Cyclooxygenase blockade attenuates responses of group III and IV muscle afferents to dynamic exercise in cats

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Submitted 2 December 2005; accepted in final form 3 January 2006

Hayes, Shawn G., Angela E. Kindig, and Marc P. Kaufman. Cyclooxygenase blockade attenuates responses of group III and IV muscle afferents to dynamic exercise in cats. Am J Physiol Heart Circ Physiol 290: H2239–H2246, 2006. First published January 6, 2006; doi:10.1152/ajpheart.01274.2005.—Cyclooxygenase products accumulate in statically contracting muscles to stimulate group III and IV afferents. The role played by these products in stimulating thin fiber muscle afferents during dynamic exercise is unknown. Therefore, in decerebrated cats, we recorded the responses of 17 group III and 12 group IV triceps surae muscle afferents to dynamic exercise, evoked by stimulation of the mesencephalic locomotor region. Each afferent was tested while the muscles were freely perfused and while the circulation to the muscles was occluded. The increases in group III and IV afferent activity during dynamic exercise while the circulation to the muscles was occluded were greater than those during exercise while the muscles were freely perfused (P < 0.01). Indomethacin (5 mg/kg iv), a cyclooxygenase blocker, reduced the responses to dynamic exercise of group III afferents by 42% when the circulation to the triceps surae muscles was occluded (P < 0.001) and by 29% when the circulation was not occluded (P = 0.004). Likewise, indomethacin reduced the responses to dynamic exercise of group IV afferents by 34% when the circulation was occluded (P < 0.001) and by 18% when the circulation was not occluded (P = 0.026). Before indomethacin, the activity of the group IV, but not group III, afferents was significantly higher during postexercise circulatory occlusion than during rest (P < 0.05). After indomethacin, however, group IV activity during postexercise circulatory occlusion was not significantly different from group IV activity during rest. Our data suggest that cyclooxygenase products play a role both in sensitizing group III and IV afferents during exercise and in stimulating group IV afferents during postexercise circulatory occlusion.

Moderate dynamic exercise increases cardiac output, ventilation, and mean arterial blood pressure (30). Two neural mechanisms, namely, central command and the exercise pressor reflex, have been proposed to cause these effects. The first mechanism, central command, consists of the parallel activation of the cardiovascular and respiratory systems as well as the motor pathways that induce locomotion (5, 36). The second mechanism, the exercise pressor reflex, arises from mechanical and metabolic stimuli that activate group III and IV afferents in the working muscles (14, 19, 22). Mechanical stimuli appear to be transduced primarily by group III muscle afferents, whereas metabolic stimuli appear to be transduced primarily by group IV muscle afferents (1, 2, 10).

Static and rhythmic contractions, induced by electrical stimulation of ventral roots or peripheral nerves, have provided us with important information about the discharge properties of group III and IV muscle afferents (16, 17, 20). Nevertheless, contractions evoked by electrical stimulation of muscle nerves should not be considered “exercise” for three reasons. First, during electrical stimulation of ventral roots or peripheral nerves, α-motoneurons with the fastest conduction velocities are recruited first, whereas during exercise, α-motoneurons with the fastest conduction velocities are recruited last (11). Second, during electrical stimulation of ventral roots or peripheral nerves, α-motoneurons discharge synchronously, whereas during exercise, α-motoneurons discharge asynchronously (13). Third, contraction induced by stimulation of ventral roots or muscle nerves evokes muscle tetany, a condition that can be considered noxious. Consequently, the responses of group III and IV afferents to intermittent static contraction induced by peripheral nerve or ventral root stimulation may be different from the responses of these afferents to dynamic exercise.

Cyclooxygenase products of arachidonic acid metabolism are produced by contracting skeletal muscles (12, 23, 28, 32). These products are likely to have two effects on the discharge properties of group III and IV muscle afferents during exercise. First, they can stimulate these afferents to discharge (18, 27). Second, they can sensitize group III and IV afferents, lowering their thresholds to mechanical and metabolic stimuli arising in the exercising muscles (26, 27, 29). Although there is evidence that cyclooxygenase products sensitize the muscle mechanoreflex in humans (21) as well as evoke part of the exercise pressor reflex in cats (31), there is no electrophysiological evidence that cyclooxygenase products affect the responses of group III and IV muscle afferents to either dynamic exercise or postexercise circulatory occlusion.

