A Combined Stress Hormone Infusion Decreases In Vivo Protein Synthesis in Human T Lymphocytes in Healthy Volunteers

Anna Januszkiewicz, Pia Essén, Margaret A. McNurlan, Olle Ringdén, Peter J. Garlick, and Jan Wernerman

In vivo protein synthesis decreases in mononuclear cells following a combined stress hormone infusion given to healthy volunteers as a human trauma model. Here, the purpose was to further investigate this finding and to measure in vivo protein synthesis in isolated T lymphocytes. Furthermore, the effects of stress hormones on the lymphocyte subpopulations and mononuclear cells, characterized by flow cytometry and phytohemagglutinin (PHA)-induced and unstimulated proliferative responses in vitro, were elucidated. Healthy volunteers (n = 16) were randomized into 2 groups to receive either a stress hormone or a saline infusion for 6 hours. In vivo protein synthesis was studied before and after the treatment by measuring the incorporation of stable isotopically-labeled phenylalanine into lymphocyte and mononuclear cell proteins. Protein synthesis decreased after stress hormone infusion in both cell populations: in T lymphocytes from 13.0% ± 0.7%/d (mean ± SD) to 8.6% ± 2.1%/d ($P < .01$) and in mononuclear cells from 13.3% ± 1.2%/d to 6.3 ± 2.0%/d ($P < .001$). No change in proliferative responsiveness in vitro was observed. The stress hormone infusion produced a decrease in the percentage of T helper CD3/CD4 from 41% to 18% ($P < .001$), T cytotoxic CD3/CD8 from 27% to 15% ($P < .001$), as well as total T CD3 cells from 69% to 35% ($P < .001$). There was an increase in the percentage of natural killer (NK) cells CD16/CD56 from 17% to 55% ($P < .001$). Determination of phenotypes expressed on activated T lymphocytes showed that CD3/HLA-DR was unchanged and CD3/CD25 decreased from 14% to 7% ($P < .01$) in the stress hormone group. The study showed that the decrease of in vivo protein synthesis was 34% in T lymphocytes as compared with 53% in mononuclear cells, when determined immediately after a 6-hour stress hormone infusion. This change was associated with a pronounced decrease in all lymphocyte subpopulations, except for the NK cells, which increased substantially.

SURGICAL TRAUMA AFFECTS immune competence, which is not easily characterized by any single marker. A number of tests are used to determine the level of activation of circulating immune cells: in vitro proliferative responses with or without stimulation, characterization of cell populations and subpopulations by assessment of surface cell markers, expression of activity markers, and pattern of secreted cytokines.[1-4] In vivo determination of protein synthesis enables the measurement of the ongoing metabolic activity in circulating immune cells as a measure of their activation. This is a qualitatively different approach to study activation of immune cells, which may contribute to characterization of the immune competence in patients.

A combined stress hormone infusion, consisting of epinephrine, cortisol, and glucagon, is a well–defined stress model in healthy volunteers, which mimics a number of metabolic changes observed after surgery or trauma.[5-7] With this model, in vivo protein synthesis in mononuclear cells decreases by 60% immediately after a 6-hour stress hormone infusion.[8] On the other hand, cortisol alone, given as a 6-hour infusion, does not change the rate of in vivo protein synthesis in T lymphocytes of healthy volunteers, either immediately or 18 hours after the end of the infusion.[9]

In the present study, the effect of a 6-hour combined stress hormone infusion on in vivo protein synthesis in T lymphocytes, as well as mononuclear cells, was determined. Furthermore, the lymphocyte subpopulations and the activation markers were characterized, and in vitro proliferative responses were studied.

MATERIALS AND METHODS

Materials

L-[$^2H_5$-ring] phenylalanine, 99 atom%, (Mass Trace, Woburn, MA) was dissolved in sterile water together with unlabelled phenylalanine (Ajinomoto Company, Tokyo, Japan) to a concentration of 20 g/L and an appropriate isotopic enrichment. The solutions were prepared, heat sterilized, and stored in sterile containers.

Subjects and Experimental Protocols

Healthy male volunteers (n = 16) participated in the study (age, 32 ± 7 [mean ± SD]; weight, 79 ± 11 kg; height, 181 ± 9 cm; body mass index [BMI], 24 ± 3 kg/m^2) and were randomized into 2 groups. There were no differences between the groups concerning age, weight, height, and BMI. Group 1 received a stress hormone infusion: epinephrine at 0.5 nmol/kg/min (Adrenalin NM Pharma, NM Pharma, Stockholm, Sweden), cortisol at 6 μg/kg/min (Solu-Cortef, Upjohn, Puurs, Belgium), and glucagon at 3 ng/kg/min (Glucagon Novo Nordisk, Novo Nordisk, Malmö, Sweden) for 6 hours in saline at 3 mL/kg/h (Natriumklorid, Pharmacia, Stockholm, Sweden). Group 2 served as a control group and received saline at 3 mL/kg for 6 hours. The study started in the morning after an overnight fast. Protein synthesis of lymphocytes and mononuclear cells was determined twice: before the start of the infusion and during the last 90 minutes of the infusion. Blood samples were collected for determination of protein synthesis rate, lymphocyte proliferative responses, and characterization of T-lymphocyte subpopulations.

