arterial pressure above baseline levels both before and after tempol.

Table 1. The tension-time indexes for static contraction and tendon stretch in rats whose hindlimbs were freely perfused and in rats whose femoral artery had been ligated 72 h before the start of the experiment before and after intra-arterial tempol (10 mg) or tiron (0.2 g/kg) injection

<table>
<thead>
<tr>
<th>Group</th>
<th>TTI, kg·s</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td><strong>Tempol</strong></td>
<td></td>
</tr>
<tr>
<td>Freely perfused group</td>
<td></td>
</tr>
<tr>
<td>Static contraction</td>
<td>10</td>
</tr>
<tr>
<td>Tendon stretch</td>
<td>8</td>
</tr>
<tr>
<td>Ligated (72 h) group</td>
<td></td>
</tr>
<tr>
<td>Static contraction</td>
<td>9</td>
</tr>
<tr>
<td>Tendon stretch</td>
<td>8</td>
</tr>
<tr>
<td><strong>Tiron</strong></td>
<td></td>
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<tr>
<td>Freely perfused group</td>
<td></td>
</tr>
<tr>
<td>Static contraction</td>
<td>6</td>
</tr>
<tr>
<td>Tendon stretch</td>
<td>6</td>
</tr>
<tr>
<td>Ligated (72 h) group</td>
<td></td>
</tr>
<tr>
<td>Static contraction</td>
<td>6</td>
</tr>
<tr>
<td>Tendon stretch</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n \); no. of rats. TTI, tension-time index. *\( P < 0.05 \), after vs. before tempol.
tempol \((P < 0.05; \text{Fig. 2A})\). However, the magnitude of the pressor response to stretch was attenuated by tempol \((P < 0.05; \text{Fig. 2B})\). The magnitude of the cardioaccelerator response to stretch was not attenuated by tempol \((P = 0.51; \text{Fig. 2B})\). The tendon stretch tension-time index was slightly higher after tempol \((P < 0.05; \text{Table 1})\).

### Intravenous Control for Tempol

We found that intravenous injection of tempol had no effect on the pressor responses to static contraction in rats whose hindlimbs were freely perfused \((n = 6)\) or in rats whose hindlimbs had been ligated 72 h before the start of the experiment \((n = 6)\) \((P > 0.74; \text{Table 2})\). In two of the six freely perfused rats, intravenous injection of tempol attenuated the pressor response to contraction.

In addition, we found that intravenous injection of tempol had no effect on the pressor responses to tendon stretch in rats whose hindlimbs were freely perfused \((n = 6)\) or in rats whose hindlimbs had been ligated 72 h before the start of the experiment \((n = 5)\) \((P > 0.12; \text{Table 3})\). In two of the six freely perfused rats and in one of the five ligated rats, intravenous injection of tempol attenuated the pressor response to stretch. In the rats whose hindlimbs were freely perfused, tempol decreased baseline heart rate and the peak cardioaccelerator and pressor responses to stretch \((P < 0.05; \text{Table 3})\).

### Effect of Tiron on Responses to Contraction

In six rats whose hindlimbs were freely perfused, static contraction increased mean arterial pressure and heart rate above baseline levels both before and after tiron \((P < 0.05; \text{Fig. 3A})\). The magnitudes of the pressor and cardioaccelerator responses to contraction were not attenuated by tiron \((P > 0.19; \text{Fig. 3})\). The static contraction tension-time index was not different before and after tiron \((P = 0.48; \text{Table 1})\).

We found that the pressor response to static contraction was greater in rats whose femoral artery had been ligated 72 h before the start of the experiment \((n = 6)\) than that in rats whose hindlimbs were freely perfused \((P < 0.05; \text{Fig. 3A})\). There was no difference in the cardioaccelerator response to contraction between the two groups \((P = 0.55; \text{Fig. 3B})\). In the rats whose femoral artery was ligated, static contraction increased mean arterial pressure above baseline levels both before and after tiron \((P < 0.05; \text{Fig. 3A})\). Unlike tempol, however, the magnitude of the pressor response to contraction was not attenuated by tiron \((P = 0.74; \text{Fig. 3A})\). The magnitude of the cardioaccelerator response to contraction was not attenuated by tiron \((P = 0.55; \text{Fig. 3B})\). The static contraction tension-time index was not different before and after tiron \((P = 0.52; \text{Table 1})\).

