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Stress-induced changes of cytoplasmic free calcium in lectin-simulated mouse T lymphocytes

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SUMMARY

Extreme environmental or homeostatic changes provoke an adaptive stress-response of the organism. In our present work we have investigated the effect of "overcrowd-stress" on the Concanavalin-A stimulated cytoplasmic free Ca concentration of mouse splenic T lymphocytes. Mice were kept under "normal" (68 cm^2/animal) or "overcrowded" (22 cm^2/animal) conditions for 10 days. No change was observed in resting Ca after overcrowd-stress. The lectin-induced rise in intracellular Ca level of splenic T lymphocytes, however, was three times higher ($p < 0.01$) in stressed mice compared to the control group.

INTRODUCTION

A significant rise in intracellular calcium concentration is one of the first signals of T lymphocyte activation [1,2]. Overcrowding and other forms of psychosocial stress were shown to modulate the immune response in mice and rats [3-6]. In our present study we have investigated the effect of "overcrowd-stress" on resting and stimulated cytoplasmic free Ca concentration of mouse splenic T lymphocytes.

MATERIALS AND METHODS

Reagents and cells
Concanavalin-A (type IV), digitonin, dimethyl-sulfoxide (DMSO), EGTA,

foetal calf serum (FCS), Hepes and the RPMI 1640 medium were from Sigma. fura-2 acetoxymethyl ester (fura-2 AM) was from Calbiochem. Adult (16.5 months old) CBA/CA mice were kept under "control" (59 cm^2/animal) or "overcrowded" (22 cm^2/animal) conditions for 10 days. 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 [7]. Cells (5 x 10^6 cells/ml) were incubated with fura-2 AM at a final concentration of 2 microM 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 Na_2SO_4, 5 mM KCl, 1 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose and 10 mM Hepes, pH 7.45) and the experiment was completed within 45 minutes. Fluorescence measurements were performed at a cell density of 5 x 10^6 cells/ml with gentle stirring using a PTI Deltascan V-1048 D101 type interfaced fluorimeter at 37 °C. Excitation and emission wavelengths were 340 (380) and 520 nm-s, respectively with 5 nm slits. Samples were calibrated with the digitonin (10 microM) EGTA (5 mM) method and the intracellular calcium concentration was calculated as described earlier [7]. Data were corrected for the aspecific lysis of fura-2 [see ref. 7].

Table I.
Resting and Concanavalin A-stimulated intracellular calcium concentration of splenic T lymphocytes in control and stressed mice Isolated splenic T lymphocytes were treated with Concanavalin A ("stimulated" cells) and intracellular calcium concentration was measured as described in Materials and Methods. n = number of animals; [Ca] = intracellular calcium concentration in nM; SEM = standard error of mean. The levels of significance (calculated by the Student's t test) are the following groups 1 and 3: $p < 0.3$ (not significant); groups 2 and 4: $p < 0.01$; groups 1 and 2: $p < 0.05$; groups 3 and 4: $p < 0.001$.

Group No.	Mice	Experiment	n	[Ca] (nM)	± SEM
1	control	resting Ca	9	93.0	± 13.8
2	control	stimulated Ca	8	125.8	± 11.2
3	stressed	resting Ca	23	104.8	± 6.0
4	stressed	stimulated Ca	16	197.5	± 12.9

RESULTS AND CONCLUSIONS

Resting intracellular calcium concentration was not significantly different in control and in stressed animals (cf. groups 1 and 3 in Table I.). Due to the routine correction of the minimal loss of fura-2 from the cells during measurement different experimental protocols (changes in the order of measurement of various samples) did not cause any difference in the final data (data not shown).

If splenic T lymphocytes were stimulated with Concanavalin-A at a final concentration of 5 microg/ml, cells from control animals show a moderate increase in cytoplasmic calcium (+ 32.8 nM) while cells from stressed animals responded with an almost three-fold higher (+ 92.7 nM) increase in intracellular calcium concentration after lectin treatment (see Table I. and Figure 1.). Similar differences were gained if we used phytohemagglutinin (PHA-L, Sigma, 2 microg/ml) as stimulant (data not shown).

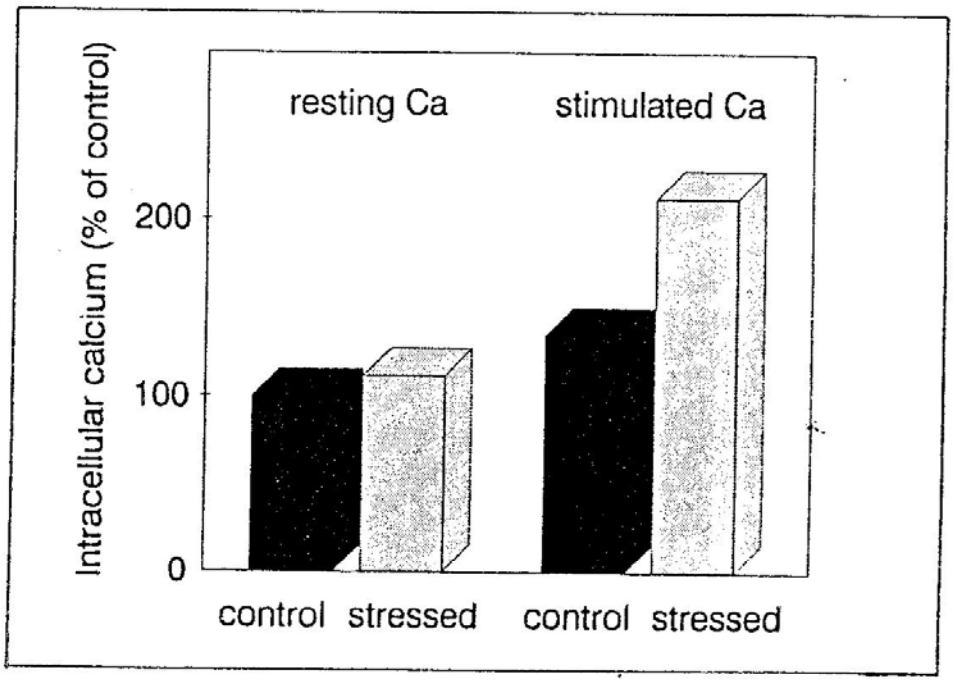

Figure 1.
Differences of resting and lectin-stimulated intracellular calcium concentration in splenic T lymphocytes of control and stressed mice
Intracellular calcium concentration was measured as described in Materials and Methods. Data are expressed as percentages of the control resting intracellular calcium level (93 nM).

Chelation of extracellular calcium (by the addition of extracellular EGTA at a final concentration of 5 mM) abolished the lectin-induced increase in intracellular calcium levels in splenic T lymphocytes from both control and stressed animals (data not shown). Thus, the observed differences in changes of intracellular calcium concentration reflect stress-induced changes in lectin-induced calcium influx to splenic T lymphocytes.

Acute stress induces an elevated intracellular calcium concentration and inositol trisphosphate levels as well as significant increases in both phospholipase A_2 and phospholipase C activities [8,9]. Overcrowding and other forms of psychosocial stress were shown both to stimulate [5] and suppress the immune response in mice and rats [3-6]. Our results

demonstrate that changes in calcium influx during stimulation of T lymphocytes may participate in the immunomodulation of chronic psychosocial stress.

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