Heart rate and electrocardiogram were continuously monitored with S&W Medico Teknik Diascope device (Simonsen & Weel, Taastrup, Denmark), and blood pressure was controlled by auscultation every second hour.

From the Departments of Anaesthesiology and Intensive Care and Clinical Immunology, Huddinge University Hospital, Karolinska Institutet, Stockholm, Sweden; and the Department of Surgery, State University of New York, Stony Brook, NY.

Submitted November 13, 2000; accepted April 12, 2001.

Address reprint requests to Anna Januszkiewicz, MD, Department of Anaesthesiology and Intensive Care, Huddinge University Hospital, SE - 141 86 Stockholm, Sweden.

0026-0495/01/5011-0010$35.00/0
doi:10.1053/meta.2001.27204

The nature, purpose of the study, and possible risks were explained to all volunteers before obtaining their consent. The research protocol was approved by the Ethical Committee of the Huddinge University Hospital, Karolinska Institutet, Stockholm, Sweden.

Determination of Protein Synthesis Rate in Peripheral Blood T Lymphocytes and Mononuclear Cells

An intravenous injection of L-[$^2H_5$]phenylalanine (45 mg/kg, 10 mol% excess [MPE], first determination and 20 MPE, second determination) was given into an antecubital vein over 10 minutes. Venous blood samples were taken from the opposite arm before (time 0), and at 5, 10, 15, 30, 50, 70, and 90 minutes after injection for measurement of the isotopic enrichment of phenylalanine in plasma. For the measurement of the enrichment of L-[$^2H_5$]phenylalanine in protein of T lymphocyte and mononuclear cells (MNC), 70 mL of blood was drawn at 90 minutes after the start of the isotope injection during the first determination and at time 0 and 90 minutes after the start of the isotope injection during the second determination.

Isolation of MNC From Peripheral Blood and Separation of T Lymphocytes

A total of 70 mL of blood (20 mL for MNC isolation and 50 mL for further separation of T lymphocytes) was poured into 7 heparinized, 10-mL tubes containing cycloheximide (0.5 mmol/L, Sigma, St Louis, MO) each. Blood was diluted 1/1 with 70 mL of phosphate-buffered saline (PBS), pH 7,4 (Unimedic, Matfors, Sweden) at 18°C and transferred to 7 50-mL Falcon tubes. The MNC were isolated with a Ficoll-Isopaque gradient (Lymphoprep, Nycomed, Oslo, Norway) by centrifugation at 800×*g* for 20 minutes at room temperature. MNC were harvested from the interface and washed 3 times with PBS.[10] At this step, the pellet of cells provided for determination of protein synthesis in MNC was stored at -80°C, whereas the pellet collected from the other 5 tubes was resuspended with PBS to a final concentration of 10^7 cells/mL for further processing. Count and viability of cells were determined by microscopic inspection following eosin staining. T lymphocytes were separated by the rosette method with 1% 2-aminoethylisothiuronium bromide (AET)-treated sheep red blood cells (SRBC). SRBC rosette-forming cells were separated on Ficoll-Isopaque.[11] SRBC were lysed with 1% ammonium oxalate for 5 minutes at room temperature. The cells were washed 3 times with PBS and stored at -80°C.

Analytical Methods

The determination of L-[$^2H_5$]phenylalanine enrichment in plasma, as well as in hydrolyzed protein from isolated T lymphocytes and MNC, was made by gas chromatography-mass spectrometry (GCMS) under electron impact, selective ion monitoring. The enrichment in plasma was measured from ions m/z 336 and 431 of the tertiary butyldimethylsilyl derivative as described previously.[12] Protein from the lymphocytes and MNC samples was precipitated with cold 2% perchloric acid. The protein precipitate was washed extensively, solubilized with 0.3 mol/L sodium hydroxide, and reprecipitated, followed by hydrolysis in 40% hydrochloric acid at 110°C for 24 hours. After enzymatic decarboxylation to phenylethylamine, the enrichment of the heptafluorobutyryl derivative was determined by GCMS at m/z 106 and 109 as described previously.[13] The determinations were performed on a MD 800 quadrupole GCMS (Fisons Instruments, Beverly, MA).

Calculations of Protein Synthesis Rate in T Lymphocytes and MNC

The rate of the protein synthesis was calculated as described previously: $FSR = (P_{(t)} - P_{(0)}) \times 100/A$, in which FSR is the fractional synthesis rate of protein (% per day).[12] $P_{(0)}$ and $P_{(t)}$ are the enrichments of phenylalanine in protein of T lymphocytes and MNC at the beginning and end of the incorporation period (MPE), and A is the area under the curve for plasma phenylalanine enrichment versus time (MPE × time in days).

