ACTIVITY AND DISTRIBUTION OF BIOGENIC AMINES IN STRUCTURES OF THE THYMUS AND SPLEEN IN RESPONSE TO INJECTION OF ISOLOGOUS AND HETEROLOGOUS RED CELLS

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Injection of heterologous red cells was shown to lead after 15 min to the displacement of noradrenalin from efferent nerve endings, to a marked reorganization of the metabolism of the central follicular cells and the peripheral monoamine-containing cells of the marginal sinuses of the spleen, and cortical luminescent and mast cells of the thymus. Inversion of the medullary substance into the cortex was observed in the thymus. These changes increased in intensity until 2 h. KEY WORDS: noradrenalin; serotonin; thymus; spleen; heterologous red cells; luminescence analysis.

The practical needs of immunology, allergology, and transplantology have made it necessary to study and clarify the neurohumoral mechanisms of immunogenesis so as to be able to correct immune reactions in experimental and clinical practice [4, 9, 10, 13].

In the modern view, the idea of a role of the nervous system in immune reactions was formulated by Ado [1]. The concept of the systemic character of the neuroregulation of immunogenesis has recently been defined [10]. However, no investigations into the peripheral efferent component of the system has yet been described in the literature [13].

The writers previously studied the morphology of efferent adrenergic nervous structures and also adrenalin-containing tissue structures of lymphoid organs [7, 12], seasonal changes in adrenergic nerves in certain animals [5], and transformations of tissue and nervous structures under the influence of neurotropic agents [7].

The object of the present investigation was a qualitative study of the histochemical and luminescence-morphological changes arising during the first minutes and hours after injection of an antigen in all the adrenalin-containing structures of the thymus and spleen discovered and described by the writers previously.

EXPERIMENTAL METHOD

Tissues of the spleen and thymus of 50 Wistar-2 rats were used. Twelve intact rats (group 1) served as the control. Washed red cells from animals of the same strain were injected into the caudal vein of 24 rats (group 2). Another 14 rats received an injection of a suspension of washed sheep's red cells (group 3). Material was taken under ether anesthesia 15 min and 1 and 2 h after the procedure.

The following methods were used to process the material: Falck and Hillarp's luminescence-histochemical method in Krokhina's modification [11, 15]. Staining with polychrome toluidine blue by Unna's method, staining with alcian blue and safranin for simultaneous detection of heparin and heparin-free granules in mast cells, the Masson-Fontana argentaffin reaction to detect serotonin (5-HT), and staining by the Romanovsky–Giemsa method as a general morphological survey stain. Qualitative spectral analysis of luminescent preparations by means of the FMEL-1 photometric attachment was carried out at a voltage of 900 V with a 0.5 probe (reflecting the area of the photometric field) in order to detect the spectra of catecholamines at 480 nm and 5-HT at 525 nm in the luminescent structures.

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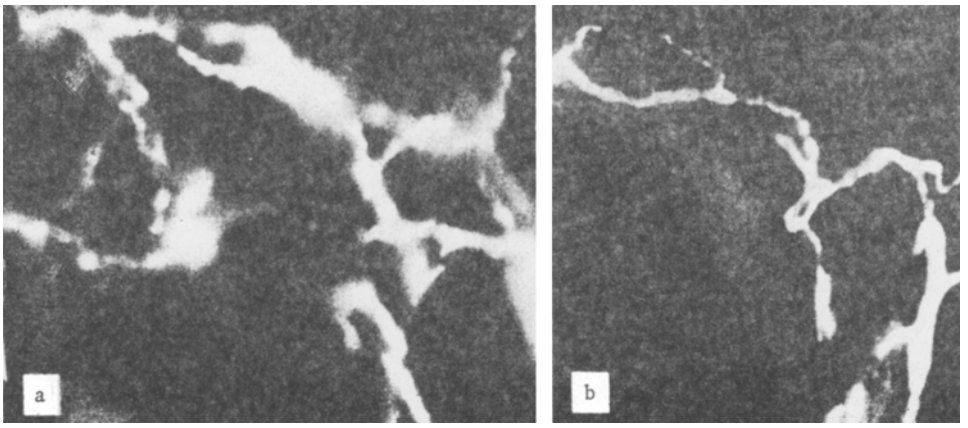

Fig. 1a. "Spreading" of noradrenalin in adrenergic nerve plexuses in thymus of Wistar-2 rats 2 h after injection of sheep's red cells. Falck's luminescence-histochemical method, ML-2 microscope, objective 90, homal 1.7.

