

The Effect of Steroids on the Circulating Lymphocyte Population

IV. The effect of stress on the thoracic duct lymphocyte population in normal and neonatally thymectomized rats

L.A. Hedman

Department of Pathology, University of Gothenburg, Sahlgren's Hospital, Gothenburg, Sweden

Summary

The influence of stress on the circulating lymphocyte population in rats was studied at different times after stress stimuli and correlated to the concentration of corticosterone in serum. A rapid increase of the corticosterone level, with a duration of about one hour, was seen in both normal and thymectomized animals. Female rats showed higher serum levels than male rats. Depression of thoracic duct lymphocyte cell levels in normal animals seemed to be maximal about 3 hours after exposure to stress and restitution to pretreatment levels was completed within 1–2 days. The lymph cell population in neonatally thymectomized animals did not show any significant changes after stress.

The endogenous corticosterone secretion after stress gave the same changes in the circulating lymphocyte population in normal rats as after injection of a high dose of prednisolone. Because of the absence of effect on thymectomized animals, the effect of endogenous secreted corticosteroids seems to be mainly on T-lymphocytes. The findings suggest the existence of a circulating highly steroid-sensitive thymus-dependent lymphocyte population. This population seems normally to be depressed by stress mechanisms mediated by adrenal corticosteroid secretion. There also seems to be a difference between circulating T- and B-lymphocytes with respect to sensitivity to low doses of naturally secreted corticosteroids.

Key-Words: Lymphocyte – Thoracic duct – Blood – Stress – Rat – Thymectomy – Corticosterone

Introduction

A syndrome consisting of rapid thymus involution and adrenal enlargement, with loss of cortical lipids, after different noxious stimuli

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was described by *Selye* as the adaptation syndrome (1). Changes in lymphoid tissue with disintegration and disappearance of lymphocytes as well as inhibition of the recirculation of lymphocytes could be induced by endogenously secreted adrenal corticosteroids after different stress stimuli or by injection of natural or synthetic corticosteroids (2, 3). It is very difficult to compare different stress situations because the various models (e.g. pain, immobilization, centrifugation, overcrowding, physical work, anaesthesia, injection of formalin) entail different stimuli and thus differ with respect to the amount of physical and psychological stress. But there is no doubt that a stress situation leads to an increase in endogenous corticosteroid production, with effect on both the lymphoid system and immune functions (4, 5). Further, a stress situation produces not only corticosteroid secretion but probably also several other endocrine substances affecting the homeostasis and perhaps also the lymphoid system (6, 7).

The effect of exogenously administered corticosteroids on lymphoid tissue and circulating lymphocytes is rather well known in both steroid-sensitive and steroid-resistant species (8, 9). A single high dose of prednisolone causes rapid and extensive changes in the circulating lymphocyte population, with rapid disappearance of cells from the circulation followed by restitution to normal levels within 24 hours. This rapid restitution of the circulating cell level with unchanged size distribution (10) and label index profile (*Hedman, Lundin*. To be published) supports the hypothesis of lymphocyte "trapping" and redistribution as

a major effect of a single steroid dose. Both rats and guinea-pigs show the same basic changes but they are more pronounced in the sensitive species.

The steroid dose (10 mg/100 g body weight) used in our earlier studies may be regarded as suprphysiological. To find out if the endogenous corticosteroid secretion also could induce the same changes in the steroid-sensitive rat, the stress model was used in this work. A further aim was to correlate the serum corticosteroid level after stress to changes in the circulating lymphocyte population.

Material and Methods

Rats of the Sprague-Dawley strain were neonatally thymectomized by the same method as in earlier work (8). Altogether 80 animals, aged 8–11 weeks, were used. The sex distribution was equal and nonthymectomized rats served as controls. The rats were exposed to an acute stress situation by swimming for 30 minutes in water at 28 degrees centigrade with a lead weight attached to the tails. Depending on the animal's ability to swim, the tail weight was changed between 4, 8, 12 and 16 grams during the stress period in order to achieve not only maximal physical stress without killing the animal but also psychological stress.

