Effect of adrenergic blockade on lymphocyte cytokine production at rest and during exercise

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Starkie, R. L., J. Rolland, and M. A. Febbraio. Effect of adrenergic blockade on lymphocyte cytokine production at rest and during exercise. Am J Physiol Cell Physiol 281: C1233–C1240, 2001.—To examine the effect of exercise and adrenergic blockade on lymphocyte cytokine production, six men ingested either a placebo (control) or an α- (prazosin hydrochloride) and β-adrenergic antagonist (timolol maleate) capsule (blockade, or BLK) 2 h before performing 19 ± 1 min of supine bicycle exercise at 78 ± 3% peak pulmonary uptake. Blood was collected before and after exercise, stimulated with phorbol 12-myristate 13-acetate and ionomycin, and surface stained for T (CD3+), and natural killer (NK (CD3–CD56+)) lymphocyte surface antigens. Cells were permeabilized, stained for the intracellular cytokines interleukin (IL)-2 and interferon (IFN)-γ, and analyzed using flow cytometry. BLK had no effect on the resting concentration of stimulated cytokine-positive T and NK lymphocytes or the amount of cytokine they were producing. Exercise resulted in an increase (P < 0.05) in the concentration of stimulated T and NK lymphocytes producing cytokines in the circulation, but these cells produced less (P < 0.05) cytokine postexercise than preexercise. BLK attenuated (P < 0.05) the elevation in the concentration of lymphocytes producing cytokines during exercise; however, BLK did not affect the amount of IL-2 and IFN-γ produced. These results suggest that adrenergic stimulation contributes to the exercise-induced increase in the concentration of lymphocytes in the circulation; however, it does not appear to be responsible for the exercise-induced suppression in cytokine production.

T cells; natural killer cells; interleukin-2; interferon-γ

INTERLEUKIN (IL)-2 and interferon (IFN)-γ are cytokines that are produced primarily by T and natural killer (NK) lymphocytes, and they have a key role in both humoral and cellular inflammatory responses (7, 34). Exercise has been demonstrated to suppress T (26, 43) and NK (29–31, 41) lymphocyte function, and it is possible that it may compromise cytokine production by these cells. The effect of exercise on IL-2 and IFN-γ production is, however, unclear. A decrease in the concentration of IL-2 and IFN-γ in the supernatant of in vitro mitogen-stimulated blood has often (1, 19, 28, 33, 41, 48), but not always (13), been observed post-exercise. It is important to note that the suppression of stimulated IFN-γ production postexercise has been hypothesized to be the prominent cause of a functional break in defense, resulting in an increase in the risk of infection (28, 48). It is possible, therefore, that this could provide a mechanism for the increased risk of infection often reported following strenuous exercise (25). Given this suggestion and the conflict in the existing literature, the effect of acute exercise on IFN-γ production from NK and T lymphocytes at an individual cell level warrants further investigation. The method employed in the current study measures intracellular cytokine production at a single-cell level, allowing determination of changes in the concentration of lymphocytes producing the measured cytokine to be ascertained. In addition, a measurement of the amount of cytokine produced by each cell can also be obtained.

Epinephrine and norepinephrine have been demonstrated to increase the concentration of circulating lymphocytes (9, 10, 15, 16, 42, 47) but to decrease T (42) and NK (16) lymphocyte activity. Furthermore, epinephrine and norepinephrine have been demonstrated to decrease in vitro stimulated IL-1, IL-6, and tumor necrosis factor (TNF)-α production by whole blood (4, 11, 36, 46). Catecholamines exert many of their effects through α- and β-adrenergic receptors. Binding of catecholamines to these receptors increases the concentration of intracellular cAMP, leading to activation of protein kinase A that, in turn, phosphorylates other intracellular enzymes, ultimately influencing cell function (21). Lymphocytes have α- and β-adrenergic receptors on their surface membranes (18), and it is through these receptors that epinephrine leads to an increase in lymphocyte concentration in the circulation (17). Lymphocytes are distributed in vivo in circulating and marginal pools that are in dynamic exchange with one another (32). Administration of catecholamines has been demonstrated to interfere with β-adrenergic receptors on NK lymphocytes (3), thereby causing a decrease in their adherence to the vascular endothelium. Because lymphocytes have adrenergic re-

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ceptrons, and because catecholamines have been demonstrated to decrease the production of cytokines predominantly produced by monocytes, it is possible that these hormones also could affect lymphocyte IL-2 and IFN-γ production.

