Effects of high-intensity cycle exercise on sympathoadrenal-medullary response patterns

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KRAEMER, WILLIAM J., JOHN F. PATTON, HOWARD G. KNUTTGEN, CHARLES J. HANNAN, THOMAS KETTLER, SCOTT E. GORDON, JOSEPH E. DZIADOS, ANDREW C. FRY, PETER N. FRYKMAN, AND EVERETT A. HARMAN. Effects of high-intensity cycle exercise on sympathoadrenal-medullary response patterns. J. Appl. Physiol. 70(1): 8-14, 1991.—Plasma proenkephalin peptide F immunoreactivity and catecholamines were examined on separate days in nine healthy males before and after maximal exercise to exhaustion at four intensities (36, 55, 73, and 100% of maximal leg power [MLP]) by use of a computerized cycle ergometer. The mean duration of 36, 55, 73, and 100% MLP was 3.31, 0.781, 0.370, and 0.1 min, respectively. All intensities were greater than those eliciting peak %27VO2 uptake for the individual subjects. Blood samples were obtained before, immediately after exercise, and 5 and 15 min after exercise. Significant (P < 0.05) increases in plasma peptide F immunoreactivity (i.e., from mean resting value of 0.18 to 0.43 pmol/ml) were observed immediately after exercise at 36% MLP. Significant increases in plasma epinephrine were observed immediately after exercise at 36% MLP (i.e., from mean resting value of 2.22 to 3.11 pmol/ml) and 55% MLP (i.e., from mean resting value of 1.67 to 2.98 pmol/ml) and 15 min after exercise at 100% MLP (i.e., from mean resting value of 1.92 to 3.88 pmol/ml). Significant increases for plasma norepinephrine were observed immediately after exercise (36, 55, 73, and 100% MLP), 5 min after exercise (36, 55, and 73% MLP), and 15 min after exercise (36% MLP). Increases in whole blood lactate were observed at all points after exercise for 36, 55, and 73% MLP and 5 min after exercise for 100% MLP. These data show that brief high-intensity exercise results in differential response patterns of catecholamines and proenkephalin peptide F immunoreactivity.

proenkephalins; opioid peptides; epinephrine; norepinephrine; anaerobic exercise; skeletal muscle morphology

MANY STUDIES have demonstrated that endurance exercise at intensities ranging from submaximal to peak %27VO2 uptake stimulates the sympathoadrenal-medullary system as evidenced by increases in plasma catecholamines (7, 10, 14, 22). Less clear, however, are the exercise-induced responses of proenkephalin peptides secreted from the adrenal medullary chromaffin cells, which have been shown to be responsive to the same stimuli that induce epinephrine release (16, 29, 35). Studies examining the smallest proenkephalin biosynthetic end products (i.e., Met-enkephalin and Leu-enkephalin) have typically demonstrated no significant increases above rest after endurance exercise stress (7, 21). Conversely, increases above rest have been observed for larger proenkephalin fragments (e.g., peptide F) consequent to exercise stress (22, 23, 25).

Limited data concerning the adrenal medullary plasma responses to short-term high-intensity exercise (greater than peak %27VO2 uptake) are available. Previous studies have demonstrated significant exercise-induced increases in plasma epinephrine and norepinephrine after short-term exercise tasks ranging from a 20-s sprint to repetitive interval runs (3, 9, 17, 34). To our knowledge, no attempt has been made to examine plasma catecholamine and proenkephalin peptide F (e.g., peptide F) responses to a wide range of exercise intensities at very high power outputs. Because enkephalin-containing polypeptides are responsive to the same stimuli that induce epinephrine secretion, it was hypothesized that enkephalin-containing peptides would also be responsive to high-intensity exercise stress. Thus the primary purpose of this study was to examine the plasma responses of catecholamines and peptide F [preproenkephalin (107-140)] to various exhaustive bouts of high-intensity cycle exercise.

METHODS

Subjects. Nine of the 10 subjects who previously participated in our previous study examining %27beta%-endorphin responses to high-intensity exercise bouts (24) were the subjects for this investigation to examine adrenal medullary responses to the identical high-intensity exercise loads. Thus, nine normally active healthy men provided written informed consent before their participation. All were medically screened by a physician, and none was taking any medications or had a history of endocrine disorders. The subjects’ physical characteristics were (mean ± SD): age, 23.9 ± 4.0 yr; height, 178.5 ± 5.0 cm; weight, 78.9 ± 7.3 kg; percent body fat, 14.8 ± 4.9; maximal %27VO2 uptake (Vo2 max), 47.3 ± 7.7 ml·kg⁻¹·min⁻¹. The muscle fiber characteristics of the subjects are presented in Table 1.

