Effect of arterial occlusion on responses of group III and IV afferents to dynamic exercise

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Adreani, Christine M., and Marc P. Kaufman. Effect of arterial occlusion on responses of group III and IV afferents to dynamic exercise. J. Appl. Physiol. 84(6): 1827–1833, 1998.—Our laboratory has shown previously that a low level of dynamic exercise induced by electrical stimulation of the mesencephalic locomotor region (MLR) stimulated group III and IV muscle afferents in decerebrate unanesthetized cats (C. M. Adreani, J. M. Hill, and M. P. Kaufman. J. Appl. Physiol. 83: 1811–1817, 1997). In the present study, we have extended these findings by examining the effect of occluding the arterial supply to the dynamically exercising muscles on the afferents' responses to MLR stimulation. In decerebrate cats, we found that arterial occlusion increased the responsiveness to a low level of dynamic exercise in 44% of the group III and 47% of the group IV afferents tested. Occlusion, compared with the freely perfused state, did not increase the concentrations of either hydrogen ion or lactate ion in the venous effluent from the exercising muscles. We conclude that arterial occlusion caused some unspecified substance to accumulate in the working muscles to increase the sensitivity of equal percentages of group III and IV afferents to dynamic exercise.

muscular contraction; sensory nerves; decerebrate cats; ischemia

The cardiovascular and respiratory responses to dynamic exercise include increases in cardiac output, ventilation, and sympathetic tone to the vascular tree. Two neural mechanisms are thought to evoke these responses. The first of these mechanisms is central command (7, 12), and the second is a reflex arising from the exercising muscles (2, 5). The afferent arm of the reflex is composed of thinly myelinated group III and unmyelinated group IV fibers (13).

Recently, our laboratory showed that group III and IV afferents with endings in the triceps surae muscles of decerebrate cats responded to dynamic exercise, which in turn was induced by stimulation of the mesencephalic locomotor region (1, 16). Specifically, we showed that a high percentage of these thin-fiber afferents was stimulated by a moderate to low level of exercise, as evidenced by the fact that the oxygen consumption of the triceps surae muscles increased only 2.5 times over resting levels. Similarly, blood flow to these hindlimb muscles increased only two times over resting levels (1).

Our interest has now turned to the effect that inadequate blood supply might have on the responses of these thin-fiber afferents to dynamic exercise. Previous work done in anesthetized cats has led to the conclusion that an inadequate blood supply to the hindlimb muscles increased the responses to tetanic contraction of more group IV afferents than group III afferents (11, 15). In the present study, we compared the responses of group III and IV afferents to dynamic exercise while the hindlimb muscles were freely perfused with the responses of these afferents to exercise while the muscles were not perfused. Our findings suggest that this conclusion needs to be modified.

METHODS

General surgical preparation and setup. Twenty-seven adult cats weighing between 1.9 and 5.1 kg (mean ± 0.1 kg) were anesthetized with a mixture of halothane and O2-N2O. A common carotid artery and external jugular vein were cannulated for monitoring blood pressure and introducing fluids, respectively. Arterial blood pressure was measured by connecting the carotid arterial cannula to a Statham transducer (P23 XL). Blood pressure and other physiological variables were displayed on a monitor (Gould V 1000) throughout the experiment. The cervical trachea was exposed and cannulated, and the lungs were ventilated mechanically (Harvard Apparatus) with the gaseous anesthetic. Arterial blood gases were periodically monitored (Radiometer ABL-3) and maintained within normal limits by adjusting ventilation or by administering sodium bicarbonate (8.5%; iv).

We fixed each cat's head in a Kopf stereotaxic frame and performed a craniotomy. After the brain was exposed, the dura mater was reflected to the sides, and the left and right cerebral hemispheres were removed. A transverse section was made just anterior to the superior colliculi at a 45° angle, and all neural tissue rostral to this section was removed. At this time, we removed the gaseous anesthetic, and the cat's lungs were ventilated with room air supplemented with 100% O2.