Half of the animals were taken for analysis of corticosterone in serum. The blood was collected by aortic bleeding at different times after the stress (0.5, 1.5, 17 and 40 hours). The corticosterone serum level was measured with a fluorimetric method described by *Mattingly* (11), using cortisol as standard. The other 40 animals were subjected to thoracic duct drainage with the same method as previously used (8). The animals were anaesthetized (Pentobarbitone 60 mg/ml intraperitoneally at 6 mg/100 g body weight) and thoracic duct drainage was started 3, 17 or 40 hours after the stress with the open-neck technique (12). The minimum requirements were the same as previously used. The lymph was collected in heparinized glass tubes. Lymph volume was measured and a sample taken for mononuclear cell count and the total flow was

then calculated in cells per hour. A venous blood sample was taken for mononuclear and polymorphonuclear cell count. Lymph and blood smears were made for microscopical examination. The animals were killed and the spleen, lymph nodes (thymic, para-aortic, axillary and mesenteric) and adrenals were dissected out, weighed and fixed in neutral formalin for histological examination.

Cell count and cell flow are given as the mean and standard error of the mean. Student's t-test is used to compare the different groups.

Results

The serum corticosterone level showed a significant increase in both normal and thymectomized animals only 30 minutes after the stress period. A decrease was seen one hour later, followed by normalisation during the next 2 days (Fig. 1). There was no difference in serum levels between normal and thymectomized rats. When male and female animals were compared, the females showed higher resting serum levels and a greater increase after the stress period (Fig. 2).

The neonatal thymectomy gave the same morphological changes in lymphoid tissue and the same reduction in the circulating lymphocyte population as was previously found (8).

The average lymph cell count (cells/ μ l) and lymph cell flow (cells/hour) in the thoracic duct of both normal and thymectomized ani-

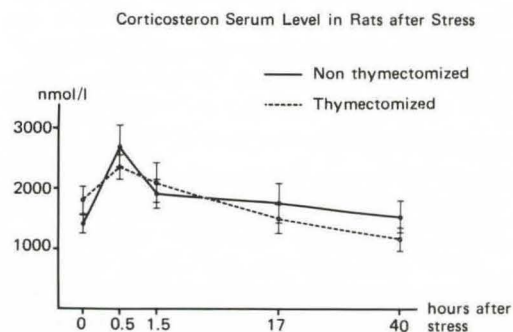


Fig. 1 The corticosterone serum level in normal and neonatally thymectomized rats at different times after stress

Corticosteron Serum Level in Rats after Stress

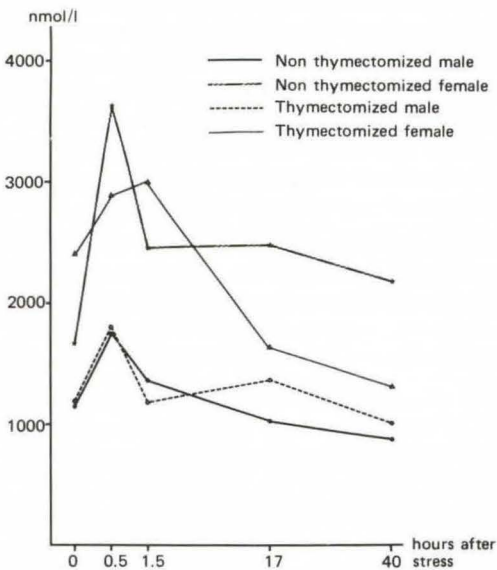


Fig. 2 The corticosterone serum level in normal and neonatally thymectomized male and female rats at different times after stress

Total Cell Count in Thoracic Duct Lymph in Rats

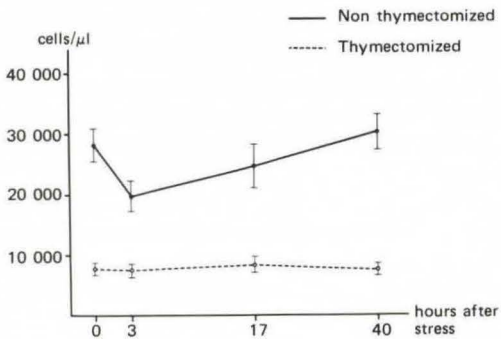


Fig. 3 The thoracic duct lymph cell count in normal and neonatally thymectomized rats at different times after stress

mals are shown in Figs. 3 and 4. In the normal animals there was a marked fall in the lymphocyte count after stress. Three hours after the stress period the reduction was 30% of the original cell level expressed as cells/ μ l and 33% expressed as cells/hour. Within 1–2