### Table 2. Baseline and peak responses in MAP, HR, and TTI for static contraction in rats whose hindlimbs were freely perfused and in rats whose femoral artery had been ligated 72 h before the start of the experiment before and after intravenous tempol injection

|                  | MAP, mmHg | HR, beats/min | TTI, kg·s
|------------------|------------|---------------|----------------
|                  | \(n\) Base | Peak | \(\Delta\) | \(n\) Base | Peak | \(\Delta\) |                      |
| **Freely perfused group** |            |       |          |            |       |          |                      |
| Control          | 6          | 85 \pm 4 | 102 \pm 5* | 17 \pm 2   | 414 \pm 18 | 433 \pm 17* | 19 \pm 3 | 21.8 \pm 2.5 |
| Tempol (10 mg iv)| 6          | 77 \pm 5 | 93 \pm 7* | 16 \pm 2   | 388 \pm 14 | 409 \pm 14* | 21 \pm 4 | 22.8 \pm 2.4 |
| **Ligated (72 h) group** |            |       |          |            |       |          |                      |
| Control          | 6          | 92 \pm 5 | 129 \pm 11* | 37 \pm 7† | 396 \pm 17 | 424 \pm 13* | 27 \pm 8 | 19.8 \pm 1.7 |
| Tempol (10 mg iv)| 6          | 95 \pm 4 | 131 \pm 6* | 36 \pm 5† | 348 \pm 55 | 368 \pm 56* | 20 \pm 5 | 18.5 \pm 1.6 |

Values are means \(\pm\) SE, \(n\), no. of rats. MAP, mean arterial pressure; HR, heart rate. *\(P < 0.05\), peak vs. baseline; †\(P < 0.05\), 72 h ligated vs. freely perfused.
Effect of Tiron on the Responses to Stretch

In six rats whose hindlimbs were freely perfused, tendon stretch increased mean arterial pressure and heart rate above baseline levels both before and after tiron ($P < 0.05$; Fig. 4). The magnitudes of the pressor ($P = 0.74$) and cardioaccelerator ($P = 1.00$) responses to tendon stretch were not attenuated by tiron (Fig. 4). The tendon stretch tension-time index was not different before and after tiron ($P = 0.08$; Table 1).

We found that the pressor response to tendon stretch was greater in rats whose femoral artery was ligated 72 h before the

Table 3. Baseline and peak responses in MAP, HR, and TTI for tendon stretch in rats whose hindlimbs were freely perfused and in rats whose femoral artery had been ligated 72 h before the start of the experiment before and after intravenous tempol injection

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>TTI, kg·s</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Baseline</td>
<td>Peak</td>
</tr>
<tr>
<td>Freely perfused group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>87 ± 3</td>
<td>102 ± 4*</td>
</tr>
<tr>
<td>Tempol (10 mg iv)</td>
<td>6</td>
<td>77 ± 7</td>
<td>89 ± 8*</td>
</tr>
<tr>
<td>Ligated (72 h) group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>96 ± 8</td>
<td>119 ± 14*</td>
</tr>
<tr>
<td>Tempol (10 mg iv)</td>
<td>5</td>
<td>93 ± 5</td>
<td>113 ± 9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of rats. *$P < 0.05$, peak vs. baseline; †$P < 0.05$, control vs. intravenous tempol within same time point.

Effect of Tiron on the Responses to Stretch

In six rats whose hindlimbs were freely perfused, tendon stretch increased mean arterial pressure and heart rate above baseline levels both before and after tiron ($P < 0.05$; Fig. 4). The magnitudes of the pressor ($P = 0.74$) and cardioaccelerator ($P = 1.00$) responses to tendon stretch were not attenuated by tiron (Fig. 4). The tendon stretch tension-time index was not different before and after tiron ($P = 0.08$; Table 1).

We found that the pressor response to tendon stretch was greater in rats whose femoral artery was ligated 72 h before the
start of the experiment \((n = 6)\) than it was in the rats \((n = 6)\) whose hindlimbs were freely perfused \((P < 0.05; \text{Fig. 4A})\). There was no difference in the cardioaccelerator response to stretch between the two groups \((P = 0.50; \text{Fig. 4B})\). In the rats whose femoral artery was ligated, stretch increased mean arterial pressure above baseline levels both before and after tiron \((P < 0.05; \text{Fig. 4A})\). Unlike the effect of tempol, the magnitude of the pressor response to stretch was not attenuated by tiron \((P = 0.74; \text{Fig. 4A})\). Likewise, the magnitude of the cardioaccelerator response to stretch was not attenuated by tiron \((P = 0.16; \text{Fig. 4B})\). The tendon stretch tension-time index was no different before and after tiron \((P = 0.16; \text{Table 1})\).