It has previously been demonstrated that the exercise-induced increase in circulating NK lymphocytes (17) and lymphocyte concentration and function (24) is abolished by the β2-receptor antagonist propanolol. In addition, incubation of blood samples with a β2-receptor antagonist results in an increase in stimulated IL-2 production compared with control samples (20, 22, 23). Together, these previously reported data demonstrate that catecholamines can affect IL-2 production in basal (resting) blood samples when concentrations are low. To our knowledge, no studies have examined the effect of adrenergic receptor blockade on cytokine production during exercise. This is important given the marked increase in sympathetic activity during exercise. This is important given the marked increase in sympathetic activity during exercise. We hypothesized that exercise would decrease cytokine production due to elevations in epinephrine but that adrenergic blockade would attenuate these alterations.

METHODS

Subjects

Six endurance-trained men (age 26 ± 5 yr, weight 71 ± 6 kg, peak pulmonary oxygen uptake (VO2 peak) 4.18 ± 0.59 l/min, means ± SD) volunteered to participate in this study. Screening before the study included a medical history, physical examination, 12-lead electrocardiogram, and full blood examination. Subjects were nonsmokers who had been free of symptoms of respiratory illnesses, and were not on any medication necessary to use supine exercise on a modified cycle ergometer as the mode of exercise. Subjects were also instructed to adhere to the diet but to consume water ad libitum.

Experimental Procedures

At least 7 days before the first experimental trial, VO2 peak was determined during a supine cycle ergometer exercise test to volitional exhaustion. The subjects lay on a bed attached to a modified electronically braked cycle ergometer (Lode, Groningen, The Netherlands). The work rate commenced at 80 W and increased 20 W every minute until exhaustion. VO2 peak was the highest VO2 achieved and was accompanied by a respiratory exchange ratio > 1.1 with a heart rate that was close to the age-predicted maximum (220 – age).

Subjects participated in two single-blind trials, conducted at least 1 wk apart. Subjects were supplied with food packages to consume for the 24 h preceding the trial, providing 15.6 MJ and comprising 71% CHO, 15% protein, and 14% fat. Subjects were instructed to abstain from alcohol, caffeine, and strenuous exercise. Subjects were also instructed to adhere to the diet but to consume water ad libitum.

Trials commenced at a set time (7:00 AM) to avoid circadian variations in hormones. Subjects arrived in the laboratory after a 10- to 12-h overnight fast to participate in the experimental trial. Subjects were fitted with a three-lead electrocardiograph for continuous monitoring of heart rate. An indwelling Teflon catheter (Ohmeda, Wiltshire, UK) with an attached valve (Safe-site, Braun, PA) was positioned into the brachial artery under local anesthetic (1% lidocaine) for collection of arterial blood samples and continual measurement of blood pressure. The catheter was kept patent by flushing with 0.5 ml NaCl after each sample collection. Two hours before exercise commenced, an arterial blood sample was collected (predrug), and subjects ingested either a placebo capsule (control) or a capsule (blockade, or BLK) containing 5 mg of prazosin hydrochloride (an α-adrenergic antagonist) and 5 mg of timolol maleate (a β-adrenergic antagonist). Pilot data revealed that subjects had variable tolerance to the BLK capsule with respect to exercise; therefore, the BLK trial was always conducted first. Given the effects of combined α- and β-adrenergic blockade, it was necessary to use supine exercise on a modified cycle ergometer as the mode of exercise. After the subjects had rested quietly for 2 h in a supine position, a preexercise arterial blood sample was collected.

Subjects then commenced a 20-min supine cycling trial at a power output eliciting 81.9 ± 2.5% VO2 peak; however, because of the effect of the drugs in the BLK, it was necessary to decrease the power output to 73.4 ± 2.9% VO2 peak at 10 min. This workload was maintained until the 20-min time point. Two subjects were unable to complete the 20-min BLK trial, fatigueing at 16 and 19 min, respectively. On average, subjects cycled for 19.2 ± 0.6 min at 78 ± 3% VO2 peak. The workload and total cycling time for each subject were replicated in the subsequent control trial.