A lumbosacral laminectomy was performed to expose the dorsal roots from L3 to the cauda equina. Each cat was then positioned over a motor-driven treadmill and secured in a Kopf spinal unit. The left triceps surae muscles, calcaneal tendon, and sciatic nerve were exposed. The tendon was severed from its bone and attached to a force transducer (Grass FT-10). A resting tension of ~100 g was placed on the tendon. The triceps surae muscles were isolated from the surrounding tissue so that the receptive fields of afferents from these muscles could be accurately located. The tibial nerve was isolated from surrounding tissue, and a hook stimulating electrode was placed under it. The remainder of the sciatic nerve was isolated, and all accessible branches were cut. These included the peroneal, sural, biceps femoris, and gluteal nerves. In addition, the femoral and obturator nerves were cut. A small incision was made in the skin over the right lateral gastrocnemius, and two platinum electrodes (Grass Instruments) were implanted in the muscle. The electrodes were secured in place with suture. Electromyographic activity was observed by amplifying and filtering the signal (Grass P511 AC Pre Amplifier; ×5,000 amplification, 0.1–1.0 kHz band pass).

Dynamic exercise. Dynamic exercise was evoked by electrical stimulation (20 Hz; 0.7 ms; 80–100 μA) of the mesencephalic locomotor region. A Grass Instruments model S88 stimulator placed in series with a constant-current unit
(Grass P5IU6) was used to stimulate this region of the brain. The stimulus-isolation unit was connected to a monopolar stainless steel electrode (Rhodes; SNEX-300), stereotaxically positioned 4 mm lateral to the midline of the brain, 1 mm caudal to the sulcus between the superior and inferior colliculi, and 2 mm below the surface of the midbrain. While the treadmill was running at a speed of 0.45 m/s (27 m/min), the stimulating electrode was lowered in 0.5-mm increments until rhythmic locomotion was evoked. Rhythmic locomotion was evident in all four limbs but was monitored in the hindlimb from which afferent activity was recorded by observing “step by step” the isometric tension developed by the contracting triceps surae muscles. The discharge patterns and recruitment order of alpha motoneurons activated by stimulation of the mesencephalic locomotor region have been shown to be almost identical to those evoked by dynamic exercise (8, 20, 21). Consequently, even though the triceps surae muscles were contracting isometrically, this will be referred to as dynamic exercise.

Electromyographic activity from the lateral gastrocnemius muscle of the contralateral hindlimb confirmed the presence of rhythmic locomotion. The duration of the step cycle, although largely fixed by the speed of the treadmill, varied according to cat size. Step cycle duration was calculated from the start recorded by measuring the time from the start of the contraction phase of one step to the start of the contraction phase of the subsequent step. For the 27 cats used in these experiments, the average step cycle duration was 0.60 ± 0.02 s/step.

Recording single-unit activity from group III and IV afferents. We recorded single-unit activity from the distal cut end of the L7 or S1 dorsal roots. We covered the exposed lumbosacral spinal cord with warm mineral oil, reflected the dura of the L7 or S1 dorsal roots. We covered the exposed lumbosacral spinal cord with warm mineral oil, reflected the dura of the L7 or S1 dorsal roots. We covered the exposed lumbosacral spinal cord with warm mineral oil, reflected the dura of the L7 or S1 dorsal roots. We covered the exposed lumbosacral spinal cord with warm mineral oil, reflected the dura of the L7 or S1 dorsal roots.

We classified afferents as either group III or group IV by their conduction velocity. Afferents with a conduction velocity between 2.5 and 30 m/s were classified as group III, and afferents with a conduction velocity 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 dorsal root filament. Sequential filaments were placed on the recording electrode until activity with receptive fields in the triceps surae muscles was located. This filament was then further dissected so that single-unit activity of the afferents could be distinguished. An afferent’s receptive field was identified as being in the triceps surae muscles if a single impulse or burst of impulses was fired in response to probing of this muscle group.

Data analysis. The discharge rate of each afferent during the 60-s period immediately preceding exercise (i.e., rest), during 1 min of dynamic exercise, and during 30 s of recovery was counted in 2-s bins. Comparisons of discharge rates at rest and during exercise were made by using paired t-tests. Values for developed tension, conduction velocity, mean afferent discharge, duration of step cycle, blood lactate concentration, and other parameters were analyzed for lactate concentration (model 23L Lactate Analyzer, Yellow Springs Instruments), pH, Pco2, and Po2 (Radiometer, ABL3).
centrations, pH, PCO2, and PO2 are expressed as means ± SE. The comparisons of lactate concentrations, pH, PCO2, and PO2 under all conditions (rest, exercise, femoral artery occluded and not occluded) were made by using a repeated-measures analysis of variance. The criterion for statistical significance was P < 0.05.

