Plasma interleukin-6 during strenuous exercise: role of epinephrine

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Received 29 January 2001; accepted in final form 13 April 2001

Plasma interleukin-6 (IL-6) has been shown to increase during prolonged exercise (17), sepsis (5), and major trauma (18). Monocytes are thought to be the source of IL-6 during sepsis, whereas it has been demonstrated that blood monocytes are not the source of IL-6 during exercise (15, 20). Thus it was demonstrated that circulating monocytes expressed less IL-6 during than before exercise (20). Interestingly, IL-6 mRNA was markedly elevated in muscle biopsies obtained from the quadriceps muscle immediately after a marathon race compared with preexercise (15), and in agreement with this finding, IL-6 mRNA was also elevated in rat muscle subjected to electrically stimulated contractions (8). Recently, we showed that during one-legged knee extension exercise, the net production of IL-6 in contracting skeletal muscles could account for the observed exercise-induced increase in plasma IL-6 (21). Thus IL-6 is produced in large amount in contracting skeletal muscles and is released to the circulation. During exercise, the plasma epinephrine levels are elevated (7). Interestingly, correlational relationships between the levels of plasma epinephrine and plasma IL-6 during exercise have been reported (16). Furthermore, both skeletal muscles (3) and immune cells (1) express large amounts of β-adrenergic receptors. Intravenous infusion of epinephrine enhances the plasma levels of IL-6 in rats (6) and in humans (19). However, whether the exercise-induced increase in plasma IL-6 is mediated by epinephrine has not been examined.

Selective administration of epinephrine for 1 h has been shown to induce lymphocytosis followed by lymphopenia, closely mimicking the effect of exercise (9, 24). In contrast, epinephrine infusion did not mediate the neutrocytosis (10), as seen during exercise.

The present study investigated whether infusion of epinephrine, reaching concentrations similar to those during strenuous exercise, could induce an increase plasma IL-6. In addition, changes in leukocyte subpopulations were detected. Healthy male subjects participated in two trials separated by ≥1 mo. The first experiment consisted of 2.5 h of treadmill running at 75% of maximal O2 consumption (V\textsubscript{\text{O2 max}}), whereas the second experiment consisted of epinephrine infusion for 2.5 h. The amount of epinephrine infused was calculated to reach the same level measured during treadmill running.

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MATERIALS AND METHODS

Subjects. Seven endurance-trained male runners (median age 30 yr, range 24–50 yr) with VO2 max of 4.7 l/min (3.61–5.03 l/min), corresponding to 60.1 ml·kg⁻¹·min⁻¹ (52.2–68.3 ml·kg⁻¹·min⁻¹), were recruited for the study. The subjects were not taking any medication. The study was approved by the local ethics committee for Copenhagen and Frederiksberg Communities. Subjects were informed of the risks of the experiment before their informed voluntary written consent was obtained.

Experimental protocol. All subjects participated in one exercise experiment. At least 1 mo later they participated in an epinephrine infusion trial. At 8:00 AM, subjects reported at the laboratory after an overnight fast, but they were allowed to drink water ad libitum. They were instructed to arrive well rested and not to have performed any extraordinary training in the last week and no training for 2 days before the experiment.

Exercise protocol. For each subject, VO2 max was determined ~1 wk before the exercise experimental day by an incremental exercise test on the same treadmill (model HC1200, Technogym) and CPX express (MedGraphics) used in the experiment. The subjects ran for 2.5 h at a speed determined in the VO2 max test to give an O2 consumption of 75% of VO2 max. Actual O2 consumption during the experiment was 75.6 ± 1.4% (SD) of VO2 max. Blood was sampled before and after 0.5 and 1.5 h of running. During exercise, the blood sampling was done by lowering the speed of the treadmill to walking speed (median duration 3 min, range 2–4 min). Thereafter, the subject ran for the last hour and the next blood sample was taken. For the next hours the subject stayed at the laboratory at rest, and blood was sampled at 0.5, 1, 1.5, and 2 h after running. Blood was sampled from the antecubital vein of both arms.

