Histochemical Study of Mast Cells from the Thymus of Mice Receiving $ACTH_{1-24}$

E. M. Naumova and V. E. Sergeeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 7, pp. 107-110, July, 2004
Original article submitted December 8, 2003

Synthetic $ACTH_{1-24}$ analogue administered in a daily dose of 0.01 mg/kg decreased the number and size of mast cells and increased intracellular serotonin concentration. $ACTH_{1-24}$ induced degranulation of young mast cells and release of undersulfated heparin. Correlation analysis showed that hormonal imbalance produced by $ACTH_{1-24}$ was accompanied by redistribution of bioamines.

Key Words: *mast cells; degranulation; metachromasia; $ACTH_{1-24}$; biogenic amines*

A large body of evidence indicates the existence of close relationships between the nervous, endocrine, and immune system. Cells of these systems secrete similar regulatory factors (interleukin, interferon, and endorphins) and carry the corresponding receptors [1-3,8]. Neurotransmitters play an important role in the central and local mechanisms for regulation of the immune system. The system for primary response, signaling, and protection of the organism regulates the entry of various exogenous substances. This system includes nervous, endocrine, and local immune components. Mast cells (MS) of the thymus respond even to small changes in biogenic amine content in the organism and are in close contact with nerves and macrophages. MS are convenient for studying of the interaction between the nervous, endocrine, and immune systems. Much attention was given to the effect of exogenous ACTH on immune reactions. The preparation in high doses suppresses the humoral immune response [6,7]. Conflicting conclusions are related to differences in animals spicies, immunological models of hormonal preparations, and conditions for treatment used in this experiments.

Here we studied neurotransmitter content and morphological changes in MS of the central immune organ during an experimental hormonal imbalance produced by exogenous ACTH.

MATERIALS AND METHODS

Experiments were performed on 90 laboratory male mice weighing 20-22 g. The animals were divided into 3 groups: intact mice (n=30); control mice receiving 0.01 ml isotonic solution (n=30); and treated mice intraperitoneally receiving synthetic analogue of $ACTH_{1-24}$ in a daily dose of 0.01 mg/kg (n=30, 0.01 ml solution of Synacten Depot, Ciba-Geigy Limited). The thymus was isolated on days 7, 14, 21, and 28 under ether anesthesia.

Cryostat sections of the thymus were treated as follows: luminescence-histochemical method of Cross, Even, and Rost for histamine-containing structures of the thymus; luminescence-histochemical method of Falk and Hillarp with modification of E. M. Krokhina for adrenergic nerve fibers and biological amines in morphofunctional structures of the thymus; morphometry of MS (μ); and cytospectrofluorometry with a FMEL-1A head for identification and measurement of biogenic amine content (serotonin, 525 nm; catecholamines, 480 nm). The results were recorded from an amplifier display and expressed in arbitrary units. Correlation analysis was used to study the relationship between luminescence of neurotransmitters in amine-containing structures. The serotonin index was cal-

Laboratory of the Department for Medical Biology, Chuvash State University, Cheboksary. ***Address for correspondence:*** nema@chuvsu.ru. Naumova E. M.

0007-4888/04/1381-0093

culated as the sum of serotonin+catecholamines in MS (mean value). Sections were stained with Unna's polychromic toluidine blue to visualize tissue mucopolysaccharides and heparin in MS. Non-sulfated "immature" heparin was α-orthochromatic (blue), β_1-metachromatic (ink-blue), and β_2-metachromatic (ink-violet). More sulfated ("maturing") heparin and "mature" heparin produced β-metachromatic (ink-cherry) and γ-metachromatic staining (purple), respectively [3]. By the degree of degranulation, MS were divided into 4 groups: T_0 cells with the non-visualized nucleus and densely packed unidentified granules; T_1 cells with individual identified granules and incompletely "masked" nucleus; T_2 cells with clearly identified inner and outer granules and distinct nucleus; and T_3 exhausted cells with individual inner and outer granules.

RESULTS

Luminescence-histochemical study of the thymus showed that administration of $ACTH_{1-24}$ into MS significantly increased the contents of serotonin and norepinephrine. These changes were observed at various terms after treatment. Visually, we revealed an increase in the number of white-to-yellow granules exhibiting strong luminescence in the cell cytoplasm (Table 1). Histamine concentration in MS decreased by 5.8 times on day 7, but increased by 1.6 times on day 28. Serotonin has immunosuppressive activity [9]. Therefore, treatment with substances increasing serotonin level is accompanied by suppression of the primary and secondary immune response.

