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Changes of immunoregulatory cells induced by psychological and physical stress: relationship to plasma catecholamines

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SUMMARY

Lymphocyte subpopulations were measured before and after physical and psychological stress in 15 healthy subjects and correlated with plasma catecholamine and cortisol levels. During psychological stress monocytes ($P < 0.05$), NK ($P < 0.01$), B cells ($P < 0.05$) and heart rate ($P < 0.001$) increased, while catecholamines remained unchanged. With physical stress granulocytes, monocytes and all lymphocyte subsets increased significantly, although B cells rose more than T cells and T (suppressor) cells more than T (helper) cells. Thus the ratio of T/B cells and of Th/Ts cells decreased ($P < 0.001$ and $P < 0.01$). Adrenaline and noradrenaline concentrations increased ($P < 0.001$), while cortisol remained unchanged. There was a negative relationship between adrenaline and the Th/Ts cell ratio before and after stress ($P < 0.05$). Lymphocyte subpopulations from a different group of 4 healthy subjects were analysed before and after isoproterenol infusion. There was a small increase in Ts and B cells only ($P < 0.1$) and a decrease of the T/B cell ratio ($P < 0.05$). The predominant enrichment of circulating B, Ts and NK cells during short lasting adrenergic activation, as well as the relationship of the T cell changes to plasma adrenaline, suggest an immunoregulatory effect of the symphathetic nervous system in stress.

Keywords immunoregulatory cells stress catecholamines

INTRODUCTION

Psychological and physical stress is known to alter the functioning of the immune system (Riley, 1981; Hedfors, Holm & Öhnell, 1976; Eskola *et al.*, 1978). A reduced lymphocyte proliferative response, impairment of antibody synthesis as well as blood leucocyte changes have been associated with bereavement in man and monkeys (Bartrop *et al.*, 1977; Reite, Harbeck & Hoffman, 1981), after exposure to loud noise or overcrowding in mice (Monjan & Collector, 1977; Gisler, 1974), after application of inescapable electric shock in rats (Laudenslager *et al.*, 1983) or during physical exercise in man (Hedfors *et al.*, 1983).

The adrenocortical hormones and the sympathetic nervous system have been postulated as mediators of the immunosuppressive effect of stress (Sorkin, Del Rey & Besedovsky, 1981; Keller *et al.*, 1983). Thus, in both humans and animals a reduced cellular or humoral immune response *in vitro* (Crary *et al.*, 1983; Indiveri *et al.*, 1983; Depelchin & Letesson, 1981), as well as distribution changes of circulating leucocytes (Bishop *et al.*, 1968; Fauci & Dale, 1974; Slade & Hepburn, 1983; Eriksson & Hedfors, 1977; Gader & Cash, 1975) have been measured after cortisol and adrenaline administration. However, phenotypic changes in lymphocyte subtypes occurring after physiologi-

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cal activation of the adrenergic nervous system have not been studied in relation to the stimulating neurotransmitters. Therefore blood leucocyte subtype distributions were investigated before and after psychological and physical stress and related to plasma catecholamine and cortisol levels.

MATERIALS AND METHODS

Subjects. Psychological and physical stress testing was performed in 15 normal subjects, 11 males and four females, aged 17–25 years (median 20 years). Fifteen millilitres of blood for leucocyte analysis and determination of catecholamine and cortisol levels in plasma was taken after an initial resting period of 20 min, exactly at the end of an 8 min cognitive conflict test and after bicycle ergometry which was carried out to submaximal work capacity. Our mental stress test was similar to Bjorkvall's modification (Bjorkvall, 1966) of Stroop's colour word conflict test (Stroop, 1935). In each subject maximal physical work capacity was determined at least 24 h prior to the study by exercising the subject to exhaustion on increasing work loads of 25 W each. Ergometry on the day of the study was carried out with work loads of 25%, 50% and 75% of the previously determined maximal work capacity, each lasting 5 min. The procedures were started at 10 a.m., electrocardiogram and blood pressure were registered during the whole test. Heart rate was calculated from the electrocardiogram R–R intervals. To differentiate between stress induced changes and those due to the circadian rhythm, plasma cortisol was determined in four healthy resting controls (three females, 19–22 years) at the same time intervals as in stressed subjects. Isoprenaline sensitivity was tested in four healthy subjects (two males, 21–30 years old) as previously described (Bertel *et al.*, 1980). Bolus injections of isotonic saline as a control followed by isoprenaline were given i.v. through an indwelling cannula placed 30 min. prior to the study. The starting dose of 0·1 μg isoprenaline/m^2 body surface was gradually increased until the chronotropic dose$_{25}$ (CD_{25}), i.e. a rise of 25 beats/min from the resting heart rate) was reached. Heart rate was measured from the electrocardiogram after each dose. Blood for leucocyte subtype analysis, plasma catecholamines and cortisol was collected at the beginning, after NaCl administration and at the CD_{25}.