RESULTS

Responses of group III afferents to dynamic exercise. We recorded afferent activity from 25 group III afferents with receptive fields in the left triceps surae muscles (mean conduction velocity: 14.4 ± 1.7 m/s; range 2.7–28.2 m/s). Each of the afferents responded to a nonnoxious probe of the triceps surae muscles, and six were mildly stimulated by stretching the calcaneal tendon to develop a tension of 2 kg. Most of the group III afferents observed were silent under resting conditions (Fig. 1); four, however, had low baseline discharge frequencies (mean resting discharge frequency for all 25 group III afferents: 0.03 ± 0.01 impulses/s).

Nineteen of the twenty-five group III afferents were stimulated by 1 min of dynamic exercise (Figs. 1 and 2). The afferents that responded to exercise began to increase their discharge rates within 2 s after the onset of exercise and maintained the increased rate of firing for the duration of the exercise bout. The mean discharge rate for all 25 group III afferents during 1 min of exercise was 0.5 ± 0.1 impulses/s. After cessation of exercise, the afferents’ discharge rates rapidly returned to their resting levels (mean recovery discharge frequency: 0.04 ± 0.02 impulses/s) (Fig. 2).

Occlusion of the femoral artery resulted in a complete cessation of the blood flow measured in the popliteal artery (n = 5). Eleven of the twenty-five group III afferents displayed augmented responses to exercise during occlusion. Of these 11 afferents, 3 did not respond to exercise when the femoral artery was freely perfused. Five of the eleven group III afferents, responding more to exercise when the femoral artery was occluded than when it was not occluded, displayed an augmented discharge with latencies of 8–30 s after the start of exercise. On the other hand, the remaining six group III afferents displayed an augmented response that had no obvious onset latency. Except for their magnitudes, the responses of these six afferents could not be distinguished from those evoked when the femoral artery was not occluded.

Fourteen group III afferents displayed the same response to exercise whether the femoral artery was occluded or was freely perfused. During occlusion, the mean resting discharge frequency for the 25 group III afferents was 0.08 ± 0.04 impulses/s. The mean discharge frequency of the 25 afferents during exercise with the femoral artery occluded was 0.8 ± 0.2 impulses/s. The recovery discharge frequency was 0.09 ± 0.04 impulses/s (Fig. 2).

Responses of group IV afferents to dynamic exercise. We recorded afferent activity from 19 group IV afferents with receptive fields in the left triceps surae muscles (mean conduction velocity: 1.3 ± 0.1 m/s; range 0.5–2.4 m/s). Each of the 19 group IV afferents responded to firm noxious probing of the triceps surae muscles. None, however, responded to stretching of the calcaneal tendon to develop a tension of 2 kg. Twelve of the nineteen group IV afferents tested displayed some baseline discharge under resting conditions (mean discharge frequency: 0.20 ± 0.04 impulses/s).

Sixteen of the nineteen group IV afferents responded to 1 min of dynamic exercise. Like the group III afferents that responded to exercise, the group IV afferents responded to exercise within 2 s of the onset of exercise; their increased firing rate was maintained until exercise stopped. The mean discharge rate for the
19 afferents during the 1-min exercise bout was 0.48 ± 0.12 impulses/s. After dynamic exercise, discharge rates of the 19 group IV afferents returned to resting levels (0.19 ± 0.07 impulses/s) (Fig. 2).

Nine of the nineteen group IV afferents tested displayed augmented responses to exercise when the femoral artery was occluded (Figs. 3 and 4). Of these nine afferents, two did not respond to exercise when the femoral artery was freely perfused. Four of the nine group IV afferents, responding more to exercise when the femoral artery was occluded than when it was not occluded, displayed an augmented discharge with latencies of 24–40 s after the start of exercise. The remaining five group IV afferents displayed an augmented response that had no obvious onset latency. Except for their magnitudes, the responses of these five afferents could not be distinguished from those evoked when the femoral artery was not occluded. The resting discharge frequency was 0.26 ± 0.09 impulses/s, the response to exercise (mean discharge frequency during exercise

Fig. 2. Histograms of responses of all 44 afferents to dynamic exercise while femoral artery was freely perfused (hatched bars) and while it was occluded (solid bars). *Significantly different from rest and recovery, P < 0.05. Brackets indicate values that are significantly different between freely perfused and occluded conditions.

