Responses of group III and IV muscle afferents to dynamic exercise

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Adreani, Christine M., J aneen M. Hill, and Marc P. Kaufman. Responses of group III and IV muscle afferents to dynamic exercise. J. Appl. Physiol. 82(6): 1811–1817, 1997.—Tetanic contraction of hindlimb skeletal muscle, induced by electrical stimulation of either ventral roots or peripheral nerves, is well known to activate group III and IV afferents. Nevertheless, the effect of dynamic exercise on the discharge of these thin fiber afferents is unknown. To shed some light on this question, we recorded in decerebrate cats the discharge of 24 group III and 10 group IV afferents while the mesencephalic locomotor region (MLR) was stimulated electrically. Each of the 34 afferents had their receptive fields in the triceps surae muscles. Stimulation of the MLR for 1 min caused the triceps surae muscles to contract rhythmically, an effect induced by an α-motoneuron discharge pattern and recruitment order almost identical to that occurring during dynamic exercise. Eighteen of the 24 group III and 8 of the 10 group IV muscle afferents were stimulated by MLR stimulation. The oxygen consumption of the dynamically exercising triceps surae muscles was increased by 2.5-fold over their resting levels. We conclude that low levels of dynamic exercise stimulate group III and IV muscle afferents.

mesencephalic locomotor region; reflex control of the circulation; C fibers; Aα fibers; autonomic nervous system

DYNAMIC EXERCISE INCREASES sympathetic nerve discharge, cardiac rate, cardiac contractility, and ventilation. If severe enough, it also increases mean arterial pressure (13, 29). Two neural mechanisms have been postulated to cause these effects, namely central command and the muscle reflex. The first mechanism, central command, is believed to consist of the parallel activation of brain stem locomotor, autonomic, and ventilatory circuits at the onset of exercise (5, 6, 16). The second mechanism, the reflex, is believed to consist of the activation of these brain stem autonomic and ventilatory circuits by the exercise-induced stimulation of group III and IV muscle afferents (1, 4, 17).

Substantial evidence exists demonstrating that both neural mechanisms are likely to play important roles in causing the cardiovascular and respiratory responses to dynamic exercise (13, 29). Nevertheless, the effect of dynamic exercise on the impulse activity of group III and IV muscle afferents has received little attention. Previously, these thin fiber afferents have been shown to be stimulated by both maintained (14, 15) and intermittent (19) tetanic contraction, which, in turn, was evoked by electrical stimulation of either the ventral roots or the peripheral nerves.

This method of electrical stimulation has provided important information about the discharge properties of group III and IV muscle afferents. However, electrical stimulation of peripheral nerves or ventral roots recruits α-motoneurons with the fastest conduction velocities first, whereas dynamic exercise recruits these α-motoneurons last (9). In addition, electrical stimulation causes motoneurons to discharge synchronously, whereas exercise causes them to discharge asynchronously (12). The mechanical forces distorting receptive fields of group III and IV afferents during tetanic contraction and those distorting receptive fields during dynamic exercise might be different. Similarly, the metabolites produced during tetanic contraction and those produced during dynamic exercise might be different. Consequently, the responses of group III and IV afferents to tetanic contraction, which have been previously documented (14, 15, 19), might be different from the responses of these afferents to dynamic exercise.

This possibility prompted us to determine the effect of dynamic exercise on the impulse activity of group III and IV afferents with endings in the triceps surae muscles of decerebrate cats. Dynamic exercise was evoked by electrical stimulation of the mesencephalic locomotor region, which is located in the cuneiform nucleus of the midbrain (27). The impulse activity of these thin fiber muscle afferents was recorded from the dorsal roots while the triceps surae muscles were contracting rhythmically, an effect induced by an α-motoneuron recruitment order and discharge pattern almost identical to that observed during dynamic exercise.

METHODS

General. Nineteen adult cats, weighing between 1.9 and 3.8 kg, were anesthetized by inhalation of a mixture of halothane (5%) and oxygen and nitrous oxide (4:1). A common carotid artery and an external jugular vein were cannulated for monitoring blood pressure and administering fluids, respectively. The trachea was cannulated, and the lungs were ventilated with the anesthetic gas mixture. Arterial blood pressure was measured by connecting the carotid arterial cannula to a Statham P23XL transducer. Arterial Po2, Pco2, and pH were measured periodically (model ABL-3, Radiometer) and were maintained within normal limits (see Table 1) either by adjusting ventilation or by administering sodium bicarbonate (8.5% iv). Dexamethasone (4 mg) was injected intravenously to reduce swelling of the brain stem after the decerebration procedure (see below).

