This study was designed to test the hypothesis that elevated plasma noradrenaline concentrations contribute to the exercise-induced modulation of the activity and percentage of the natural killer (NK) cells, and the leucocyte concentration. In a single blind, controlled, cross-over study, eight healthy men had noradrenaline infused for 1 h and achieved plasma noradrenaline concentrations comparable (20-fold increment) to those previously observed in cycle ergometer exercise (75% of maximal oxygen uptake for 1 h). The noradrenaline infusion increased the unstimulated, the interleukin-2 and interferon-α stimulated NK cell activity, and the percentage of CD16+ cells. The natural lytic activity per CD16+ cell however, did not change. The concentration of neutrophils, lymphocytes and CD16+ cells increased during the infusion. The neutrophil concentration remained elevated 2 h after infusion, at which time the lymphocyte count was back to normal. These results are comparable with the effects in the exercise model, and it is suggested that the augmented plasma noradrenaline concentrations, seen during extreme exercise, may participate in the exercise-induced immune changes.

**Keywords**  Exercise · Noradrenaline · Natural killer cells · Lymphocytes · Neutrophils

**Introduction**

It has been found that physical exercise modulates the human immune system (Gannon et al. 1995; Rhind et al. 1995; Bousquet et al. 1996; Pedersen and Nielsen 1997). A concomitant stimulation of activity in the sympathetic nervous system and thereby an increase in the plasma catecholamine concentrations has been shown (Kappel et al. 1991b; Kjaer and Dela 1996; Brenner et al. 1997). There has been substantial evidence for a link between the neurohormonal and the immune system (Hoffman-Goetz and Pedersen 1994; Madden and Felten 1995; Madden et al. 1995); β-adrenergic receptors have been found to be highly prevalent on natural killer (NK) cells, compared to CD4+ cells, CD8+ cells, B-cells, and monocytes (Maisel et al. 1990), and in vitro adrenaline and noradrenaline has been found to inhibit the NK cell activity, mediated via β-2-adrenoreceptors (Hellstrand and Hermodsson 1989; Takamoto et al. 1991). The NK cells express β-2, α-1 and α-2 but not β-1 adrenoreceptors, and adrenaline but not noradrenaline infusion has been found to decrease the numbers of β-2 and α-1 receptors (Jetschmann et al. 1997). Noradrenaline has been demonstrated to exert its effects via α- and β-1 adrenoreceptors and to a lesser degree via β-2 receptors (Motulsky and Insel 1982). Sanders and Munson (1985) have found that in vitro noradrenaline enhanced the antibody response, via both α- and β-adrenoreceptors.

In vivo administration of adrenaline to healthy persons to achieve plasma adrenaline concentrations comparable to those seen during cycle ergometer exercise (75% of maximal oxygen uptake, $\dot{V}O_{2\text{max}}$ for 1 h) has been shown to mimic the exercise effect on NK cell activity, lymphokine activated killer cell activity, subpopulations of blood mononuclear cells (BMNC) and lymphocyte proliferation (Kappel et al. 1991b; Pedersen et al. 1997a). The exercise induced effects on T-suppressor cells, NK cells, and lymphocyte activation have been shown to be mediated via β-2 adrenoreceptors (Murray et al. 1992), β-adrenoreceptors being crucial in
the recruitment of neutrophils and lymphocytes (Ahlborg and Ahlborg 1970). Catecholamine administration has induced alterations in the lymphocyte trafficking via β-2 adrenergceptors, whereas granulocyte trafficking has been found to be mediated via α-adrenergceptors (Benshop et al. 1996; Schedowski et al. 1996).

Several studies have shown that exercise at an incremental work intensity and duration concomitantly augmented the catecholamine concentrations, while the effects on immune parameters diverged (Shephard and Shek 1996; Moyna et al. 1996; Kjaer and Dela 1996; Pedersen and Nielsen 1997). Brenner et al. (1996) have found that the fluctuations in CD16+ and CD56+ cells during repetitive moderate exercise (50% of $\dot{V}O_{2\text{max}}$ for 2 × 30 min) were primarily related to the elevated noradrenaline concentrations and blood flow rather than to the increased adrenaline concentrations. However, the role of elevated plasma noradrenaline concentrations in extreme exercise-induced immune changes has not been well described.

The objective of the present experiment was to examine the effects of noradrenaline infusion, to achieve plasma concentrations similar to those reached during intense cycle ergometer exercise (75% of $\dot{V}O_{2\text{max}}$ for 60 min), on NK cell activity, subpopulations of BMNC and leucocyte concentrations.