Additional blood samples were collected after 10 min of exercise, at the completion of exercise (postexercise), and 2 h into recovery (2 h postexercise). Predrug as well as preexercise, postexercise, and 2-h postexercise samples were analyzed for alterations in leukocyte counts, T lymphocyte IL-2 and IFN-γ production, and NK cell IFN-γ production. Catecholamines were measured predrug, preexercise, at 10 min, and postexercise. Samples collected preexercise, at 10 min, and postexercise were analyzed for cortisol. Heart rate was recorded at 5-min intervals during exercise.

Leukocyte Counts

Blood (3 ml) was placed into sterile EDTA vacutainer tubes and kept at room temperature until analysis for total and differential white blood cell (WBC) counts as routinely performed by the hematology laboratory at The Alfred Hospital. Thus, in addition to total WBC concentration, we determined neutrophil, monocyte, and lymphocyte concentrations for each blood sample.

Intracellular Cytokines

Blood (2 ml) was placed in sterile sodium heparin vacutainer tubes and kept at room temperature until the end of the trial for measurement of lymphocyte intracellular IL-2 and IFN-γ production. Whole blood (1 ml) was incubated in 1.0 ml of RPMI 1640 medium (Life Technologies, Melbourne, Victoria, Australia) for 10 h and stimulated with 25 ng/ml phorbol 12-myristate 13-acetate (Sigma Aldrich, Castle Hill, New South Wales, Australia) and 1 μg/ml ionomycin (Sigma Aldrich) at 37°C and 5% CO2. Brefeldin-A (10 μg/ml) was added to all cultures at the commencement of the incubation to inhibit intracellular transport of proteins, thus retaining cytokines produced inside the cell.
Staining for T and NK lymphocyte IL-2 and INF-γ production. Aliquots (300 μl) of stimulated and unstimulated blood were incubated for 30 min with CD3 (peridinin chlorophyll protein) and CD56 (R-phycocerythrin; PE)-conjugated monoclonal antibodies (Becton Dickinson, San Jose, CA) for staining of T (CD3⁺) and NK (CD3⁻/CD56⁺) lymphocytes. Red blood cells were lysed (0.15 M ammonium chloride, 10 mM potassium bicarbonate, and 1 mM EDTA) for 10 min, and the samples were spun in a centrifuge (350 g) for 5 min. The supernatant was decanted, and the pellet was resuspended in 500 μl of 4% paraformaldehyde for 20 min. Samples were again spun (350 g) for 5 min, and the supernatant was decanted. The fixed cells were permeabilized with 500 μl of permeabilizing solution (Becton Dickinson) for 20 min, washed (1% fetal calf serum, phosphate-buffered saline, and 0.02 M sodium azide), and spun (350 g) for 5 min, and then the supernatant was decanted. The cells were then incubated with IL-2 (PE), IFN-γ (fluorescein isothiocyanate; FITC), or control (FITC/PE) monoclonal antibodies (Becton Dickinson) for 30 min, making the final combinations of antibodies (CD3⁺/IL-2/FITC/γ; IFN-γ; CD3⁻/CD56⁺/FITC). After the samples had been washed and spun (350 g) for 5 min, the pellet was resuspended in 500 μl of wash buffer. All incubations took place at room temperature in the dark.

Analysis. Analysis was performed using flow cytometry (FACSscan; Becton Dickinson). Lymphocytes were separately gated on a side scatter vs. forward scatter cytogram. Data were acquired for 1 × 10⁴ events within this gate. A subset of T cells expresses CD56; therefore, only CD3⁺ was placed in tubes containing 30 μl of lymphocytes (from the flow cytometer) and the concentration of T and NK lymphocytes by the concentration of peripheral blood using the percentage of T and NK lymphocyte, and monocyte concentrations in the differential leukocyte concentration in the circulation was less (P < 0.01) with BLK postexercise; however, at 2 h postexercise, it was higher (P < 0.01) compared with the control (Table 1).

A Student-Newman-Keuls post hoc test was used to compare data. A Student-Newman-Keuls post hoc test was used to locate differences when the ANOVA revealed a significant interaction. A Statistica (StatSoft, Tulsa, OK) software package was used to compute these statistics. The level of significance to reject the null hypothesis was set at P < 0.05.

