Sympathetic nerve activity during prolonged rhythmic forearm exercise

BRIAN A. BATMAN, J. CULLEN HARDY, URS A. LEUENBERGER, MICHAEL B. SMITH, QING X. YANG, AND LAWRENCE I. SINOWAY

Division of Cardiology/Department of Medicine and Department of Radiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

Sympathetic nerve activity during prolonged rhythmic forearm exercise. J. Appl. Physiol. 76(3): 1077–1081, 1994.—Exercise is a potent stimulus to activate the sympathetic nervous system. Previous work suggests that metabolite-sensitive muscle afferents are activated near the point of fatigue, and, when activated, they determine the muscle sympathetic nerve activity (MSNA) response to isometric forearm exercise. Yet, studies using a more prolonged rhythmic exercise paradigm suggest that the sympathetic nervous system can be activated in a more graded fashion. The purpose of this study was to determine whether metaboreceptor stimulation would also be responsible for MSNA responses to prolonged rhythmic isotonic forearm exercise. Subjects (n = 16) performed rhythmic isotonic forearm exercise at 25% maximal voluntary contraction for 30 min as we measured MSNA (microneurography). We observed progressive increases in MSNA with a peak increase of 161 units from a baseline value of 180 units. We also performed posthandgrip circulatory arrest (PHG-CA) in nine of these subjects. This maneuver isolates the metaboreceptor contribution to MSNA. During PHG-CA, AMSNA values were not different from those observed during a freely perfused recovery period (n = 7). We also compared MSNA responses during the rhythmic paradigm with those seen during a static protocol at 40% of maximal voluntary contraction in five subjects. The two types of exercise caused similar increases in MSNA, but only the static paradigm was associated with a sustained MSNA response during PHG-CA. Finally, $^{31}$P-nuclear magnetic resonance was used to evaluate muscle metabolic responses during rhythmic and static forearm exercise (n = 6). Static exercise caused muscle acidosis and an increase in $\text{H}_2\text{PO}_4^-$, whereas rhythmic exercise had no effect on muscle metabolism. We conclude that sympathoexcitation during rhythmic exercise is not dependent on engagement of muscle metabolite-sensitive afferents.

EXERCISE IS A POTENT STIMULUS for sympathetic nervous system activation (3). Victor et al. (20) used partial neuromuscular blockade to increase the central command contribution to the muscle sympathetic nerve response (MSNA) to forearm exercise. These studies suggested that near-maximal levels of central command are necessary to evoke an increase in MSNA. On the basis of these and other data, the activation of a muscle-based reflex is thought to be primarily responsible for the increase in sympathetic discharge during exercise (6–8, 12–14, 19).

Mark et al. (6) showed that static exercise increases MSNA by stimulating chemically sensitive afferents in the exercising muscle. In these experiments, static exercise caused a striking increase in MSNA. During a period of posthandgrip circulatory arrest (PHG-CA), a maneuver isolating the effects of chemical stimulation of muscle afferents, MSNA remained well above baseline. Other investigators (14, 19) showed that increases in MSNA occur only when the volunteer begins to fatigue and the working muscle cells become acidotic.

Studies performed during short bouts of rhythmic forearm or arm-crank ergometry exercise also suggest that muscle metaboreceptor activation is a prerequisite for increasing sympathetic nerve traffic (19, 21, 22). However, recent studies utilizing norepinephrine spillover during longer bouts of submaximal bicycle leg exercise suggest that sympathoexcitation occurs in a more graded fashion well before the onset of fatigue (5). These conflicting results suggested to us that different neural mechanisms may be regulating sympathetic nerve responses to short-term, fatiguing, and longer-duration steady-state exercise.

In the present experiments, we measured peroneal nerve recordings of sympathetic nerve traffic during a 30-min period of rhythmic isotonic forearm exercise at 25% of maximal voluntary contraction (MVC) and a period of PHG-CA. These results were then contrasted with those observed during rhythmic exercise without PHG-CA and static exercise at 40% MVC (2 min) followed by a period of PHG-CA. Our results suggest that activation of muscle metaboreceptors does not play a major role in evoking MSNA responses to prolonged rhythmic exercise.

