Sensitization of the Thymus to Hypoplastic Effect of Glucocorticoids after Long-Term Hypokinetic Stress

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Long-term hypokinesia increases sensitivity of the thymus to the hypoplastic effect of glucocorticoid preparation triamcinolone acetonide by potentiating the proapoptotic effect of the glucocorticoid.

Key Words: *hypokinesia; thymus; apoptosis; triamcinolone acetonide*

Stress exposure caused by limitation of motor activity is associated with involution of the thymus, despite high level of cytokines capable of limiting the hypoplastic effect of glucocorticoids on the lymphoid tissue [1,2]. The development of this phenomenon can be explained by increased sensitivity of thymocytes to the proapoptogenic effect of glucocorticoids. In order to verify this hypothesis, we studied the effect of preliminary 30-day hypokinetic stress on the resistance of the thymus to the hypoplastic effect of long-acting glucocorticoid triamcinolone acetonide (kenalog).

MATERIALS AND METHODS

The study was carried out on 24 male and female Wistar rats. Hypokinesia (HK) was induced by placing the animals into narrow low boxes limiting their motor activity for 30 days. Twenty-four hours after HK the animals were subcutaneously injected with long-acting glucocorticoid triamcinolone acetonide (TA; Berlin-Chemie) in a dose of 2 mg/kg; controls received an equivalent volume of 0.9% NaCl. Two more groups received similar injections without preliminary stress.

The count of nuclear cells in the thymus was evaluated by common hematological methods. The thymuses were plunged in 0.15 M phosphate buffer saline (pH 7.2) and carefully homogenized in a glass homogenizer. The thymocytes were resuspended in hypotonic propidium iodide (Sigma) solution [5]. The percentage of apoptotic hypochromatic cells (sub G_0/G_1; M1 peak), cells in the G_0/G_1 phase (G_1 peak, M2), and content of actively dividing cells (S-G_2-M; M3 peak) was evaluated in propidium iodide-stained thymocytes on a FACS-Calibur flow cytofluorometer (Becton Dickinson) using CellQuest software. The significance of differences was evaluated using Mann—Whitney's nonparametric *U* test.

RESULTS

The development of thymic hypoplasia in HK is caused by suppression of thymocyte proliferation paralleled by apoptosis activation (Table 1). Hypoplasia of the thymus caused by long-term HK was transitory and completely leveled 96 h after hypokinetic stress: apoptosis rate decreased and the content of G_0/G_1 phase thymocytes increased. Additional injection of exogenous glucocorticoid to stres-

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TABLE 1. Effect of HK on Thymus Sensitivity to Glucocorticoid-Dependent Hypoplasia

Group	Thymocyte count, 10^6 cells	Count of apoptotic thymocytes, sub G_1/G_0 peak	Count of thymocytes in $S+G_2+M$ phases, $S+G_2+M$ peak	Count of thymocytes in G_0/G_1 phases, G_0/G_1 peak
Control (NaCl without stress; *n*=5)	384.77±42.90	0.816±0.219	17.33±1.76	78.552±1.282
TA (*n*=4)	165.6±15.9*	3.311±1.238**	7.943±1.290*	78.861±5.700
HK+NaCl 0.9% (*n*=5)	171.1±21.8^{+}	0.1350±0.0651^{++}	12.507±0.900	85.877±0.827^{+}
HK+TA (*n*=7)	76.04±4.50^{o}	7.2960±1.1937	3.444±0.795^{o}	64.618±7.801

Note. *p=0.006, **p=0.026 compared to the control, ^{+}p=0.014, ^{++}p=0.027 compared to TA, ^{o}p=0.005 compared to TA.

sed animals led to more pronounced induction of apoptosis and more intensive suppression of proliferative activity in the thymus in comparison with animals not exposed to stress. The count of sub G_0/G_1 peak cells was increased, while that of $S+G_2+M$ peak cells decreased in stressed animals treated with TA in comparison with unstressed animals treated with TA (Fig. 1). Importantly that injection of TA to unstressed animals was associated with a several-fold increase in apoptosis rate and several-fold reduction of proliferation rates.

Thus, the results indicate sensitization of lymphoid tissue to the hypoplastic effect of glucocorticoid hormones. It is noteworthy that preliminary HK exposure stimulated the antiproliferative and proapoptogenic effects of exogenous glucocorticoid. Though the first report about proapoptogenic effect of glucocorticoids towards thymocytes was published in 1980 [6], the molecular mechanisms of glucocorticoid-dependent apoptosis in the thymus remained not completely deciphered. However, it was proven that the expression of glucocorticoid receptors increased in apoptotic thymocytes [5]. Despite the absence of information about the intermediate transmitters of the signals from glucocorticoid hormones to "death genes", caspase-9 and caspase-8 were identified as the final effectors activating, in turn, caspase-3 with subsequent fragmentation of thymocyte nuclei [3]. Hence, the glucocorticoid-dependent effect of HK can be linked with increased content of glucocorticoid receptors on thymocyte membrane.

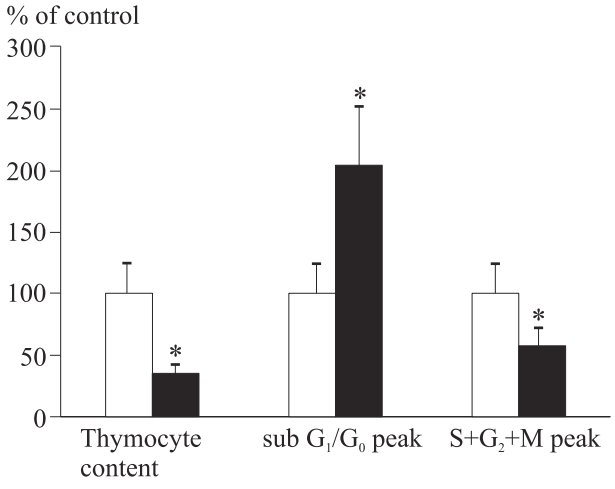

Fig. 1. Effect of HK on thymocyte count and apoptosis/proliferation ratio in the thymus. Light bars: control; dark bars: 30-day HK. *p<0.05 compared to the control.

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