Measurement of Proliferative Responses of MNC

Blood samples were drawn before the first and second determination of protein synthesis in EDTA/Venoject tubes (Terumo Europe N.W., Leuven, Belgium) and diluted 1/1 with PBS. MNC were isolated with a Ficoll-Isopaque gradient by centrifugation at 800×*g* for 20 minutes at room temperature, followed by wash in PBS. Viability was checked by eosin exclusion. Cells were suspended in culture medium consisting of RPMI 1640 medium, supplemented with 25 mmol Hepes and 2 mmol L-glutamine (Gibco BRL, Life Technologies, Paisley, Scotland), Pest (penicillin + streptomycin 100 μg/mL, Gibco BRL), and 10% heat-inactivated fetal bovine serum (Gibco BRL). A total of 100 μL of cell suspension (1×10^5 cells) was cultured in wells of flat-bottomed plates with either 100 μL of culture medium (spontaneous proliferation) or 100 μL of phytohemagglutinin (PHA-M, Sigma) 100 μg/mL (mitogenic stimulation). Cells were cultured for 72 hours in humidified air and 5% CO_2 at 37°C. For the last 24 hours of culture, 2 μCi of ^{3}H-thymidine (Radiochemical Centre, Amersham, UK) was added to each well. Cells were harvested by a Skatron Combi Cell Harvester (Skatron Instruments, Suffolk, UK), and radioactivity was measured with a Liquid Scintillation Counter (Wallac, Turku, Finland). All wells were cultured in triplicates × 2 and results expressed as mean counts per minute (cpm).

Characterization of Lymphocyte Subpopulations

For characterization of lymphocyte subpopulations, the whole blood was incubated with 1 of the combinations of murine antihuman monoclonal antibodies: CD3/CD4, CD3/CD8, CD3/CD56+16, CD3/HLA-DR (Simultest, Becton Dickinson, San Jose, CA) and CD2 fluorescein isothiocyanate (FITC), CD19 phycoerythrin (PE), CD3 FITC, CD25 PE (Becton Dickinson). The Simultest Control (Becton Dickinson) was used as a negative control tool. FACS Lysing Solution (Becton Dickinson) was used to lyse erythrocytes, and the stained cells were analyzed by flow cytometry Simulsetprogram (Becton Dickinson). The light-scatter gate was defined by anti-CD45/anti-CD14 (Simultest LeukoGATE, Becton Dickinson) to distinguish lymphocytes from granulocytes, monocytes, and unlysed red blood cells. Lymphocyte subset absolute counts were calculated as total lymphocyte count × lymphocyte subset percentage. The CD4/CD8 ratio was calculated as the fraction of cells positive to the CD4 antibody (helper cells) divided by the fraction of cells positive to the CD8 antibody (cytotoxic cells).

White blood cell counts were determined with a Hx3 blood cell analyzer (Bayer Diagnostics, Tarrytown, NY).

Statistical Analysis

Data are presented as means ± SD. Comparison of mean values for 2 measurements was performed by Student's *t* test for paired or unpaired samples. Differences associated with physiologic responses were tested with analysis of variance. One-way analysis of variance with repeated measures was used to compare differences within each group. Two-way analysis of variance with repeated measures was used to compare differences between 2 groups. *P* values less than .05 were considered statistically significant.

RESULTS

Physiological Responses

All subjects who received the combined stress hormone infusion experienced enhanced heartbeat for several minutes

after the start of the infusion. Heart rate was increased from 71 ± 6 beats/minute at the beginning of the study to the 95 ± 9 beats/minute at the end of the study. This increase was different compared with the control group: 63 ± 11 versus 59 ± 8 ($P < .001$). Systolic blood pressure remained unchanged throughout the study in both groups, whereas diastolic blood pressure dropped in the stress hormone group from 74 ± 6 mm Hg at the beginning of the study to 59 ± 12 mm Hg at the end of the study ($P < .05$). Values for the control group were 69 ± 8 mm Hg and 71 ± 11 mm Hg, respectively.

Protein Synthesis in T Lymphocytes and MNC

The protein synthesis rate in T lymphocytes was 12.8% ± 1.0%/d as the mean value of the first determination in the 2 groups combined. It decreased by 34% ± 17% (mean ± SD) ($P < .01$) during the 6-hour stress hormone infusion (Table 1). In the MNC, the rate of protein synthesis as the mean value of the first determination in the 2 groups combined was 12.7% ± 1.6%/d. Infusion of stress hormones caused a decrease in protein synthesis by 53% ± 16% ($P < .001$) (Table 2). Protein synthesis did not change in the control groups, either in T lymphocytes or in MNC throughout the study. One subject in each group was excluded from the study due to the failure with separation of T lymphocytes.

Proliferation of Unstimulated and PHA-Induced MNC

In vitro proliferative responsiveness in unstimulated and PHA–stimulated MNC was determined at time points 0 hour and 4.5 hours (Fig 1). In the unstimulated cells, there was no change in the control group, whereas in the stress hormone group, there was an increased responsiveness at 4.5 hours in 2 of 5 subjects. Following PHA stimulation, the proliferative response decreased marginally in controls, as well as in the stress hormone group. From each group, 3 subjects were excluded due to technical problems with the isolation and culture of MNC, therefore, these data were not included in the statistical analysis.

