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Adrenalectomy does not prevent thymic involution induced by stress in mice

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Abstract

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Thymic involution in response to immobilization stress is studied in C57BL/6 mice subjected to adrenalectomy. Thus, adrenalectomized mice show a decrease of thymus weight which is similar to that observed in stressed animals with adrenal glands. This stress-induced change mainly affects the thymic population of immature thymocytes. Corticosteroids seem not to be responsible for the thymic involution by stress.

Key words: Stress, thymus, corticosterone, adrenals, thymocytes.

Resumen

PÉREZ-MERA, M.L. & REY-MÉNDEZ, M. (1993). La adrenalectomía no previene en ratones la involución tímica inducida por el estrés. *Nova Acta Científica Compostelana (Bioloxía)*, **4**: 179-184

La involución tímica en respuesta a estrés de inmovilización fue estudiada en ratones C57BL/6 sometidos a adrenalectomía. Los ratones adrenalectomizados mostraron un decrecimiento del peso del timo que era similar al observado en animales con glándulas adrenales y estresados. Este cambio inducido por el estrés afecta principalmente a la población inmadura de timocitos. Los corticosteroides parecen no ser responsables de la involución tímica inducida por el estrés.

Palabras clave: Estrés, timo, corticosterona, adrenales, timocitos.

INTRODUCTION

Stress has been shown to exert significant effects on immune system, in which the thymus gland plays a central role (KELLER *et al.*, 1981). Thymus involution which is an age-related process has been associated with stress and its effects on the modulation of immune system (MONJAN, 1981). This involution was described as early as 1936 by Selye and has been related to lymphopenia, adrenal hypertrophy and involution of spleen (STEIN *et al.*, 1985).

The processes that may mediate stressful influences on immune function are unknown. The endocrine system is highly responsive to stress and probably has a significant effect on

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immune processes. The secretion of corticosteroids has long been considered to be the mechanism of stress-induced modulation of immunity and related disease processes (SELYE, 1937, 1973; DANTZER, 1991).

Glucocorticoids have been recognized for their dramatic suppression of the immune system, including thymic involution and lysis or redistribution of circulating lymphocytes (CUPPS & FAUCI, 1982; KIRSCHBAUM *et al.*, 1992). It has been shown that adrenalectomy results in thymic enlargement (SANTISTEBAN & DOUGHERTY, 1954) and that lymphocyte function, which is suppressed by stress-induced adrenal activity, can be prevented by adrenalectomy (KELLER *et al.*, 1983). On the other hand, COMPTON & CIDLOWSKI (1986) have found that corticosteroids induce thymocyte death through DNA degradation, since in vivo treatment of adrenalectomized rats with glucocorticoids results in the rapid breakdown of the thymocyte genome at internucleosomal sites (COMPTON & CIDLOWSKI, 1987). Thymocytes DNA degradation during the cell death period after dexamethasone injection has been also shown recently by SCHWARTZ *et al.* (1993).

These facts suggest that thymic involution can be related to the stress-induced increase in the level of corticosteroids and tended to support the hypothesis that adrenal hormones are the primary mediators of stress effects on the immune system. However, previous results obtained in our laboratory (PÉREZ-PARALLÉ MERA, 1990) indicate that this increase in the level of corticosteroids in stress conditions (eight times higher than control) does not produce a breakdown of thymocyte DNA. Moreover, KELLER *et al.* (1983) have found that lymphocyte stimulation by mitogens in stress conditions is suppressed in adrenalectomized rats.

These findings have lead us to investigate the effects of immobilization stress in adrenalectomized C57BL/6 mice to determine if adrenals are required for stress-induced thymic involution. For this reason, immobilization stress was applied to adrenalectomized C57BL/6 mice and its effects were studied by measuring the weight of lymphatic organs (spleen and thymus), the number of mature and immature thymocytes, and body weight gain.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (35±2 days old), weighing about 16-19g were housed in a temperature and light-controlled room with free access to food and water. Lights were on for 12h/day begining at 08.00h; temperature was 22±1°C.