Subjects were thoroughly familiarized with all testing equipment and procedures before the study. Body density
TABLE 1. Muscle fiber characteristics of experimental subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Type II fibers</td>
<td>51.5 ± 8.8</td>
</tr>
<tr>
<td>%Type Ila fibers</td>
<td>41.2 ± 6.9</td>
</tr>
<tr>
<td>%Type IIb fibers</td>
<td>10.4 ± 8.9</td>
</tr>
<tr>
<td>Type I area, μm² × 100</td>
<td>54.2 ± 8.6</td>
</tr>
<tr>
<td>Type II area, μm² × 100</td>
<td>63.0 ± 14.0</td>
</tr>
<tr>
<td>%Type II area</td>
<td>54.8 ± 8.1</td>
</tr>
<tr>
<td>Capillaries, mm²</td>
<td>282.4 ± 62.1</td>
</tr>
<tr>
<td>Capillaries/fiber</td>
<td>2.64 ± 0.47</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 9.

(8, 36) and VO₂ max (4, 30) were measured ~2 wk before the determination of maximal power production.

Muscle biopsy samples were performed to further characterize the experimental subjects. Muscle biopsy samples were obtained ~10 days before maximal power testing. Muscle tissue samples were obtained from the superficial portion of the vastus lateralis muscle of the dominant leg by the percutaneous needle biopsy technique of Bergstrom (1) as modified by Evans et al. (24). Special care was taken to approximate the same biopsy location in all subjects with a depth of ~2 cm. Data from repeat biopsies (randomly performed) demonstrated nonsystematic and insignificant interbiopsy variations in fiber-type distributions. Standard methods for determination of muscle fiber characteristics were utilized and were identical to previously described methodologies (24).

Maximal leg power (MLP) and exercise intensity determinations. MLP was determined with a specially constructed cycle ergometer and computerized data collection-processing system. Subjects were tested for maximal power for one revolution (i.e., highest score of 5 revolutions) on the cycle ergometer at 60 rpm. Three consecutive tests with a minimum of 20 min rest between tests were performed. MLP was operationally defined as the mean of the highest two scores of the three tests to avoid the influence of an aberrant result. Subjects were seated in a rigid metal armchair behind the crank to provide back support. The distance from the chair to the pedal crank was established for each subject according to leg length and was kept constant for all testing. Force transducers on the pedals and position transducers on the pedals and crank allowed calculation of cycling power. The component of foot-on-pedal force perpendicular to the crank arm times the crank arm length equaled instantaneous pedaling torque. The torque times concurrent crank angular velocity equaled instantaneous cycling power.

In addition to the MLP (100%), subjects were tested at exercise intensities of 36, 55, and 73% MLP (test-retest reliability, r > 0.90). These intensities were ~115, 175, and 230% of those that elicited VO₂ max, respectively; MLP was at an intensity of 318% VO₂ max. Each test involved setting cycle resistance at the percentage of MLP at which the subject was to exercise. The pedal crank was then run at 60 rpm by the motor and the subject moved his legs freely without trying to push against the pedals. An investigator orally signaled the subject to begin pedaling at the same time as the ergometer motor reduced the pedal crank rpm by 10%. The subject had to generate power at a level predetermined by the investigator to maintain ergometer speed at 60 rpm. An analog panel meter in front of the subject indicated whether speed was being maintained. An audible electric metronome set at 120 beats/min gave the subject additional assistance in maintaining pedaling cadence. Warnings were given when revolutions per minute dropped. When the subject experienced extreme difficulty maintaining pedaling cadence, he was verbally encouraged to continue. The test ended when a subject became exhausted, as operationally defined by the inability to keep power output from dropping >3% below the set speed for 7 s. The 7 s were subtracted from the total time of exercise. At the end of each exercise test, subjects remained seated and were allowed to recover passively for 15 min, during which time blood was obtained. Table 2 shows the cardiorespiratory responses and duration of each exercise intensity.

Blood collection procedures. Each subject performed the four exercise intensities in random order on 4 separate days. Testing was conducted between 0800 and 1000 h, with each subject being tested at the same time of day to reduce the influence of diurnal variation. Before testing, subjects refrained from food for 8 h and from exercise and caffeine for 24 h. None of the subjects used tobacco products. A 20-gauge Teflon cannula was placed into an antecubital arm vein and was kept patent with a continuous flow of isotonic saline (~30 ml/h) before exercise. After the cannula was inserted, subjects rested in the seat position and two preexercise resting blood samples (R1 and R2) were obtained 20 min apart. Blood samples were also taken immediately after each exercise bout to exhaustion and 5 and 15 min postexercise. Blood for catecholamines was collected in precooled (4°C) plastic monovettes (Sarstedt, Princeton, NJ) containing heparin sodium, immediately transferred into precooled glass vacutainers containing appropriate preservatives (i.e., EGTA and reduced glutathione), mixed gently, and centrifuged at 1,500 g and 4°C for 15 min. Blood to be used for subsequent radioimmunoassay for proenkephalin F was collected into precooled plastic monovettes (Sarstedt) containing heparin sodium and 25 μl of aprotinin per milliliter of whole blood (Sigma Chemical, St. Louis, MO). Plasma samples were stored at ~70°C until analyzed. Samples were thawed only once for analysis.