Analysis of Cell Surface Phenotype

Lymphocyte phenotyping for each group was made at time points 0 hour and 4.5 hours. Data for leukocytes, lymphocytes, T-lymphocyte subpopulation counts, and CD4/CD8 ratio are shown in Table 3. Values demonstrate leukocytosis in the stress hormone group ($P < .001$). A decrease in lymphocytes, as well as T helper CD3/CD4, T cytotoxic CD3/CD8, and total T CD3 counts was observed in both groups ($P < .01$ in the stress hormone group, $P < .05$ in the control group), whereas natural killer (NK) CD16/CD56 count increased in the stress hormone group ($P < .05$).

Table 1. Rates of Protein Synthesis (%/d) in T Lymphocytes Determined at Two Time Points (Before and After 6 Hours of Stress Hormones or Saline Infusion)

	Group 1 Stress Hormones		Group 2 Saline	
Subject	0 Hour	6 Hours	0 Hour	6 Hours
1	12.9	6.1	12.7	13.8
2	13.1	11.0	12.3	12.2
3	12.9	10.0	10.4	10.8
4	12.9	5.5	13.8	12.4
5	14.5	9.2	12.5	10.9
6	12.0	10.0	12.2	11.4
7	12.8	8.1	13.8	12.2
Mean ± SD	13.0 ± 0.7	8.6 ± 2.1*†	12.5 ± 1.2	12.0 ± 1.0

*$P < .001$ within the group.
†$P < .01$ between the groups.

Table 2. Rates of Protein Synthesis (%/d) in MNC Determined at Two Time Points (Before and After 6 Hours of Stress Hormones or Saline Infusion)

	Group 1 Stress Hormones		Group 2 Saline	
	0 Hour	6 Hours	0 Hour	6 Hours
1	14.6	6.1	13.4	11.5
2	11.8	9.0	12.8	12.6
3	13.1	3.6	11.4	10.3
4	14.1	8.4	9.9	10.1
5	13.5	4.9	9.6	12.1
6	11.7	4.8	13.4	11.9
7	14.5	7.0	13.7	12.5
Mean ± SD	13.3 ± 1.2	6.3 ± 2.0*†	12.0 ± 1.7	11.6 ± 1.0

*$P < .001$ within the group.
†$P < .001$ between the groups.

Figure 2 demonstrates changes in the percentage of lymphocyte subpopulations. There was a reduction in the percentage of $CD4^+$ cells from 41% ± 9% to 18% ± 8% ($P < .001$), $CD8^+$ cells from 27% ± 6% to 15% ± 4% ($P < .001$), total $CD3^+$ cells from 69% ± 7% to 35% ± 10% ($P < .001$) in the stress hormone group. By contrast, there was a 3–fold increase in the percentage of $CD16/CD56^+$ cells from 17% ± 10% to 55% ± 12% ($P < .001$) after 4.5 hours of stress hormone infusion. CD4/CD8 ratio remained unchanged.

Results for the activated T lymphocytes are presented in Table 4. $CD3^+$/HLA-DR expression did not change, but $CD3^+/CD25^+$ expression decreased after 4.5 hours of the stress hormone infusion ($P < .01$) and increased slightly, but significantly in the control group ($P < .01$).

DISCUSSION

A combined stress hormone infusion resulted in an increased peripheral count of leukocytes and a decreased count of lymphocytes. Among the T-lymphocyte subpopulations, helper and cytotoxic cells were reduced, while NK cells increased in number. The in vivo protein synthesis rate of T lymphocytes and MNC decreased by 34% and 53%, respectively. In contrast, the in vitro proliferative responses were not altered significantly.

The in vivo protein synthesis rate was determined in 2 cell populations: in T lymphocytes, separated with the rosette method, which in our hands gives a purity of 90% to 95% as determined by flow cytometry, and in MNC, isolated by the density gradient and consisting of 60% to 80% lymphocytes and 20% to 35% monocytes.[9] Protein synthesis rate in the basal state in the combined stress hormone and control groups was 12.8% ± 1.0%/d in T lymphocytes and 12.7% ± 1.6%/d in the MNC. This rate of protein synthesis is relatively high, as compared with human skeletal muscle with a rate of only 2%/d, and human liver, which has a fractional synthesis rate of total (both