### Methods

#### Protocol

After having given their informed consent, eight healthy male volunteers [median age 25 years (range 20–28), median mass 75.5 kg (range 66–83), median height 1.84 m (range 1.76–1.90)], participated in the study, which had been approved by the local Ethics Committee of Copenhagen and Frederiksberg Communities (L 92028). The experiment was part of a study in which on 2 different days the subjects received adrenaline and noradrenaline in smaller concentrations than has been described previously (Kappel et al. 1998).

**Experiment design**

In a random order, with at least a 2-week interval:

1. Noradrenaline (Noradrenalin, 1 mg · ml⁻¹, Danish Hospital Pharmacies, Copenhagen, Denmark) diluted in isotonic saline at a rate of 16 µg · min⁻¹, or
2. Isotonic saline was infused for 1 h. A total volume of 30 ml was administered.

Ascorbic acid (Danish Hospital Pharmacies) to a final concentration of 100 mg · ml⁻¹, was added to all infusates.

**Blood sampling**

The baseline blood sample was drawn at approximately 8 a.m., after at least 30 min of rest. The infusion was then begun and lasted for 1 h. The second sample was drawn during the last minutes of infusion and 2 h after finishing the infusion the last sample was drawn. Blood samples (100 ml) were immediately transferred to heparinized (Heparin, Danish Hospital Pharmacies; final concentration 25 U · ml⁻¹) vacutainers (Becton Dickinson, Mountain View, Calif, USA) and shortly after BMNC were prepared.

**Assays**

The BMNC preparation, analysis of cell surface markers and leucocyte counts, detection of plasma adrenaline and noradrenaline concentrations were carried out as previously described (Kappel et al. 1998).

**NK activity**

The NK cell activity was measured using K562 target cells in a 51Cr release assay as previously described (Kappel et al. 1998) using effector-to-target cell ratios: 100:1, 50:1, 25:1, 12.5:1. The lytic units (LU) were calculated as the number of effector cells required to lyse 10% of 10 000 target cells, and the results were presented as the number of LU contained in 10⁷ cells in accordance with Eq. 10 in Bryant et al. (1992; except that the slope was calculated for each person). The LU per CD16+ cell (LU/CD16 +) were calculated as

$\text{LU/CD16+} = \text{LU} / (10^7 \times (\text{CD16%}/100))$.

### Statistical methods

The standard errors in this study cannot be used to judge significance between means, since these were all calculated for the same eight persons. The differences between the results obtained before treatment and those obtained during or after treatment were calculated for each person and type of treatment, including the controls. The significance of these changes was ascertained using two-way analysis of variance (the GLM procedure in SAS, version 5; SAS Institute, N.C., USA) by person and type of treatment. Multiple regressions with noradrenaline and adrenaline concentrations as independent variables, tested the correlations with the immune parameters. Wilcoxon’s signed paired rank sum test was used for comparison of plasma adrenaline and noradrenaline concentrations (Table 1). A $P < 0.05$ was considered significant and the degree of significance is indicated by different symbols.

### Results

The mean plasma noradrenaline concentrations increased 20-fold [3.566 (SEM 0.343) vs 0.171 (SEM 0.014) ng · ml⁻¹] during noradrenaline infusion but were not different from the control values 2 h afterwards (Table 1). The plasma adrenaline concentrations were elevated three-fold [0.045 (SEM 0.005) vs 0.013 (SEM 0.003) ng · ml⁻¹] during noradrenaline infusion and returned to baseline values 2 h later (Table 1).

#### Table 1 The concentrations of noradrenaline and adrenaline in relation to infusion of noradrenaline or isotonic saline (control) for 1 h. The values before, during the last minutes of infusion, and 2 h after are shown

<table>
<thead>
<tr>
<th>Noradrenaline</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>SEM</strong></td>
</tr>
<tr>
<td>Noradrenaline (ng · ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.171</td>
</tr>
<tr>
<td>During</td>
<td>3.566</td>
</tr>
<tr>
<td>After</td>
<td>0.324</td>
</tr>
<tr>
<td>Adrenaline (ng · ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.013</td>
</tr>
<tr>
<td>During</td>
<td>0.045</td>
</tr>
<tr>
<td>After</td>
<td>0.026</td>
</tr>
</tbody>
</table>

*P < 0.05 from control and baseline values