Leucocyte and hormone analysis in stress tests and isoprenaline sensitivity testing. Total leucocytes in blood and in the buffy coat harvested from 10 ml of blood were counted with Gentian violet, 29 ± 3% (mean ± s.e.) of the blood leucocytes were harvested in the buffy coat. One million buffy coat cells/sample were taken up in 150 μl of phosphate-buffered saline (PBS) with 10 mM sodium azide (NaN_3) and incubated for 30 min at 4°C according to a slight modification of the method described by Hoffman *et al.* (1980) with 6 μg of the biotinylated monoclonal antibodies (MoAb) Leu 1, Leu 2a and Leu 3a (Becton Dickinson, Sunnyvale, California, USA), with 10 μl of OKM1 (marker for NK cells, monocytes and granulocytes from Ortho Diagnostic Systems, Raritan, New Jersey, USA) or with PBS containing 10 mM NaN_3. After the first incubation, samples were treated with lysing buffer for 3 min at 20°C, washed and resuspended in balanced salt solution (BSS with NaN_3). T cells were incubated for 30 min at 4°C with 6 μg of avidin coupled fluorescein isothiocyanate (FITC) (Becton Dickinson), OKM1 treated cells with 50 μl of 1:10 diluted rabbit anti-mouse immunoglobulin (Ig) FITC (Nordic, Tilburg, Netherlands) and B cells with 50 μl of 1:5 diluted goat anti-human F(ab')$_2$ Ig FITC (Cappel Labs Inc., Cochranville, Pensylvania, USA). After washing, all cell samples were resuspended in PBS with NaN_3 at a concentration of 2×10^6 cells/ml.

Leucocyte and lymphocyte subpopulations were analyzed in a Cytofluorograf 50HH combined with the Computer 2150 (Ortho Instruments, Westwood, Maine, USA). By excitation of the cells with Argon laser light at 488 nm (500 mW, light mode), 90° scatter, forward narrow angle scatter and green fluorescence could be measured simultaneously; the fluorescence signals were logarithmically amplified. The numbers of lymphocytes, monocytes and granulocytes were counted in the different regions of the scatter cytogram. Erythrocytes, platelets, dead cells and debris were eliminated from the scatter or fluorescence analysis by electronically gating out cells with low scatter signals. Within the lymphocyte cluster the spectra of the immunofluorescent cells (Leu 1, Leu 2a, Leu 3a, Ig and OKM1) were recorded. Gating was kept identical in the samples from each subject

before and after stress. The 50,000 cells acquired/sample together with the total leucocyte count from buffy coat served to calculate the absolute number of the different cell types in the buffy coat. Lymphocytes were measured with an intraindividual variation coefficient ranging from 1 to 2·8%; for monocytes this value ranged from 2·5 to 5·6% and for granulocytes it was between 1·25 and 6·8% (seven samples each of 10 subjects).

The OKM1 MoAb was used both for labelling monocytes in the monocyte cluster and NK cells in the lymphocyte cluster (Zarling *et al.*, 1981). In preliminary studies the NK activity of cells sorted from the lymphocyte cluster was determined in a ^{51}Cr release assay with K-562 target cells. Lysis of lymphocytes prior to sorting with OKM1 and complement reduced NK activity to $30 \pm 7\%$ and $12 \pm 3\%$ (effector to target ratio 33:1 and 11:1, respectively) of its value in untreated lymphocytes. Catecholamines were measured in the supernatant plasma of the buffy coat preparation by a radioenzymatic assay (Da Prada & Zürcher, 1976) and plasma cortisol was determined with a radioimmunoassay kit.