Table 1. Circulating total WBC, neutrophil, lymphocyte, and monocyte concentrations in peripheral blood during exercise with or without α- and β-adrenergic blockade

<table>
<thead>
<tr>
<th></th>
<th>Predrug</th>
<th>Preexercise</th>
<th>Postexercise</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs</td>
<td>4.65 ± 0.36</td>
<td>5.32 ± 0.47</td>
<td>8.97 ± 0.55</td>
<td>7.25 ± 0.6*</td>
</tr>
<tr>
<td>BLK</td>
<td>4.82 ± 0.46</td>
<td>5.23 ± 0.49</td>
<td>7.95 ± 0.61</td>
<td>8.54 ± 0.69†</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.51 ± 0.33</td>
<td>2.82 ± 0.46</td>
<td>3.88 ± 0.56</td>
<td>5.15 ± 0.54*</td>
</tr>
<tr>
<td>BLK</td>
<td>2.66 ± 0.48</td>
<td>3.14 ± 0.5</td>
<td>4.02 ± 0.62</td>
<td>6.69 ± 0.69†</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.47 ± 0.08</td>
<td>1.82 ± 0.07</td>
<td>4.02 ± 0.46</td>
<td>1.44 ± 0.07</td>
</tr>
<tr>
<td>BLK</td>
<td>1.49 ± 0.11</td>
<td>1.53 ± 0.06</td>
<td>3.16 ± 0.23</td>
<td>1.21 ± 0.04</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.35 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>0.52 ± 0.03</td>
<td>0.42 ± 0.04*</td>
</tr>
<tr>
<td>BLK</td>
<td>0.33 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td>0.38 ± 0.03</td>
<td>0.42 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are circulating total white blood cell (WBC), neutrophil, lymphocyte, and monocyte concentrations (1 × 10⁹ cells/l) in peripheral blood with (BLK) or without (control, Con) α- and β-adrenergic blockade. Blood samples were collected before treatment (predrug) and before commencement of (preexercise), immediately following (postexercise), and 2 h into recovery from (2 h postexercise) 19.2 min of supine cycling. Values are means ± SE; n = 6 subjects. *Significantly different main time effect from predrug and preexercise (P < 0.01). †BLK significantly different from control (P < 0.01).

RESULTS

In resting subjects, BLK had no effect on total and differential leukocyte concentration in the circulation or on the concentration of T and NK lymphocytes producing cytokines spontaneously or upon stimulation.

There was an increase (P < 0.01) in circulating lymphocyte, monocyte, and neutrophil concentrations postexercise compared with preexercise, resulting in an increase in total circulating leukocyte concentrations (Table 1). Leukocyte concentrations remained elevated 2 h postexercise (P < 0.01), and this was due to maintained monocyte and neutrophil concentrations (Table 1). Lymphocyte concentration returned to preexercise levels postexercise (Table 1). Compared with the control, BLK suppressed (P < 0.05) the increase in circulating lymphocyte and monocyte concentrations postexercise; however, neutrophil concentration was elevated (P < 0.01) 2 h postexercise (Table 1). As a result, the concentration of total leukocytes in the circulation was less (P < 0.01) with BLK postexercise; however, at 2 h postexercise, it was higher (P < 0.01) compared with the control (Table 1).

There was an increase (P < 0.01) in the concentration of T lymphocytes (CD3⁺) and NK lymphocytes (CD3⁻/CD56⁺) in the circulation postexercise compared with preexercise (Fig. 1). As a percentage of total lymphocytes, NK lymphocytes increased (P < 0.01), whereas T lymphocytes decreased postexercise. BLK resulted in a decrease (P < 0.05) in the concentration of NK lymphocytes in the circulation postexercise compared with the control.
pared with the control (Fig. 1). There was a treatment effect in that there were less \((P, 0.05)\) T lymphocytes in the circulation postexercise in BLK compared with the control (Fig. 1).

The concentration of stimulated T lymphocytes positive for IL-2 was increased \((P, 0.01)\) postexercise compared with preexercise (Fig. 2) because of the increase in T lymphocytes in the circulation at this time point. Although there was an increase in the concentration of cells producing IL-2 upon stimulation, there was a decrease \((P, 0.05)\) in the amount that these cells were producing postexercise compared with preexercise (Table 2). There was a treatment effect in that the concentration of stimulated T lymphocytes positive for IL-2 was lower \((P, 0.05)\) with BLK compared with the control (Fig. 2) because the BLK caused a suppression in the concentration of T lymphocytes entering the circulation during exercise. BLK had no effect on the amount of IFN-\(\gamma\) produced upon stimulation (Table 2).

