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EFFECT OF CHRONIC STRESS ON THE SYMPATHETIC COMPONENT OF THE RAT THYMUS

MILEVA MIĆIĆ*, IRENA ŽIVKOVIĆ*, DANICA DJERGOVIĆ*, JOVANA KNEŽEVIĆ*, MIRJANA LOVREN** and N. UGREŠIĆ***

Immunology Research Center "Branislav Janković", Institute for Biological Research**, Faculty of Pharmacy****

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It is well known that different stressing agents, such as sound exposure, rotation, intermittent shock or forced immobilization, produce alterations of the immune system. Having in mind recent studies about the effects of stress on the immune system, we investigated whether chronic stress induced by a forced swimming procedure alters the sympathetic component of the autonomic nervous system in the rat thymus. For that purpose we exposed AO strain male rats, 22 days old, to a swim test during 21 days. The experimental animals were divided into two groups, and sacrificed by decapitation, the first group the day after the last treatment, and the second group one month later. Their thymuses were used for determination of the distribution and density of sympathetic nervous profiles, as well as the concentrations of noradrenaline (NA), dopamine (DA), and serotonin (5-HT). When compared with controls, the results for the first group showed that chronic stress did not affect the distribution of sympathetic nervous profiles, but significantly reduced the density and intensity of fluorescence,as well as the concentrations of NA, DA and 5-HT. The results for the second group showed the same changes but of lower degree. These changes indicate that chronic stress might influence thymus development and T cell maturation by altering the sympathetic component, and that these altrations persist for one month.

Key words: rat thymus, chronic stress, sympathetic nervous profiles, monoamines.

INTRODUCTION

Through an extremlely complicated equlibrium called homeostasis, all living organisms maintain their survival in the face of both externally and internally generated "stressors".This harmony is constantly challenged by different stressors which can induce disharmony or threaten homeostasis (Fricchione and Stefano, 1944). As a response to stressor agents organisms activate the sympathetic nervous system (Brown, 1986) and the hypothalamic-pituitary-adrenal

axis (Rivier et al., 1984). After brain perception of the stressor, the crucial event in neuroendocrine activation of stress responses is triggering of corticotropin releasing factor (CRF) release from the hypothalamus (Lombardi et al., 1995). CRF serves as an integrator of the behavioral, neuroendocrine and autonomic responses to stress (Owens and Nemeroff, 1991). Central CRF has been found to reduce cellular immune function and specific antibody responses, and alter autonimic outflow via activation of the sympathetic nervous system (Irwin et al.,1992).

The sympathetic nervous system is one of the pathways for communication between the brain and cells of the immune system (Irwin, 1994). The presence of nerve fibers in the thymus, innervating the vasculature and the parenchyma tissues, provides the anatomical basis for a dialogue between the CNS and the lymphoid tissues (Felten et al., 1985). Neurotransmitters present in sympathetic fibers that innervate the thymus, may act both as a paracrine hormone available to receptors on thymic cells and as a local transmitters in nerve terminals that directly contact cortical thymocytes (Felten et al., 1987).

It is well known that stress induces a marked increase in circulating levels of plasma monoamines, alters the distribution of T-cell subpopulations, reduces lymphocyte response to mitogen stimulation and suppresses cellular immune function through acute activation of the sympathetic nervous system, most likely via beta 2-adrenergic receptor mechanisms (Irvin, 1994). One the other hand, how stressors influence the sympathetic component of the autonomic nervous system in the thymus, a key organ for the differentiation of T lymphocytes, has not been analyzed. Therefore we think that it might be useful to investigate in the first place whether chronic stress induced by swimming alters the sympathetic component in the thymus of pubertal rats and secondly whether such alterations remain until the adult period. We prolonged the swimming test in order to obtain changes not only in the CNS, but also in lymphatic organs.

MATERIALS AND METHODS

Animals. Inbred AO female rats with pups, obtained from the vivarium of the Military Medical Academy (Belgrade, Yu) were used in these experiments. The animals were housed at temperature of 22 ± 2^0C in a controlled room, and received food and water ad libitum. The male pups remained with their mothers until day 21 and were then separated and housed five per cage.