Effect of catecholamines in vitro. Two to five million mononuclear leucocytes (MNL) prepared by Ficoll density gradient centrifugation from blood of two resting subjects were incubated in the dark for 15 min at 37°C in BSS, containing 4% heat-inactivated fetal calf serum (FCS) and 10 mM NaN_3 with four concentrations of either adrenaline ranging from 15 to 500 pg/ml or noradrenaline ranging from 80 to 2,500 pg/ml. The catecholamines were dissolved in 1 mM hydrochloric acid immediately prior to use. After incubation the samples were washed twice with BSS and processed for immunofluorescent staining of T cell subpopulations and B cells as described above. No phenotypic changes could be induced within the subsets by incubation of MNL with adrenaline (Leu 1 $70·0 \pm 0·6\%$, Leu 2a $16·1 \pm 0·1\%$, Leu 3a $51·8 \pm 0·3\%$, Ig $11·5 \pm 0·3\%$) or noradrenaline (Leu 1 $73·8 \pm 0·4\%$, Leu 2a $34·6 \pm 0·1\%$, Leu 3a $41·0 \pm 0·1\%$, Ig $8·1 \pm 0·2\%$) at any of the concentrations employed.

Statistical analyses. Cellular, biochemical and haemodynamic parameters before and after stress showed a normal distribution and were statistically compared by the paired *t*-test and linear regression analysis.

RESULTS

Quantitative cellular changes

As shown in Table 1, in buffy coat the mean number of leucocytes as well as lymphocytes increased only slightly after psychological but significantly ($P < 0·001$) after physical stress. Six subjects showed a pronounced increase in the number of granulocytes, while there was little or no change in the granulocyte count in the other nine; nevertheless, the mean granulocyte number was significantly higher after physical stress ($P < 0·001$). Contrary to the T lymphocytes, which only increased slightly after cognitive stress testing, monocytes (OKM1 positive, $P < 0·05$), NK OKM1 positive lymphocytes, $P < 0·01$) and B (Ig) cells showed a moderate but significant ($P < 0·05$) increase after psychological stress. After physical excercise all lymphocyte subtypes were found to be enriched in the circulation, although B cells increased more than T cells (Leu 1) and the increase of Ts (suppressor) (Leu 2a) cells was higher than that of Th (helper) (Leu 3a) cells. Thus the ratio of T/B cells was significantly ($P < 0·001$) decreased by 46% and the ratio of Th/Ts cells was decreased by 19% ($P < 0·001$) after physical stress.

Qualitative cellular changes

A representative example of fluorescent spectra registered from one individual before and after physical exercise is presented in Fig. 1. The spectra of Leu 1 cells had a mean fluorescence intensity in channel 338 ± 27 (mean $\pm$ s.d.) before and 339 ± 28 after ergometry; similarly, the spectra of the Leu 3a cells showed a normal distribution before and after physical stress with mean values in channel 277 ± 27 and 274 ± 21, respectively. Two populations of fluorescent cells could be distinguished in the spectra of both B cells and Leu 2a cells. After physical stress only the strongly fluorescent B cells increased, whereas among the Leu 2a cells the weakly fluorescent population of cells increased and the strongly fluorescent cells decreased. In the spectra of the OKM1 positive

Table 1. Leucocyte subpopulations (absolute numbers were measured in buffy coat obtained from 10 ml of blood of 15 subjects) before (control) and after psychological and physical stress

Cells ($\times 10^6$)	Control	Stress	
		Psychological	Physical
Total leucocytes	14·6±1·3	15·5±1·1	33·7±4·0‡
Granulocytes	5·2±0·5	5·6±0·5	12·7±2·0‡
Monocytes scatter	0·37±0·04	0·40±0·03	0·93±0·09‡
OKM1	0·11±0·07	0·14±0·07*	0·32±0·23‡
Lymphocytes	6·9±0·8	7·4±0·7	14·1±1·5‡
B cells (Ig)	1·4±0·1	1·8±0·2*	4·5±0·4‡
T cells (Leu 1)	4·9±0·7	5·1±0·5	0·3±1·1‡
T helper (Leu 3a)	3·1±0·4	3·1±0·3	4·9±0·6†
T suppressor (Leu 2a)	1·8±0·3	1·9±0·2	3·7±0·5‡
NK (OKM1)	0·30±0·05	0·47±0·07†	1·36±0·33‡
Ratio			
T/B	3·43±0·31	3·09±0·31	1·86±0·20‡
Leu 3a/Leu 2a	1·79±0·21	1·70±0·18	1·38±0·11‡

Monocytes were defined by their scatter, and with the OKM1 MoAb, after analysis in the monocyte cluster. The number of OKM1 positive cells was also measured in the lymphocyte cluster to label NK cells. Mean±s.e.; *$P<0·05$, †$P<0·01$, ‡$P<0·001$ comparing stress results with control values as calculated with the paired t-test.