8-Isoprostane Concentrations

In 19 rats whose hindlimbs were freely perfused, static contraction increased the concentration of 8-isoprostaglandin \(F_{2\alpha}\) in muscle interstitial fluid above its baseline level \((P < 0.05; \text{Fig. 5})\). Likewise, in 22 rats whose femoral artery had been ligated 72 h before the start of the experiment, static contraction increased the concentration of 8-isoprostaglandin \(F_{2\alpha}\) in muscle interstitial fluid above its baseline level \((P < 0.05; \text{Fig. 5})\). However, there was not a difference in the magnitude of the increase between the two groups of rats \((P = 0.89; \text{Fig. 5})\). The tension-time index was not different between freely perfused \((12.6 \pm 1.1 \text{ kg·s})\) and 72-h-ligated \((11.0 \pm 0.8 \text{ kg·s}; P = 0.14)\) groups.

Ten of the 19 freely perfused and 10 of the 22 ligated rats were further tested with static contraction after tempol had been administered. Tempol did not attenuate the magnitude of 8-isoprostaglandin \(F_{2\alpha}\) increase evoked by contraction in either the freely perfused or the ligated hindlimbs \((P > 0.30; \text{Fig. 6})\). The tension-time indexes were not different before and after tempol in either group (freely perfused: before 13.8 ± 1.2, tempol 13.4 ± 0.9 \text{ kg·s}; 72 h ligated: before 11.4 ± 1.1, tempol 11.9 ± 1.0 \text{ kg·s}; \(P > 0.45\)).

**DISCUSSION**

We tested the hypothesis that oxidative stress played a greater role in evoking the exercise pressor reflex in rats whose femoral arteries were ligated 72 h before the start of the experiment than it did in evoking the reflex in rats whose hindlimb muscles were freely perfused. We attempted to reduce oxidative stress by injecting either tempol or tiron, both of which are superoxide dismutase mimetics, into the arterial circulation of the muscles being contracted. We found that tempol, but not tiron, attenuated the exercise pressor reflex in the rats whose femoral arteries were ligated; neither of the two mimetics attenuated the reflex in rats whose hindlimbs were freely perfused. Despite the fact that tempol attenuated the exercise pressor reflex in rats whose femoral arteries were occluded, this superoxide dismutase mimetic did not significantly reduce muscle interstitial levels of 8-isoprostaglandin \(F_{2\alpha}\), an index of oxidative stress, during rest or contraction in either group of rats. Taken together, these findings suggest that tempol attenuated the exercise pressor reflex in the rats whose femoral arteries were ligated by a mechanism other than that which reduced oxidative stress.

The mechanism by which tempol attenuated the exercise pressor reflex in rats whose femoral arteries were ligated may involve potassium channels. Specifically, tempol has been found to open calcium-activated potassium \((\text{BK})\) \((45, 48)\) and ATP-sensitive potassium \((\text{K}_{\text{ATP}})\) channels \((4)\), both of which are superoxide dismutase mimetics, into the arterial circulation of the muscles being contracted. We found that tempol, applied topically, decreased renal sympathetic nerve activity \((37)\). Likewise, this mechanism may have been responsible for the vasodilation and subsequent decrease in arterial blood pressure caused by tempol \((45, 47)\). In our experiments, this mechanism may have been responsible for the attenuation of the exercise pressor reflex induced by tempol in rats with occluded femoral arteries. Moreover, this mechanism is consistent with our finding that tiron, a superoxide dismutase mimetic that does not open potassium channels \((48)\) and that does not relax vascular smooth muscle \((45)\), had no effect on the exercise pressor reflex in the rats whose femoral arteries were occluded.