The cat’s head was then placed in a Kopf stereotaxic frame, and much of the skull was removed. The dura mater was reflected to the sides, and both cerebral hemispheres were removed. A transverse section was made 1 mm anterior to the superior colliculi at a 45° angle, and all neural tissue rostral to the section was removed. The gaseous anesthetic was discontinued, and the lungs were ventilated with room air supplemented with 100% oxygen.

A lumbarosacral laminectomy was performed to expose the spinal roots from L5 to the cauda equina. The cat was then
Dynamic exercise. Dynamic exercise (i.e., locomotion) was evoked by electrical stimulation (20 Hz, 0.7 ms, 80–100 µA) of the mesencephalic locomotor region (MLR). A monopolar stainless steel electrode (model SNEX-300, Rhodes) was positioned stereotaxically 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. The treadmill was turned on at a speed of 0.45 m/s, and then the midbrain was stimulated electrically. The electrode was lowered in 0.5-mm increments until locomotion was evoked. The criteria for locomotion included rhythmic alternating movement of the three limbs, which were placed on the treadmill, and rhythmic contractions of the left triceps surae muscles, which were fixed in place. Locomotion was evidenced both by recording the isometric tension developed by the contracting left triceps surae muscles and by recording the EMG activity of the right gastrocnemius muscle. The duration of the step cycle, although largely determined by the speed of the treadmill, varied among the cats depending on their size. Step cycle duration was calculated 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. The average step cycle duration, when the treadmill was hand driven, was 0.60 ± 0.02 s per step (n = 19).

Table 1. Effect of dynamic exercise, induced by stimulation of mesencephalic locomotor region, on blood flow, oxygen consumption, and metabolism of left triceps surae muscles

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popliteal arterial blood flow, ml/min</td>
<td>12</td>
<td>3.5 ± 0.3</td>
<td>6.5 ± 0.7*</td>
</tr>
<tr>
<td>Arterial lactate concentration, mmol/l</td>
<td>9</td>
<td>3.7 ± 0.8</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>Venous lactate concentration, mmol/l</td>
<td>9</td>
<td>3.9 ± 0.7</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Lactate production, mmol/min</td>
<td>9</td>
<td>0.7 ± 0.5</td>
<td>0.9 ± 0.7</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>9</td>
<td>7.41 ± 0.03</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>Venous pH</td>
<td>9</td>
<td>7.34 ± 0.04</td>
<td>7.31 ± 0.04</td>
</tr>
<tr>
<td>Arterial PCO₂, Torr</td>
<td>9</td>
<td>35.0 ± 3.0</td>
<td>37.2 ± 2.4</td>
</tr>
<tr>
<td>Venous PCO₂, Torr</td>
<td>9</td>
<td>44.7 ± 2.9</td>
<td>46.4 ± 2.8</td>
</tr>
<tr>
<td>Arterial PO₂, Torr</td>
<td>9</td>
<td>168.7 ± 7.1</td>
<td>163.5 ± 10.1</td>
</tr>
<tr>
<td>Venous PO₂, Torr</td>
<td>9</td>
<td>44.3 ± 5.7</td>
<td>37.6 ± 4.2</td>
</tr>
<tr>
<td>Arterial oxygen content, ml O₂/100 ml blood</td>
<td>12</td>
<td>12.7 ± 0.9</td>
<td>12.9 ± 1.1</td>
</tr>
<tr>
<td>Venous oxygen content, ml O₂/100 ml blood</td>
<td>9</td>
<td>9.2 ± 0.9</td>
<td>7.1 ± 1.1</td>
</tr>
<tr>
<td>Arteriovenous oxygen difference, ml O₂/100 ml blood</td>
<td>9</td>
<td>3.5 ± 1.6</td>
<td>5.8 ± 2.7*</td>
</tr>
<tr>
<td>Triceps surae oxygen consumption, ml O₂/min</td>
<td>9</td>
<td>0.13 ± 0.03</td>
<td>0.32 ± 0.04*</td>
</tr>
<tr>
<td>Triceps surae developed tension, g/step</td>
<td>12</td>
<td>0</td>
<td>365 ± 30*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of observations. *Values that are significantly higher during exercise than during rest, P < 0.05.

placed over a treadmill, which could be either motor or hand driven, and secured in a Kopf spinal unit (Fig. 1). The left hindlimb was fixed in place 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 ~100 g. The left triceps surae muscles were isolated from surrounding tissue so that the receptive fields of group III and IV afferents could be accurately located. The left peroneal, sural, and gluteal nerves, as well as the muscular branch of the sciatic nerve, were cut. Similarly, the left femoral and obturator nerves were cut. A small incision was made in the skin overlying the right lateral gastrocnemius muscle, and two electromyogram (EMG) electrodes were implanted into its substance. The EMG activity of the right gastrocnemius muscle was amplified (model PS11, Grass Instruments), filtered (0.1–1.0 kHz), and recorded on a Gould ES1000 recorder.