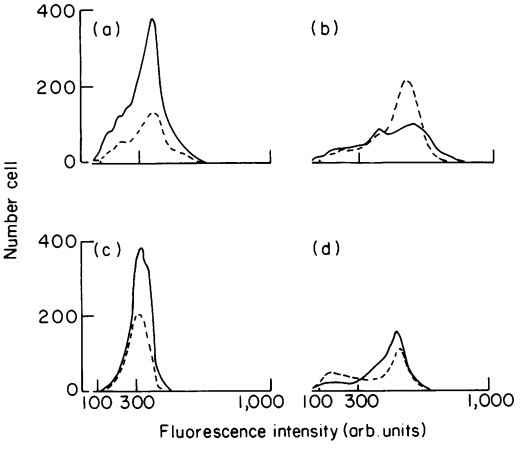

Fig. 1. Fluorescence spectra of antibody labelled lymphocyte subpopulations before (solid lines) and after physical stress (dashed lines) measured in one subject. (a) Leu 1, (b) Ig, (c) Leu 3a and (d) Leu 2a.

lymphocytes, which showd a normal distribution, the mean fluorescence intensity rose from a control value of 298±37 to 348±32 after physical stress.

Hormonal and hemodynamic changes

As shown in Table 2, after cognitive stress testing only heart rate and blood pressure amplitude were significantly ($P<0·001$) increased. After exercise a significant ($P<0·001$) increase was observed for plasma adrenaline and especially noradrenaline as well as for heart rate and blood pressure amplitude. Plasma cortisol was slightly decreased after the psychological stress and there was no

Table 2. Biochemical and haemodynamic responses to psychological and physical stress testing in 15 subjects

		Stress	
	Control	Psychological	Physical
Adrenaline (pg/ml)	46±5	52±5	109±11*
Noradrenaline (pg/ml)	279±21	288±20	1,094±107*
Cortisol (nmol/l)	465±53	378±49	386±48
Heart rate (beats/min)	65±2	73±2*	165±5*
Blood pressure (mmHg)			
systolic	111±3	124±3*	150±4*
diastolic	71±3	81±3*	58±4*

Mean±s.e.; *$P<0.001$; paired t-test for data before and during stress tests.

further change after ergometry. In the four subjects examined at the same time intervals without stress there was a decrease in cortisol concentration from a mean value of 493±32 to 362±61 nmol/l, which was not significantly different from the results obtained after stress. The six subjects mentioned above who exhibited granulocytosis had significantly higher cortisol levels (531±150, 448±220, 481±155 nmol/l, mean±s.e., measured before and after psychological and physical stress) than the subjects without granulocytosis (314±176, 220±101, 202±88 nmol/, $P<0.05$). The percentage increase of granulocytes was significantly correlated with the basal cortisol levels ($r=0.643$, $P<0.05$, data not shown). Interestingly, an inverse relationship was found between the plasma adrenaline level and the ratio of Th/Ts cells before (Fig. 2a, $r=-0.544$, $P<0.05$) and after psychological stress ($r=0.693$, $P<0.01$, Fig. 2b). The % increase of adrenaline during ergometry was also inversely related to the % change of the ratio Leu 3a/Leu 2a cells ($r=-0.555$, $P<0.05$, Fig. 3). An inverse relationship was observed between the increase of adrenaline and the increase of heart

