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Running Elevates Plasma β-Endorphin Immunoreactivity and ACTH in Untrained Human Subjects (41225)

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Abstract. Twenty minutes of submaximal treadmill running was associated with an elevation in plasma levels of β-endorphin immunoreactivity ($P < 0.02$). This increase was greater in men (14.9 ± 3.4 fmole/ml) than women (2.6 ± 1.2 fmole/ml) ($P < 0.05$). Plasma levels of ACTH and growth hormone also increased after running. ACTH increased more in men (7.8 ± 1.1 fmole/ml) than in women (1.1 ± 0.44 fmole/ml) ($P < 0.02$). There was a similar growth hormone response in both sexes. No correlation can at this time be made with levels in the central nervous system. Changes in plasma levels of β-endorphin immunoreactivity may be responsible for some of the euphoria and analgesia anecdotally associated with running.

Exercise alters peripheral levels of a variety of hormones in man. Prolonged exercise results in a marked increase in growth hormone and in significant elevations in plasma concentrations of catecholamines, glucagon, and cortisol (1). The magnitude of these hormonal responses to exercise appears to be dependent on the amount of work performed (2) and the degree of previous physical training (2–4). One recent report suggests that maximal treadmill exercise is accompanied by a concomitant increase in circulating plasma β-endorphin and ACTH (5). The mechanism through which exercise modifies endocrine function, however, remains unknown.

Anecdotally, individuals who exercise regularly report an exercise-induced euphoria and analgesia. Since the administration of β-endorphin results in alterations of mood and in physical addiction (6), we measured plasma β-endorphin immunoreactivity before and after moderate exercise. Subjects from both sexes were studied to determine whether response was different. We also measured plasma growth hormone to determine a known hormonal response to exercise, and ACTH which originates with β-endorphin from a common precursor (7) and is secreted with β-endorphin following a variety of stimuli (5, 8, 9).

Materials and Methods. Five men and four women volunteers ranging in age from 24 to 36 years were studied. All were healthy, and none was taking medications. One of the women ran 2 miles five times a week, but none of the others exercised regularly. After a 4- to 6-hr period without food and a 20-min rest period, basal blood samples were obtained. Testing occurred between 1300 and 1500 hr for all subjects. The subjects ran on a treadmill with continuous electrocardiographic monitoring for 20 min at a speed and elevation sufficient to maintain their heart rates at 80% of predicted maximum. After exercise, a second blood sample was obtained. Blood pressure was measured every 5 min during exercise.

Two men were restudied 2 weeks after their first run. In this second study, an indwelling 16-gauge catheter was placed in a peripheral vein and normal saline given slowly (15 cc/hr). The initial study was then repeated except blood samples were obtained at intervals during and following the exercise period. In addition, heart rate was maintained during the exercise period at 80% of predetermined maximum heart rate.

Blood samples were obtained in lightly heparinized plastic syringes (1 unit/cc) and placed on ice. They were immediately transferred to cold plastic centrifuge tubes

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and spun at 8000 rpm at 5° for 20 min. The plasma was separated, frozen, and stored at −25° for one day. All samples from a given individual were processed at the same time.

β-Endorphin immunoreactivity. β-Endorphin immunoreactivity was extracted from plasma prior to assaying using a modification of the method originally described by Krieger *et al.* (10) with an average extraction efficiency of 43%. Five milliliters of plasma was placed in a glass siliconized tube containing 240 mg of silicic acid (60−200 mesh) (Sigma, St. Louis, Mo.) and mixed every 5 min four times at 4°. Following centrifugation at 2000 rpm at 4° for 10 min, the precipitate was washed with 4 ml of cold 0.01 *M* phosphate-buffered saline, pH 7.5, and then 4 ml of distilled water. β-Endorphin was eluted twice with 4 ml of acetone and 0.1 *N* HCl (40:60 v/v). The elutes were combined and allowed to evaporate overnight under a warm airstream. The final residue was reconstituted in 0.5 ml of β-endorphin assay buffer and a radioimmunoassay performed as previously reported (11). The antibody utilized in the assay has a 20% cross-reactivity on a weight basis and a 60% cross-reactivity on a molar basis with β-lipotropin and no cross-reactivity with ACTH, β-MSH, or enkephalins. The intraassay coefficient of variation was <4%.

ACTH. ACTH was extracted by the method of Ratcliffe and Edwards (12) with an average extraction efficiency of 65%. One milliliter distilled water containing 100 mg vycor (Corning, N.Y.) was added to 0.5−3.0 ml plasma in polystyrene tubes. After gentle agitation for 30 min at room temperature, the tubes were centrifuged at 2000 rpm for 5 min at 4° and the supernatant was discarded. The pellet was washed with 2 ml of distilled water and ACTH was desorbed from the vycor precipitate by the addition of 1 ml 50% acetone−1 ml HCl with gentle agitation for 30 min at room temperature. After centrifugation at 2000 rpm for 5 min at 4°, the supernatant was decanted into 12 × 75-mm polystyrene tubes and evaporated under a warm airstream. The resulting residue was resuspended in 1 ml of ACTH assay buffer. The radioimmunoassay utilized was modified from the method of Galskov (13). $^{1-39}$ACTH was generously provided by the NPA/NIAMDD Hormone Distribution Program and was used as standard and tracer. ^{125}I-$^{1-39}$ACTH was prepared using the method of Hunter and Greenwood (14). The antibody employed was produced in a rabbit against a $^{1-24}$ACTH-human albumin conjugate as described by Rose and Newsome (15). This antibody cross-reacts in an equimolar fashion with $^{1-39}$ACTH. The assay sensitivity was 40 pg/ml, and the intraassay coefficient of variation was 3.4%.