Animals were assigned to the following experimental groups, four per cage:

(CO) Control: mice were left undisturbed in the animal house for the experimental time.

(CA) Adrenalectomized control: mice were operated, adrenal glands removed, and left undisturbed in their cages for the experimental time.

(SA) Sham adrenalectomized: mice were operated under ether anesthesia but adrenal glands were not removed.

(IA) Immobilization stress and adrenalectomized: mice were bilaterally adrenalectomized under ether anesthesia and, after three days of rest, were stressed by immobilization for 14 days.

After adrenal surgery all mice were provided with a 0.15M NaCl solution in their drinking water.

Controls and stressed animals were maintained in recomended conditions for these studies (RILEY, 1981).

Stress protocols

Immobilization stress was performed during 14 days between 10.00 and 12.00 a.m. Mice were immobilized by taping their four limbs in supine position to a board for 90min a day.

General procedures

Adrenalectomy and sham operation were performed by a lateral subcostal approach using ether anesthesia (WAYNFOTH, 1980). Absence of adrenal tissue was confirmed by both necropsy and corticosterone levels.

Mice were weighed at the start and the end of the experimental period in order to asses their rate of weight gain. After the stress or control period, animals were immediately killed by decapitation, after ether anesthesia, and the trunk

blood collected and centrifuged (1500xg, 10min) at 4°C. Serum was frozen at -20°C and adrenals, spleen and thymus were removed and weighed. Plasma corticosterone levels in trunk blood were measured by the method of ZENKER & BERNSTEIN (1958).

Cell preparation

Thymuses were minced in cold phosphate-buffered saline (PBS), and dispersed by pressing through a stainless-steel mesh to obtain a single cell suspension. Erytrocytes were removed by treatment with a lysing buffer (17mM Tris-HCl and 144 mM NH4Cl, pH 7.2) at 0°C for 10 min. Cells were washed twice in PBS containing 1% (w/v) bovine serum albumin (BSA; SIGMA, USA), and resuspended in the same buffer.

Fractionation of thymocytes

The fractionation was carried out essentially according to the method decribed by REISNER & SHARON (1984). $2x10^8$ cells in 0.25ml of PBS were mixed with 0.25ml of peanut agglutinin (PNA; SIGMA, USA) solution (1.0mg/ml PBS) in a small plastic tube, left at room temperature for 20min, and then layered on top of 20% (v/v) heat inactivated fetal calf serum (FCS; GIBCO, Europe) in PBS. After 30min at room temperature, the fraction settled to the bottom of the tube (PNA^+), and the upper layer containing PNA^- thymocytes was carefully aspirated and transferred to tubes containg 5ml of 0.2M D-Galactose (SIGMA, USA) in PBS to dissociate agglutinated cells. After 15min at room temperature, 5ml of PBS were added and cells collected by centrifugation (200xg, 5min), resuspended in 5ml of 0.2M D-Galactose in PBS, pelleted again and finally washed twice with PBS. In all the experiments cell viability was determined by trypan blue exclusion and only cell suspensions with a viability of 90% or more were used.

Statistics

The results are expressed as the mean values ± S.E.M. Data were obtained from eight mice (n=8). Statistical evaluation was performed by one-way analysis of variance. Comparisons between groups were made by Student-Newman-Keuls (SNK) test and $p \leq 0.05$ was considered significant.

RESULTS

The analysis of variance of experimental data shows significant differences among groups for all the studied parameters (data not shown). Results for each parameter are summarized in Table I.

All the experimental groups show a significantly lower body weight gain than control group ($p \leq 0.01$). Immobilization adrenalectomized was the most affected group since for this group body weight was significantly lower than the other groups (showing a decrease of about 63.06%, 45.62% and 55.54% to CO, CA and SA groups, respectively).

Both thymus and spleen weight were lower in IA group ($p \leq 0.01$) than in control groups (CO, CA, and SA), however no differences were found in these parameters among control groups.