Stress procedure. Water stress was induced as described by Qersolt et al. (1977) but we modified the stressing time. The stress paradigm was administred once daily (10 am). Male rats, 22 days old at the start were treated for 21 consecutive days. Briefly, the rats were plunged individually into a vertical plexiglass cylinder containing 15 cm of water maintained at 24^0C, under the red light, and watched constantly for the entire time during which they were in water. The dynamics of the experiment was 15 minutes on the first day and 5 minutes the following days. At the end of each test the rats were dried for 15 minutes in a

heated enclosure (32^0C) before being returned to their cages. Control animals were transported from the animal room to the laboratory along with the stressed rats. The experimental animals were divided into two groups and sacrificed by rapid decapitation: the first group one day after the last stress session and the second group one month later together with their corresponding controls. Stressed treatment groups and controls contained 12 rats. The thymus glands were removed carefully, in aseptic conditions, cleaned of fat and connective tissue and weighed. All thymuses were snap frozen for fluorescence histochemistry and biochemical determination of monoamines.

Fluorescence histochemistry. Monoaminergic innervation of the thymus was examined using the glyoxylic - acid - induced fluorescent histochemical method described by de la Torre (1979). Serial sections of 20 μm thickness were cut through the thymus, on a cryostat at -20^0C and mounted on clean slides. The slides with five levels were immediately dipped in to a solution containing 2% glyxylic acid, 0,2 M sucrose, and 0,236 M monobasic potassium phosphate (pH 7,4) for ten minutes and dried under a gentle warm airflow. The sections were then covered with immersion oil, heated at 95^0C for 2,5 minutes, and immediately coverslipped. All sections were analysed on the same day to prevent diffusion decompensation of fluorescence. Sections were examined and photographed on an Olympus BH2 fluorescence photomicroscope equipped with exciting filter BP-405 and barrier filter Y-475.

Determination of monoamine content. The monoamine content was determined according to the fluorometric method described by Laverty and Taylor (1968). Thymuses from 8 animals from each experimental group were homogenized in acidified butanol, and then monoamines (NA, DA,5-HT) were extracted into 0.1 N HCl. The fluorophores were formed using the hydroxyindole tehnique,and the intensity of fluorescence was measured on an Amico-Bowman spectrophotofluorimeter. Alfa-noradrenaline hydrochloride, 3-hydroxytyramine hydrochlorifde and serotonin creatinine sulphate (Koch-light, England) were used as standards.

Statistical analysis. The results are expressed as mean æ SE. The standard Student's test was used for statistical analysis.

RESULTS

Thymic weight. The absolute weight of the thymus in rats exposed to chronic stress for 21 days starting from day 22, of age, and sacrificed the day after the last treatment or on day 75, was significantly decreased compared with age matched controls (Figure 1).

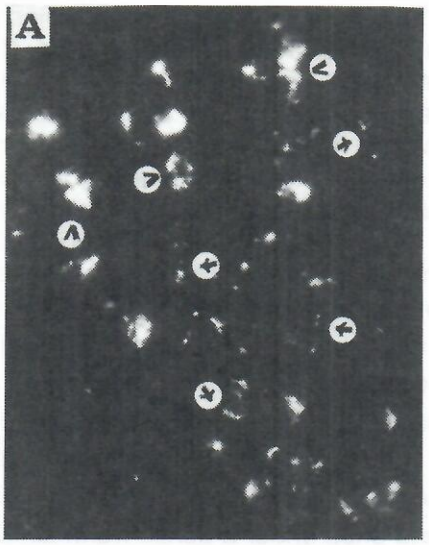

Figure 1. The mass of thymuses of rats exposed to the swim test and the corresponding control groups. K1-44 day old control group, K2-75 day old control group, E1-first experimental group, E2-second experimental group.

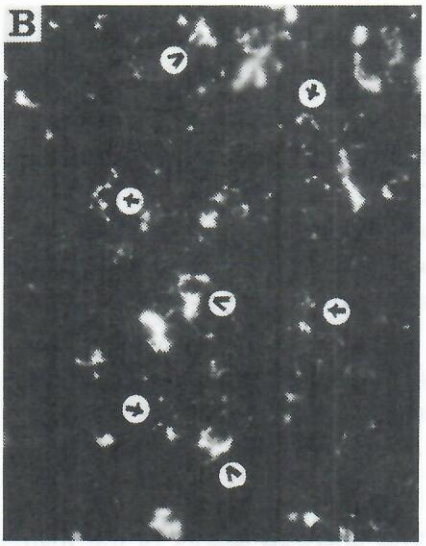

Figure 2. Sympathetic nerve profiles (arrow) and autofluorescent cells (arrowhead in the thymus of control groups: (A) K1-44 day old and (B) K2-75 day old; and experimental groups: (C) E1-first group and (D) E2-second group.