The serotonin index in MS sharply increased on days 7 and 14 after treatment (compared to control animals). This reflects the prevalence of serotonin above catecholamines in MS of intact, control, and treated mice (Table 2).

The immunosuppressive effect of $ACTH_{1-24}$ was indirectly confirmed by the decrease in the number and size of MS in the field of view (Table 3).

Correlation analysis was performed for the contents of serotonin, catecholamines, and histamine in the thymus. A positive correlation was found between serotonin concentration in the following cells: MS and thymocytes of the medullar layer (r=0.997); and MS and thymocytes of the cortical layer (r=0.992). These relationships were not observed in control animals (0.811 and 0.744, respectively). Probably, $ACTH_{1-24}$ stimulates serotonin production in MS and its absorption by thymocytes of the cortical and medullar layer in the thymus. A positive correlation between serotonin concentration in MS and luminescent granular cells of the corticomedullary zone (LGC CMZ) was not found after treatment with $ACTH_{1-24}$. The corre-

TABLE 1. Contents of Serotonin, Catecholamines, and Histamine in MS of the Thymus after Administration of $ACTH_{1-24}$ ($M \pm m$)

Period, days	Serotonin			Catecholamines			Histamine		
	I	C	T	I	C	T	I	C	T
7	0.134±0.003	0.190±0.005$^+$	0.271±0.003*	0.012±0.001	0.019±0.006$^+$	0.019±0.012	0.143±0.008	0.319±0.013$^+$	0.055±0.006*
14	0.136±0.002	0.209±0.002	0.535±0.002*	0.023±0.001	0.024±0.007^{++}	0.043±0.004*	0.091±0.001	0.268±0.007	0.248±0.005**
21	0.121±0.003	0.215±0.003$^+$	0.318±0.002	0.030±0.004	0.021±0.001$^+$	0.091±0.003**	0.101±0.001	0.251±0.006$^+$	0.307±0.003
28	0.124±0.003	0.200±0.003$^+$	0.242±0.008*	0.026±0.003	0.020±0.001	0.177±0.008*	0.108±0.001	0.217±0.008$^+$	0.350±0.041*

Note. Here and in Tables 2 and 3: I, intact mice; C, control mice; T, treated mice. $^+p<0.01$ and $^{++}p<0.05$ compared to control mice; $^+p<0.01$ and $^{++}p<0.05$ compared to intact mice.

TABLE 2. Serotonin Index in MS of Thymus in Control and Experimental Mice on Days 7, 14, 21, and 28 of Daily Treatment with $ACTH_{1-24}$

Period, days	I	C	T
7	6.2	1.0	14.3
14	5.9	8.7	12.4
21	4.0	10.2	3.5
28	4.8	10.0	1.4

lation coefficients in control and treated mice were -0.987 and 0.346, respectively. These changes reflect the impairment of serotonin-absorbing function in LGC CMZ. In the thymus of control animals, strong positive correlations were revealed between histamine concentration in MS and thymocytes of the cortical layer (r_C=0.981), MS and LGC CMZ (r_C=0.923), and MS and luminescent granular cells of the subcapsular zone (r_C=0.966). These data show that MS are the major histamine-producing cells in the thymus. After treatment with $ACTH_{1-24}$, a negative correlation was revealed for MS and luminescent granular cells of the subcapsular zone (r_T=-837). These changes were probably accompanied by impairment of histamine absorption in granular cells of the subcapsular zone. A strong positive correlation was found between histamine concentration in MS and thymocytes of the medullar layer (r_T=0.989). The data illustrate intensive absorption of histamine by thymocytes of the medullar layer (r_T=0.989).