Growth hormone. Human growth hormone was measured by a double-antibody radioimmunoassay using materials provided by the NPA/NIAMDD Hormone Distribution Program with the exception of the antibody which was a gift from Dr. A. M. Lawrence, Chicago. This antibody was produced in rabbits immunized against a purified growth hormone preparation obtained from the NIH. It has no cross-reactivity with human TSH and only 1.7% cross-reactivity against human prolactin. The intraassay coefficient of variation was 3.4%.

Statistical analysis. All samples for a given individual were processed into the same assay. Nonparametric analysis was performed utilizing the sign test and Wilcoxon signed-rank test. Statistical comparisons between groups were performed using the nonpaired and paired *t* tests where appropriate.

Results. Twenty minutes of running was associated with an increase in plasma levels of β-endorphin immunoreactivity ($P < 0.02$). This increase was greater in men (14.9 ± 3.4 fmole/ml; 51.7 ± 13 pg/ml) (mean ± SEM) than in women (2.6 ± 1.2 fmole/ml; 9.0 ± 0.4 pg/ml) (mean ± SEM) ($P < 0.05$) (Fig. 1). Plasma levels of ACTH and growth hormone also increased after exercise. The ACTH increment was similar to that of β-endorphin immunoreactivity in that plasma levels increased more in men (7.8 ± 1.1 fmole/ml; 31.2 ± 6.0 pg/ml) than in women (1.1 ± 0.44 fmole/ml, 5.2 ± 2.0 pg/ml) ($P < 0.02$), though no correlation in the response of these peptides could be made for a given individual. No difference in response could be determined between

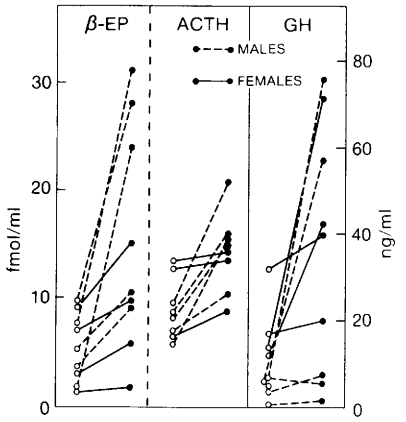

FIG. 1. Response of plasma β-endorphin immunoreactivity, ACTH, and GH to moderate exercise in male and female subjects. Open circles represent basal levels. Closed circles represent postexercise levels.

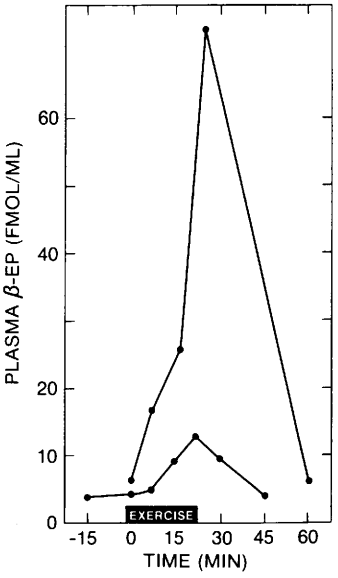

FIG. 2. Time course for plasma β-endorphin immunoreactivity in two male subjects before, during, and after a period of moderate exercise.

the woman who exercised and the other women studied. Exercise was associated with an elevation of plasma growth hormone levels in eight of nine subjects ($P < 0.005$) with five of the eight increasing greater than 5 ng/ml. There was a similar GH response in both sexes (Fig. 1).

The time course for the rise in plasma β-endorphin immunoreactivity during exercise is depicted in Fig. 2. β-Endorphin immunoreactivity continued to rise during the 20 min of exercise and fell rapidly upon cessation. Changes in blood pressure and heart rate did not correlate with changes in levels of the hormones measured (data not shown).

Discussion. The data reported herein suggest that submaximal treadmill running is associated with an elevation of plasma β-endorphin immunoreactivity and ACTH in human subjects. This finding is in agreement with the recent report showing a rise in β-endorphin and ACTH in eight athletes running on a treadmill to maximum aerobic capacity (5). Although the antibody to β-endorphin in this latter study showed a complete cross-reactivity with human β-lipotropin, chromatographic analysis confirmed the elevation of β-endorphin after exercise. The number of subjects in our study was small; however, the response of β-endorphin immunoreactivity to running was more pronounced in men than women. This finding is consonant with animal data showing a larger β-endorphin rise in male rats after immobilization as compared to female rats (16). One must question whether the response is correlated to estrogen levels. In our study, the rise in plasma β-endorphin immunoreactivity occurred rapidly and levels of β-endorphin immunoreactivity returned quickly to preexercise values when the exercise stopped. The surprising lack of a significant growth hormone response in four subjects did not relate to the magnitude of β-endorphin immunoreactivity or ACTH response in those subjects.

A recent report using affinity chromatography demonstrated the usefulness of this technique for separating β-endorphin from β-lipotropin in assays using an antiserum with a large cross-reactivity between the two (17). Our individual plasma extracts were not subjected to chromatography, and our antibody has a significant cross-reactivity with β-lipotropin (60%). Our studies confirm, however, the results of Fraioli *et al* (5) which reported a treadmill exercise induced rise in plasma β-endorphin immunoreactivity further determined to be β-endorphin after chromato-

graphic separation from β-lipotropin. The physiologic role of the endogenous opioids is unknown. It now appears that they may not function tonically, but may be of significance in the adaptation to certain endogenous or exogenous stimuli (18). Our data suggest that moderate exercise is capable of causing an elevation in the levels of plasma β-endorphin immunoreactivity and ACTH. Men appear to respond to a greater degree than women though additional studies must be done to better define differences in response. Whether circulating levels of β-endorphin have any central action remains to be established, as does the source of circulating β-endorphin.

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