Infusion protocol. Epinephrine was infused for 2.5 h. The amount infused was based on the mean plasma concentrations of epinephrine found during the exercise experiment. Pilot experiments showed that an epinephrine infusion of 8 ng·kg⁻¹·min⁻¹ for 1.5 h and 14 ng·kg⁻¹·min⁻¹ for the last 1 h would result in the same plasma epinephrine concentrations attained during 2.5 h of running at 75% of VO2 max. Before the 2.5 h of epinephrine infusion, two venous catheters were placed in two forearm veins in the right and left arm for blood sampling and epinephrine infusion, respectively. Blood was sampled at the same time points as during the exercise.

Measurements of leukocyte subpopulations. Leukocyte subpopulations were determined by the Central Laboratory, University Hospital of Copenhagen, Rigshospitalet, using standard laboratory procedures.

Measurements of epinephrine. Blood samples for measurement of epinephrine were drawn into ice-cold glass tubes containing glutathione (1.3 mg/ml blood) and EGTA (1.5 mg/ml blood), pH 6–7, and spun immediately. Plasma was stored at ~80°C until analyzed by high-performance liquid chromatography (Hewlett-Packard, Waldbronn, Germany) with electrochemical detection.

Measurement of IL-6. Blood samples for cytokine measurement were drawn into precooled glass tubes containing EDTA. The tubes were spun immediately at 2,200 g for 15 min at 4°C. The plasma was stored at ~80°C until analyses were performed. For IL-6 measurement, high-sensitivity enzyme-linked immunosorbent assay kits (ELISA kit, R&D Systems, Minneapolis, MN) were used. According to R&D Systems, the IL-6 ELISA kit is insensitive to the addition of the recombinant forms of the soluble receptor (sIL-6R), and the measurements, therefore, correspond to both soluble and receptor-bound cytokine.

Statistics. Lymphocyte and neutrophil counts, log plasma IL-6, and log epinephrine were normally distributed; therefore, these data are shown as means ± SE. Changes over time and between groups were tested using 2 × 8 repeated-measure ANOVA (2 × 4 for epinephrine data). If significance was indicated, Newman-Keuls post hoc tests were used to test for significant differences between pre- and exercise/infusion values and between exercise and infusion values at the different time points. P < 0.05 was accepted as the level of significance.

Statistical calculations were performed using SigmaStat for Windows (version 2.03).

RESULTS

The concentration of epinephrine increased nearly threefold in response to exercise in the first experiment. The epinephrine infusion in the second experiment was calculated to give the same increase in plasma epinephrine, and the results showed no significant difference between the plasma concentrations of epinephrine during exercise and epinephrine infusion (Fig. 1).

The plasma concentration of IL-6 increased 29-fold during exercise to reach peak levels at the end of exercise (Fig. 2). The increase in plasma IL-6 during epinephrine infusion was only about sixfold, with the peak value at 1 h after exercise. A highly significant difference was demonstrated in plasma IL-6 concentrations during the two experiments (2-way ANOVA, P < 0.001). Plasma IL-6 was nearly eightfold (P < 0.001) higher at the end of exercise than at the end of infusion. Interestingly, at 2 h after exercise and after infusion, the difference was only about twofold (P < 0.001).

The lymphocyte concentration was increased after 0.5 h of exercise and declined below preexercise values in the recovery period (Fig. 3). The lymphocyte concentration during epinephrine infusion closely mimicked that during exercise, but the lymphocyte count was lower after exercise than after epinephrine infusion.

The neutrophil concentration increased approximately threefold during exercise and remained elevated during the 2 h of recovery, while epinephrine concentration...
infusion induced no changes in neutrophil counts (Fig. 4).