Recording of impulse activity from group III and IV afferents. Single-unit activity of group III and IV afferents with receptive fields in the left triceps surae muscles was recorded from fine filaments split from the left L₁ or S₁ dorsal roots. These filaments were dissected from the roots and placed on one foot of a bipolar hook electrode. The other foot was grounded to the cat with a thin string soaked in saline. The neural signals were passed through a high-impedance probe (model HIP511, Grass Instruments), amplified, and filtered.

**Fig. 1.** Schematic representation of preparation used to record responses of group III and IV afferents during mesencephalic locomotor region (MLR) stimulation. Afferent activity was recorded from either left L₁ or S₁ dorsal root. Blood flow was recorded from left popliteal artery. EMG was recorded from right gastrocnemius muscle. Tension was recorded with a strain gauge, which was attached to left calcaneal tendon. Cat was induced "to run" on treadmill by passing current through electrode stimulating MLR.
(100–3,000 Hz). Action potentials were displayed on a monitor (model VI1000, Gould) and a storage oscilloscope (model HP 54603B, Hewlett-Packard).

Conduction velocity of an afferent was calculated by dividing the conduction distance between the recording electrode on the dorsal root and the stimulating electrode on the tibial nerve by the conduction time, which was measured on the storage oscilloscope. Afferents conducting impulses between 2.5 and 30 m/s were classified as group III fibers. Afferents conducting impulses at <2.5 m/s were classified as group IV fibers (21). The receptive fields of the afferents were located by applying pressure to the triceps surae muscles. Pressure was applied by squeezing the muscles between the experimenter’s thumb and index finger in both a nonnoxious and noxious manner. The pressure was graded according to the subjective sensation reported by a fellow experimenter when similar pressure was applied to the forearm. The responses of the afferents to “tendon stretch” was assessed by turning the rack and pinion, which in turn lengthened the triceps surae muscles and the calcaneal tendon. Group I and II afferents were easily identified by their conduction velocities and their responses to stretch and twitch contraction; these thick fiber afferents were discarded.

Once a group III or IV afferent with its receptive field in the triceps surae muscles was identified and a resting level of discharge was established, the response of this afferent to 60 s of dynamic exercise (i.e., locomotion) was recorded. Moreover, the afferent’s activity during the 30 s after the exercise period was recorded.

Blood flow, oxygen consumption, and lactate production measurements. Blood flow was measured by placing a 1.0-mm ultrasonic flow probe (model 1RS, Transonic Systems) around the popliteal artery. The space between the vessel and the probe was filled with acoustic couplant (Aquasonic ultrasound transmission gel), and the probe was fixed to the surrounding tissue with suture. The probe was connected to a flowmeter (model T206, Transonic Systems).

In nine cats, oxygen consumption of the triceps surae muscles at rest and during locomotion was calculated to quantify the intensity of exercise performed. Oxygen consumption (VO₂) was calculated by using the Fick equation: 

\[ \text{VO}_2 = \frac{Q}{C_v - C_a} \]

where Q is popliteal blood flow, Ca is arterial oxygen content, and Cv is venous oxygen content. Arterial oxygen content was calculated after blood was obtained from the carotid artery (model ABL-3, Radiometer). A side branch of the popliteal vein (i.e., the caudal genicular vein) was cannulated such that the tip of the cannula was in the popliteal vein just after its exit from the triceps surae muscles. Venous oxygen content was calculated by obtaining and analyzing samples from the popliteal vein (model ABL-3, Radiometer). Blood flow measurements and arterial as well as venous blood samples were taken at rest and just before the conclusion (i.e., the last 5 s) of dynamic exercise. Arterial and venous blood samples were also analyzed for lactate concentrations (model 2300, Yellow Springs Instruments). In addition, the tension developed by the contracting triceps surae muscles during each step of dynamic exercise was calculated.