We tested the hypothesis, therefore, that cyclooxygenase products of arachidonic acid metabolism increase the responsiveness of group III and IV afferents to dynamic exercise and that these responses will be attenuated by indomethacin, a cyclooxygenase blocker. We further hypothesized that cyclooxygenase products of arachidonic acid metabolism stimulate group IV, but not group III, afferents during postexercise circulatory occlusion. We tested these hypotheses by recording the impulse activity of group III and IV muscle afferents during dynamic exercise while the circulation to the working muscles was occluded and while the circulation to the muscles was intact both before and after the administration of indomethacin.

METHODS

General

The Institutional Animal Care and Use Committee of the University of California, Davis, approved all procedures. Twenty-eight adult...
cats, weighing between 2.5 and 4.5 kg, were anesthetized by inhalation of a mixture of halothane (5%) and oxygen. The trachea was cannulated, and the lungs were ventilated with the anesthetic gas mixture. A common carotid artery and an external jugular vein were cannulated for monitoring blood pressure and administering fluids, respectively. Arterial blood pressure was measured by connecting the carotid arterial cannula to a Statham P23 XL transducer. Arterial Po2, Pco2, and pH were measured periodically (model ABL 700 Series, Radiometer) and were maintained within normal limits either by adjusting ventilation or by administering sodium bicarbonate (8.5% iv). Before the decerebration procedure, dexamethasone (4 mg) was injected intravenously to reduce swelling of the brain stem. The cat was then placed in a Kopf stereotaxic frame and spinal unit that were located over a treadmill (Fig. 1). A precocilicular-postmamillary decerebration was performed. The gaseous anesthetic was gradually discontinued, and the lungs were ventilated with room air.

A lumbosacral laminectomy was performed to expose the L6 to S2 spinal roots. The left hindlimb was fixed in place at the ankle and knee by clamps, and the left triceps surae muscles, calcaneal tendon, and sciatic nerve were exposed. The tendon was severed from the calcaneal bone, attached to a force transducer (model FT-10, Grass Instruments), and stretched with a rack and pinion so that it developed a resting tension of ~150 g. The left peroneal, sural, gluteal, femoral, and obturator nerves, as well as the muscular branch of the sciatic nerve, were cut. A small incision was made in the skin overlying the right lateral gastrocnemius muscle, and two right-angled electromyogram (EMG) electrodes (Grass Instruments) were implanted and sutured into the muscle itself. The EMG activity of the right gastrocnemius muscle was amplified (model P511, Grass Instruments), filtered (0.1–1.0 kHz), and recorded with Spike2 (CED) data acquisition system.

Dynamic Exercise

Locomotion was evoked by electrical stimulation (40 Hz; 0.5 ms; 40–110 μA) of the mesencephalic locomotor region with a monopolar stainless steel electrode (SNEX-300, Rhodes), which was stereotaxically positioned 5 mm lateral to the midline of the brain, 2 mm caudal to the sulcus between the superior and inferior colliculi, and 2 mm below the surface of the midbrain. While the treadmill was hand driven at a speed of 0.40 m/s (24 m/min), the stimulating electrode was lowered in 0.5-mm increments until locomotion arising from the four limbs was observed. Locomotion was monitored by measuring the tension developed by the left triceps surae muscles and the EMG from the right triceps surae muscles. The discharge patterns and recruitment order of α-motoneurons activated by stimulation of the mesencephalic locomotor region have been shown to be almost identical to those evoked by dynamic exercise (33, 34).

Recording Single-Unit Activity From Groups III and IV Afferents

We recorded the impulse activity of individual group III and IV triceps surae muscle afferents from the distal cut end of the left L7 or S1 dorsal roots. The neural signals were passed through a high-impedance probe (Grass HIP511), amplified (Grass P511), and filtered (0.1- to 3-kHz band pass). The action potentials were displayed on a monitor as well as on a storage oscilloscope (Hewlett-Packard). The receptive field of an afferent was identified as being in the triceps surae muscles if a burst of impulses were discharged in response to either noxious or nonnoxious probing of this muscle group. Noxious probing consisted of vigorously pinching the muscles with the fingers, whereas nonnoxious probing consisted of either gently stroking the triceps surae with a blunt rod or gently squeezing the muscles with the fingers.