Total Cell Flow in Thoracic Duct Lymph in Rats

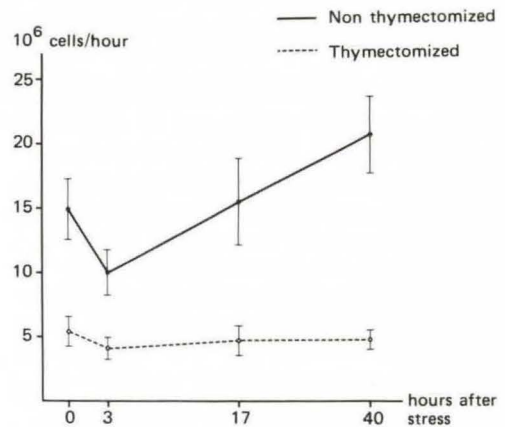


Fig. 4 The thoracic duct lymph cell flow in normal and neonatally thymectomized rats at different times after stress

days the original cell level was restored, with a slight, but not significant, overcompensation in cell flow 40 hours after the stress. The neonatally thymectomized animals did not show any change in cell count in the thoracic duct lymph. A slight decrease in cell flow seen at 3 hours was not significant.

Mononuclear cells in venous blood in normal rats followed a similar pattern as in thoracic duct lymph, but for thymectomized animals there was a gradual decrease in cell count up to 40 hours (Fig. 5). The polymorphonuclear cell count in venous blood was increased in normal animals during the 40 hours studied (Fig. 6).

There were no changes in body or organ weights after stress and the lymphoid organs (spleen, thymus and lymph nodes) did not histologically show any sign of acute involution, measured as increased cytolysis. The spleen and lymph nodes showed in unstressed thymectomized animals a depletion of small lymphocytes in the so called thymus-dependent areas and the same was seen also after the stress period.

Discussion

The main natural corticosteroid secreted from the adrenals in the rat is corticosterone (13),

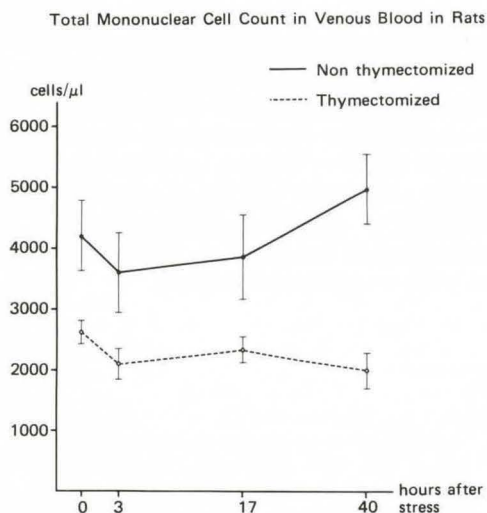


Fig. 5 The venous mononuclear cell count in normal and neonatally thymectomized rats at different times after stress

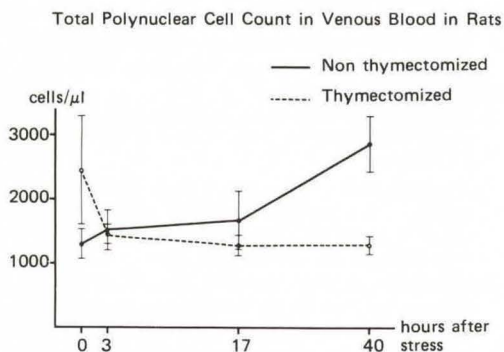


Fig. 6 The venous polymorphonuclear cell count in normal and neonatally thymectomized rats at different times after stress

an 11-hydroxycorticoid. The analytical method used (11) measures specifically cortisol and corticosterone, which makes it suitable for determination of adrenal cortical activity in the rat.