Data analysis. The discharge rate of each afferent was counted and placed in 2-s bins for the 1-min period immediately preceding dynamic exercise, for the 1 min of dynamic exercise, and for the first 30 s of recovery. The phase of the step cycle (i.e., contraction or relaxation) during which the afferent fired was recorded. The contraction phase of the step cycle was defined as the period of time during the generation of developed tension, and the relaxation phase was defined as the period of time before the generation of developed tension by the left triceps surae muscles. Comparisons of discharge rates during rest with those during exercise were done with paired t-tests. Similarly, comparisons of oxygen consumption and lactate production during rest with those during exercise were done with paired t-tests. The criterion for statistical significance was P < 0.05. All values are expressed as means ± SE.

RESULTS

Responses of group III afferents to dynamic exercise. We recorded the impulse activity of 24 group III afferents with receptive fields in the left triceps surae muscles (conduction velocity: 14.6 ± 1.8 m/s; range: 2.7–28.2 m/s). Each of the 24 afferents responded to nonnoxious probing of the triceps surae muscles. Six of the 24 group III afferents responded weakly to stretching the calcaneal tendon with the rack and pinion. The tendon stretch was mild, increasing tension by only 2 kg. No attempt was made to investigate the responses of the afferents to either moderate or severe levels of stretch. Twenty of the group III afferents were silent during resting conditions, with the remaining 4 displaying firing rates of 0.3 impulses/s or less. During rest, the discharge frequency of the 24 group III afferents averaged 0.03 ± 0.01 impulses/s.

Eighteen of the 24 group III afferents were stimulated by 60 s of dynamic exercise induced by electrical stimulation of MLR. Of the 18 group III afferents stimulated by exercise, 12 discharged synchronously with the contraction phase of the step cycle (Fig. 2). The afferents that responded to exercise displayed an increase in discharge within 2 s of its onset and maintained the increase throughout the exercise period (Fig. 3). When averaged for the entire 60 s of exercise, the mean discharge rate of the 24 group III afferents was 0.5 ± 0.1 impulses/s (Fig. 4), a level of firing that was significantly greater than that during rest (P < 0.05). After the exercise period ended, the afferent’s discharge rapidly returned to their resting levels (i.e., 0.04 ± 0.02 impulses/s; Figs. 3 and 4). During exercise, 68 ± 5% of the action potentials were discharged during the contraction phase of the step cycle.

Responses of group IV afferents to dynamic exercise. We recorded the impulse activity of 10 group IV afferents with receptive fields in the left triceps surae muscles (conduction velocity: 1.3 ± 0.2 m/s; range: 0.7–2.4 m/s). Each of the 10 group IV afferents responded to noxious probing of the muscles; they did not, however, respond to nonnoxious probing. Similarly, none of the 10 responded to mild tendon stretch, which was induced by turning the rack and pinion. Eight of the 10 afferents displayed some spontaneous activity during rest, their discharge frequency averaging 0.5 ± 0.2 impulses/s.

Eight of the 10 group IV afferents were stimulated by dynamic exercise (Figs. 5 and 6). Of the eight group IV afferents stimulated by exercise, only two discharged synchronously with the contraction phase of the step cycle (Fig. 5). Like the group III afferents that responded to exercise, the group IV afferents responded to exercise within the first 2 s of its onset. Moreover, this
Intensity of dynamic exercise. Blood flow to the triceps surae muscles during exercise was almost twice that during rest \( (n = 12; P < 0.05; \text{Table 1}) \). Similarly, oxygen consumption by this muscle group during exercise was almost 2.5 times that during rest \( (n = 9; P < 0.05; \text{Table 1}) \). Furthermore, the arteriovenous oxygen difference during exercise was significantly greater than that during rest \( (n = 9; P < 0.05; \text{Table 1}) \). Other metabolic variables did not change significantly from rest to exercise \( (\text{Table 1}) \). The triceps surae muscles developed, on average, \( 365 \pm 30 \text{ g} \) of tension per step.

**DISCUSSION**

We have shown that group III and IV muscle afferents were stimulated by rhythmic contractions, which, in turn, were induced by stimulation of the MLR. The recruitment order and discharge pattern of the α-moto-
neurons activated by stimulation of the MLR are almost identical to the recruitment order and discharge pattern of these motoneurons during dynamic exercise (10–12, 28, 31). Moreover, the level of dynamic exercise used in our experiments to stimulate these thin fiber muscle afferents was low. This was evidenced by the fact that the oxygen consumption of the dynamically exercising triceps surae muscles increased over resting levels by only 2.5-fold. In contrast, oxygen consumption of cat hindlimb muscles during forceful intermittent contractions can increase over resting levels by 10-fold (2, 3).