We classified afferents as either group III or group IV by their conduction velocities. Afferents with conduction velocities between 2.5 and 30 m/s were classified as group III, and afferents with conduction velocities of <2.5 m/s were classified as group IV. We calculated conduction velocity by measuring the conduction time and distance from a stimulating electrode placed under the tibial nerve close to its exit from the triceps surae muscles and the recording electrode placed under the dorsal root filament. The criterion for a response to dynamic exercise by a group III or IV afferent was an increase ≥0.2 impulses (imp)/s.

Protocols

Once we identified a group III or IV afferent with a receptive field in the triceps surae muscles and established its resting level of activity, we recorded the response of the afferent to two perturbations, namely, dynamic exercise with the left hindlimb freely perfused and dynamic

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Fig. 1. Schematic representation of preparation used to record responses of group III and IV triceps surae muscle afferents to dynamic exercise. Thin fiber afferent discharge was recorded from either the left L7 or S1 dorsal root. Electromyogram (EMG) activity was recorded from right gastrocnemius muscles. Tension developed by left triceps surae muscles was recorded by a strain gauge attached to left calcaneal tendon. Dynamic exercise was evoked by electrical stimulation of mesencephalic locomotor region (MLR). [Adapted from Adreani et al. (1).]
The two afferents that did not respond to dynamic exercise while the muscles were freely perfused also did not respond to dynamic exercise while their circulation was occluded; consequently, the two group III afferents were discarded. Thirteen of the 15 afferents responding to exercise increased their discharge within the first 2 s of exercise and discharged at a higher rate for the entire exercise period. Of the remaining two, one responded within the first 4 s of exercise and the other responded within the first 10 s of exercise. On average, group III afferents responding to dynamic exercise discharged significantly more impulses during the contraction phase of the step cycle than during the resting phase of the step cycle (Table 1; $P < 0.002; n = 13$). Six of the 15 group III afferents were silent during resting conditions, whereas nine discharged at 0.8 imp/s or less.

Indomethacin (5 mg/kg iv) significantly attenuated the responses of the 15 group III afferents to dynamic exercise (Fig. 2; $P = 0.004; n = 15$). Specifically, dynamic exercise before indomethacin increased activity from 0.2 ± 0.06 to 1.4 ± 0.3 imp/s (Fig. 2; $P < 0.001; n = 15$), whereas dynamic exercise after indomethacin increased activity from 0.1 ± 0.1 to 0.9 ± 0.2 imp/s (Fig. 2; $P < 0.001; n = 15$). Indomethacin significantly attenuated the responses to exercise of the 15 group III afferents by 29% (Fig. 2; $P < 0.05$). Baseline activity was not decreased.

### Table 1. Percentage of impulses occurring during contraction phase of step cycle during dynamic exercise

<table>
<thead>
<tr>
<th></th>
<th>Freely Perfused</th>
<th>Circulatory Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80±3</td>
<td>75±3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>78±3</td>
<td>81±3</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>65±4</td>
<td>68±3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>68±4</td>
<td>68±3</td>
</tr>
</tbody>
</table>

Values are means ± SE (in %); $n$, no. of afferents.

exercise with the left hindlimb circulation occluded. Both perturbations were performed before and 30 min after indomethacin was injected intravenously (5 mg/kg). During dynamic exercise with the hindlimb freely perfused, we recorded the afferent activity for 1 min before exercise, during the exercise bout, and for 1 min immediately after the exercise bout. During dynamic exercise with the hindlimb circulation occluded, we recorded the afferent activity for 1 min before and during 5 min of circulatory occlusion before dynamic exercise. In addition, we recorded the activity during the entire exercise bout and during the 1 min of postexercise circulatory occlusion. We randomized the order of the maneuvers, i.e., circulatory occlusion and freely perfused. Circulatory occlusion was achieved by tightening a ligature placed around both the iliac artery and vein.