It is not relevant to compare the serum corticosteroid levels after prednisolone injection and after stress, because of the different involuntary effect on lymphoid tissue of different synthetic and natural corticosteroids

(14). However, the effect on the thoracic duct lymphocytes was basically the same after prednisolone injection (8) and after stress. But in our stress model a relatively weaker effect, measured as cell level depression, was found in the stress situation. The transient increase in serum corticosterone level was of the same magnitude as found earlier in mice after acceleration stress (15), but the decrease was perhaps somewhat faster in our work. The higher initial serum level in female animals and the possibly greater increase after stress (Fig. 2) may be related to the relatively higher adrenal weight in females but may also be explained by corticosteroid synthesis in gonadal tissue.

Our results agree quite well with those in some earlier studies. *Spry* (3) found a significant depression of thoracic duct cell flow in normal rats within 2 hours after ether stress or during infusion of prednisolone. The operative method used was an abdominal approach with a cannula in the thoracic duct.

Steroid-sensitive animals, such as rats and mice, are often used to study stress mechanisms but our knowledge about species differences is very limited (16). The suppressive effect of different stress situations on different immune reactions has been well documented (5, 17, 18) and a suppression of the immune responsiveness in spleen cells to sheep red blood cells (direct plaques) could be observed up to 3 days after acceleration stress in mice (19). The theory of a "homing or trapping" mechanism during steroid treatment is also supported by work with stress models but the results vary somewhat. In mice, theta-positive lymphocytes increase in bone marrow after stress (20), but infusion of Cr^{51} -labeled lymph node cells showed homing of both T- and B-lymphocytes in bone marrow and spleen (4).

In our work we found that the effect on thoracic duct lymphocytes after stress was absent in T-lymphocyte-depleted (neonatally thymectomized) animals. In contrast, high doses of prednisolone gave the same changes in normal and thymectomized rats, with depression of the circulating lymphocyte level, followed by restitution to pretreatment level within one

day. The original and the returning thoracic duct lymphocyte populations seem to be identical with respect to size distribution and label index profile. For normal rats, these findings support the theory of a "trapping" mechanism both after injection of a high dose of prednisolone and after endogenous secretion of corticosteroids (stress). The lack of reaction in thymectomized animals after stress could partly be explained by differing sensitivity between T- and B-lymphocytes to different steroid doses. The T-lymphocytes might have a higher sensitivity than B-lymphocytes to lower doses of endogenously secreted corticosteroids. A high steroid dose, on the other hand, seems to have an effect on both T- and B-lymphocytes.

Our earlier work (8) showed, in normal rats, a marked increase in the number of thoracic duct lymphocytes after adrenalectomy. This effect was absent in neonatally thymectomized animals. In the same way as for the stress situation, this could be explained by differing steroid sensitivity between T- and B-lymphocytes. It could also be explained by differing sensitivity between different subpopulations of T-lymphocytes.

Different numbers of specific cytoplasmic glucocorticoid receptors have been found in different non-lymphoid and lymphoid tissues in rats and rabbits (21). In the rat thymus and spleen these steroid receptors change in number during the animal's life (21, 22). Difference in receptor numbers in various lymphocyte subpopulations has been suggested from experiments with leukemic cells (23) but nothing is known about this problem in relation to normal lymphocytes in man or animals. Duval suggested a different mechanism for the steroid-resistance in normal thymus cells than in malignant lymphoma cells, which seem to be resistant because of defective cytoplasmic receptors (24). Different functional subpopulations of lymphocytes have been found to differ in steroid sensitivity in both mice and rats but no correlation to cytoplasmic receptor number has been found. Another finding is the existence of different forms of steroid-binding with specific binding to the receptors and non-specific binding to sites throughout the cell (25, 26).

A very hypothetical question is whether the lymphocytes have several types of receptors, differing in affinity to different corticosteroids. This could theoretically be an alternative explanation for the absence of effect in T-lymphocyte depleted animals after stress and the quantitatively weaker effect of endogenously secreted corticosteroids compared to injection of prednisolone in normal animals.

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Lars A. Hedman, Dept. of Pathology, Univ. of Gothenburg, Sahlgren's Hospital, S-41345 Gothenburg, Sweden