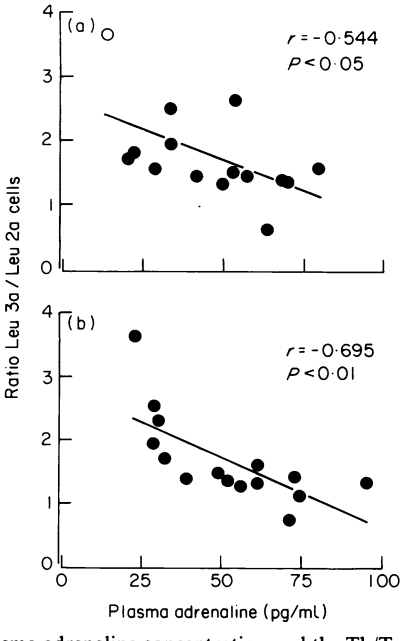

Fig. 2. Relationship between plasma adrenaline concentration and the Th/Ts cell ratio (a) before and (b) after psychological stress.

rate after physical stress ($r = -0{\cdot}517$, $P < 0{\cdot}05$, data not shown). Consequently basal heart rate was also significantly related to the basal Th/Ts ratio ($r = 0{\cdot}528$, $P < 0{\cdot}05$) while the rise of the heart rate after ergometry was significantly related to the stress-induced change in this ratio ($r = 0{\cdot}516$, $P < 0{\cdot}05$).

Isoprenaline sensitivity testing

As shown in Table 3, after isoprenaline bolus injection, which produced a heart rate increase of 25 beats/min, there was no significant alteration in the absolute values of granulocytes, monocytes,

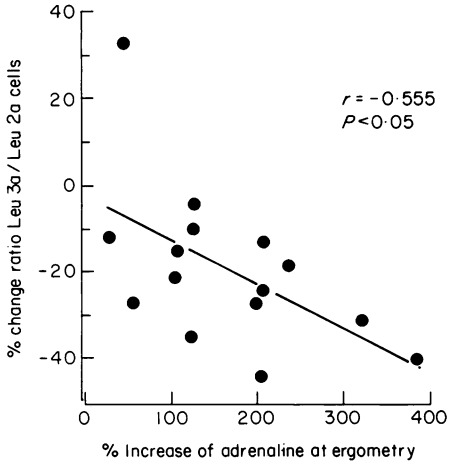

Fig. 3. Relationship between the percentage increase of adrenaline and the percentage change of the Th/Ts cell ratio after physical stress.

Table 3. Leucocyte subpopulations (absolute numbers were measured in buffy coat obtained from 10 ml of blood of four subjects) at rest (control) and at the CD_{25} (bolus injection of isoprenaline producing an increase of heart rate by 25 beats/min)

Cells ($\times 10^6$)	Control	CD_{25}
Total leucocytes	16·9±3·1	21·2±7·5
Granulocytes	7·5±1·8	8·8±3·1
Monocytes scatter	0·35±0·08	0·39±0·11
OKM1	0·16±0·04	0·11±0·05
Lymphocytes	4·8±1·5	6·5±2·7
B cells (Ig)	1·1±0·5	2·8±1·7
T cells (Leu 1)	3·0±1·1	2·9±0·09
T helper (Leu 3a)	2·2±0·8	2·1±1·0
T suppressor (Leu 2a)	1·1±0·2	1·5±0·5
NK (OKM1)	0·26±0·12	0·92±0·81
Ratio		
T/B	3·22±0·36	1·73±0·39*
Leu 3a/Leu 2a	1·93±0·47	1·36±0·29

Monocytes were defined by their scatter, and with the OKM1 MoAb, after analysis in the monocyte cluster. The number of OKM1 positive cells was also measured in the lymphocyte cluster to label NK cells. Mean±s.e., *$P < 0{\cdot}05$, in the paired t-test comparing CD_{25} with control values.

lymphocytes and NK cells. Due to the relative enrichment of B cells, the T/B ratio was significantly ($P < 0{\cdot}05$) decreased. There was also a relative increase in the number of Ts cells which led to a reduced Th/Ts cell ratio at the limit of significance ($P < 0{\cdot}1$). Neither plasma adrenaline, noradrenaline nor cortisol were significantly increased. Isotonic saline did not produce any cellular or hormonal changes (data not shown).

DISCUSSION

In the present study quantitative and qualitative changes of blood lymphocytes, monocytes and granulocytes were produced by short term psychological and physical stress. After psychological stress the numbers of circulating monocytes, B cells and NK cells were increased; after physical stress increased numbers of all leucocyte subpopulations, but preferentially of B and Ts cells were observed. The stressing procedures used produced a short lasting stimulation of symphathetic nervous activity. A β-adrenergic mechanism was probably involved as reflected by the small increase in heart rate after isoprenaline administration and psychological stress. The marked increase in heart rate, blood pressure amplitude as well as adrenaline and noradrenaline after physical stress points to a combined α- and β-adrenergic activation.