**Arachidonic Acid Injections**

Arachidonic acid is metabolized by cyclooxygenase into various vasoactive by-products, two of which, prostaglandin E2 and prostacyclin, are potent vasodilators. Intravenous injection of arachidonic acid has been shown to decrease mean arterial pressure, presumably due to the vasodilator effect of these by-products. To establish that our dose of indomethacin blocked this vasodilator response, we injected a 2-mg dose of arachidonic acid intravenously before and 30 min after administration of indomethacin (5 mg/kg iv). Arterial pressure was recorded (Spike 2) for 1 min before and subsequent to the arachidonic acid injection. In all experiments, each 10 mg of indomethacin was dissolved in 1 ml of sodium carbonate (100 mM).

**Data Analysis**

Afferent activity is expressed as impulses per second. The tension time index (24) was calculated step by step by integrating the area between the tension trace and the baseline level (Spike 2). Peak developed tension for each step was calculated by subtracting the tension at the peak of the step cycle. All values are expressed as means ± SE. Two-by-two repeated-measures ANOVA followed by Tukey post hoc tests were used to determine statistical significance. The criterion for statistical significance was set at $P < 0.05$.

**RESULTS**

**Group III Afferents**

We recorded the impulse activity of 17 group III afferents whose receptive fields were in the left triceps surae muscles (conduction velocity: 14.5 ± 1.2 m/s; range: 5.4–23.8 m/s). Each of the 17 responded to nonnoxious probing of the triceps surae muscles.

**Dynamic exercise with triceps surae muscles freely perfused.** Fifteen of the 17 group III afferents (conduction velocity: 15.6 ± 1.1 m/s; range: 9.3–23.8 m/s) responded to dynamic exercise while the triceps surae muscles were freely perfused. The two afferents that did not respond to dynamic exercise while the muscles were freely perfused also did not respond to dynamic exercise while their circulation was occluded; consequently, the two group III afferents were discarded. Thirteen of the 15 afferents responding to exercise increased their discharge within the first 2 s of exercise and discharged at a higher rate for the entire exercise period. Of the remaining two, one responded within the first 4 s of exercise and the other responded within the first 10 s of exercise. On average, group III afferents responding to dynamic exercise discharged significantly more impulses during the contraction phase of the step cycle than during the resting phase of the step cycle (Table 1; $P < 0.002; n = 13$). Six of the 15 group III afferents were silent during resting conditions, whereas nine discharged at 0.8 imp/s or less.

Indomethacin (5 mg/kg iv) significantly attenuated the responses of the 15 group III afferents to dynamic exercise (Fig. 2; $P = 0.004; n = 15$). Specifically, dynamic exercise before indomethacin increased activity from 0.2 ± 0.06 to 1.4 ± 0.3 imp/s (Fig. 2; $P < 0.001; n = 15$), whereas dynamic exercise after indomethacin increased activity from 0.1 ± 0.1 to 0.9 ± 0.2 imp/s (Fig. 2; $P < 0.001; n = 15$). Indomethacin significantly attenuated the responses to exercise of the 15 group III afferents by 29% (Fig. 2; $P < 0.05$). Baseline activity was not

AJP-Heart Circ Physiol • VOL 290 • JUNE 2006 • www.ajpheart.org

Fig. 2. Summary data of group III afferent responses to dynamic exercise before and ~30 min after indomethacin (Indo; 5 mg/kg iv). A: responses of 15 group III triceps surae muscle afferents to dynamic exercise while the hindlimb was freely perfused (FP). B: responses of 13 group III triceps surae muscle afferents to dynamic exercise while circulation to hindlimb was occluded. CO, circulatory occlusion. Open, shaded, and hatched bars represent mean discharge for a 1-min period; black bars represent mean discharge for the period of dynamic exercise (see Table 2). Imp, impulses. Vertical brackets represent SE. *Significant differences ($P < 0.05$) between adjacent means. Horizontal brackets represent significant differences ($P < 0.05$) between connected means.
significantly changed by indomethacin ($P > 0.05; n = 15$). Likewise, tension time indices and peak tensions that developed during dynamic exercise were the same before and after indomethacin (Tables 2 and 3; $P > 0.05; n = 15$). In addition, the durations of the exercise periods were not significantly different from each other before and after treatment with indomethacin (Table 4; $P > 0.05; n = 15$).

