Changes in Intracellular Calcium Metabolism in T Lymphocytes After Transient and Persistent Psychosocial Stress of Mice of Various Ages

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Abstract. Intracellular calcium level is a sensitive marker of the homeostasis of living cells and its changes are essential steps of T lymphocyte activation. Environmental stress provokes an adaptive response of the organism. In recent studies the authors have investigated the effect of transient and persistent psychosocial (overcrowd) stress on resting and lectin-stimulated cytoplasmic free Calcium (Ca) concentration of splenic T lymphocytes from young and aged mice (106 animals total). The animals were kept under "normal" (68 cm^2/animal) or "overcrowded" (22 cm^2/animal) conditions for 10 or 90 days respectively. Young animals showed no change in resting Ca after overcrowd stress. The lectin-induced rise in intracellular Ca level, however, was three times higher ($p < 0.01$) in transiently stressed young mice compared to the control group. Changes were returned to the control level after persistent overcrowd stress. T cells from aged mice displayed significantly smaller levels of resting, and lectin-stimulated intracellular calcium concentration ($p < 0.01$ each), as compared to those of the non-stressed, aged animals. This age-induced inadequate adaptation in the calcium metabolism of T lymphocytes may significantly contribute to the diminished immune response of the aged in stress.

Introduction

Intracellular calcium metabolism plays a prominent role in the homeostasis of living cells. Calcium acts as a second messenger, inducing a vast variety of changes in the cell. Elevation of intracellular and intranuclear calcium concentration accompanies activation and apoptosis of many cells, e.g. thymic lymphocytes (1-6).

Various forms of psychosocial stress, such as overcrowding, were shown to modulate the immune response in mice and rats (7-9). Huie *et al* (10) demonstrated a decrease of serum ionized calcium in stressed rats. Besides this finding, knowledge about the changes of intracellular calcium concentration after psychosocial stress is very limited.

The authors' earlier studies indicated that ageing induces a decrease in resting levels of intracellular free calcium of human T lymphocytes (11,12). Transient (10 days) "overcrowd-stress" induced an elevated Ca-response of T lymphocytes from young mice (13). In recent studies the authors also investigated the effect of persistent (90 days) "overcrowd-stress" on Ca-metabolism of T lymphocytes of young and aged mice, finding that T cells from aged mice displayed significantly smaller levels of resting, and lectin-stimulated intracellular calcium concentration, as compared to those of the non-stressed, aged animals[1].

Materials and methods

Reagents and cells

Concanavalin-A (type IV), digitonin, dimethyl-sulfoxide (DMSO), EGTA, foetal calf serum (FCS), Hepes, phytohaemagglutinin, RPMI 1640 medium and succinyl-Concanavalin-A were from Sigma. Fura-2 acetoxymethyl ester (fura-2 AM) was from Calbiochem. Young (20 weeks) and old (24 months) CBA/CA mice were kept under "control" (68 cm^2/animal) or "overcrowded" (22 cm^2/animal) conditions for 10 or 90 days respectively. Splenic T lymphocytes were separated from erythrocytes by hypotonic lysis in ice-cold distilled water and from granulocytes, monocytes and B lymphocytes by plastic adherence in RPMI 1640 medium with 10% FCS.

Measurement of intracellular calcium concentration

Intracellular calcium concentration was measured as described earlier (11,12). Splenic T lymphocytes (5 x 106 cells/ml) were incubated with fura-2 AM at a final concentration of 2 uM in RPMI 1640 medium for 30 minutes at 37°C. After a 5-fold dilution, the incubation was continued for an additional 15 minutes. Cells were washed twice in a modified Hank's medium (143 mM NaCl, 1 mM Na2SO4, 5 mM KCl, 1 mM NaH2PO4, 0.5 mM MgCl2, 1 mM CaCl2, 5 mM glucose, and 10 mM Hepes, pH 7.45). The experiment was completed within 45 minutes. Fluorescence measurements were performed