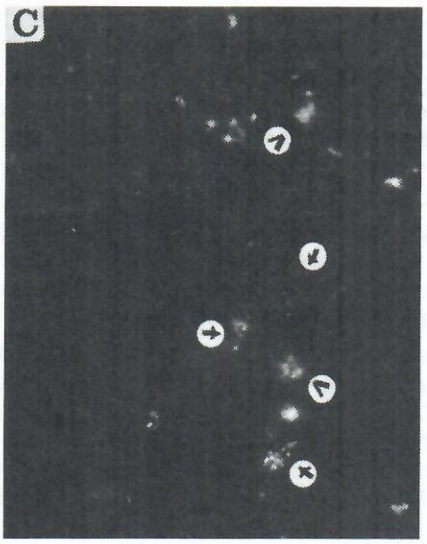

Figure 3. The intrathymic concentration of monoamines in 44 days old rats exposed to the swim test from age 22 days during 21 days (E1) and their corresponding control (K1)

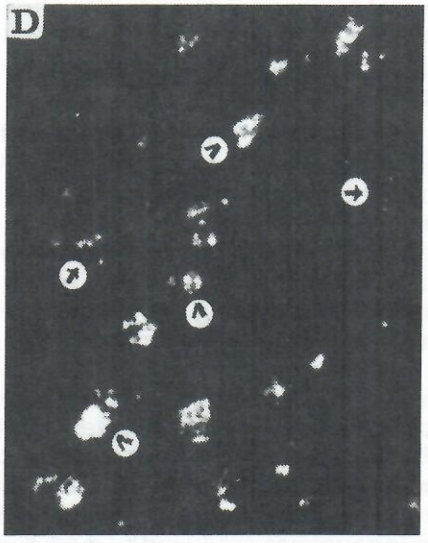

Figure 4. The intrathymic concentration of monoamines in 75 day old rats exposed to the swin test from age 22 days during 21 days (E2) and their corresponding control (K2)

Histofluorescence analyses. Chronic stress performed in sexually immature rats did not affect the intrathymic distribution of sympathetic nerve profiles in either experimental group compared with controls. The fine varicose of monoamine containing fibers were found along the blood vessels, in the capsule, interlobular septae, among parenchyma cells of the thymus, and at close proximity to a delicate network of cortical autofluorescent (CAF) cells. The density of sympathetic nerve profiles and intensity of their fluorescence in the thymic cortex were decreased in the first experimental group (Figure 2).

In the second experimental group, the density of sympathetic nerve profiles and intensity of their fluorescence were reduced, too, while the autofluorescence cells in corticomedullary borders were similar as in the control group of animals of the same age (Figure 2).

Thymic monoamine content. Chronic stress induced by the swim test in the sexually immature rats very significantly ($p<0,01$) reduced the intrathymic concentracion of NA and DA, and significantly ($p<0,05$) decreased the 5-HT content in the first experimental group (Figure 3). Moreover, in the second experimental group, treated the same way, and sacrificed 1 month later we found a significant ($p<0,05$) decrease in intrathymic concentration of NA and DA (Figure 4).

DISCUSSION

Our results demonstrate that chronic stress induced by swimming decreases the weight of the thymus in both experimental groups. This result is in agreement with the finding of thymic antrophy after stress from immobilization (Robin et al. 1987.). Loss of thymic weight may be the result of cell death and of an exodus of small cortical lymphocytes, whereas most other subsets are retained (Clarke and Kendall, 1994).

Since the stress response results in the elevation of corticosteroid levels, mediated through the hypotalamic-pituitary-adrenal (HPA) axis, which induces apoptosis of cortical thymocytes, it was suggested that certain stresses can cause reduction of the thymus weight (Clarke and Kendall, 1994). Moreover, the loss of thymic weight observed in rats under stress conditions can be explained by inhibition of migration of precursor cells from the bone marrow to the thymus, through the corticomedullary boundary, as a consequence of reduction of autonomic innervation and blood flow. (Perezamera et al., 1994). It is possible that corticostroids released during stress may also alter the adhesive properties of endothelial cells, inhibit synthesis and expression of endothelial E-selectin and ICAM-1 adhesion molecules, and alters lymphocyte endothelial interactions and the passage of lymphocytes across the thymic blood-barrier located at the corticomedullary boundary (Ottaway and Husband, 1994). However, the reaction may be modulated by other factors. Some of them may be protective, such as thimulin, one of the thymic hormones produced by medullary epithelial cells which can act as an antistress hormone to promote thymocyte differentiation (Clarke and Kendall,1994).