Fig. 1b. Normal adrenergic nerve fibers in spleen of intact rat. Falck's luminescence-histochemical method, ML-2 microscope, objective 90, homal 1.7.

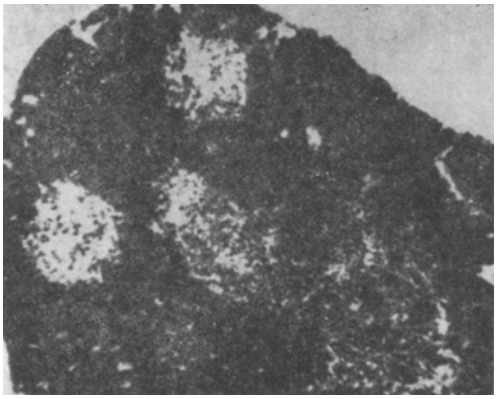

Fig. 2. Inversion of medulla into cortex in lobule of thymus of Wistar-2 rat 2 h after injection of sheep's red cells. Romanovsky–Giemsa stain, MBR-1 microscope, 35×.

EXPERIMENTAL RESULTS

Since the morphology of the thymus and spleen may differ in different strains of animals of the same species [8], the morphology of these organs was compared in Wistar-2 rats and in noninbred albino rats, on which the previous investigations had been undertaken.

The luminescence picture of the thymus of intact Wistar-2 rats was found to agree exactly with that of the thymus of noninbred rats. In both subcapsular and premedullary luminescent cells of the cortex 5-HT and catecholamines were detected spectroscopically. The only difference was that the subcapsular zone of the cortex was rather poorer in luminescent cells. In the spleen of the Wistar-2 rats the luminescence-morphological picture on the whole likewise was the same as that described by the writers in the spleen of noninbred rats. Intrafollicular and interfollicular luminescent monoamine-containing macrophages and also luminescent marginal cells were observed here. Adrenergic nerve plexuses in both organs, just as in noninbred rats, were discovered mainly along the course of the blood vessels (Fig. 1). The concentration of biogenic amines in the nerve fibers, just as in noninbred rats, showed seasonal fluctuations with a maximum in the spring and summer.

The number of luminescent granules in the premedullary luminescent cells in the thymus showed a marked decrease 15 min after injection of heterologous red cells, whereas in the parenchyma of the gland and, in particular, around the nerve fibers there was an increase in the diffuse luminescence, in which 5-HT and catechol-

amines could be detected spectroscopically. This suggests (according to existing data [14]) that at this time the noradrenalin was displaced from the nerve fibers, which accordingly appeared indistinct, wide, and dull (Fig. 2).

In the spleen the disappearance of noradrenalin from the nerve fibers was expressed more sharply than in the thymus. In the spleen, in addition, the luminescence of the marginal cells of the perifollicular sinuses was much weaker.

By two h after injection of sheep's red cells luminescence of the amine-containing marginal cells and the central intrafollicular macrophages in many follicles disappeared completely, as a result of which the follicles became difficult to distinguish under the luminescence microscope. Nerve fibers were visible after 2 h in only a few cases, they were few in number, and the intensity of their luminescence was sharply reduced. The cortex of the lobules of the thymus 2 h after injection of heterologous red cells as a rule appeared dark, but occasionally round clusters of brightly luminescent monoamine-containing cells could be found here, and were distinguished by a brighter and greener luminescence than in the ordinary cortical cells of the thymus.

Injection of isologous red cells should not have caused any changes in the normal luminescence picture. This was in fact an almost true picture of the result of the luminescence-histochemical analysis of the lymphoid organs of the corresponding group of animals, but strictly speaking exact agreement with normal was not obtained. Occasionally it was possible to see a splenic follicle with "dim" marginal and intrafollicular cells. In 1 or 2 lobules of the thymus luminescence of the cortical cells was "quenched."

Hence, after injection of heterologous red cells the phenomenon described above was partly induced by the antigen, but mainly was possibly a standard reaction to stress.

