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Changes in plasma proenkephalin peptide F and catecholamine levels during graded exercise in men

(adrenal medulla/opioid peptide)

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ABSTRACT **Proenkephalin peptide F immunoreactivity, epinephrine, and norepinephrine were measured in the plasma of endurance-trained and untrained male subjects riding on a bicycle ergometer at 28%, 54%, 83%, and 100% of maximum oxygen consumption ($\dot{V}O_2$). At rest the trained group had peptide F levels almost twice the level of the untrained group, whereas all other variables measured were the same. The maximum epinephrine and norepinephrine levels were found at 100% exercise intensity, with a precipitous drop in the levels at 5 min of recovery. In contrast, the peptide F immunoreactivity reached a maximum at 5 min of recovery and was still substantially above the initial level after 15 min of rest. In addition, the trained subjects showed another peak of peptide F immunoreactivity at 54% $\dot{V}O_{2max}$. Possible explanations for the different patterns of catecholamine and peptide F levels are presented.**

Claims of "runners high," mood alterations, and exercise analgesia all have been credited to the actions of β-endorphin (1, 2). Most of these studies observed increases from before to after exercise only (3–6). These data led to the common belief that β-endorphin increased with increasing exercise intensity. Farrell *et al.* (7), however, showed that the significant increases in β-endorphin/β-lipotropin occurred only after a 60% treadmill run as opposed to runs at 70% and 80% of maximum oxygen consumption ($\dot{V}O_{2max}$), where no further increases were observed. Thus, the exercise response of β-endorphin does not appear to occur in a linear manner.

The enkephalin-containing polypeptides (ECPs) found in the adrenal medullary chromaffin cells have been shown to be secreted in response to the same stimuli that induce epinephrine release (8–10). Since exercise stimulates catecholamine release, specifically epinephrine (11–13), it was hypothesized that the ECPs would also respond to exercise stress. This study was designed to examine the response of peptide F (14) [preproenkephalin-(107–140)] immunoreactivity (ir), epinephrine, and norepinephrine in plasma to various exercise intensities (25%, 50%, 75%, and 100% of $\dot{V}O_{2max}$) in trained (T) and untrained (UT) subjects.

MATERIALS AND METHODS

Two groups of healthy college-age males, endurance-trained and untrained, volunteered for this study. The 10 T subjects, ages 19–24 years, were middle distance runners on the University of Wyoming varsity track team. The 10 UT subjects, ages 19–30 years, had been involved in no formal exercise training during the past 2 years. Selected subject characteristics are presented in Table 1.

Subjects cycled on a Monarch bicycle ergometer calibrated prior to each test. Each subject cycled continuously at 50 rpm beginning with a 3-min workload at 0 kg·min^{-1}, which was increased every 3 min until voluntary exhaustion. Oxygen uptake was measured throughout the test by using open-circuit spirometry. The volume of inspired air was measured with a Parkinson–Cowan gas meter. The subjects breathed through a high-velocity low-resistance valve (Collins Triple-J) into an air trap, from which continuous expired % CO_2 and % O_2 values were determined, using Beckman LB-2 and OM-11 analyzers, respectively. Standard gas mixtures were calibrated by analysis with a Scholander microsample analyzer. The $\dot{V}O_{2max}$ was then used to calculate the submaximal $\dot{V}O_2$ levels (15). Maximum oxygen consumption was determined in this first session by using a modification of the methods described by Weltman and Regan (16).

In the next session a progressive four-stage (actual values of 28%, 54%, 83%, and 100% of $\dot{V}O_{2max}$) exercise test protocol was followed. To ensure steady-state exercise each subject cycled at 50 rpm for three continuous 8-min stages at 28%, 53%, and 84% (actual calculation) of their maximum oxygen consumption. The three submaximal stages were followed by a 100% of $\dot{V}O_{2max}$ stage, which lasted from 1 to 2 min. Blood samples were obtained between the 7th and 8th min of each submaximal stage. Blood also was obtained immediately after the 100% exercise intensity and at 5 and 15 min after exercise (R-5 and R-15). After the 100% of $\dot{V}O_{2max}$ exercise intensity, subjects pedaled without resistance on the bicycle for 2 min. After this active recovery the subject was seated upright in a chair for the duration of the 15-min recovery period.