We can only speculate as to why tempol in our experiments attenuated the exercise pressor reflex in rats with femoral artery
contraction-induced production of 8-isoprostaglandin F2α. We found that ligating the femoral artery had no effect on the finding which was confirmed in our experiments. Nevertheless, the occluder was released, and was followed by a 10-min period of inactivity. In contrast, Wang et al. (43) continuously infused tempol into the femoral artery and made no attempt to trap it in the vasculature of the hindlimb. In our study, the circulation was occluded only during the 5-min injection period, a duration that was far too short to produce an ischemia-induced increase in superoxide radicals, which in turn might reduce the effectiveness of tempol. Indeed, when the resting anterior tibialis muscles of rats were rendered ischemic for 4 h, no increase in superoxide radicals was found in the interstitial fluid taken from this muscle during either the ischemic or the subsequent reperfusion periods (32). Even more importantly, the continuous intra-arterial infusion of tempol, such as that done by Wang et al. (43), might have, by opening potassium channels, hyperpolarized group III and IV muscle afferents to a far greater extent than did tempol infusion in either our experiments or those of Koba et al. (22). Consequently, these thin fiber muscle afferents may have been less responsive to contraction in the experiments reported by Wang et al. (43) than they were in either ours or those of Koba et al. (22). Clearly, decreasing the excitability of the afferents would result in an attenuated exercise pressor reflex.

Exercise is well known to increase production of reactive oxygen species within skeletal muscle (9, 18, 24, 30, 35), a finding which was confirmed in our experiments. Nevertheless, we found that ligating the femoral artery had no effect on the contraction-induced production of 8-isoprostaglandin F2α, our index of oxidative stress. This latter finding might appear at first glance to conflict with that reported by Judge et al. who reported that contraction of the gastrocnemius and soleus muscles in rats with ligated femoral arteries increased oxidative stress to a greater extent than did contraction of these muscles in rats whose femoral arteries were patent (14, 15). Judge et al., however, contracted the muscles forcefully and measured oxidative stress 1 h after their 30-min intermittent contraction protocol ended, their purpose being to damage the muscles. In contrast, we contracted the muscles for only 30 s, our purpose being to determine if oxidative stress played a role in the exaggeration of the exercise pressor reflex that was caused by ligating the femoral artery. This exaggeration could only occur during the 30-s contraction period. What happened 1 h afterward was not relevant to our purpose.

8-Isoprostaglandin F2α can be generated by free radical-induced peroxidation of arachidonic acid, a reaction that is believed to be independent of cyclooxygenase, whose activity is blocked by the administration of nonsteroidal anti-inflammatory drugs such as indomethacin or meclofenamate (7, 23, 29). Although 8-isoprostaglandin F2α can be generated by a cyclooxygenase pathway, the magnitude of effect is small and in some assays nonexistent (2, 17, 21, 33). Moreover, nonsteroidal, such as indomethacin and meclofenamate, appear to have antioxidant effects that are independent of their ability to block cyclooxygenase (17). 8-Isoprostaglandin F2α concentrations have been found to be increased in numerous instances of oxidative stress and are generally accepted to be a good index of oxidative stress (13, 16, 28, 29). Specifically, the Biomarkers and Oxidative Stress Study concluded that “the lipid degradation products, such as 8-isoprostaglandin F2α, primarily constitute markers of oxidative stress” (17).

Any interpretation of our findings must be viewed with three limitations in mind. First, even though the interval between the start of either tempol or tiron injection and contraction was only 15 min, the possibility exists that the antioxidant effect of the two superoxide dismutase mimetics was either not present or reduced when we contracted the hindlimb muscles. Nevertheless, tempol in our experiments was still capable of attenuating the exercise pressor reflex in the rats whose femoral arteries were ligated, a finding that might be interpreted as evidence that tempol was still active, although the effect might have been on potassium channels rather than superoxide ions. Second, our measurement of oxidative stress was taken for the entire contraction period of 60 s and as result did not allow us to determine the time course of the effect. Third, although tempol reduces superoxide radicals, it also increases hydrogen peroxide, which in turn can form hydroxyl radicals by Fenton’s reaction. Therefore, we cannot rule out the possibility that tempol’s inability to reduce 8-isoprostaglandin F2α concentrations in our experiments was caused by a compensatory increase in hydroxyl radicals.

In summary, even though ROS are known to stimulate unmyelinated afferents innervating either hindlimb muscle or skin (8, 10), they do not appear to be responsible for the exaggerated exercise pressor reflex evoked by static contraction of hindlimb muscles with a restricted arterial blood supply. We come to this conclusion because static contraction in our experiments increased muscle interstitial concentrations of 8-isoprostaglandin F2α, an index of ROS, to the same extent in rats whose femoral arteries were ligated as it did in rats whose femoral arteries were patent. Perhaps the search for the cause of the exaggerated exercise pressor reflex in this preparation should shift from ROS to a change in number or types of receptors on the endings of the group III and IV muscle afferents whose stimulation by contraction comprise the afferent arm of the exercise pressor reflex.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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