Biochemical analyses. The methods used to purify the samples, conduct the radioimmunoassay, identify the immunoreactivity, and show cross-reactivities have been previously described in detail (21, 22, 28). Briefly, peptide F immunoreactivity was measured by radioimmunoassay in duplicate using commercially available 125I ligand and antisera (Peninsula Laboratories, Belmont, CA). The plasma immunoreactivity showed parallel displacement to peptide F. The interassay coefficient of variation was 4.9%, and the intra-assay coefficient of variation was 3.5%. Determinations of plasma immunoreactivity values were accomplished with the use of a Beckman 5500 gamma counter and on-line data reduction system.

Plasma catecholamines were determined using high-performance liquid chromatography with electrochemical detection. A preliminary aluminum oxide extraction was used to determine catecholamines from a 1-ml
plasma sample. The filtrate was injected (150 μl) onto the reverse-phase column (Altex ultrapore-octadecasyl, 5 μm). Mobile phase (50 mM sodium acetate, 20 mM citric acid, 0.2 mM sodium acetate, 0.135 mM sodium EDTA, and 5% methanol) was pumped at 1 ml/min by a Waters model 6000 pump. Detection was accomplished with an ESA Coulonem detector. The sensitivity of the assay was 0.05 ng/ml. Data were accumulated and calculated on the I&HM system 9000 computer.

Hemoglobin was analyzed in triplicate by the cyanmethemoglobin method (Sigma Chemical), and hematoctrit was determined in triplicate by microcapillary technique. Changes in plasma volume pre- to postexercise were calculated from changes in hematocrit and hemoglobin (5). Blood lactate was analyzed in triplicate with a micro blood lactate analyzer (model 640, Wolverine Medical, Alto, MI).

Statistical analysis. Statistical evaluation of the data was accomplished by an analysis of variance with repeated measures and subsequent Tukey's posthoc tests. A significant correlation (r = -0.78) existed between exercise duration and peak postexercise plasma concentrations of the various hormones for each exercise intensity. Significant correlations between duration and peak plasma concentrations were observed for norepinephrine at 55% MLP (r = -0.91) and 100% MLP (r = -0.67) and 15 min after exercise at 100% MLP. Additionally, R2 plasma epinephrine values at 100% MLP were significantly greater than R1 values. This was the only difference in resting baseline values observed for any of the blood measures.

In Fig. 3 the responses of plasma peptide F immunoreactivity are shown. The only significant increase observed above resting baseline occurred immediately after exercise at 36% MLP. Because epinephrine and peptide F immunoreactivity are found in the adrenal chromaffin cells, comparative responses of epinephrine and proenkephalin peptide F immunoreactivity are shown in Fig. 4.

Plasma concentrations of peptide F immunoreactivity showed significant inverse correlations: with epinephrine at rest for 55% MLP (r = -0.91) and 100% MLP (r = -0.67) and 15 min after exercise at 100% MLP (r = -0.99); with norepinephrine at rest for 100% MLP (r = -0.70) and immediately after exercise at 73% MLP (r = -0.77).

In the attempt to evaluate the influence of exercise duration on postexercise blood concentrations, a correlational matrix was run for the total data set and for each exercise intensity. A significant correlation (r = 0.78) between norepinephrine and exercise duration was shown when the entire data set was examined. In addition, the duration of exercise was correlated against the peak postexercise plasma concentrations of the various hormones for each exercise intensity. Significant correlations between duration and peak plasma concentrations were observed for norepinephrine at 55% MLP (r = -0.76) and at 73% MLP (r = -0.70) and for epinephrine at 36% MLP (r = 0.68).

Changes in mean plasma volume pre- to postexercise were as follows (mean ± SD): 36% MLP, -10.9% ± 8.27; 55% MLP, -5.3% ± 7.87; 73% MLP, -0.2% ± 5.97; and 100% MLP, -2.6% ± 7.32.

**DISCUSSION**

Although previous studies have examined the effects of various short-term exercise protocols on plasma catecholamine responses, limited data are available concerning the concomitant plasma responses of preproenkephalin peptides (7, 21-23, 25). Furthermore, no previous studies have examined these responses over a wide range of high-intensity exercise. Catecholamines and proen-
kephalin peptides are found in the same chromaffin cells and are sensitive to similar stimuli (16, 29, 35). To date, other than the adrenal medulla, no significant source of peptide F has been identified. Yet, plasma responses of epinephrine and peptide F immunoreactivity to exercise have demonstrated different response patterns, which have been attributed to differences in the endurance training levels of the subjects (22). The exact physiological mechanisms mediating such observations remain unknown.