Further investigations were carried out to shed light on this phenomenon. In the thymus, 15 min after injection of sheep's red cells, an increase in the number of weakly alcianophilic mast cells, which also stained β-metachromatically by Unna's method, was observed in some lobules. These facts were confirmed by the results of luminescence analysis showing an increase in the content of free catecholamines and 5-HT in the tissue, followed by their uptake by mast cells. The alcianophilic mast cells could not be detected by luminescence analysis, evidently because the 5-HT which was luminescent in them was partly screened by heparin [6]. The degranulation observed in many mast cells also was evidence of their overloading with biogenic amines.

Injection of heterologous red cells led after 1 h to the appearance of structures outwardly resembling lymphoid follicles, of various sizes, in the cortex of some lobules of the thymus. As a rule, these structures were collections of basophilic cells with round nuclei, with a wide border of cytoplasm, and giving positive histochemical reactions for 5-HT. It was evidently these collections that appeared during luminescence analysis in the cortex of the thymus in the form of luminescent islands. It was shown that this phenomenon was inversion of the medulla of the lobules into the cortex (Fig. 3). Inversion was probably the result of antigenic action, for these structures appeared only after injection of allogenic red cells. Data in the literature indicating the integrity of the thymus during exposure to the action of antigens are to some extent in disagreement with this result, but they are concerned only with the absence of a plasma-cell reaction [2].

In the spleen 15 min after injection of heterologous red cells the beginning of cell and tissue reactions was seen. In the center of the follicles partial "uncovering" of the macrophages was found. Some trabecular veins and arterial capillaries, revealed at the periphery of the lymphoid sheaths of the pulpar arteries, were filled with strongly 5-HT-positive small lymphocytes, a picture which can perhaps be interpreted as a disturbance of lymphocyte transport.

Administration of heterologous antigens thus causes displacement of biogenic amines from efferent nerve endings and the accumulation of free amines in the intercellular spaces of the thymus and spleen within the first few minutes. A rapid redistribution of biogenic amines is observed in luminescent macrophages. Mutual inversion of cortex and medulla takes place in the lobules of the thymus.

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EFFECT OF CHRONIC PYROGENAL STRESS ON THE MITOTIC REGIME AND NUMBER OF DNA-SYNTHESIZING CELLS IN THE CORNEAL AND LINGUAL EPITHELIUM OF ALBINO RATS

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The effect of prolonged stress on the mitotic regime and number of DNA-synthesizing cells in the corneal and lingual epithelium was studied in response to pyrogenal. Injection of pyrogenal for 5 days caused a decrease of 41% in the number of mitoses in the corneal and lingual epithelium. The decrease in the number of dividing cells did not correlate with changes in the rate of mitosis. The number of pathological mitoses in the corneal epithelium of intact rats remained unchanged during stress. The index of labeled nuclei in the corneal and lingual epithelium of the control rats was 12.6 and 10.8 respectively, which did not differ significantly from their values in the experimental animals (12.2 and 12.2). KEY WORDS: mitotic activity; stress; pyrogenal; DNA synthesis.

In previous investigations the writers found a decrease in the mitotic activity of the corneal and lingual epithelium following single [6] and repeated [7] injections of pyrogenal. The inhibition of cell division was found to be mediated through adrenal hormones [8], increased secretion of which is observed under the influence of pyrogenal [2, 3].

The object of the present investigation was to use pyrogenal stress as a model with which to study the effect of prolonged stress on the number of DNA-synthesizing cells and the mitotic regime of the corneal and lingual epithelium. The study of this problem is also of applied interest, for pyrogenal is used, in particular, for the treatment of dermatological diseases, an important link in the pathogenetic mechanism of which is stimulation of mitotic activity and disturbance of DNA synthesis [12].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 140-190 g. Pyrogenal was injected into the caudal vein in a dose of 5 μg/100 g body weight between 11 a.m. and 12 noon daily for five days. Intact animals served as the control. The animals were decapitated six h after the last injection, and 1 h before sacrifice the rats were given an intraperitoneal injection of thymidine-^{3}H in a dose of 0.6 μCi/g body weight. Since it was impossible to obtain satisfactory autoradiographs of the corneal epithelium after administration of this

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