Sham operated group showed no significant differences in weight adrenal againts control whereas levels of corticosterone were significantly higher in SA group than in control grou ($p \leq 0.01$).

The effect of experimental procedures on thymocytes total number is depicted in Fig. 1. Group CA showed no significant differences in cell number against group CO. However, both SA and IA groups presented a significant reduction of cell number versus control ($p \leq 0.01$). IA group showed the smallest cell number (showing a 51.31% decrease to control) that is correlated with the thymus weight.

Cell separation by PNA shows cellular variations affecting mainly ($p \leq 0.01$) the PNA^+ cells but in immobilization group both PNA^+ and PNA^- cell number were decreased in comparison to control ($p \leq 0.01$).

DISCUSSION

Our results indicate that all the studied parameters are affected by immobilization stress

TABLE I. Body weight gain, gland weights and corticosterone levels (mean±sem) for experimental groups of C57BL/6 mice (N=8/group)

Parameter Group	Body[a] (g)	Thymus (mg)	Spleen (mg)	Adrenals (mg)	Corticosterone (µg/dl)
CO	7.39±0.23	68.45±2.19	90.66±4.77	5.17±0.37	6.15±0.65
CA	5.02±0.16*#	69.64±1.19$§	98.84±5.27$§	—	—
SA	6.14±0.38*#	68.11±1,18$§	89.44±2.30$§	4.83±0.23	10.84±1.17*
IA	2.73±0.29*&	40.89±1.15*&	61.78±1.77*&	—	—

Groups: CO= control, CA= adrenalectomized control, SA= sham adrenalectomized, IA= immobilization stress and adrenalectomized group.

[a] Body weight gain was calculated as an increase of body weight in the experimental period (14 days).
* $p \leq 0.01$ and § ns ($p > 0.05$) vs. CO; & $p \leq 0.01$ vs. CA and SA.
# $p \leq 0.01$ and $ ns between CA and SA groups.

and adrenalectomy. The observed effects on thymus and spleen weight, and body weight gain agree with previous results obtained in stressed mice (DECATANZARO & MACNIVEN, 1992; RILEY, 1981).

Thus, we found that both adrenalectomy and immobilization stress produce decrease in body weight gain that seems not to be related to diminution in food intake (GAMALLO *et al.*, 1986). Although, CA and SA groups also show a decrease in body gain, this may be related to surgical stress, as several authors have pointed out (MILLS, 1985).

Furthermore, SA group shows a higher level of corticosterone than CA group, that may also be due to surgical stress. This increase of corticosterone level is not correlated with changes in adrenal weight. Our result is coincident with the finding pointed out by LEE & MCDONALD (1985) who found an increase in plasma corticosterone concentration in crowding animals while adrenal weight remained unchanged.

Acording to our data the elimination of adrenal steroids does not inhibit the process of thymic involution in stressed animals. The fact that control groups subjected to surgical stress do not present significant depression effect on the lymphoid glands may be due to two factors: one of them is the short duration of surgical stress, the other one would be the time passed after the operation (KELLER *et al.*, 1981).

The decrease in thymus weight shown in IA group is related to a decrease of both immature and mature thymocytes although cellular variations affect mainly PNA+ cells. In a previous study (PÉREZ-PARALLÉ MERA, 1990) about the changes in thymus weight induced by three different stressors we found that the number of mature cells remained constant in stressed animals. This difference may due to the fact that in the last case adrenalectomy was not performed.

Moreover, this study is not consistent with the hypothesis that considers the elevated levels of corticosteroids as responsible for the depletion of lymphocytes within the thymus that occurs with stress (SANTISTEBAN & DOUGHERTY, 1954; RILEY, 1981). However, results obtained by KELLER *et al.* (1983) demonstrate that stress-related adrenal secretion of corticosteroids is not required for the stress-induced suppression of lymphocyte stimulation by phytohemaglutinin in the rat. Our present results demonstrate that stress-induced thymic involution in mice is not prevented by adrenalectomy.