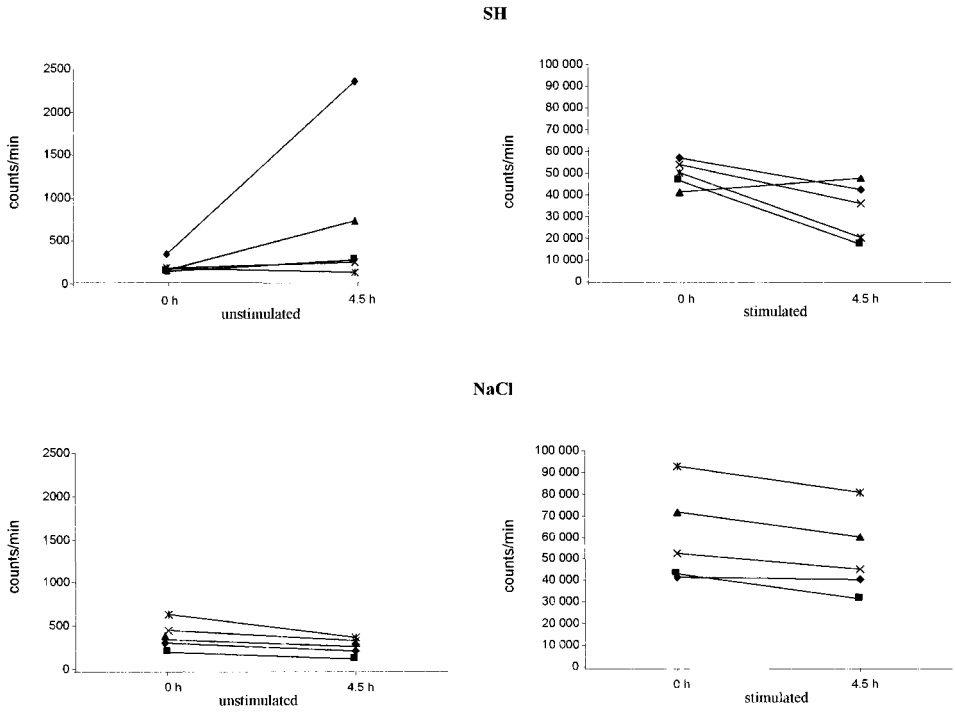

Fig 1. In vitro proliferative responsiveness in unstimulated and PHA-stimulated MNC determined before and after 4.5 hours of a combined stress hormone or saline infusion in healthy volunteers (n = 10). The lines represent values for the individual volunteers.

stationary and export) proteins of approximately 24%/d.[14,15] T lymphocytes are involved in the cell-mediated immunity, and together with monocytes, share an immunoregulatory role. In vivo determination of protein synthesis in T lymphocytes and MNC reflects the metabolic activity of these immune cells, including synthesis of cytokines and other regulatory proteins, cell division, production of prostaglandins, and cell receptors. Our results suggest that both T lymphocytes and monocytes are metabolically active even in the resting state, and it can be speculated that immune cells continuously synthesize regulating proteins and receptors necessary to preserve homeostasis of the immune system.

The combined stress hormone infusion induced a decrease of protein synthesis by 34% in the circulating T lymphocytes. By contrast a 6-hour infusion of cortisol alone does not affect the rate of protein synthesis, either immediately or 18 hours after the end of the infusion, when given to healthy volunteers.[9] The decrease observed after the combined infusion of the stress hormones may therefore be explained by the need for several stress hormones together or that hormones other than cortisol, possibly epinephrine alone, mediate responses leading to the decrease of protein synthesis.

MNC showed the same pattern as T lymphocytes following the combined stress hormone infusion, with the protein synthesis rate decreased by 53% immediately after the end of the infusion. This decrease in protein synthesis, being more accen-

Table 3. Counts of Leukocytes and Lymphocyte Subsets (Count × 10^9/L) Determined Before and After 4.5 Hours of Stress Hormone or Saline Infusion

	Group 1 Stress Hormones		Group 2 Saline	
	0 Hour	4.5 Hours	0 Hours	4.5 Hours
Leukocytes	6.3 ± 1.10	15.80 ± 2.80*†	5.80 ± 1.40	5.10 ± 1.20
Lymphocytes	2.10 ± 0.80	1.00 ± 0.20‡§	1.90 ± 0.60	1.40 ± 0.20‖
T_{helper} ($CD3^+/CD4^+$)	0.85 ± 0.41	0.17 ± 0.08‡†	0.87 ± 0.34	0.65 ± 0.16‖
$T_{cytotoxic}$ ($CD3^+/CD8^+$)	0.61 ± 0.30	0.15 ± 0.05‡†	0.53 ± 0.17	0.41 ± 0.14‖
Total T ($CD3^+$)	1.46 ± 0.59	0.33 ± 0.10‡†	1.45 ± 0.40	1.09 ± 0.22‖
NK ($CD16^+/CD56^+$)	0.35 ± 0.15	0.60 ± 0.16‖†	0.25 ± 0.12	0.17 ± 0.04
CD4/CD8 ratio	1.67 ± 0.70	1.28 ± 0.65	1.65 ± 0.74	1.62 ± 0.56

NOTE. Values are expressed as mean ± SD. The CD4/CD8 ratio is calculated as a fraction of CD4 positive cells divided by the fraction of CD8 positive cells.

*$P < .001$ within the group.

†$P < .001$ between groups.

‡$P < .01$ within the group.

§$P < .01$ between groups.

‖$P < .05$ within the group.