Prior to the progressive exercise session a Jelco 20-gauge Teflon catheter (Critikon, Tampa, FL) was inserted into an antecubital vein. The catheter was kept open by a continuous flow (25 ml·hr^{-1}) of heparin-free isotonic saline. Blood samples (10 ml) were obtained by using a plastic syringe connected to a three-way stopcock adaptor on the catheter and were immediately transferred into Vacutainers containing appropriate preservatives for the catecholamines and peptide F. After centrifugation, plasma was stored at −80°C until analyzed.

Differentiated catecholamines were determined by using the radioenzymatic assays described by Peuler and Johnson (17). Assay materials were supplied in a kit by Upjohn. A 3.0-ml blood sample was placed into a sterile Vacutainer containing EGTA and glutathione. After immediate centrifugation plasma was stored at −80°C until analyzed. All samples were measured in triplicate with paired internal standards.

For peptide F ir measurements, a 4.0-ml blood sample was immediately placed into a sterile Vacutainer containing 100 μg of aprotinin (Sigma), which served as a protease inhibitor.

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Abbreviations: ir, immunoreactivity; T, trained; UT, untrained.

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Table 1. Subject characteristics

Group	Age, yr	Fat, %	Height, cm	Weight, kg	$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	Best 10-km time, min:sec
T (n = 10)	21.40 ± 1.35	6.44 ± 0.90	178.17 ± 9.88	68.18 ± 6.39	61.10 ± 3.56	31:12 ± 0:15
UT (n = 10)	24.20 ± 3.52	13.09 ± 5.00	178.56 ± 6.57	66.06 ± 13.59	42.10 ± 9.74	—

All values were obtained at an altitude of 2200 m [585 torr (77.8 kPa) average air pressure]. Results are mean ± SD.

Peptide F was measured by a radioimmunoassay (18) using commercially available ^{125}I-labeled ligand and antisera (Peninsula Laboratories, Belmont, CA). The antisera used showed crossreactivities of less than 0.05% with any known opioid peptide. The plasma ir showed parallel displacement to peptide F (data not shown). The partially purified (see below) peptide F ir coeluted with the authentic synthetic peptide when chromatographed on a C_{18} reverse-phase HPLC column (Fig. 1). All samples were analyzed in duplicate and measured in the same assay to avoid run-to-run assay variation. To avoid nonspecific displacement in the radioimmunoassay, the peptide F from each sample was partially purified with a 3.0-ml C_{18} extraction column (Baker). A 1.0-ml plasma sample was injected onto the column, followed by a 2.0-ml initial buffer (0.5 M acetic acid/0.2 M pyridine, pH 4.0) wash. A 0.5-ml wash of initial buffer containing 30% (vol/vol) 1-propanol was injected into the C_{18} column to elute peptide F and collected in a plastic 1.5-ml Eppendorf test tube. Recovery of peptide F was determined for every sample by pipeting approximately 2000 cpm of ^{125}I-labeled peptide F into the plasma prior to extraction. Since 20,000 cpm was used in the radioimmunoassay this amount of material had no effect. Radioactivity was measured after purification and the percent recovery was determined for each sample. The mean recovery of radioactively labeled peptide F was 83%. The partially purified samples were then stored at −80°C until analyzed. No substantial degradation of peptide F was observed with these procedures by using HPLC elution time as the measurement, since the radioactivity (>90%) eluted with the authentic labeled peptide. This HPLC method is sensitive enough to separate the iodinated peptide from the native peptide.