METHODS

Exercise Paradigms

Thirty male subjects, 27 ± 1 (range 19–40) yr old, were studied. All subjects gave informed consent before being studied. The subjects were all in good health, and none was taking any medications. In addition, none of the subjects performed regular unilateral forearm exercise.

MVC was measured (kg) in both forearms of each subject. The subjects were placed in the supine position and was instrumented with electrocardiogram leads, an automated blood pressure device (Dinamap, Tampa, FL), and a pneumograph to record the stage of ventilation. After instrumentation, baseline heart rate, mean arterial blood pressure, and MSNA data were obtained for 5 min. The subjects then performed rhythmic isotonic handgrip exercise with the nondominant hand at −25% MVC at a cadence of 2 s of contraction/0 s of release. For these studies, we used a modified Ultra Grip dynamometer with a distance of 4.5 cm between the handles. We selected 25% MVC because pilot experiments demonstrated that intermittent exercise at 35% MVC could be
sustained for only 7 min \((n=5)\), whereas exercise at 15% could be continued for >1 h \((n=2)\).

In nine subjects, after 30 min of exercise, the circulation to the forearm was arrested by inflating an upper arm occlusion cuff \((250\text{ mmHg})\). At end exercise, the cuff was always inflated as the subjects held the contraction. The subjects then performed one additional 2-s forearm contraction during the ischemic conditions. This period of PHG-CA was continued for 2 min; then, recovery data were obtained while the subject remained in a relaxed state. In seven separate subjects, exercise was followed by a freely perfused rest period.

In five of the subjects who performed the rhythmic protocol followed by PHG-CA, static handgrip was performed with the opposite dominant hand at 40% MVC after a 3- to 5-min rest period. This type of exercise was previously shown to substantially increase MSNA levels in normal volunteers \((6,19)\). After 2 min, the circulation was arrested and handgrip was discontinued. PHG-CA was continued for 2 min; the occlusion cuff was then released, and recovery data were obtained for 5 min. For these experiments, a Stoeelting dynamometer \((\text{Wood Dale, IL})\) was used. In six separate subjects, the 30-min rhythmic and the 2-min static paradigms were performed while forearm nuclear magnetic resonance \((NMR)\) studies were done. In these studies, both paradigms were performed with the nondominant forearm.

In addition to the above-mentioned protocols, a time-controlled experiment was performed in four subjects. In this experiment, the subject's peroneal nerve activity was evaluated over 40 min without an exercise intervention. Finally, in five separate subjects, the perceived levels of effort during the 40% static and the 25% rhythmic protocols were compared. For these comparisons, a modified Borg range was used. The lowest level of perceived effort would correspond to a value of 6 and the highest to a value of 20 \((1,2)\).

Techniques

Microneurographic technique. This procedure has previously been described in detail \((6,17,18)\). Multunit recordings of MSNA were obtained using a tungsten electrode placed in the right peroneal nerve just below the fibular head. A reference electrode was placed in the skin a few centimeters from the active electrode. The signal then was amplified, filtered, rectified, and integrated.

On completion of the study, each neurogram was analyzed by the primary author, who manually counted the number of sympathetic bursts per minute as well as the amplitude of each burst \((\text{in mm})\) to calculate the total activity per minute. Each record was overread by one of the other investigators. The data are presented as the change in total amplitude from baseline \((\text{in arbitrary units})\).

\(^{31}\text{P-NMR spectroscopy.}\) The procedures for measuring high-energy phosphate metabolites in our laboratory have been described previously \((17)\). For these studies, we used a 1.9-T 26-cm-bore superconducting magnet \((\text{Oxford Instruments, Abbingdon, } \text{UK})\) that was interfaced to a radiofrequency transmitter/receiver \((\text{Nicolet Instrument, Madison, WI})\). We placed a 2.5-cm-diam coil over the flexor digitorum superficialis forearm muscle. Room temperature gradients were adjusted to optimize proton homogeneity on tissue water. The spectra that were obtained were collected at 32.5 MHz and represented the Fourier transformation of 32 transients averaged over 60 s.