Fig. 3. Original trace of a group IV afferent (CV = 1.2 m/s) with femoral artery freely perfused (A) and with femoral artery occluded (B). ●, Action potential (AP); thick arrowhead, onset of femoral arterial occlusion; solid vertical lines, stimulus artifact from electrical stimulation of mesencephalic locomotor region. EMG, electromyogram.
FREELY PERFUSED

EMG

Tension (g) 500

A P

OCCLUDED

EMG

Tension (g) 500

A P

1 sec

Fig. 4. Original trace of 2 group IV afferents (unmarked AP have a CV = 2.4 m/s; AP with • have a CV = 1.7 m/s) with femoral artery freely perfused (A and B) and with femoral artery occluded (C and D). Afferent with unmarked AP had similar discharge rates during rest and exercise whether femoral artery was occluded or was freely perfused. Afferent with AP marked by • did not discharge when femoral artery was freely perfused but did discharge during rest and exercise when femoral artery was occluded.

with the femoral artery occluded) was 0.62 ± 0.15 impulses/s, and the recovery firing rate was 0.30 ± 0.08 impulses/s (Fig. 2).

During exercise with the femoral artery freely perfused, the triceps surae muscles developed 420 ± 51 g of tension/step. During exercise with the femoral artery occluded, the triceps surae muscles developed 425 ± 50 g of tension/step (P < 0.05). During exercise, the triceps surae muscles did not fatigue, as determined from the tension trace when the femoral artery was either freely perfused or occluded. Blood flow through the popliteal artery before femoral arterial occlusion and before exercise averaged 4.2 ± 0.7 ml/min (n = 5). Similarly, peak blood flow through the popliteal artery after exercise and after release of the femoral arterial occlusion averaged 12.1 ± 2.6 ml/min (n = 5; P < 0.05).

Lactate concentration, pH, P CO2, and P O2 measurements during exercise. There were no significant changes in any of the arterial and venous variables tested, with the exception of a significant difference between arterial and venous PO2 (Table 1). The lactate concentration in the venous effluent from the triceps surae muscles after dynamic exercise was not greater when the femoral artery was occluded than when it was freely perfused.

DISCUSSION

We found that occluding the femoral artery increased the responses to dynamic exercise of approximately equal percentages of group III and IV afferents with endings in the triceps surae muscles. Even though this occlusion decreased blood flow in the popliteal artery from normal levels to zero, it had only modest effects on

Table 1. Effects of dynamic exercise on pH, P CO2, P O2, and lactate ion concentrations in arterial and venous blood

<table>
<thead>
<tr>
<th></th>
<th>Lactate Concentration, mmol/l</th>
<th>pH</th>
<th>P CO2, Torr</th>
<th>P O2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest, freely perfused</td>
<td>Arterial</td>
<td>1.8 ± 0.4</td>
<td>7.40 ± 0.02</td>
<td>35.4 ± 2.4</td>
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<tr>
<td></td>
<td>Venous</td>
<td>2.4 ± 0.5</td>
<td>7.36 ± 0.03</td>
<td>43.0 ± 3.9</td>
</tr>
<tr>
<td>Exercise, freely perfused</td>
<td>Arterial</td>
<td>2.8 ± 0.6</td>
<td>7.32 ± 0.02</td>
<td>43.2 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Venous</td>
<td>2.8 ± 0.5</td>
<td>7.32 ± 0.02</td>
<td>45.3 ± 1.9</td>
</tr>
<tr>
<td>Rest, occluded</td>
<td>Arterial</td>
<td>2.1 ± 0.5</td>
<td>7.34 ± 0.02</td>
<td>41.1 ± 2.9</td>
</tr>
<tr>
<td>Exercise, occluded</td>
<td>Arterial</td>
<td>2.6 ± 0.6</td>
<td>7.32 ± 0.01</td>
<td>42.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Venous 1</td>
<td>3.2 ± 0.6</td>
<td>7.26 ± 0.02</td>
<td>51.4 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Venous 2</td>
<td>3.3 ± 0.7</td>
<td>7.28 ± 0.02</td>
<td>53.4 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Venous 3</td>
<td>3.2 ± 0.7</td>
<td>7.26 ± 0.03</td>
<td>53.2 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Venous 4</td>
<td>3.1 ± 0.7</td>
<td>7.26 ± 0.03</td>
<td>53.2 ± 3.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 cats. Venous samples were taken from popliteal vein. Venous 1-4 represent 250-µl sequential samples taken immediately after release of femoral arterial occlusion (occluded).
the levels of lactate and hydrogen ions in the venous effluent from the exercising muscle. We cannot exclude the possibility, however, that a postexercise hyperemia and reactive hyperemia may have limited our ability to detect increases in lactate and hydrogen ions in the venous effluent. Nevertheless, we hesitate to claim that the triceps surae muscles were ischemic in our experiments even though there was little, if any, arterial blood flow to these muscles.