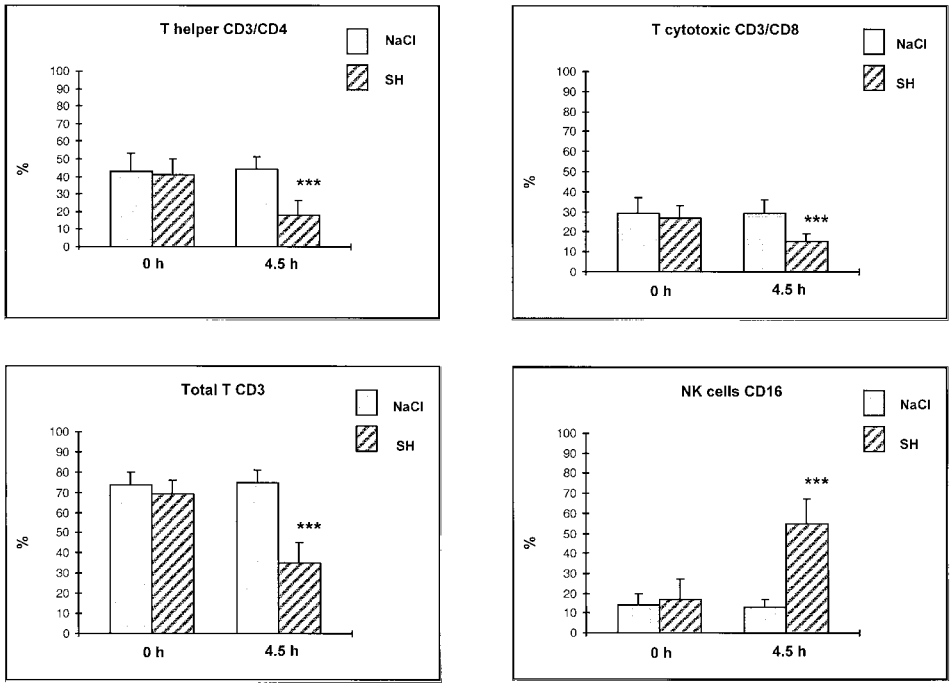

Fig 2. Changes in T-lymphocyte subpopulations determined by flow cytometry before and after 4.5 hours of a combined stress hormone or saline infusion in healthy volunteers (n = 16). Results for the selected subpopulations are expressed as a percentage of all lymphocytes (mean ± SD). *** $P < .001$ in the stress hormone group *v* 0 hour.

tuated in the MNC, means that monocytes, which are less abundant, have a metabolic activity suppressed more than T lymphocytes. The magnitude of the observed reduction in MNC is in accord with results reported earlier in healthy volunteers, in which the decrease by 60% at the end of the 6-hour combined stress hormone infusion is followed by normalization at 18 hours after cessation of the infusion.[8] A similar pattern with immediate suppression of the metabolic activity is also observed in a human sepsis model.[16] An endotoxin bolus given to healthy volunteers decreases protein synthesis in circulating T lymphocytes, whereas leukocytes, consisting of lymphocytes, monocytes, and neutrophils, show an increase, probably due to the increased protein synthesis rate in the neutrophils. On the other hand, infusion of recombinant interleukin (IL)-2, which induces proliferative and cell-mediated responses of T lymphocytes, enhances the protein synthesis rate in MNC of patients with colon cancer.[17] All of these results together show that the determination of in vivo protein synthesis may reflect immediate changes in activation of immune cells.

A combined epinephrine, cortisol, and glucagon infusion has been used as a human trauma model in a number of studies, which focused on metabolic changes following major surgery and trauma.[8,18] A triple-hormone cocktail provides plasma hormone concentrations similar to those found during abdominal surgery and is safely tolerated by healthy volunteers.[19] There are no previous data on the impact of a combined stress hormone infusion in vivo on human T lymphocytes or monocytes, even though the effects of single stress hormones on immune cell function have been extensively studied in vitro.[20-24] In the present study, no alteration of the proliferation of MNC in response to stress hormones was detected, although in the PHA-stimulated group, there was a tendency to a slight decrease of the rate of thymidine incorporation. In the unstimulated group, some individuals showed an enhanced proliferative response following the hormonal challenge, but the change was not uniform and therefore not conclusive. In particular, there was no parallelism between the 50% reduction of in vivo protein synthesis observed in all subjects and the more scattered and discrete changes seen in the in vitro proliferative response, with or without stimulation.

Table 4. Percentage of Activated T Lymphocytes Determined Before and After 4.5 Hours of Stress Hormone or Saline Infusion

	Group 1 Stress Hormones		Group 2 Saline	
	0 Hour	4.5 Hours	0 Hours	4.5 Hours
$CD3^+$/HLA-DR %	13 ± 18	5 ± 2	6 ± 5	7 ± 5
$CD3^+/CD25^+$ %	14 ± 6	7 ± 5*†	14 ± 4	16 ± 4*

NOTE. Values are expressed as mean ± SD.
*$P < .01$ within groups.
†P .01 between groups.