Exercise resulted in an increase \((P < 0.01)\) in the concentration (Fig. 3) of stimulated T lymphocytes producing IFN-\(\gamma\) postexercise compared with preexercise because more lymphocytes were in the circulation at this time. Furthermore, cells produced less \((P < 0.01)\) IFN-\(\gamma\) postexercise compared with preexercise (Table 2). BLK resulted in a decrease \((P < 0.01)\) in the concentration of cells producing IFN-\(\gamma\) postexercise compared with the control (Fig. 3) because the BLK caused a suppression in the concentration of T lymphocytes entering the circulation during exercise. BLK had no effect on the amount of IFN-\(\gamma\) produced upon stimulation (Table 2).

The concentration of NK lymphocytes producing IFN-\(\gamma\) upon stimulation was elevated \((P < 0.01)\) postexercise compared with preexercise (Fig. 4) because these cells entered the circulation. Once again, exercise resulted in a decrease \((P < 0.01)\) in the amount of IFN-\(\gamma\) produced per cell postexercise (Table 2), and this suppression was maintained 2 h postexercise. BLK resulted in a decrease \((P < 0.05)\) in the concentration of stimulated NK cells producing IFN-\(\gamma\) postexercise compared with the control (Fig. 4) because BLK reduced the concentration of these cells in the circulation. There was a difference in the amount of IFN-\(\gamma\) produced by NK cells upon stimulation predrug and preexercise between BLK and the control (Table 2), although the cause of this was unclear.

It is important to note that there was considerable variation in FI of stimulated lymphocytes when BLK and control data were compared, particularly at rest. To overcome any artifactual effect of these variations, we also performed the statistical analyses on log-transformed data for all FI analyses and on changes in FI from the previous measurement point (e.g., preexer-

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**Fig. 1.** T (CD3\(^+\)) and natural killer (NK) (CD3\(^-\)CD56\(^+\)) lymphocyte concentrations in the circulation with (BLK) and without (Con) \(\alpha\)- and \(\beta\)-adrenergic blockade. Blood samples were collected before treatment (predrug) and before commencement of (preexercise), immediately following (postexercise), and 2 h into recovery from (2 h postexercise) 19.2 min of supine cycling. Values are means \(\pm\) SE; \(n = 6\) subjects; Con, control. #Significantly different main time effect from predrug and preexercise \((P < 0.01)\). †BLK significantly different from control \((P < 0.01)\). ††Significantly different main treatment effect from control \((P < 0.05)\).

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**Fig. 2.** Stimulated T lymphocyte (CD3\(^+\)/interleukin (IL)-2\(^+\)) cells with (BLK) and without (Con) \(\alpha\)- and \(\beta\)-adrenergic blockade. Blood samples were collected before treatment (predrug) and before commencement of (preexercise), immediately following (postexercise), and 2 h into recovery from (2 h postexercise) 19.2 min of supine cycling. Values are means \(\pm\) SE; \(n = 6\). #Significantly different main time effect from predrug and preexercise \((P < 0.01)\). ††Significantly different main treatment effect from control \((P < 0.05)\).
ADRENERGIC BLOCKADE AND CYTOKINE PRODUCTION

Table 2. Mean fluorescence intensity of stimulated T cells positive for IL-2 and T cells and NK cells positive for IFN-γ during exercise with or without α- and β-adrenergic blockade

<table>
<thead>
<tr>
<th></th>
<th>Predrug</th>
<th>Preexercise</th>
<th>Postexercise</th>
<th>2 h Postexercise</th>
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</thead>
<tbody>
<tr>
<td>Stimulated CD3⁺/IL-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>106.9±37.6</td>
<td>116.9±28.8</td>
<td>61.4±9.3²</td>
<td>90.6±23.1</td>
</tr>
<tr>
<td>BLK</td>
<td>75.6±13.9</td>
<td>80.7±13.1</td>
<td>50.7±12.2⁶</td>
<td>57.3±9.2</td>
</tr>
<tr>
<td>Stimulated CD3⁺/IFN-γ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>166.4±41.8</td>
<td>171.1±27.9</td>
<td>134.4±31.3³</td>
<td>152.6±31.2</td>
</tr>
<tr>
<td>BLK</td>
<td>282.2±71.5</td>
<td>240.1±45.4</td>
<td>145.8±19.4⁶</td>
<td>197.2±15.4</td>
</tr>
<tr>
<td>Stimulated CD3⁻CD56⁻/IFN-γ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>62.9±10.8</td>
<td>61.8±7.8</td>
<td>41.8±5.5⁶</td>
<td>57.3±8.9⁶</td>
</tr>
<tr>
<td>BLK</td>
<td>185.5±60.5⁺</td>
<td>129.9±30.5⁺</td>
<td>62.3±16.1⁺</td>
<td>92.5±13.4⁺</td>
</tr>
</tbody>
</table>