Physical stress is known to increase plasma cortisol, but as shown by Moorthy & Zimmerman (1978), strenuous, prolonged exercise such as marathon running is necessary to activate adrenocortical hormones; this is confirmed by our results which show a slight decrease of the plasma cortisol level according to the circadian rhythm, but no increase after short term exercise. However, only subjects with high cortisol levels before and after stress exhibited granulocytosis and its extent was significantly related to the basal cortisol level. This is in agreement with reports on granulocytosis after cortisol administration (Bishop *et al.*, 1968) and is currently believed to result from the redistribution of granulocytes between blood and bone marrow (Fauci, 1975). We suggest that either cortisol regulates blood granulocytes independently of the adrenergic system or that cortisol has a permissive role in the mobilization of granulocytes after adrenergic activation. To our knowledge leucocyte analysis has not been performed in association with psychological stress before; on the other hand our findings after ergometry confirm previous reports on the effect of i.v. or s.c. administered adrenaline upon peripheral leucocytes. Eriksson & Hedfors (1977) as well as Yu & Clements (1976) showed that after adrenaline injection absolute numbers of T cells indentified as sheep erythrocyte rosette forming cells were increased. The preponderant increase in the number of B and NK cells after short term exercise has been described earlier by Hedfors *et al.* (1976) and Hedfors, Biberfeld & Wahren (1978). The decrease of the Th/Ts cell ratio as well as the increased numbers of monocytes observed in the present study might explain the reduced T lymphocyte proliferation response upon mitogen stimulation *in vitro* which has been reported after adrenaline adminstration and short term exercise (Hedfors *et al.*, 1976; Crary *et al.*, 1983). In addition, Sorkin *et al.* (1981) showed that an increased immune response to sheep red blood cells *in vivo* is associated with a reduced noradrenaline content in the spleen of symphathectomized animals. Our findings of the increased antigen density per cell and the high NK cell number might be related to the increased *in vitro* NK cell activity which has been found in healthy subjects after noradrenaline infusion (Kraus *et al.*, 1983).

In the present study the stress induced changes were measured in the small blood compartment consitituting only 10% of the total lymphoid cell pool; and as these did not exceed 100–200% they might be of limited significance. However, the altered distribution of lymphocytes is in good agreement with the functional effects of catecholamines *in vitro* reviewed above and might thus significantly contribute to stress induced immunosuppression. The increased blood lymphocyte numbers might indicate that the catecholamines prevent the cells from residing in the sites of antigen or target recognition and processing.

The mechanism of action of catecholamines in inducing leucocyte changes is unknown. Our data exclude an *in vitro* effect. Because cells were mobilized selectively and qualitative changes of the phenotypes of B and Ts cells accompanied the quantitative alterations, *in vivo* the catecholamines probably not only influence the flow in lymphoid organs but also act by direct binding to

immunocompetent cells. β-adrenergic receptor measurements on human lymphocyte subtypes performed earlier in our laboratory showed a predominant localization of these receptors on B cells as compared to T cells and a higher affinity for the antagonist (±)^{125}I-cyanopindolol in Ts cells than in Th cells (Landmann *et al.*, 1984). In this study the increase in the number of B cells occurred during slight adrenergic activation and was independent of any change in plasma catecholamines. Also, B and Ts cells showed the most pronounced changes after ergometry or after isoprenaline sensitivity testing. We may thus relate the cellular changes to a direct action of adrenaline upon β-adrenergic receptors on lymphoid cells. The negative relationship between adrenaline and the Th/Ts cell ratio before and after psychological and physical stress might also be interpreted as a direct effect of adrenaline upon β-adrenergic receptors in T cell subpopulations. Our hypothesis is supported by data from Ernström & Sandberg (1973) who found an increased release of lymphocytes from the spleen into the blood without any flow change in normal or sympathectomized guinea-pigs treated with catecholamines.

In summary, the present results suggest that adrenaline plays an important role in the mobilization of immunocompetent cells and might lead to a distribution pattern favouring immunosuppression during stress.

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