The data were statistically evaluated with a two-way analysis of variance (ANOVA) with repeated measures. Comparisons were made between groups (T vs. UT) and over four, five, or seven repeated measures, depending on the number of measurements obtained for the dependent variable. Subsequent post hoc analyses were computed with paired (within-group comparisons) and unpaired (between-group comparisons) Student t tests. In this study significance was chosen as $P < 0.05$.

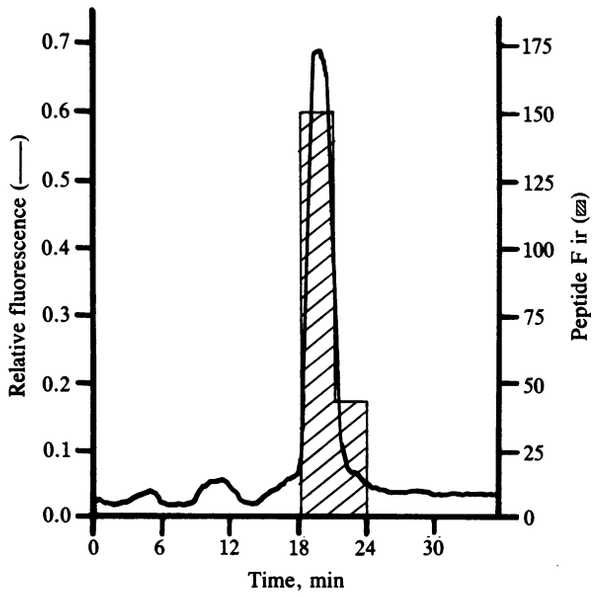

FIG. 1. Isocratic HPLC of peptide F ir from plasma. Partially purified peptide F ir (205 fmol) was isocratically eluted from an Altex C_8 column with 0.5 M acetic acid/0.2 M pyridine, pH 4.0, containing 22.7% (vol/vol) 1-propanol. The solid trace is fluorescence of synthetic peptide F detected after treatment with fluorescamine in a run immediately following the sample, and the hatched bars are the ir from the sample. Recovery of ir was 190 fmol.

RESULTS AND DISCUSSION

The data showed a typical response of epinephrine to graded exercise (Fig. 2*A*), which has been demonstrated previously

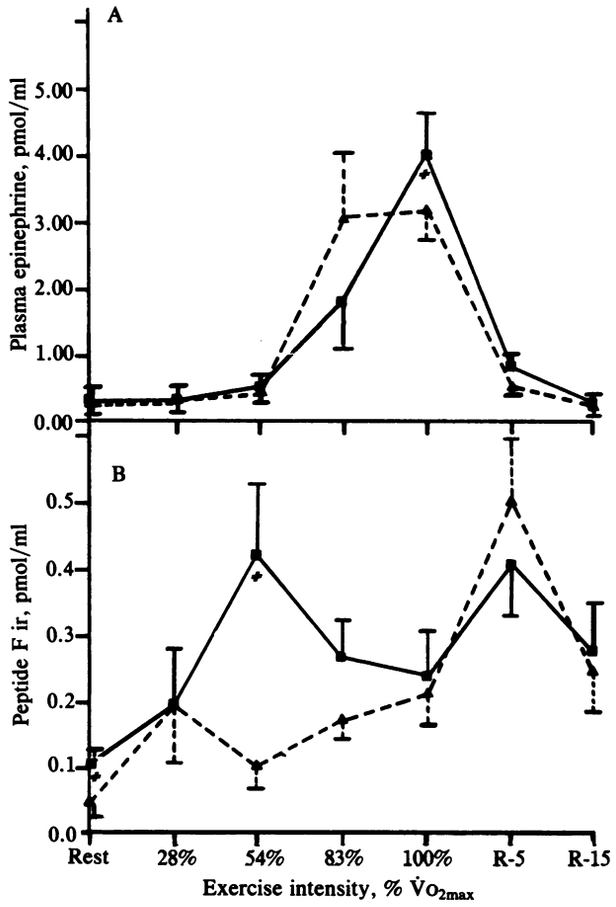

FIG. 2. Plasma epinephrine and peptide F ir as a function of exercise intensity. (*A*) Plasma epinephrine levels for the T (■—■) and UT (▲---▲) groups. (*B*) Plasma peptide F ir in the T (■—■) and UT (▲---▲) groups. The data represent the mean ± SEM (n = 10 for both groups). # indicates a difference with $P < 0.05$ between the two groups. The values for epinephrine at 83% and 100% are above resting values for both groups, as are the values for peptide F ir at 54% and above for the T group and 83% and above for the UT group.