The primary finding of this investigation was that plasma peptide F immunoreactivity and epinephrine concentrations did not follow the same pattern of response after short-duration high-intensity exercise stress. This was demonstrated by plasma peptide F immunoreactivity
increasing above resting values only immediately after exercise at 36% MLP. Concomitantly, increases were observed for epinephrine immediately after exercise at both 36 and 55% MLP and also 15 min after exercise at 100% MLP. These results are not consistent with the cosecretion from the adrenal medulla of peptide F and epinephrine.

Because the magnitude of increases observed were greater than could be explained by changes in plasma volume shifts alone, other physiological mechanisms may have influenced these results. The hormonal responses might be explained by several possible mechanisms. Differences in clearance rates might have affected both plasma epinephrine concentration and peptide F immunoreactivity. Kjaer et al. (18) demonstrated that, during long-term exercise at submaximal intensities (i.e., 30–76% VO$_2$ max), changes in clearance rates could not fully account for increases in epinephrine concentrations. What happens to clearance rates during short-term high-intensity exercise remains unknown. Catabolic metabolism in various tissues (e.g., liver and lungs) might also influence plasma concentrations of peptide F immunoreactivity. Katzenstein et al. (15) demonstrated that a half-life of ≥15 min for peptide F is possible because the amount of degradation in labeled peptide F was negligible in the peripheral tissues such as the liver and the lungs. In a study by Kraemer et al. (21), extremely low plasma

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**FIG. 3.** Responses of plasma peptide F to 4 high-intensity exercise bouts (means ± SD). *P < 0.05 from corresponding resting baseline.

**FIG. 4.** Comparison of mean responses of plasma peptide F immunoreactivity (solid line) and epinephrine (dotted line) to 4 high-intensity exercise bouts.
Met-enkephalin concentrations were observed in the heat (41.2 ± 0.5°C, 39.0 ± 1.7% relative humidity) but plasma concentrations of peptide F immunoreactivity were not reduced. These data again suggested that proenkephalin fragments may be more resistant than Met- and Leu-enkephalins to peripheral degradation even under extreme environmental conditions. The greater resistance to peripheral degradation and longer half-life exhibited by proenkephalin fragments (e.g., peptide F) than by the smaller end products of proenkephalin biosynthesis (i.e., Met- and Leu-enkephalins) may support the possible existence of physiological roles in the periphery for these opioid peptides.

Although it has been suggested that other endogenous opioid peptides are influenced by or related to “anaerobic factors” such as blood lactate, no such relationships were observed between peptide F and blood lactate in this investigation. The lack of a relationship between β-endorphin and blood lactate to the identical high-intensity exercise was also observed in our previous study utilizing essentially the same subject population (24). It is possible that because of the short duration of exercise and large distribution space, anaerobic feedback mechanisms may not be fully operational.

With the experimental design utilized in this investigation, it was not possible to consider the singular effects of either intensity or duration of exercise separately on the hormonal response patterns examined. In this study, the intensity of the exercise individually influenced, if not determined, the duration of each exercise bout. With this type of dual influence of the two exercise variables on our results as a design limitation, the data from this investigation suggest that the exercise duration was primarily related to the “peak” plasma hormonal concentrations. Furthermore, norepinephrine demonstrated a greater number of significant relationships with duration than did any other hormone examined. Peak plasma epinephrine concentrations were correlated to the duration of exercise only at 36% MLP. This probably reflected the higher metabolic demands of exercise at 36% MLP, which proved to be of longer duration than any of the other exercise intensities examined (i.e., 55, 73, and 100% MLP; see Tables 2 and 3). Future studies will need to specifically examine the effects of intensity and duration of exercise on these hormonal responses with experimental designs that consider these variables separately.

It is interesting to observe that during the time period before exercise at 100% MLP, a significant increase in plasma epinephrine was observed from R1 to R2. Previous studies have not shown this type of response. Still, no study has examined an extended recovery pattern after such brief high-intensity exercise or the simultaneous response of proenkephalin fragments. Previous studies have shown increases in peptide F immunoreactivity 5 min into recovery (22, 23) after maximal endurance exercise tests. This type of recovery pattern was not observed in the present investigation. If epinephrine and peptide F biosynthesis and release from the chromaffin cells are related, which is highly speculative, differences in the recovery pattern of peptide F could have influenced epinephrine responses.

In summary, this investigation showed that peptide F immunoreactivity, a proenkephalin fragment originating from the adrenal medulla, did not follow the response patterns of catecholamines, and more specifically epinephrine, to high-intensity exercise. This study also suggested that preparatory mechanisms for short-duration maximal exercise performance may involve activation of the adrenal medulla with increases in epinephrine secretion.
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