Dynamic exercise with circulatory occlusion. Thirteen of the 15 group III afferents responding to dynamic exercise with the triceps surae muscles freely perfused were tested for their responses to dynamic exercise with the circulation occluded. Circulatory occlusion significantly augmented the responses to dynamic exercise of nine of the 13 group III afferents. The mean discharge rate of the 13 afferents during exercise with circulatory occlusion was $1.9 \pm 0.4$ imp/s compared with $1.4 \pm 0.3$ imp/s during exercise with the hindlimb freely perfused ($P < 0.05$). Three of the 13 afferents did not change their responses to dynamic exercise, and one afferent decreased its response to exercise with circulatory occlusion. The 13 group III afferents that responded to dynamic exercise during circulatory occlusion displayed an increase in discharge within the first 2 s of exercise and continued to discharge at a higher rate than those at rest for the entire exercise period. Postexercise circulatory occlusion tended to increase the discharge of group III afferents over baseline activity from 0.2 to 0.4 imp/s, but the effect was small and not significant ($P > 0.05$).

For the 13 group III afferents responsive to dynamic exercise with circulatory occlusion, indomethacin significantly attenuated their responses by 42% (Fig. 2; $P < 0.001; n = 13$). Dynamic exercise before indomethacin (5 mg/kg iv) increased afferent activity from 0.2 ± 0.1 to 1.9 ± 0.4 imp/s ($P < 0.001; n = 13$), whereas dynamic exercise after indomethacin increased afferent activity from 0.1 ± 0.1 to 1.1 ± 0.2 imp/s (Fig. 2; $P < 0.001; n = 13$). Indomethacin did not change baseline activity of the 13 group III afferents (Figs. 2 and 3; $P = 0.50; n = 13$). Additionally, indomethacin did not significantly attenuate the discharge rate of the 13 group III afferents during postexercise circulatory occlusion. Specifically, the discharge rate of group III afferents during postexercise circulatory occlusion before indomethacin was $0.4 \pm 0.1$ imp/s, whereas after indomethacin, it was $0.2 \pm 0.1$ imp/s ($P = 0.24; n = 13$). The tension time indices and peak tensions that developed during dynamic exercise were not significantly different from each other before and after indomethacin (Tables 2 and 3; $P > 0.05; n = 13$). Likewise, the durations of the exercise periods were not significantly different from each other before and after treatment with indomethacin (Table 4; $P > 0.05; n = 13$).

Group IV Afferents

We recorded the impulse activity of 12 group IV afferents with receptive fields in the left triceps surae muscles (conduction velocity: $1.1 \pm 0.1$ m/s; range: 0.5–1.67 m/s). Each of the 12 group IV afferents responded to noxious probing of the triceps surae muscles. Nonnoxious probing of the triceps surae muscles stimulated only two of the 12 group IV afferents.

Dynamic exercise with triceps surae muscles freely perfused. Eight of the 12 group IV afferents were stimulated by dynamic exercise with the hindlimb freely perfused (conduction velocity: $1.3 \pm 0.1$ m/s; range: 0.7–1.67 m/s). None of the four group IV afferents that did not respond to dynamic exercise was stimulated by noxious probing of the triceps surae muscles. Each of the eight group IV afferents that responded to dynamic exercise did so within the first 2 s of exercise; the increase in activity was maintained throughout the exercise period. Although group IV afferents responding to dynamic exercise discharged more impulses during the contraction phase of the step cycle than during the resting phase of the step cycle, the difference was not significant (Table 1; $P = 0.101; n = 10$).

Indomethacin significantly attenuated the responses to dynamic exercise of these eight group IV afferents (Fig. 4; $P = 0.026; n = 8$), but the effect was modest (i.e., 18%). Specifically, dynamic exercise before indomethacin increased activity from $0.1 \pm 0.04$ to $1.2 \pm 0.4$ imp/s (Fig. 4; $P = 0.011; n = 8$), whereas dynamic exercise after indomethacin increased activity from $0.1 \pm 0.06$ to $1.0 \pm 0.4$ imp/s (Fig. 4; $P = 0.037; n = 8$). Baseline activity was not significantly changed by injection of indomethacin ($P = 0.64; n = 8$). Likewise, the tension time

### Table 2. Tension time indices for dynamically exercising cats both with hindlimb freely perfused and with hindlimb circulatory occlusion