The relative concentrations of \(P_1\) and phosphocreatine were calculated from the area under each respective resonance. These concentrations were expressed in arbitrary units, with the resting phosphate normalized to a value of 1. Intracellular pH was calculated from the chemical shift of \(P_1\) relative to a fixed peak position, in this case phosphocreatine \((14,17)\). The relative concentration of \(P_1\) that was present in the diprotonated form was determined from the pH, the concentration of \(P_1\), and the \(pK_a\) for the conversion of \(\text{HPO}_4^{2-}\) to \(\text{H}_2\text{PO}_4^-\) \((23)\).

Statistical Analysis

To evaluate when MSNA was greater than the baseline value during the 25% rhythmic protocol, data were meaned every 2 min, and a one-way analysis of variance was performed. Comparisons between rhythmic exercise with and without PHG-CA were performed using a two-way analysis of variance with a between-subject comparison \((\text{paradigm})\) and a within-subject comparison \((\text{time of paradigm})\). Post hoc analysis was performed using a Tukey’s test.

Comparisons of rhythmic and static exercise were performed using a two-way analysis of variance. Both main effects, paradigm \((\text{rhythmic vs. static exercise})\) and time, were within-subject variables. Because the durations of the two paradigms were very different, we expressed time as a percentage of the total duration of each paradigm. We used two exercise time points for comparisons: 50% \((\text{midpoint of grip})\) and 100% \((\text{end of grip})\) of the total time of each paradigm. The MSNA data and the other variables used in this analysis were each averaged over 1 min. Values were also compared during the period of PHG-CA and during the 5th min of recovery. Comparisons between means were performed using the simple effects. Values are expressed as means \(\pm\) SE, and \(P < 0.05\) was considered statistically significant.

RESULTS

During the rhythmic exercise protocol, the rise in MSNA was gradual and progressive, with total activity first becoming different from baseline 6 min after the onset of exercise \((\text{Fig. 1, top})\). The peak increase in MSNA was 161 units above a baseline value of 180 units. This pattern of increase was not likely a time effect, because no rise in MSNA was observed in the four subjects in whom we performed time control experiments \((\text{Fig. 1, bottom})\).
2.2 performed using 2-way repeated-measures analysis of variance. Comparisons of 2 modes of exercise at specific times were performed using simple effects. SE values are shown in Table 1. CA, circulatory arrest.

In Fig. 2, we compare the change in MSNA from baseline (AMSNA) during rhythmic exercise and the ensuing period of PHG-CA with an identical rhythmic exercise paradigm not followed by PHG-CA. The MSNA values during the period of postexercise ischemia are not statistically different from values seen during freely perfused recovery.

The mean data comparing rhythmic and static exercise are shown in Fig. 3. The key point raised by these data is that the MSNA responses at end exercise are not different in the two paradigms. However, MSNA values are different during the PHG-CA portions of the paradigms. Specifically, PHG-CA values during the rhythmic protocol are lower than those seen during the static exercise paradigm. The $^{31}$P-NMR data show that static but not rhythmic exercise caused cellular acidosis and a rise in $H_2PO_4^-$ (Fig. 3, B and C). Complete data (including standard error data) for these studies are shown in Table 1. There was no effect of paradigm (static vs. rhythmic) on the heart rate or blood pressure responses. There was a strong trend toward an interaction for blood pressure, although the simple effects method did not demonstrate higher values for blood pressure at any specific time point.

The perceived level of effort was similar for the static and rhythmic protocols, although there was a trend toward a greater level of perceived effort during the static paradigm (end exercise: rhythmic 15 ± 1.5, static 18 ± 0.5 arbitrary units; $P = 0.098$).

**DISCUSSION**

We have shown that the pattern of increase in MSNA during “prolonged” rhythmic exercise is different from that during “short-term” static exercise. Moreover, although nerve traffic was similar at end exercise, the MSNA response during the PHG-CA phase of the study is far lower after rhythmic than after static exercise. Finally, for similar levels of MSNA, the level of cellular acidosis and the increase in calculated dihydrogen phosphate are less during rhythmic than during static exercise. These findings suggest that the excitation of muscle metaboreceptors is not a primary determinant of sympatoexcitation during long periods (30 min) of rhythmic exercise.