Staining of thymus sections with Unna's toluidine blue revealed a 1.8-fold decrease in the number of MS on day 7 of daily treatment with $ACTH_{1-24}$. γ-Metachromatic and β_3-metachromatic MS were absent in the thymus of experimental mice. β_3-Metachromatic MS were presented by β_1- and β_2-metachromatic cells. β_1-Metachromatic cells prevailed under these conditions. By the degree of degranulation, we observed an increase in the ratio of degranulating T_2 and T_3 cells (26 and 32%, respectively; *vs.* 13 and 25% in the control, respectively) and decrease in the count of T_0 cells (20 *vs.* 34% in the control). The number of MS in mouse thymus decreased by 2.3 times on day 14 after treatment. During this period, signs of metachromasia in MS of the thymus were similar to those observed on day 7 after administration of $ACTH_{1-24}$. We revealed an increase in the ratio of T_2 and T_3 cells (37 and 33%, respectively; *vs.* 24 and 15% in the control, respectively) and significant decrease in the count of T_1 cells. After treatment with $ACTH_{1-24}$ for 21 days, individual MS in the capsule were characterized by β_2-metachromasia. It is important that in control animals we found only γ- and β_3-metachromatic cells. The ratio of MS differing in the state of heparin underwent pronounced changes. We observed an increase in the ratio of degranulating T_3 cells (37 *vs.* 13% in the control) and decrease in the count of T_1 cells (22 *vs.* 33% in the control).

On day 28 we found only individual MS in the capsule and septa. The number of MS decreased by 3.5 times compared to the control. The ratio between MS changed towards accumulation of β_2-metachromatic cells. We detected only individual β_1- and β_3-cells. The ratio of T_3 cells sharply increased (43 *vs.* 4% in the control), while the count of T_1 cells decreased (13 *vs.* 69% in the control). Published data show that ACTH stimulates the synthesis of heparin in MS [4,5]. We found that $ACTH_{1-24}$-produced metabolic changes are accompanied by the release of undersulfated heparin into the intercellular space. The action of endogenous ACTH mediated by cholecystokinin-4 is accompanied by stimulation of merocrine secretion in MS (granulolysis) over the first 15 min after treatment [4]. In our experiments MS were characterized by apocrine secretion of heparin (degranulation) with destruction of the cell membrane. The absence of granulolysis was probably associated with long-term administration of the preparation and material sampling in the delayed period.

Our findings suggest that $ACTH_{1-24}$ produces a variety of morphological changes in MS, which includes a decrease in the number and size of cells, increase in intracellular serotonin concentration, degranulation of young cells, and release of undersulfated heparin. The disappeared and newly formed relationships between MS and other amine-containing structures of the thymus reflect the redistribution of biogenic amines under conditions of hormonal imbalance produced by chronic $ACTH_{1-24}$ treatment .

This work was supported by the "Russian Universities" program (UR 11.01.029).

TABLE 3. Size and Number of MS in the Field of View after Administration of $ACTH_{1-24}$

Index	Day 7		Day 14		Day 21		Day 28	
	C	T	C	T	C	T	C	T
Number	6.05±0.80	3.45±0.70*	4.1±0.4	1.8±0.4*	3.7±0.6	1.35±0.40	3.8±0.8	1.1±0.3*
Size, μ	20.3×13.1	7.8×6.6	15.3×11.6	7.3×4.2	10.5×5.9	6.7×5.6	12×7.8	7.2×5.6

REFERENCES

1. I. G. Akmaev, *Morfologiya*, Nos. 9-10, 36 (1993).
2. I. G. Akmaev and V. V. Grinvich, *Byull. Eksp. Biol. Med.*, **131**, No. 1, 22-32 (2001).
3. T. L. Petrova and V. E. Sergeeva, *Neurotransmitter Supply to Microstructures of the Thymus during Ovariectomy and Estrogen Treatment* [in Russian], Cheboksary (2002).
4. E. A. Smirnova, B. A. Umarova, G. N. Kopylova, and E. L. Goncharova, *Byull. Eksp. Biol. Med.*, **135**, No. 1, 17-20 (2003).
5. F. V. Shapiro, B. A. Umarova, and S. M. Srukova, *Ibid.*, **120**, No. 10, 349-352 (1995).
6. I. Aebischer, M. R. Stampfli, A. Zurcher, *et al.*, *Eur. J. Immunol.*, **24**, No. 8, 1908-1913 (1994).
7. A. Armario, M. Giralt, O. Marti, *et al.*, *Endocr. Res.*, **20**, No. 2, 139-149 (1994).
8. C. Delrue-Perollet, K. S. Li, S. Vitiello, and P. J. Neveu, *Brain Behav. Immun.*, **9**, No. 2, 149-162 (1995).
9. L. D. Van de Kar, M. C. Alvarez Sanz, and J. M. Yracheta, *Pharmacol. Biochem. Behav.*, **48**, No. 2, 429-436 (1994).