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Before Indomethacin</th>
<th>After Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freely perfused</td>
<td>15</td>
<td>53±6</td>
<td>57±7</td>
</tr>
<tr>
<td>Circulatory occlusion</td>
<td>13</td>
<td>50±5</td>
<td>54±5</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freely perfused</td>
<td>10</td>
<td>49±8</td>
<td>52±10</td>
</tr>
<tr>
<td>Circulatory occlusion</td>
<td>10</td>
<td>45±7</td>
<td>47±8</td>
</tr>
</tbody>
</table>

Values are means ± SE (in kg/s); n, no. of afferents. There were no significant differences ($P > 0.05$) between corresponding means before and after indomethacin injection (5 mg/kg iv).

### Table 3. Peak developed tension during dynamic exercise induced by stimulation of mesencephalic locomotor region

<table>
<thead>
<tr>
<th></th>
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<th>Circulatory Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0±0.2</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.0±0.2</td>
<td>1.3±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE (in kg); n, no. of afferents.

### Table 4. Exercise duration induced by stimulation of mesencephalic locomotor region

<table>
<thead>
<tr>
<th></th>
<th>Freely Perfused</th>
<th>Circulatory Occlusion</th>
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</thead>
<tbody>
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<td>Group III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>56±2</td>
<td>56±5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>58±7</td>
<td>57±7</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>53±7</td>
<td>56±7</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>57±7</td>
<td>57±6</td>
</tr>
</tbody>
</table>

Values are means ± SE (in s). There were no significant differences in duration between any two values.
indices developed during dynamic exercise were not significantly different from each other before and after treatment with indomethacin (Table 2; *P* > 0.05; *n* = 8). Likewise, the durations of the exercise periods were not significantly different from each other before and after treatment with indomethacin (Table 4; *P* > 0.05; *n* = 8).

**Dynamic exercise with circulatory occlusion.** Each of the 12 group IV afferents responding to dynamic exercise with the hindlimb freely perfused was tested for its response to dynamic exercise with the hindlimb circulation occluded. Ten of the 12 afferents were stimulated by dynamic exercise with the hindlimb circulation occluded; two of the 10 did not respond to dynamic exercise with the hindlimb freely perfused. Each of the 10 group IV afferents responding to dynamic exercise during circulatory occlusion displayed an increase in discharge within the first 2 s of exercise, and each continued to discharge over baseline levels for the entire exercise period.

Overall, the responses of the 10 group IV afferents to dynamic exercise while the hindlimb circulation was occluded were significantly (*P* < 0.05) greater than the responses to dynamic exercise while the hindlimb was freely perfused. Specifically, eight of the 10 afferents displayed augmented responses; the mean discharge rate of the 10 afferents during exercise with circulatory occlusion was 1.7 ± 0.5 imp/s, whereas the discharge rate during exercise with the hindlimb freely perfused was 1.2 ± 0.4 imp/s. Two of the 10 afferents did not change their responses to dynamic exercise with circulatory occlusion.

For the 10 group IV afferents responding to dynamic exercise with circulatory occlusion, indomethacin significantly attenuated their responses by 34% (Fig. 4; *P* < 0.001). Dynamic exercise before indomethacin increased afferent activity from a baseline of 0.1 ± 0.1 to 1.7 ± 0.4 imp/s (*P* < 0.001; *n* = 10), whereas dynamic exercise after indomethacin increased afferent activity from 0.1 ± 0.1 to 1.1 ± 0.2 imp/s (Fig. 4; *P* = 0.003; *n* = 10). Indomethacin did not significantly change baseline activity of the group IV afferents (Figs. 4 and 5; *P* = 0.97; *n* = 10). However, indomethacin did significantly attenuate the discharge rate during postexercise circulatory occlusion. Specifically, during postexercise circulatory occlusion before indomethacin, the discharge rate of the group IV afferents was 0.3 ± 0.1 imp/s, whereas after treatment with indomethacin, it was 0.07 ± 0.03 imp/s (*P* = 0.04; *n* = 10). The time indices developed during dynamic exercise were not significantly different before and after treatment with indomethacin (Table 4; *P* > 0.05; *n* = 10). Likewise, the durations of the exercise periods were not significantly different from each other before and after treatment with indomethacin (Table 4; *P* > 0.05; *n* = 10).