Our findings are at odds with previous reports that suggested that rhythmic exercise increases nerve traffic in a manner similar to static exercise (21, 22). Specifically, these reports suggested that MSNA only increases when stimulus intensity is sufficient to result in muscle fatigue within a few minutes of the onset of exercise. These prior studies suggested that the stimulation of chemically sensitive muscle afferents was an absolute prerequisite for the increase in sympathetic nerve traffic seen with forearm exercise. Part of the difference in results may be due to a difference in protocols. The prior reports used shorter exercise paradigms than that used in this report. This is likely to be an important issue, because we did not observe an increase in nerve traffic until the subjects had exercised for 6 min.

Our NMR experiments also suggest that the level of muscle acidosis and the magnitude of the increase in...
H$_2$PO$_4^-$ were less during rhythmic than during static exercise and below the level of muscle acidosis previously shown to stimulate metabosensitive afferents in humans (9, 14, 15, 17, 19). Accordingly, these findings also support our contention that a non-metaboreceptor-mediated process is involved in evoking the increase in MSNA.

The precise mechanisms responsible for the increase in nerve traffic during the rhythmic exercise protocol are not clear. The results of our experiments do not allow us to differentiate between augmented central command and stimulation of mechanically sensitive afferents. On first observation, the progressive rise in MSNA would seem more consistent with a central command-mediated process, because the deformation of the mechanoreceptors was not likely to change during the 30-min protocol. However, work byVictor et al. (20) suggests that central command is not an important determinant of MSNA responses during forearm exercise in humans.

We would suggest that a more likely explanation is that group III mechanically sensitive afferents become sensitized by metabolic byproducts of contractions (4, 11, 16). Because pH and H$_2$PO$_4^-$ did not change, we suggest that a prostaglandin product of arachidonic acid metabolism may have been released at work loads unassociated with cellular acidosis and sensitized these muscle afferents in the exercising forearm, and this led to the progressive rise in MSNA. Wilson and colleagues (24, 25) showed that rhythmic forearm flexion at up to 0.4 W causes no change in exercising skeletal muscle pH but large increases in the release of various prostaglandins. Rotto and colleagues (11) showed that group III muscle afferents can be sensitized to static contraction by arachidonic acid. Further studies will be necessary to further evaluate this issue in human subjects.

In the studies shown in Fig. 2, the freely perfused recovery period was compared with a post-rhythmic handgrip ischemic period. This comparison of postexercise periods was performed in different groups of subjects. This approach is less statistically powerful than if we made these comparisons in the same individuals. We did not perform two rhythmic exercise paradigms in each subject because of the total time involved and because we believed that the first bout of exercise would potentially modify the MSNA response during the second bout of exercise. However, we do not believe that our findings from the rhythmic protocol were due solely to the statistic used. For example, if we had used only a one-way analysis of variance and a post hoc Tukey’s test to analyze the rhythmic exercise/PHG-CA paradigm (n = 9), we would have found that the MSNA values during exercise were greater than baseline whereas the MSNA values during PHG-CA were not greater than baseline.

Along similar lines, it could be argued that if the data in Fig. 2 were expressed differently, we would have reached different conclusions. Specifically, if the value at end exercise were considered the “maximal MSNA response” (i.e., a 100% change), then the values during PHG-CA would represent 33% of this value, whereas the value during freely perfused recovery would represent only 20% of the respective peak exercise value. However, even if we expressed our data in this manner and performed a two-way analysis of variance, we would not have found a statistically significant difference in the PHG-CA and freely perfused recovery values.

Finally, our results may not be applicable to all types of rhythmic exercise. Ray (10) recently showed that one-legged dynamic exercise is unassociated with an increase in MSNA. Under these circumstances, elevations in filling pressures may disengage cardiopulmonary receptors and oppose the sympathoexcitation due to muscle reflexes.