Cells counts, as well as cell populations and subpopulations, assessed by the expression of surface markers, are often used as a measure of cellular immune status. In the present study, the increase in the white cell count and the decrease in the number of lymphocytes after 4.5 hours of stress hormone infusion was observed. This is in accord with the pattern observed in healthy volunteers who received a bolus dose of epinephrine and hydrocortisone together, as well as postoperatively in surgical patients.[22,25] In addition, distinct changes in the lymphocyte subpopulations were observed during the stress hormone infusion. There was an 80% decrease of helper lymphocytes ($CD4^+$), a 75% decrease of cytotoxic lymphocytes ($CD8^+$), and an almost 80% decrease of total T lymphocytes ($CD3^+$) presented as the absolute number of cells. The analysis of the

percentage of lymphocyte subpopulations revealed the reduction to half of that in the basal state in all of those cell populations. The effects of a short exposure to catecholamines with or without a bolus dose of hydrocortisone on T-lymphocyte subpopulations are well known, however, the impact of the combined stress hormone infusion has not been described earlier.[22,26-28]

After 4.5 hours of saline infusion, there was a decrease in count of lymphocytes, with a consequent drop of $CD4^+$, $CD8^+$, and $CD3^+$ T lymphocytes, without changes in the percentage of these populations. Probably it depends on the insignificant decrease in the percentage of lymphocytes (data not shown) and the total white cell count, as the lymphocyte count was calculated from those values. Then, in turn, all lymphocyte subpopulation counts were calculated from the absolute number of lymphocytes.

NK cells showed a distinct response to the combined stress hormones. A 3-fold increase in the percentage of NK cells out of the total lymphocytes, corresponding to a 2-fold increase in the absolute count, was demonstrated after 4.5 hours of the infusion. This is in accord with the increased count of NK cells seen following a short pharmacologic stress exerted by intravenously administered epinephrine and hydrocortisone.[22,26-28] An unexpected observation was the statistical correlation between the change in the fraction of NK cells and the change in protein synthesis in total T lymphocytes ($r^2 = .60$, $P < .05$, Fig 3). A possible explanation is that circulating NK cells have a lower rate of protein synthesis as compared with other T-cell subpopulations, such as helper and cytotoxic cells. However, more direct measurements are needed to establish in vivo protein synthesis in the different T-lymphocyte subpopulations.

Expression of activation markers, such as HLA-DR or $CD25^+$, is another measure of increased activity of immune cells. In the present study, we could demonstrate a 50% decrease in the percentage of T lymphocytes expressing $CD25^+$. Expression of the HLA-DR receptor showed a big scatter, thus a possible decrease in the stress hormone group might have been observed. There are no data in the literature concerning the effects of single or combined stress hormones on the expression of these activity markers. However, in the early postoperative period, there is a transient increase in the number of $CD4^+$ cells expressing HLA-DR.[2]

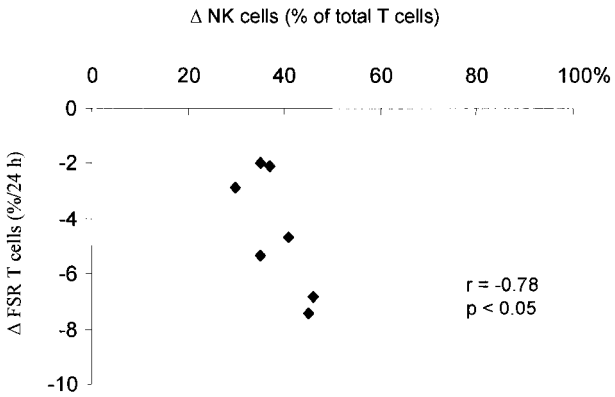

Fig 3. The decrease in the protein synthesis rate in total T lymphocytes as related to the change in the proportion (%) of NK cells out of total lymphocytes after a combined stress hormone infusion in healthy volunteers (n = 7). A statistically significant correlation was found ($r = -.78$, $P < .05$).

In conclusion, the decrease of in vivo protein synthesis in circulating T lymphocytes was 34% as compared with 53% in MNC immediately after a 6-hour combined stress hormone infusion. This metabolic response to the hormonal challenge was accompanied by pronounced changes in the lymphocyte subpopulations and in the expression of the activity markers. Measurement of protein synthesis reflects the metabolic activity and activation of immune cells. When evaluating immune competence in patients, measurement of the protein synthesis rate gives the opportunity to add an in vivo assessment of cellular activity to the markers of immune function.

ACKNOWLEDGMENT

We would like to thank Viveka Gustavsson and Peder Winnbrant for excellent nursing assistance; George Casella, Charlotte Tammik, and Eva Skoog for the expert technical assistance; and Associate Professor Birger Christensson and Anette Jonsson for performing the immunofluorescence analysis.