**Intravenous injection of arachidonic acid.** In five cats, the effectiveness of indomethacin in blocking cyclooxygenase was challenged by measuring the depressor response to arachidonic acid. The dose of indomethacin used was the same as that used in the afferent recording portion of our experiments (i.e., 5 mg/kg iv). Before indomethacin, a 2-mg intravenous injection of arachidonic acid significantly decreased mean arterial blood pressure from 140 ± 4 to 77 ± 5 mmHg (*P* < 0.05; *n* = 5), whereas after indomethacin, this dose of arachidonic acid decreased mean arterial blood pressure from 125 ± 7 to only 111 ± 11 mmHg (*P* < 0.05). The difference in the depressor responses to arachidonic acid before and after indomethacin was significant (*P* < 0.05).

**DISCUSSION**

We investigated the role played by cyclooxygenase products of arachidonic acid metabolism in activating group III and IV muscle afferents during dynamic exercise. These thin fiber afferents are important because they comprise the afferent arm of the exercise pressor reflex (19), which is well known to play a critical role in increasing sympathetic discharge during ex-
the responses of group III, but not group IV, afferents to static contraction of the triceps surae muscles (26, 29). Finally, static contraction of these muscles in cats has been shown to increase intramuscular levels of arachidonic acid and its cyclooxygenase products (28, 32).

Although the role played by cyclooxygenase products of arachidonic acid metabolism in evoking the cardiovascular responses to exercise has been investigated in healthy humans, the findings have been controversial. For example, cyclooxygenase blockers given orally have been shown in two studies to have no effect on the cardiovascular responses to handgrip exercise performed either statically (3) or rhythmically (4). Moreover, both studies found that cyclooxygenase blockade had no effect on the pressor or muscle sympathetic nerve responses to postexercise circulatory occlusion (3, 4). These findings led these investigators to conclude that cyclooxygenase metabolites of arachidonic acid played no role in stimulating group III and IV muscle afferents during exercise.

In contrast, two other studies found that cyclooxygenase blockers, given through the vasculature, did attenuate the cardiovascular responses to exercise. The first study (6) found that intravenous ketoprofen, a cyclooxygenase blocker, attenuated the pressor, cardioaccelerator, and ventilatory responses to static handgrip in healthy men. The second study (21) found that brachial arterial infusion of indomethacin, another cyclooxygenase blocker, abolished the muscle sympathetic nerve response to low-level rhythmic handgrip, whereas brachial arterial infusion of aminophylline, which blocked adenosine receptors, had no effect on this sympathetic response to handgrip. The indomethacin-induced attenuation of the muscle sympathetic nerve response to low-level rhythmic handgrip was attributed to the muscle mechanoreflex because posthandgrip circulatory occlusion did not increase muscle sympathetic nerve activity after brachial arterial injection of saline, the vehicle for indomethacin (21).

The route of administration of the cyclooxygenase blocker might in part explain the contrasting findings of the studies described above. For example, in one of studies that gave the blocker orally (4) and in one that gave the blocker intravenously (6), venous concentrations of thromboxane B₂, a stable product of cyclooxygenase metabolism, were measured. In the study giving the blocker orally (4), thromboxane B₂ concentrations decreased from 36 ± 6 to 22 ± 3 pg/ml, whereas in the study giving the blocker intravenously (6), thromboxane B₂ concentrations decreased from 57.5 ± 7 to only 1.6 ± 0.4 pg/ml. On the basis of these reports, we offer the speculation that oral administration of cyclooxygenase blockers did not decrease prostaglandin and thromboxane production by the contracting muscles to a sufficient level to prevent activation of group III and IV afferents by these cyclooxygenase metabolites during exercise.

We (1, 2, 25) have shown previously that dynamic exercise increased the activity of group III and IV afferents in decerebrated cats. This increase displayed two characteristics. First, approximately two-thirds of the group III afferents tested, but only one-fifth of the group IV afferents tested, discharged synchronously with the contraction phase of the step cycle (1, 25). Second, occlusion of the circulation to the triceps surae muscles increased the responses to dynamic exercise of approximately equal percentages of group III afferents (44%) and group IV afferents (47%) (2). The present study has for the exercise (14, 22, 35). We found that cyclooxygenase products played a role in activating both group III and IV muscle afferents during dynamic exercise; this was the case both while the triceps surae muscles were freely perfused and while their circulation was occluded. We also found that cyclooxygenase products played a role in activating group IV, but not group III, muscle afferents during postexercise circulatory occlusion.