A substantial number of group IV afferents are thought to be nociceptors (18). Others are thought to be metaboreceptors, signaling that the oxygen and/or blood supply in the exercising muscles is not satisfying demand. In our experiments, the group IV afferents stimulated by dynamic exercise did not appear to signal either nociceptive or metaboreceptive events, the latter being caused by a lack of blood and/or oxygen supply. Specifically, the amount of dynamic exercise performed by the cats was low and, therefore, probably not noxious; moreover, it probably did not create a mismatch between oxygen supply and demand in the working muscles. These facts make our finding that group IV afferents were responsive to a low level of exercise surprising. Because most of the group IV afferents did not discharge in synchrony with the rhythmic contractions, their sensitivity to mechanical distortion did not appear to be high. These findings lead to the speculation that these unmyelinated afferents responded to metabolic stimuli that were unrelated to a mismatch between blood supply and demand in the exercising muscle.

Group III muscle afferents, in contrast to group IV afferents, are sensitive to mechanical distortion of their receptive fields. For example, group III afferents respond vigorously to maneuvers such as tendon stretch and nonnoxious probing of their receptive fields (14, 19). Moreover, group III afferents often respond briskly at the onset of tetanic contraction (14, 25). The sensitivity to mechanical distortion possessed by group III afferents probably explains our finding that during dynamic exercise these thinly myelinated afferents frequently discharged in synchrony with the contraction phase of the step cycle.

We do not know whether the discharge rates of the group III and IV afferents stimulated by the low level of dynamic exercise used in our experiments were sufficient to evoke reflex autonomic and ventilatory effects. Nevertheless, some previous studies performed in anesthetized animals lead us to speculate that this might be the case. Sato et al. (26), for example, measured cardioaccelerator responses to different frequencies of electrical stimulation applied to hindlimb muscle nerves of chloralose-anesthetized, vagotomized cats. When the currents were sufficient to recruit group IV afferents, Sato et al. found that the minimum effective frequency needed to increase heart rate was only 0.25 Hz. In addition, Mizumura and Kumazawa (22) found that 2-Hz electrical stimulation of hindlimb muscle nerves with current intensities that recruited group IV afferents increased ventilation in barbiturate-anesthetized dogs. These investigators did not report the ventilatory effects of stimulation frequencies lower than 2 Hz.

In our preparation, the triceps surae muscles from which afferent activity was recorded were fixed in place and, consequently, did not participate in the locomotion evoked by stimulation of the MLR. The peak tensions developed by the restrained triceps surae muscles in our experiments were similar but lower than those measured during walking in unrestrained intact cats (30). This lower developed tension can be attributed to two causes. First, the triceps surae muscles were partially denervated in our preparation and as a result received less than normal spindle support. Second, in unrestrained cats, peak muscle tension occurs when the contracting triceps surae muscles are lengthening [i.e., contracting eccentrically (7, 30)]. Muscle tension developed during eccentric contraction has been shown...
to exceed that developed during isometric contraction (8, 23), the latter of which was measured in our preparation. Based on these facts, we think it is reasonable to speculate that the mechanical stimulus (i.e., developed tension) to the group III and IV afferents having activity that was recorded in our experiments would have been greater if the triceps surae muscles had been allowed to move freely. Despite this restraint, 75% of the group III afferents and 80% of the group IV afferents tested in our experiments were stimulated by a low level of dynamic exercise.

In our experiments, the dynamically exercising triceps surae muscles developed only a small amount of tension (i.e., 365 g); nevertheless, a large percentage (i.e., 75%) of group III afferents were stimulated. This finding contrasts with that reported previously by our laboratory (14) in which a similar percentage of group III afferents appeared to require at least 2 kg of developed tension to stimulate them during tetanic (static) contractions. One explanation for this discrepancy might be that during dynamic exercise, such as that induced by stimulation of the mesencephalic locomotor center, muscle contraction is caused by an $\alpha$-motoneuron recruitment order and discharge pattern that are physiological, whereas during tetany, such as that induced by electrical stimulation of the ventral roots, muscle contraction is caused by an $\alpha$-motoneuron recruitment order and discharge pattern that are not
physiological. This difference in α-motoneuron discharge might result in greater mechanical stimulation of the receptive fields of group III afferents during dynamic exercise than during tetany.

In conclusion, we have shown for the first time that muscle afferents are stimulated by low levels of dynamic exercise. This stimulation did not appear to depend on the production of a metabolite signaling that blood supply did not satisfy demand in the exercising muscles. Our present and past findings (24) are consistent with the hypothesis that the exercise pressor reflex (20), the afferent arm of which is composed of group III and IV muscle afferents (17), plays a role in the cardiovascular and respiratory adjustments to low levels of muscular activity.

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