(11–13). Both the UT and T groups showed the same pattern of response for epinephrine levels, with a significant difference between the two groups occurring only at the maximal exercise intensity (T > UT, $P < 0.04$). The same overall pattern was observed for norepinephrine, with the only significant difference between groups also at 100% $\dot{V}O_{2max}$ ($P < 0.001$, data not shown).

In contrast, the two groups showed substantially different peptide F ir patterns in response to exercise (Fig. 2*B*). The resting levels of peptide F ir differed between the two groups, with T > UT ($P < 0.01$). Whether this difference reflects an increased normal output of peptide F is not clear. However, there were no differences observed between the groups at rest in any of the other variables examined, suggesting that it is an increased normal output. In the UT group peptide F ir increased along with epinephrine, whereas in the T group peptide F peaked at 54%, significantly above the UT group ($P < 0.008$) and declined thereafter, while epinephrine was increasing above 54%. The recovery response of peptide F ir was similar for both T and UT subjects—i.e., increased levels after cessation of exercise (significantly above the levels at rest and 100%). The increased levels of peptide F ir in recovery does not correlate to the response of epinephrine, which declines abruptly after exercise (at 5 min of recovery).

These differences in the plasma levels of peptide F ir and epinephrine and norepinephrine in response to the graded exercise are not consistent with cosecretion from the adrenal medulla. These results can be explained by several possible mechanisms. The first would be a change in the plasma clearance rates of these substances after secretion from the adrenal medulla. Although this could explain the differences observed during the recovery period it cannot explain the increase at 54% for the trained group. Thus, this mechanism seems unlikely and a change in clearance rate with exercise intensity has not been observed for any bioactive peptide to our knowledge. The second mechanism would be processing of a precursor secreted from the adrenal gland to generate peptide F in the blood. This possibility cannot be eliminated, but as above that cannot explain the increase at 54% in the absence of an increase in epinephrine or norepinephrine. The third mechanism requires a source of peptide F ir in addition to the adrenal medulla. These data cannot be used to judge this possibility, but no peripheral source of substantial quantities of peptide F ir has to date been identified. The fourth mechanism would involve different pools of adrenal chromaffin granules containing either peptide F ir and low amounts of catecholamines or peptide F ir and high amounts of catecholamines. Immunohistochemical studies demonstrate that the proenkephalin peptides coexist with the catecholamines in the same cell but cannot rule out the possibility that cells contain substantially differing ratios of the two (19, 20). Evidence from studies of human pheochromocytoma cells in culture (21) and reserpine-treated dogs (22) suggests that heterogenous pools of cells do exist. Whichever mechanism is operating, these data suggest that the trained group has the ability to activate that mechanism at lower (54%) exercise intensity. Since the physiologic roles of the proenkephalin peptides from the adrenal medulla are unknown it is not possible to explain why this should occur. However, it is interesting to note that it occurs just below the level at which this group trains (typically 65–85% $\dot{V}O_{2max}$).

Although the mechanism by which the differential response to peptide F ir and catecholamines in exercise is achieved remains to be elucidated, these data clearly reveal this differential response under normal conditions. These studies also demonstrate the response of a proenkephalin-derived peptide, peptide F, to graded exercise and the differences in response of T and UT subjects. For further understanding of these data, the response of other proenkephalin-derived peptides to exercise will need to be examined. These studies may provide information relating to the physiologic roles of these peptides, which have yet to be determined.

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