In summary, the regulation of the immune function in response to stress may not be limited to corticosteroids. It may well be that there is an adrenal independent stress-induced depletion of thymocytes or a selective redistribution to lymphoids tissues (STEIN *et al.*, 1985). It has been suggested that thymic involution is not, as has been thought, due to lymphocyte destruction but

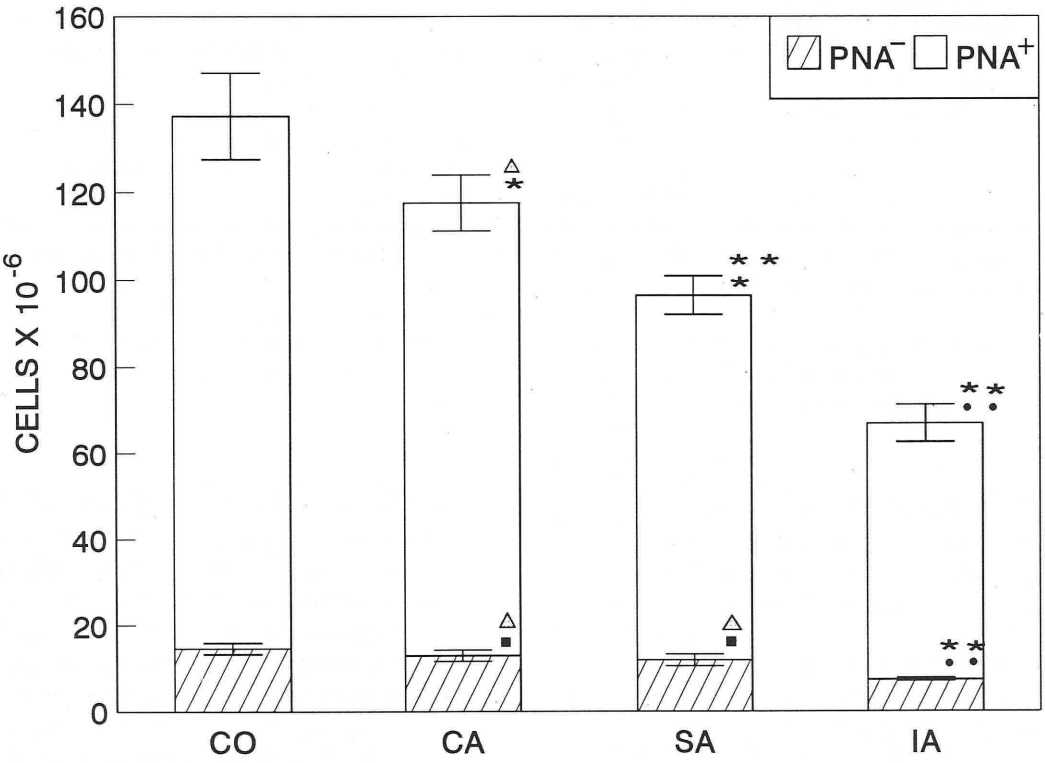

Fig.1. Effect of adrenalectomy and immobilization stress on PNA fractionated thymocytes. Columns indicate the cell number of PNA^+ and PNA^- thymocytes from: control (CO), adrenalectomized control (CA), sham adrenalectomized (SA) and immobilization stress adrenalectomized (IA). Values are means±S.E.M. (n=8). Significant differences from both PNA^+ and PNA^- control cells by SNK test: ** $p \leq 0.01$ and △ ns vs. CO; •• $p \leq 0.01$ vs. CA and SA; and * $p \leq 0.05$ and ■ ns between CA and SA groups.

results from a process of thymocytes leaving the blood and sequestering in tissues, especially in the bone marrow (Cohen & Crnic, 1985). In any case, it is more probable that the effects of stress on the thymus and for that matter the immune system, in general, represent the influence of a variety of factors, involving several, if not a great many mechanisms.

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