The density of intrathymic sympathetic components was decreased in the sexually, immature rats exposed to chronic stress by swimming. It is well known that disruption of the sympathetic autonomic nervous system alters normal development of the thymus, maturation of the thymic cells and the ability of lymphocytes to accumulate in lymphoid tissues of rodents (Ottaway and Husband, (1994). As in the brain these changes may be a consequence of deleterious effects of the glucocorticoids (Gcs) secreted during stress (Sapolsky., 1996). Recent papers have shown that as little as 3 weeks of stress and/or stress levels of Gcs can reversibly decrease the number of apical dendrites in the hipocampus (Watanabe et al., 1992). Having in mind, that water immersion stress caused aloss of CA3 and CA4 neurons (Mizoguchi et al., 1992), there is good reason to suppose that the decrease of the density of sympathetic nervous profile in the thymus, during chronic stress induced by swimming might result from actions of the high concentration of corticosteroids. These studies showed that the intrathymic concentration of monoamines was significantly reduced in the rats exposed to swimming during 3 weeks. Significant decreases of intrathymic concentrations of monoamines were found in castrated rats (Leposavic et al., 1995), and during pregnancy (Clarke and Kendall., 1994). Thus, reduced amounts of monoamines were available for interactions with the cells bearing receptors for neurotransmitters (Singh and Owen, 1976). Catecholomines have an inhibitory effect on lymphocytes proliferation through beta-2 adrenoceptors, mediated by cAMP (Felten, 1990), so reduction in the thymic concentration of noradrenaline might cause diminution of the level of cyclic nucleotides in lymphocytes, and it may be associated with a greater proliferation of lymphocytes, or increasing density of beta adrenergic receptors (DeRobertis et al., 1974), as a feed back control mechanism to limit the extent of cellular proliferation. It is well known that lymphocytes from mice spleen deprived of sympathetic innervation display increased beta-adrenergic receptor density as a stabilizing strategy to increase cellular sensitivity in response to the diminished presence of neurotransmitters (Miles et al., 1985). Thus, at least partly, the reduction of the catecholamine content may be ascribed to changes in the presynaptic action of IL 1 on symphathetic nerves in immun organs (Berkenbash et al., 1989). In conclusion, it is clear that chronic stress induced by swimming affects the density of sympathetic nervous profiles in the thymus and the concentration of monoamines and that these changes remained throughout the period of these experiments. However, the biological relevance of these observations on the process of maturation of T-lymphocytes still represents a great challenge for further investigations.

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EFEKAT HRONIČNOG STRESA NA SIMPATIČKU KOMPONENTU PACOVSKOG TIMUSA

MILEVA MIĆIĆ, IRENA ŽIVKOVIĆ, DANICA ĐERGOVIĆ, JOVANA KNEŽEVIĆA, MIRJANA LOVREN i N. UGREŠIĆ

SADRŽAJ

Poznato je da različiti stresni agensi, kao što je muzika, rotacija, diskontinuiran šok ili prisilna imobilizacija, kod životinja izazivaju promene različitih imunoloških parametara. Imajući u vidu do sada poznate rezultate o efektu stresa na imuni sistem u ovom radu smo želeli da ispitamo da li hronični stres izazvan prisilnim plivanjem utiče na simpatičku komponentu autonomnog nervnog sistema u timusu. U tom cilju, mužjaci pacova AQ soja, stari 22 dana, su podvrgavani prisilnom plivanju u trajanju od 21-og dana. Životinje su podeljene u dve grupe u žrtvovane. Prva grupa je žrtvovana dan nakon poslednjeg tretmana, a druga grupa se odmarala od tretmana do 75 dana starosti kada je žrtvovana. Kao kontrole korišćeni su netretirani pacovi iste starosti. Timusi su korišćeni za ispitivanje rasporeda i gustine nervnih vlakana, kao i za određivanje koncentracije noradrenalina (NA), dopamina (DA) i serotonina (5-HT). Dobijeni rezultati su pokazali da hronični stres nema efekat na raspored simpatičkih nervnih vlakana u timusu, ali značajno smanjuje njihovu gustinu, intenzitet fluorescencije kao i koncentracije NA, DA i 5-HT kod prve grupe u odnosu na odgovarajuću kontrolu. Slične, ali manje izražene, promene su nađene i kod druge grupe eksperimentalnih životinja. Dobijeni rezultati pokazuju da hronični stres kod mladih pacova menja simpatičku komponentu u timusu, što može uticati na njegov razvoj i procese sazrevanja T limfocita, kao i da se zapažene promene kod mladih pacova odražavaju do 75 dana.