The role played by cyclooxygenase products of arachidonic acid metabolism in evoking the exercise pressor reflex has been previously investigated in anesthetized cats. Specifically, the exercise pressor reflex was reduced by more than half when cyclooxygenase blockers were injected into the arterial supply of statically contracting hindlimb muscles. Moreover, the reflex was restored toward its original level when prostaglandin 

E₂, a product of cyclooxygenase metabolism, was injected into the arterial supply of the hindlimb (31). The role played by cyclooxygenase products in causing the responses of group III and IV muscle afferents to static contraction has also been determined, and the results appear to parallel those reported for the reflex. Specifically, cyclooxygenase blockade attenuated the responses of both group III and IV muscle afferents to static contraction. Moreover, femoral arterial injection of arachidonic acid in cats not treated with cyclooxygenase blockers increased
most part confirmed these findings. In addition, the present study has shown for the first time that postexercise circulatory occlusion, a maneuver that was not performed in our previous studies (1, 2, 25), significantly increased over (preexercise) baseline levels the discharge of group IV, but not group III, afferents.

An important limitation of our study is that we selected thin fiber muscle afferents on the basis of the fact that they displayed at least some mechanical sensitivity to probing of their receptive fields in the triceps surae muscles. Thus we may have excluded afferents that innervated the triceps surae muscles but had no mechanical sensitivity. The presence of mechanically insensitive afferents can be revealed both by electrical stimulation of their axons as they exit the muscles and by exogenous chemical stimulation. However, both approaches have limitations. First, the strong currents required to activate group IV afferents when measuring conduction times from stimulating to recording electrodes leave open the possibility that one is activating the axons of afferents that innervate nearby structures, such as joints, bone, and other muscles. This possibility caused us to test only group III and IV afferents that we were sure had their receptive fields in the triceps surae muscles. Second, chemical stimulation of otherwise silent afferents can alter their sensitivity to dynamic exercise, especially when the interval between injection of a chemical and dynamic exercise is short, which is often the case because the recording life of group III and IV afferents can be limited.

A second limitation is that we did not measure the changes in plasma prostaglandin or thromboxane levels before and after indomethacin. However, we did challenge the effectiveness of cyclooxygenase blockade by measuring the depressor response to arachidonic acid injection. In addition, this dose and its route of administration were the same as those used in the afferent recording experiments. Moreover, the interval between indomethacin injection and arachidonic acid injection was the same as the interval between indomethacin injection and dynamic exercise.

In conclusion, our present and previous findings concerning the responses of group III and IV muscle afferents to dynamic exercise enable us to offer the following speculation. First, group III afferents appear for the most part to respond to mechanical stimuli during dynamic exercise, and cyclooxygenase products of arachidonic acid metabolism appear to increase their sensitivity to this stimulus. Because group III afferents discharged mostly during the contraction phase of the step cycle, we speculate that the mechanical stimulus originated from shortening of muscle fibers and did not originate from increases in blood flow, which would be expected to be greater during the relaxation phase of the step cycle than during the contraction phase (8, 9). On the other hand, we speculate that group IV muscle afferents responded to metabolic stimuli during dynamic exercise; cyclooxygenase products of arachidonic acid may either sensitize these unmyelinated afferents to other metabolic stimuli such as bradykinin, ATP, and lactic acid (7, 27) or stimulate them directly (15, 16, 27). Even though a few group IV afferents displayed some mechanical sensitivity (e.g., Fig. 5), most did not discharge during dynamic exercise in synchrony with the step cycle. Nevertheless, we cannot rule out the possibility that group IV afferents are responding at least in part to the increase in blood flow caused by dynamic exercise (1). This mechanism has been suggested as a stimulus to thin fiber muscle afferents during exercise (8).
ACKNOWLEDGMENTS

We thank Yao Dong for technical expertise.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-30710.

REFERENCES