One might argue that, although the femoral artery was occluded, the exercising triceps surae muscles received much of their usual arterial blood flow from collateral sources. We think this possibility was unlikely because we were unable to draw any blood from the veins draining the triceps surae muscles while the femoral artery was occluded. We were able to obtain this venous blood only after the femoral arterial occlusion was released.

One explanation for our findings is that the lack of blood flow to the triceps surae muscles caused some substance to accumulate in them during exercise. This substance, in turn, may have increased the responsiveness of some group III and IV afferents to dynamic exercise. In our experiments, we did not investigate the nature of this substance and, therefore, can only speculate about its identity. One possibility is bradykinin, which has been shown to increase the sensitivity of both group III and IV afferents to intermittent static (i.e., tetanic) contraction (14). A second possibility is a cyclooxygenase product of arachidonic acid, which has been shown to increase the sensitivity of group III but not group IV afferents to maintained static contraction (17, 18).

Comparisons made between the responses of group III and IV afferents to static contraction and those of these afferents to dynamic exercise yield some surprising findings. Specifically, static muscular contraction stimulated a lower percentage of group III and IV afferents than did dynamic exercise, even though static contraction resulted in more tension development than did dynamic exercise (1, 9, 10, 16). Three factors may explain this discrepancy. First, the experiments using static contraction were performed on barbiturate-anesthetized cats (9, 10), whereas the experiments using dynamic exercise were performed on decerebrate unanesthetized cats (1, 16). Second, the experiments using static contraction employed electrical stimulation of peripheral nerves or ventral roots to cause muscle tetany, whereas the experiments using dynamic exercise employed electrical stimulation of the mesencephalic locomotor region to cause rhythmic contraction. The former method does not activate and recruit alpha motoneurons in a physiological manner, whereas the latter method does (8, 20, 21). Third, static contractions, which usually fatigue the working muscles, may be less potent mechanical stimuli to group III and IV afferents than is rhythmic contraction (i.e., dynamic exercise), which provides a constantly oscillating stimulus to these afferents.

We did not calculate the oxygen consumption of the exercising triceps surae muscles in our experiments. Previously, however, our laboratory found that these muscles increased their oxygen consumption by 2.5 times over resting levels in a preparation identical to that used in the present study (1). Moreover, the speed of “walking,” the peak tension developed by the triceps surae muscles, as well as the duration of the exercise period in the present study, was almost identical to that of our previous study in which we measured oxygen consumption (1). As a result, we think it is reasonable to speculate that the oxygen consumption of the triceps surae muscles in the present study was similar if not identical to that reported previously (1).

If our speculation proves to be accurate, it may provide a clue about the inability of femoral arterial occlusion to increase substantially the concentrations of hydrogen and lactate ions in the venous effluent of the triceps surae muscles during dynamic exercise. The level of oxygen consumption of the triceps surae muscles in the present study was probably about one-quarter of their maximum (3, 4). This moderately low level of dynamic exercise, which lasted only 1 min, may not have placed a sufficient metabolic demand on these muscles to produce substantial amounts of lactic acid when the femoral artery was occluded. Nevertheless, the lack of blood flow to the triceps surae muscles appeared to have increased the responses of approximately equal percentages of group III and IV afferents to dynamic exercise.

One interpretation of our findings is that hydrogen ion is not the sole metabolic byproduct of contraction that increases the sensitivity of group III and IV afferents to dynamic exercise. This interpretation has support in the literature on humans, in which postexercise circulatory arrest evoked larger sympathetic effects in nonconditioned than in conditioned forearm muscles, even though the intracellular hydrogen ion concentrations were identical in both groups (19). Speculation was offered that the metabolite causing the reflex increase in sympathetic discharge to the nonconditioned forearm was either a prostaglandin or thromboxane (19).

Previously, our laboratory has shown that arterial occlusion increased the responsiveness to static contraction in 47% of the group IV and 12% of the group III afferents tested (11). Presently, we report that arterial occlusion increased the responsiveness to dynamic exercise in 47% of the group IV and 44% of the group III afferents tested. Two factors may explain the differences reported in the two studies. First, in the previous study (11), arterial occlusion increased the lactic acid levels in the statically contracting triceps surae muscles, whereas in the present study occlusion did not increase lactic acid levels in the dynamically exercising triceps surae muscles. Second, in the previous study (11), arterial occlusion caused the statically contracting muscles to fatigue, whereas in the present study occlusion did not cause fatigue in the dynamically exercising muscles. These two factors will result in different metabolic and mechanical stimuli impinging on the group III and IV muscle afferents. Different stimuli
will, in turn, evoke different responses from these afferents.

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