Values are mean fluorescence intensity (FI; in arbitrary units) of stimulated T cells (CD3⁺) positive for interleukin (IL)-2 and T cells and natural killer (NK) cells (CD3⁻CD56⁻) positive for interferon (IFN)-γ during exercise with (BLK) or without (Con) α- and β-adrenergic blockade. Blood samples were collected before treatment (predrug) and before commencement of (preexercise), immediately following (postexercise), and 2 h into recovery from (2 h postexercise) 19.2 min of supine cycling. Values are means ± SE; n = 6. *Significantly different main time effect from predrug and preexercise (P < 0.01). †BLK significantly different from control (P < 0.01).

cycle — predrug) (Table 2). Both analyses revealed no differences when BLK and control data were compared (range: P = 0.21–0.77).

Plasma epinephrine and norepinephrine concentrations were elevated (P < 0.01) after 10 min of exercise and postexercise (Table 3). BLK resulted in greater (P < 0.05) catecholamine levels at 10 min and greater (P < 0.01) epinephrine levels postexercise (Table 3). Cortisol concentration was increased (P < 0.01) postexercise (Table 3), and BLK resulted in lower levels (P < 0.05) at 10 min and postexercise (Table 3). BLK resulted in a treatment effect of lowering (P < 0.05) heart rate in response to exercise (Table 4).

DISCUSSION

The results from the present study demonstrate that strenuous exercise resulted in an increase in the concentration of stimulated T lymphocytes producing IL-2 and of T and NK lymphocytes producing IFN-γ, but this increase was attenuated by adrenergic blockade, suggesting that adrenergic stimulation contributes to exercise-induced leukocytosis. In contrast, exercise caused a suppression in the amount of IFN-γ and IL-2 produced by stimulated lymphocytes, thereby providing a potential mechanism for the often-reported postexercise immunosuppression. However, since adrenergic blockade did not affect the exercise-induced decrease in the amount of IFN-γ and IL-2 production, it appears that adrenergic stimulation is not a mechanism for this decrease.

Despite an increase in the concentration of cells producing cytokines, stimulated lymphocytes produced less IL-2 and IFN-γ postexercise compared with preexercise. This suppression in NK lymphocyte IFN-γ production was maintained 2 h postexercise. These observations support previous studies that have observed a decrease in supernatant IL-2 and IFN-γ concentrations.
achieved, because the exercise-induced elevation in heart rate and lymphocyte concentration was not completely suppressed in BLK. If complete blockade was not achieved, then a lesser amount of epinephrine could have bound to circulating lymphocytes, and it is possible that this may have resulted in the reduced amount of cytokine produced by stimulated lymphocytes. If this occurred, then further suppression in stimulated cytokine production may not be observed during the control trial. It is possible, however, that complete blockade of the adrenergic receptors was achieved and that other factors caused lymphocyte concentration and heart rate to increase during exercise. Previous studies reported similar reductions in heart rate with β-blockade during strenuous exercise as observed in the present study (14, 39). Furthermore, Murray et al. (24) also observed elevations in lymphocyte concentration during exercise with adrenergic blockade. It is important to note, however, that three other studies did report complete suppression in NK lymphocyte concentration with β-blockade after mental stress (3), parachute jumping (2), and head-up tilt (17).

Plasma cortisol concentration was increased during exercise, and cortisol has been reported to suppress stimulated IL-1, IL-6, and IFN-γ production by monocytes (8). Therefore, it is possible that elevated cortisol levels may have caused the suppression in the amount of IL-2 and IFN-γ produced by stimulated lymphocytes.