In conclusion, our results suggest that the sympathetic activation associated with prolonged nonfatiguing exercise is not dependent on a reduction in muscle cell pH. The results of these experiments are consistent with the hypothesis that the neural mechanism responsible for the increase in sympathetic nerve traffic during prolonged rhythmic forearm exercise is different from that noted during short-term static exercise. This suggests that, under certain specific conditions, central command and/or muscle mechanoreceptor activation may contrib-

### Table 1. MAP, HR, AMSNA, H$_2$PO$_4^-$, and pH during rhythmic and static exercise

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Midpoint Grip</th>
<th>End Grip</th>
<th>PHG-CA</th>
<th>Recovery</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP. mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhythmic</td>
<td>90±2</td>
<td>100±5</td>
<td>103±3</td>
<td>93±3</td>
<td>86±5</td>
<td>F = 0.825 F = 52.350 F = 2.972</td>
</tr>
<tr>
<td>Static</td>
<td>90±3</td>
<td>99±3</td>
<td>112±5</td>
<td>102±5</td>
<td>83±4</td>
<td>P = 0.415 P &lt; 0.001 P = 0.052</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhythmic</td>
<td>66±1</td>
<td>82±2</td>
<td>83±2</td>
<td>70±3</td>
<td>66±3</td>
<td>F = 0.003 F = 32.125 F = 2.234</td>
</tr>
<tr>
<td>Static</td>
<td>63±1</td>
<td>84±3</td>
<td>90±4</td>
<td>67±4</td>
<td>64±2</td>
<td>P = 0.957 P &lt; 0.001 P = 0.111</td>
</tr>
<tr>
<td><strong>AMSNA, total activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhythmic</td>
<td>98±40</td>
<td>139±45</td>
<td>44±22</td>
<td>33±10</td>
<td></td>
<td>F = 0.028 F = 6.374 F = 5.688</td>
</tr>
<tr>
<td>Static</td>
<td>-1±83</td>
<td>168±69</td>
<td>133±21</td>
<td>-5±31</td>
<td></td>
<td>P = 0.876 P &lt; 0.008 P &lt; 0.012</td>
</tr>
<tr>
<td><strong>H$_2$PO$_4^-$ arbitrary units</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhythmic</td>
<td>0.31±0.01</td>
<td>0.65±0.08</td>
<td>0.67±0.12</td>
<td>0.64±0.13</td>
<td>0.24±0.04</td>
<td>F = 18.506 F = 36.468 F = 16.812</td>
</tr>
<tr>
<td>Static</td>
<td>0.33±0.01</td>
<td>1.07±0.14</td>
<td>1.35±0.24*</td>
<td>2.05±0.31*</td>
<td>0.34±0.04</td>
<td>F &lt; 0.008 F &lt; 0.001 F &lt; 0.001</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhythmic</td>
<td>7.10±0.03</td>
<td>7.01±0.05</td>
<td>7.03±0.06</td>
<td>7.00±0.06</td>
<td>7.12±0.03</td>
<td>F = 17.466 F = 15.255 F = 6.278</td>
</tr>
<tr>
<td>Static</td>
<td>7.07±0.03</td>
<td>6.94±0.04</td>
<td>6.67±0.06*</td>
<td>6.59±0.07*</td>
<td>6.88±0.08*</td>
<td>P &lt; 0.009 P &lt; 0.001 P &lt; 0.002</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 for H$_2$PO$_4^-$ and pH and 5 for others. MAP, mean arterial pressure; HR, heart rate; AMSNA, change in muscle sympathetic nerve activity from baseline. Data for hemodynamic parameters and nuclear magnetic resonance were obtained from different subjects. * P < 0.05 vs. rhythmic.
ute to the level of sympathetic discharge seen with exercise in humans.

This research was supported by National Heart, Lung, and Blood Institute First Independent Research Support and Transition Award HL-44667 (L. I. Sinoway) and Clinical Investigator Development Award HL-02654 (U. A. Leuenberger) and an American Heart Association Established Investigator Award (L. I. Sinoway).

Address for reprint requests: L. I. Sinoway, Div. of Cardiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, PO Box 850, Hershey, PA 17033.

Received 15 April 1993; accepted in final form 24 September 1993.

REFERENCES