DISCUSSION

The major finding was that epinephrine infusion, inducing a plasma epinephrine level comparable to that obtained during 2.5 h of exercise, did not mimic the exercise-induced increase in plasma IL-6. However, the epinephrine infusion induced a small and consistent increase in plasma IL-6, with the peak at 1 h after cessation of infusion. This is in agreement with other studies (6, 19). The IL-6 release during epinephrine infusion has previously been shown to be inhibited by addition of a $\beta_2$-receptor antagonist (6); therefore, epinephrine must exert its effect through $\beta_2$-receptors. It is, however, not known whether the release or the clearance of IL-6 (or both) mechanisms is responsible for the increase in plasma IL-6 during epinephrine infusion comparable to that during exercise. The present data do not support the idea that a correlational relationship between plasma epinephrine and plasma IL-6 during exercise, found by Papanicolaou et al. (16), is due to a causal relationship. The peak plasma IL-6 during the exercise was almost eightfold more pronounced than peak plasma IL-6 obtained during epinephrine infusion. In a recent study by Steensberg et al. (21), the exposure of epinephrine to the resting and the exercising legs was the same. However, only the exercising leg released detectable amounts of IL-6 into the circulation. Thus it is not likely that epinephrine mediates the IL-6 release from contracting skeletal muscles during exercise. Furthermore, Starkie et al. (20) demonstrated that circulating monocytes do not contribute to the increase in plasma IL-6 during exercise. Therefore, epinephrine-mediated monocyte release is not likely to occur. On the basis of the exclusion of circulating monocyte- and skeletal muscle-derived IL-6, it is reasonable to suggest that the clearance of IL-6 in the plasma is decreased during the epinephrine infusion as a result of an epinephrine-induced reduced splanchnic blood flow. In addition, epinephrine induces increased systemic energy expenditure (14). In the study by Steensberg et al., it was suggested that IL-6 was released from contracting skeletal muscles as a consequence of low energy status in the muscles. Thus it has been demonstrated that injection of recombinant human IL-6 to humans increases the fasting blood glucose concentration (23) and liver glucose output (22). It has also been shown that consuming carbohydrate during exercise attenuates the exercise-induced increase in IL-6 (11–13). Thus the elevated plasma IL-6 levels during epinephrine infusion may reflect an increase in IL-6 release from different tissue as a result of low energy status.

At 2 h after exercise, plasma IL-6 was elevated only about twofold in the exercise experiments compared with after epinephrine infusion. Furthermore, the concentration of plasma IL-6 peaked 1 h after cession of infusion. At this time, it is not likely that the splanchnic blood flow is affected by epinephrine. Therefore, the elevated plasma IL-6 levels are probably due to augmented IL-6 releases. Even though the plasma levels of epinephrine are not augmented 2 h into recovery, epinephrine may exert its metabolic effect with a time lag (4).
Strong evidence exists that epinephrine mediates the exercise effect on the lymphocyte concentration. Thus intravenous administration of epinephrine for 1 h has been demonstrated to induce lymphocytosis followed by lymphopenia, closely mimicking the effect of exercise (9, 24). Furthermore, Ahlborg and Ahlborg (2) showed that, after administration of propranolol, exercise resulted in practically no increase in lymphocytes. Also, β-receptor blockade inhibited head-up tilt-induced lymphocytosis, but not neutrocytosis (10). In the present study, epinephrine infusion resulted in an increase in lymphocyte counts comparable with that seen during exercise. However, in the recovery period, the lymphocyte counts were lower 1–2 h after exercise than after infusion. The present study investigated 2.5 h of exercise, in contrast to previous studies investigating only 1 h; therefore, it is likely that the more pronounced lymphopenia after exercise is due to a cortisol effect, inasmuch as cortisol exerts its effect with a time lag of 2–3 h (17).

Previous studies failed to show that epinephrine infusion was able to mimic the exercise effect on neutrophils (9, 24). Furthermore, β-receptor blockade did not abolish the head-up tilt-induced neutrocytosis (10). In agreement, the present study clearly demonstrated that epinephrine did not mimic the exercise effect on neutrophils. The immediate exercise-induced increase in neutrophils is likely to be mediated by a combination of catecholamines and growth hormone, whereas cortisol mediates the prolonged neutrocytosis (17).

In conclusion, exercise-induced increase in plasma IL-6 cannot be mimicked by epinephrine infusion. Moreover, epinephrine has no effect on muscle-derived IL-6 and does not stimulate blood monocytes to produce IL-6. In theory, exercise-induced increase in epinephrine may augment the level of plasma IL-6 though a decreased clearance. In addition, increased metabolic demands due to epinephrine-mediated augmented energy expenditure are likely to be a stimulus for an increased IL-6 release.

The present study verifies previous findings showing that epinephrine infusion mimics the exercise-induced lymphocytosis and has no effect on neutrophils. However, it is novel that the postexercise lymphopenia is not solely mediated by epinephrine.

The excellent technical assistance of Ruth Rousing and Hanne Willumsen is acknowledged.

The study was supported by a scholarship from H:S Denmark and National Research Foundation Grant 504-14 to The Copenhagen Muscle Research Centre.

REFERENCES