As stated previously, suppression of stimulated IFN-γ production postexercise has been hypothesized to cause an increase in the risk of infection (28, 48). In the present study, exercise resulted in a decrease in the amount of cytokine produced by stimulated lymphocytes, providing a potential mechanism for the frequently reported postexercise immunosuppression. It is important to note, however, that exercise also resulted in an increase in the concentration of lymphocytes within the circulation. The overall impact on immune function, as well as whether cytokine production is a limiting factor in immune protection postexercise, is complex. From data in the present study, it cannot be determined whether having more lymphocytes in circulation is beneficial, since these cells cannot respond to stimuli optimally.

The elevated concentrations in plasma epinephrine and norepinephrine during BLK, compared with the control, support previous investigations (12, 38–40) and are most likely to be caused by decreased clearance by β-receptors (5, 6). These elevated levels of catecholamines, therefore, suggest successful blockade of the adrenergic receptors. It is possible, however, that complete blockade of the adrenergic receptors was not

Table 3. Plasma epinephrine, norepinephrine, and cortisol during exercise with or without α- and β-adrenergic blockade

<table>
<thead>
<tr>
<th></th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>2.2 ± 0.4</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>0.5 ± 0.0</td>
<td>0.8 ± 0.2</td>
<td>8.9 ± 2.4†</td>
<td>6.0 ± 1.7††</td>
</tr>
<tr>
<td>Cortisol</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>10.5 ± 2.4†</td>
<td>11.4 ± 3.5†</td>
</tr>
<tr>
<td></td>
<td>0.9 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>22.9 ± 5.1††</td>
<td>15.8 ± 5.5†</td>
</tr>
<tr>
<td></td>
<td>364 ± 34</td>
<td>425 ± 49</td>
<td>552 ± 59*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>335 ± 36</td>
<td>377 ± 53†</td>
<td>436 ± 41*††</td>
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</tr>
</tbody>
</table>

Values are plasma epinephrine, norepinephrine, and cortisol concentrations during exercise with (BLK) or without (Con) α- and β-adrenergic blockade. Blood samples were collected before treatment (p predrug) and before commencement of (p a predexercise), 10 min into the exercise bout (10 min), and immediately after (p postexercise) 19.2 min of supine cycling. Values are means ± SE; n = 6; nm, not measured. †Significantly different main time effect from predrug and preexercise (P < 0.01). †BLK significantly different from Con (P < 0.01).

after a strenuous bout of exercise (1, 19, 28, 33, 41, 48). Given the marked exercise-induced increase in circulating catecholamines and the fact that catecholamines decrease in vitro stimulated monocyte IL-1, IL-6, and TNF-α production by whole blood (4, 11, 36, 46), it is reasonable to suggest that adrenergic stimulation may provide a mechanism for the current and previous (1, 19, 28, 33, 41, 48) observation. Adrenergic blockade did not, however, alter the suppressive effect of exercise on cytokine production. To our knowledge, this is the first study to investigate such a mechanism, and our data provide strong evidence to suggest that the decrease in IL-2 and IFN-γ produced by stimulated lymphocytes as a result of strenuous exercise is not mediated by α- and/or β-adrenergic receptor stimulation.

Table 4. Heart rate during exercise with or without α- and β-adrenergic blockade

<table>
<thead>
<tr>
<th></th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>155 ± 4</td>
<td>172 ± 6</td>
<td>168 ± 5</td>
<td>167 ± 5</td>
</tr>
<tr>
<td>BLK</td>
<td>134 ± 7</td>
<td>141 ± 8</td>
<td>136 ± 8</td>
<td>145 ± 8*</td>
</tr>
</tbody>
</table>

Values are heart rate (beats/min) during exercise with (BLK) or without (Con) α- and β-adrenergic blockade. *Significantly different main treatment effect from control (P < 0.01).
nism for the immunosuppressive effect of strenuous exercise. Because the increased concentration of T lymphocytes producing IL-2 and of T and NK lymphocytes producing IFN-γ upon stimulation was attenuated by adrenergic blockade, it appears that sympathoadrenal activity mediates, in part, the exercise-induced increase in cell concentration. However, because α- and/or β-adrenergic blockade did not affect the exercise-induced amount of IL-2 and IFN-γ produced by stimulated lymphocytes, it is unlikely that adrenergic receptor stimulation mediates this response.

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