REFERENCES

1. Cheadle WG, Pemberton RM, Robinson D, et al: Lymphocyte subset responses to trauma and sepsis. J Trauma 6:844-849, 1993

2. Hensler T, Hecker H, Heeg K, et al: Distinct mechanisms of immunosuppression as a consequence of major surgery. Infect Immun 6:2283-2291, 1997

3. O'Sullivan ST, Lederer JA, Horgan AF, et al: Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished IL-12 production associated with decreased resistance to infection. Ann Surg 222:482-490, 1995

4. Schäffer M, Barbul A: Lymphocyte function in wound healing and following injury. Br J Surg 85:444-460, 1998

5. Bessey PQ, Watters JM, Aoki TT, et al: Combined hormonal infusion stimulates the metabolic response to injury. Ann Surg 200: 264-281, 1984

6. Wilmore DW: Are the metabolic alterations associated with critical illness related to the hormonal environment? Clin Nutr 5:9-19, 1986

7. Wernerman J, Hammarqvist F, Botta D, et al: Stress hormones alter the pattern of free amino acids in human skeletal muscle. Clin Physiol 13:309-319, 1993

8. McNurlan MA, Sandgren A, Hunter K, et al: Protein synthesis rates of skeletal muscle, lymphocytes, and albumin with stress hormone infusion in healthy man. Metabolism 45:1388-1394, 1996

9. Januszkiewicz A, Essén P, McNurlan MA, et al: In vivo protein synthesis of circulating human T lymphocytes does not respond to a cortisol challenge within 24 hours. Acta Anaesthesiol Scand 44:202-209, 2000

10. Bøyum A: Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 5:9-15, 1976 (suppl 5)

11. Parker JW: Immunologic basis for the redefinition of malignant lymphomas. Am J Clin Pathol 72:670-686, 1979

12. McNurlan MA, Essén P, Thorell A, et al: Response of protein synthesis in human skeletal muscle to insulin: An investigation with L-[$^2H_5$]phenylalanine. Am J Physiol 267:E102-108, 1994

13. Essén P, McNurlan MA, Thorell A, et al: Determination of protein synthesis in lymphocytes in vivo after surgery. Clin Sci 91:99-106, 1996

14. Essén P, McNurlan MA, Wernerman J, et al: Uncomplicated surgery, but not general anesthesia, decreases muscle protein synthesis. Am J Physiol 262:E253-E260, 1992

15. Barle H, Nyberg B, Essén P, et al: The synthesis rates of total liver protein and albumin determined simultaneously in vivo in man. Hepatology 25:154-158, 1997

16. Januszkiewicz A, Essén P, Andersson B, et al: In vivo protein synthesis in T lymphocytes and leukocytes is affected in response to an endotoxin challenge in healthy volunteers. Clin Nutr 19:14, 2000 (suppl 1, abstr)

17. Park KGM, Heys SD, McNurlan MA, et al: Lymphocyte protein synthesis in vivo: A measure of activation. Clin Sci 86:671-675, 1994

18. Rosenfeld BA, Faraday N, Campbell D, et al: Hemostatic effects of stress hormone infusion. Anesthesiology 81:1116-1126, 1994

19. Smeets HJ, Kievit J, Harinck HI, et al: Differential effects of counterregulatory stress hormones on serum albumin concentrations and protein catabolism in healthy volunteers. Nutrition 11:423-427, 1995

20. Deitch EA, McIntyre Bridges R: Stress hormones modulate neutrophil and lymphocyte activity in vitro. J Trauma 10:1146-1154, 1987

21. Crary B, Borysenko M, Sutherland DC, et al: Decrease in mitogen responsiveness of mononuclear cells from peripheral blood after epinephrine administration in humans. J Immunol 2:694-697, 1983

22. Brohee D, Vanhaeverbeek M, Kennes B, et al: Leukocyte and lymphocyte subsets after a short pharmacological stress by intravenous epinephrine and hydrocortisone in healthy humans. Intern J Neurosci 53:53-62, 1990

23. Chernish SM, Davidson JA, Brunelle RL, et al: Response of normal subjects to a single 2-miligram dose of glucagon administered intramuscularly. Arch Int Pharmacodyn Ther 218:312-327, 1975

24. Mihas AA, Gibson RG, Mihas TA: Effects of gastrointestinal hormonal peptides on the transformation of human peripheral lymphocytes. Res Commun Chem Pathol Pharmacol 73:123-126, 1991

25. Hansbrough JF, Bender EM, Zapata-Sirvent R, et al: Altered helper and suppressor lymphocyte populations in surgical patients. A measure of postoperative immunosuppression. Am J Surg 148:303-307, 1984

26. Crary B, Hauser SL, Borysenko M, et al: Epinephrine-induced changes in the distribution of lymphocyte subsets in peripheral blood of humans. J Immunol 3:1178-1181, 1983

27. Schedlowski M, Falk A, Rohne A, et al: Catecholamines induce alterations of distribution and activity of human natural killer (NK) cells. J Clin Immunol 5:344-351, 1993

28. Tønnesen E, Christensen NJ, Brinkløv MM: Natural killer cell activity during cortisol and adrenaline infusion in healthy volunteers. Eur J Clin Invest 17:497-503, 1987